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**PROCEDIMIENTO PARA PRODUCIR PROPILENO A PARTIR DE GAS DE
SÍNTESIS MEDIANTE PRODUCCIÓN FERMENTATIVA DE PROPANOL Y
DESHIDRATACIÓN**

5 [Campo de la invención]

La presente invención se refiere a la producción de propileno mediante deshidratación de propanol sobre ventajosamente un catalizador ácido, por la cual el propanol se obtiene por conversión metabólica de gas de síntesis, que se produce por gasificación de biomasa, residuos, carbón, residuos líquidos, 10 efluentes gaseosos de hornos de acero o por reformado de gas natural, en propanol (tanto n-propanol como isopropanol). El suministro limitado y el coste creciente del petróleo crudo han provocado la búsqueda de procedimientos alternativos para producir productos de hidrocarburo tales como propileno. El propanol puede obtenerse por conversión metabólica de gas de síntesis por 15 microorganismos. Constituida por materia orgánica de organismos vivos, la biomasa es la fuente de energía renovable más importante del mundo.

[Antecedentes de la invención]

La producción de propanoles es limitada en comparación con la producción de propileno. En 2005, la producción mundial de n-propanol fue aproximadamente 20 140 kta y de isopropanol superior a 2000 kta, mientras que la producción mundial de propileno supera 65000 kta.

Históricamente, el isopropanol se ha producido por hidratación de propileno sobre catalizador ácido (Kirk-Othmer Encyclopedia of Chemical Technology, 2007 & Ullmann's Encyclopedia of Industrial Chemistry, 2002). En la hidratación 25 indirecta se forman ésteres de sulfato de isopropilo a partir de propileno y ácido

sulfúrico, que posteriormente se hidroliza en isopropanol. En la hidratación directa, el propileno se convierte a alta presión y baja temperatura sobre un catalizador de lecho fijo ácido. El n-propanol puede obtenerse por hidroformilación de etileno mediante el propanal intermedio que se hidrogena adicionalmente en n-propanol.

- 5 Condiciones típicas empleadas en el procedimiento oxo catalizado por fosfina sustituida con rodio a baja presión son 90-130 °C y menos de 28 bar (2,8 MPa), mientras que las condiciones para el procedimiento oxo catalizado por cobalto son 110-180 °C y por encima de 200 bar (20,0 MPa). El propanal se hidrogena posteriormente en n-propanol en presencia de hidrógeno en exceso sobre un
- 10 catalizador metálico, normalmente compuesto de compuestos de cobre, cinc, níquel y cromo.

El propanol también se ha producido por la hidrogenación de acetona sobre catalizador metálico en isopropanol. La acetona es el subproducto en la producción de fenol a partir de cumeno (Ullmann's Encyclopedia of Industrial

15 Chemistry, 2002).

La acetona, como uno de los tres productos en la tecnología de fermentación ABE (acetona-butanol-etanol) basada en azúcares, también podría hidrogenarse en isopropanol ("Bacterial acetone and butanol production by industrial fermentation in the Soviet Union: use of hydrolyzed agricultural waste for

20 biorefinery", Appl. Microbiol. Biotechnol., 71, pág. 587-597, 2006; "History of the Acetone-Butanol-Ethanol Fermentation Industry in China: Development of Continuous Production Technology", J. Mol. Microbiol Biotechnol., 13, pág. 12-14, 2007; "Acetone-butanol fermentation revisited", Microbiol. Rev. 50, pág. 484-524, 1986 y Jones, D., "Applied acetone-butanol fermentation", en "Clostridia.

25 Biotechnology and medical application", pág. 125-168, 2001, Wiley-VCH Verlag

GmbH, Weinheim, Alemania). La fermentación de acetona-butanol-etanol (ABE) por *Clostridium acetobutylicum* es una de las fermentaciones industriales conocidas más antiguas. Se clasificó segunda solo con respecto a la fermentación del etanol por levadura en su escala de producción, y es uno de los mayores procedimientos biotecnológicos jamás conocidos. Sin embargo, desde los años 50, la fermentación ABE industrial ha disminuido gradualmente, y casi toda la acetona se produce ahora mediante rutas petroquímicas. Más recientemente están siendo desarrolladas rutas metabólicas bioquímicas para producir propanol a partir de hidratos de carbono. La patente de EE.UU. 2009/0246842 describe un procedimiento para la fermentación de azúcares en isopropanol.

El propanol también puede producirse a partir de gas de síntesis (mezcla de CO, H₂ y CO₂) por un procedimiento catalítico similar a Fischer-Tropsch, produciendo una mezcla de alcoholes superiores, aunque frecuentemente se produce una formación preferencial de propanol (Applied Catalysis A, general, 186, pág. 407, 1999 y Chemiker Zeitung, 106, pág. 249, 1982). Todavía otra ruta para obtener propanol es la condensación de Guerbet catalizada por base de metanol con etanol (J. of Molecular Catalysis A: Chemical 200, 137, 2003, J. Chem. Soc. Chem. Commun, 22, pág. 1558, 1990 y Applied Biochemistry and Biotechnology, 113-116, pág. 913, 2004).

Mientras que en el pasado los propanoles se han producido habitualmente a partir de etileno o propileno, que son productos químicos de alto valor añadido, hay una necesidad de producir propanoles a partir de otras fuentes de carbono de manera que los propanoles respectivos puedan deshidratarse en propileno.

Desde hace muchos siglos, los azúcares simples son fermentados en etanol con la ayuda de *Saccharomyces cerevisiae*. Las nuevas rutas de

la última década a partir de celulosa y hemicelulosas se han desarrollado para fermentar hidratos de carbono más complejos en etanol. Por lo tanto, los hidratos de carbono necesitan liberarse de la biomasa lignocelulósica. La biomasa consiste en aproximadamente 30% de celulosa, 35% de hemicelulosas y 25% de lignina.

- 5 La fracción de lignina no puede valorizarse como etanol, a partir de su naturaleza aromática, sino que solo puede usarse como fuente de energía que presenta en muchos casos un exceso para poner en marcha una planta industrial. Recientemente están desarrollándose rutas más eficaces que producen gas de síntesis a partir de materiales que contienen carbono y que posteriormente se
- 10 fermenta en etanol ("Bioconversion of synthesis gas into liquid or gaseous fuels", K. Klasson, M. Ackerson, E. Clausen, J. Gaddy, *Enzyme and Microbial Technology*, 14(8), pág. 602, 1992; "Fermentation of Biomass-Generated Producer Gas to Ethanol", R. Datar, R. Shenkman, B. Cateni, R. Huhnke, R. Lewis, *Biotechnology and Bioengineering*, 86 (5), pág. 587, 2004; "Microbiology of
- 15 synthesis gas fermentation for biofuel production", A. Hemstra, J. Sipma, A. Rinzema, A. Stams, *Current Opinion in Biotechnology*, 18, pág. 200, 2007; "Old Acetogens, New Light", H. Drake, A. GoBner, S. Daniel, *Ann. N Y. Acad. Sci.* 1125: 100-128, 2008). El gas de síntesis puede producirse por gasificación de la biomasa completa sin necesidad de liberarla de ciertas fracciones. El gas de
- 20 síntesis también puede producirse a partir de otra materia prima mediante gasificación: (i) carbón, (ii) residuos municipales (iii) residuos plásticos, (iv) coque de petróleo y (v) residuos líquidos de refinerías o de la industrial del papel (licor negro). El gas de síntesis también puede producirse a partir de gas natural mediante reformado con vapor o reformado autotérmico (oxidación parcial). Para
- 25 la síntesis de metanol convencional, la síntesis de alcoholes superiores o Fischer-

Tropsch se requiere una relación de hidrógeno con respecto a monóxido de carbono de aproximadamente 2. En el caso de gasificación de materia prima pobre en hidrógeno, esta relación será inferior a 1 y de ahí que se requiera un desplazamiento de agua-gas ($\text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + \text{H}_2$) para ajustar la relación. La

5 ruta bioquímica para transformar gas de síntesis en etanol es mucho menos rigurosa considerando la relación de hidrógeno con respecto a monóxido de carbono.

La ruta bioquímica de la conversión del gas de síntesis se describe por la ruta de Wood-Ljungdahl. La fermentación del gas de síntesis ofrece varias

10 ventajas tales como alta especificidad de biocatalizadores, menores costes de energía (debido a las condiciones de bioconversión a baja presión y baja temperatura), mayor resistencia al envenenamiento del biocatalizador y casi ninguna limitación para una relación de H_2 con respecto a CO prefijada ("Reactor design issues for synthesis-gas fermentations" M. Bredwell, pág. Srivastava, R. Worden, Biotechnology Progress 15, 834-844, 1999; "Biological conversion of

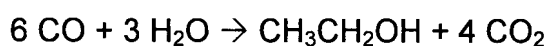
15 synthesis gas into fuels", K. Klasson, C. Ackerson, E. Clausen, J. Gaddy, International Journal of Hydrogen Energy 17, pág. 281, 1992). Los acetógenos son un grupo de bacterias anaerobias que pueden convertir componentes de gas de síntesis como CO, CO_2 y H_2 en acetato mediante la ruta de acetil-CoA reductora o

20 de Wood-Ljungdahl.

Se han aislado varias bacterias anaerobias que tienen la capacidad para fermentar gas de síntesis en etanol, ácido acético y otros productos finales útiles. *Clostridium ljungdahlii* y *Clostridium autoethanogenum* fueron dos de los primeros organismos conocidos por convertir CO, CO_2 y H_2 en etanol y ácido acético.

25 Comúnmente conocidos como acetógenos, estos microorganismos tienen la

capacidad para reducir CO₂ en acetato con el fin de producir energía requerida y producir masa celular. La estequiometría global para la síntesis de etanol usando tres combinaciones diferentes de componentes de gas de síntesis es la siguiente (J. Vega, S. Prieto, B. Elmore, E. Clausen, J. Gaddy, "The Biological Production of Ethanol from Synthesis Gas", Applied Biochemistry and Biotechnology, 20-1, pág. 781, 1989):



Las bacterias acetogénicas son anaerobios obligados que utilizan la ruta de acetil-CoA como mecanismo predominante para la síntesis reductora de acetil-CoA a partir de CO₂ (Drake, H. L. (1994). Acetogenesis. Nueva York: Chapman & Hall). Este grupo de microorganismos es incluso más versátil en el sentido de que pueden usar gases simples como CO₂/H₂ y CO, además de azúcares, ácidos carboxílicos, alcoholes y aminoácidos.

Clostridium ljungdahlii, uno de los primeros microorganismos autotróficos conocidos por fermentar gas de síntesis en etanol, se aisló en 1987; como acetógeno favorece la producción de acetato durante su fase de crecimiento activa (acetogénesis)) mientras que el etanol se produce principalmente como un producto no relacionado con el crecimiento (solventogénesis) ("Biological conversion of synthesis gas into fuels", K. Klasson, C. Ackerson, E. Clausen, J. Gaddy, International Journal of Hydrogen Energy 17, pág. 281, 1992). *Eubacterium limosum* es un acetógeno, aislado de hábitats como el intestino humano, panza, aguas residuales y tierra, presenta alta tasa de crecimiento bajo altas concentraciones de CO, produciendo acetato, etanol, butirato e isobutirato (I.

Chang, B. Kim, R. Lovitt, J. Bang, "Effect of CO partial pressure on cell-recycled continuous CO fermentation by *Eubacterium limosum* KIST612", *Process Biochemistry*, 37(4), pág. 411, 2001).

Peptostreptococcus productus es un coco anaerobio Gram-positivo mesófilo encontrado en el intestino humano y puede metabolizar CO₂/H₂ o CO para producir acetato (W. Lorowitz, M. Bryant, "Peptostreptococcus productus Strain That Grows Rapidly with CO as the Energy-Source", *Applied and Environmental Microbiology*, 47(5), pág. 961, 1984).

Clostridium autoethanogenum es una bacteria móvil tipo bacilo formadora de esporas Gram-positiva estrictamente anaerobia que metaboliza CO para formar etanol, acetato y CO₂ como productos finales, además tiene capacidad para usar CO₂ y H₂, piruvato, xilosa, arabinosa, fructosa, ramnosa y L-glutamato como sustratos (J. Abrini, H. Naveau, E. Nyns, "Clostridium autoethanogenum, Sp-Nov, an Anaerobic Bacterium That Produces Ethanol from Carbon-Monoxide", *Archives of Microbiology*, 161 (4), pág. 345, 1994).

Clostridium carboxidivorans P7 es un anaerobio productor de disolvente que se aisló del sedimento de una pileta de decantación agrícola. Es móvil, Gram-positivo, formador de esporas y principalmente acetogénico, que forma acetato, etanol, butirato y butanol como productos finales (J. Liou, D. Balkwill, G. Drake, R. Tanner, "Clostridium carboxidivorans sp. nov., a solvent-producing Clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen Clostridium scatologenes strain SL1 as Clostridium drakei sp. nov.", *International Journal of Systematic and Evolutionary Microbiology*, 55(5), pág. 2085, 2005).

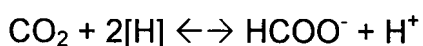
Los acetógenos son bacterias anaerobias obligadas que usan la ruta de acetil-CoA reductora como (i) mecanismo predominante para la síntesis reductora

de acetil-CoA a partir de CO₂, (ii) procedimiento de conservación de energía
 aceptor de electrones terminales, y (iii) mecanismo para la síntesis de carbono
 celular a partir de CO₂ (Drake, H. L. (1994). Acetogenesis. Nueva York: Chapman
 & Hall). Al igual que otros anaerobios, los acetógenos requieren un aceptor de
 5 electrones terminales diferente del oxígeno. En la ruta de acetil-CoA, el CO₂ sirve
 de aceptor de electrones y el H₂ sirve de donante de electrones. La síntesis de
 acetil-CoA a partir de CO₂ e H₂ requiere una reducción de 8 electrones de CO₂
 que implica las tres siguientes etapas:

- Formación del precursor de carbonilo de acetil-CoA
- 10 Formación del precursor de metilo de acetil-CoA
- Condensación de los dos precursores anteriores para formar acetil-CoA.

La rama de metilo de la ruta de acetil-CoA (véase la Figura 1)

- Esta parte de la ruta produce la formación de la proteína metil-corrinoide
 que se combina con el producto de la rama de carbonilo, para formar acetil-CoA.
- 15 En la primera etapa de esta rama, el CO₂ se reduce en formiato (HCOO⁻) como se
 muestra en la siguiente ecuación:

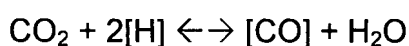


- Esta reacción reversible está catalizada por la enzima formiato
 deshidrogenasa (FDH). La ferredoxina es el aceptor de electrones más
 20 comúnmente empleado, entre acetógenos, la NADH frecuentemente actúa de
 donante de electrones. Para acetógenos cultivados sobre CO, Ljungdahl sugirió
 que el CO debía primero oxidarse en CO₂ por la enzima monóxido de carbono
 deshidrogenasa (CODH) y posteriormente reducirse a formiato por FDH (L.
 Ljungdahl, "The autotrophic pathway of acetate synthesis in acetogenic bacteria",
 25 Annual Review of Microbiology, 40, 415, 1986).

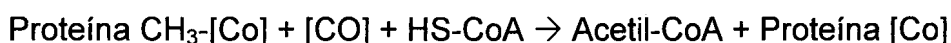
El formiato se activa con tetrahidrofolato (THF) para formar 10-formil-THF por la enzima formil-THF sintetasa en una condensación dependiente de ATP. Este grupo formilo es entonces reducido por una serie de 3 enzimas en un grupo metilo unido (metil-THF). En la etapa final de esta rama, el grupo metilo se transfiere a una proteína que contiene corrinóide proteína [Co].

La rama de carbonilo (véase la Figura 1)

Esta rama de la ruta produce la formación de un grupo carbonilo unido que luego se fusiona con el grupo metilo unido formado en la rama de metilo para formar acetil-CoA. La monóxido de carbono deshidrogenasa (CODH) desempeña una función muy esencial con una doble funcionalidad. Primero, cataliza la oxidación de CO en CO₂, la reducción de CO₂ en carbonilo unido, mediando finalmente en la síntesis de acetil-CoA a partir de los grupos metilo y carbonilo. Por el último motivo, la CODH también se conoce como acetil-CoA sintasa. En la rama de carbonilo, el CO₂ se reduce primero a [CO] ([] indica que el monóxido de carbono está unido a enzima) del siguiente modo:



El resto carbonilo unido se condensa con el resto metilo unido de la rama de metilo para formar un resto acetil-CODH unido. En la etapa final, la CODH condensa el acetilo unido con coenzima A libre formando acetil-CoA del siguiente modo:



Los microorganismos usan enzimas hidrogenasa tanto para desecarbón animal electrones acumulados durante la fermentación mediante formación de hidrógeno o captación de hidrógeno como oxidación para producir energía. La reacción en la que participa el hidrógeno es una reacción reversible catalizada por

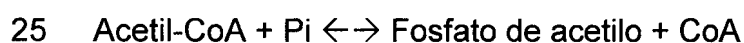
hidrogenasa:



La enzima CODH funciona en combinación con hidrogenasa para formar el precursor de carbonilo de acetil-CoA (L. Ljungdahl, "The autotrophic pathway of acetate synthesis in acetogenic bacteria", Annual Review of Microbiology, 40, 415, 1986).

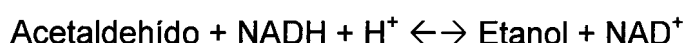
La acetil-CoA es un producto intermedio fundamental en la ruta metabólica de acetógenos ya que es un precursor versátil de alcoholes, ácidos carboxílicos, diácidos, hidroxiácidos, dioles, lípidos, aminoácidos, nucleótidos e hidratos de carbono (Ljungdahl, L., "The autotrophic pathway of acetate synthesis in acetogenic bacteria", Annual Review of Microbiology, 40, pág. 415-450, 1986): (i) para material celular, formado mediante la ruta anabólica, en la que acetil-CoA es reductoramente carboxilada en piruvato por la enzima piruvato sintasa (Diekert, G., "Metabolism of Homoacetogens", Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 66(1-3), pág. 209-221, 1994) que posteriormente se convierte en fosfoenolpiruvato (PEP) que es un producto intermedio en la conversión de biomasa y (ii) para la conservación de energía, la acetil-CoA pasa por la ruta catabólica con el fin de preparar ATP. Además del producto intermedio esencial PEP que es la base de muchas rutas bioquímicas, la acetil-CoA puede condensarse adicionalmente con restos de hidrocarbilo de cadena más larga.

En el caso de homoacetógenos, la acetil-CoA se convierte en acetato catalizado por fosfotransacetilasa y acetato cinasa mientras que produce ATP por fosforilación a nivel de sustrato:



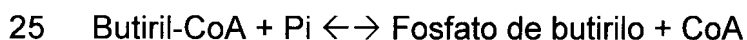


En la primera reacción, la unidad de CoA se elimina de la acetil-CoA y un grupo fosfato es añadido por la enzima fosfotransacetilasa, produciendo formación de fosfato de acetilo. En la segunda reacción, el fosfato de acetilo se convierte en acetato, a la vez que una molécula de adenosin difosfato (ADP) es fosforilada para formar ATP. Esta parte de la ruta de conservación de energía es normalmente favorecida con respecto a la ruta de formación de alcohol durante su fase de crecimiento exponencial del microorganismo ya que proporciona energía a la célula en forma de ATP, conocida como la fase acidogénica del metabolismo, que también produce una disminución en el pH del medio debido a la producción de ácido. La segunda fase de la fermentación es la fase solventogénica, en la que principalmente se produce etanol:



En la rama solventogénica de la ruta, el microorganismo utiliza NADH como potencial de reducción para primero formar acetaldehído por la enzima acetaldehído deshidrogenasa, seguido de la posterior reducción en etanol por la enzima alcohol deshidrogenasa.

Muchos acetógenos (*Clostridium acetobutylicum*) tienen la capacidad para producir productos de 4 carbonos como butanol y ácido butírico por condensación de 2 moléculas de acetil-CoA para formar acetoacetil-CoA que se isomeriza adicionalmente en butiril-CoA. Análogamente a la acetil-CoA, la posterior transformación en ácido butírico produce ATP, mientras que la formación de butanol produce el consumo de equivalentes reductores:



Fosfato de butirilo + ADP $\leftrightarrow$ Butirato + ATP

Butiril-CoA + NADH + H⁺ $\leftrightarrow$ Butiraldehído + CoA-H + NAD⁺

Butiraldehído + NADH + H⁺ $\leftrightarrow$ Butanol + NAD⁺

Rutas para la producción de compuestos oxigenados que tienen tres carbonos:

1. Producción de ácido propiónico

Especies de *Propionibacterium* (*Propionibacterium acidipropionici*, *Propionibacterium acnes*, *Propionibacterium cyclohexanicum*, *Propionibacterium freudenreichii*, *Propionibacterium freudenreichii shermanii*) y varias otras bacterias anaerobias tales como *Desulfobulbus propionicus*, *Pectinatus frisingensis*, *Pelobacter propionicus*, *Veillonella*, *selenomonas*, *Fusobacterium* y *Clostridium*, en particular *Clostridium propionicum*, producen ácido propiónico como producto de fermentación (Playne M., "Propionic and butyric acids", En: Moo-Young M, editor. Comprehensive biotechnology, Nueva York: Pergamon Press, vol. 3, pág. 731-759, 1985; Seshadri N, Mukhopadhyay S., "Influence of environmental parameters on propionic acid upstream bioprocessing by *Propionibacterium acidipropionici*", J. Biotechnology 29, pág. 321-328, 1993). En quesos tipo suizo, las propionibacterias consumen lactato y producen ácido propiónico, ácido acético y CO₂. En general, un amplio intervalo de sustratos puede convertirse en ácido propiónico, como glucosa, lactosa, sacarosa, xilosa, glicerol y lactato. Las propionibacterias son anaerobias mesófilas, con forma de bacilo, no esporulantes, no móviles Gram-positivas. El género *Propionibacterium*, que pertenece a la clase de actinobacterias de alto G+C, se divide en dos grupos: las propionibacterias "cutáneas" y las "lácteas", basándose en su hábitat (Stackebrandt, E., Cummins, C, Johnson, J., "The Genus *Propionibacterium*", en The Prokaryotes, E. Balows, H.

Truper, M. Dworkin, W. Harder, K. Scheifer, eds., 2006).

a. Ruta dicarboxílica

Las propionibacterias convierten fuentes de carbono para producir ácido propiónico como producto principal mediante principalmente la ruta de ácido dicarboxílico (también llamada el ciclo de Wood-Werkman o la ruta de metil-malonil-CoA), como se muestra en la Figura 2. La ruta de la glucólisis cataboliza glucosa en fosfoenolpiruvato (PEP), un metabolito rico en energía. Existen dos rutas de glucólisis alternativas: ruta de Embden-Meyerhorf-Parnaz (EMP) y ruta de hexosa monofosfato (HMP). En la ruta de EMP, 1 mol de glucosa se convierte en 2 moles de PEP y 2 moles de NADH, mientras que en la ruta de HMP 1 mol de glucosa proporciona 5/3 moles de PEP y 11/3 moles de NADH. El PEP se convierte adicionalmente en dos posibles productos intermedios, piruvato y oxaloacetato. La mayoría de los PEP se convierten en piruvato mientras que los PEP restantes se convierten en oxaloacetato. Para la producción de piruvato, 1 mol de PEP se convierte en 1 mol de piruvato y 1 mol de ATP obtenido de una transferencia de un resto fosforilo de PEP en ADP. El ATP total obtenido de las rutas de EMP y HMP por mol de glucosa es 2 y 5/3 moles, respectivamente. La glucólisis mediante la ruta de EMP proporciona una menor cantidad de NADH (EMP: HMP = 2: 11/3), pero una mayor cantidad de ATP (EMP: HMP = 2: 5/3). La relación de la contribución de la ruta de EMP con respecto a HMP en la glucólisis depende de las especies de propionibacteria, sustratos y condiciones de fermentación. En el nodo de piruvato, el piruvato es dirigido hacia tres rutas principales. La mayor parte del piruvato se convierte en ácido propiónico mediante el ciclo de Wood-Werkman. Algo del piruvato se convierte en acetato, mientras que algo se incorpora en biomasa. En la ruta de formación de propionato, el

piruvato entra en el ciclo de Wood-Werkman, mediante una transcarboxilación de un resto carboxilo de metilmalonil-CoA en piruvato, catalizada por oxaloacetato transcarboxilasa en una reacción acoplada de piruvato con oxaloacetato y metilmalonil-CoA con propionil-CoA. En esta reacción acoplada, el grupo carboxilo transferido de metilmalonil-CoA a piruvato para formar propionil-CoA y oxaloacetato nunca es liberado de la reacción o no se observa intercambio entre este grupo carboxilo con el CO_2 disuelto en el caldo de fermentación (Wood HG., "Metabolic cycles in the fermentation of propionic acid", en Current Topics in Cellular regulation, Estabrook y Srera RW, eds., Nueva York: Academic Press, vol 18, pág. 225-287, 1981). Debido a esta reacción de transcarboxilación, la fijación de CO_2 es mínima y solo se usa para producir cantidades catalíticas de oxaloacetato para reiniciar el ciclo cuando, por ejemplo, se acumula succinato como producto final. Bajo tales circunstancias, el oxaloacetato se genera por condensación de CO_2 con fosfoenolpiruvato catalizado por una PEP carboxilasa. Posteriormente, el oxaloacetato se convierte en maleato por maleato deshidrogenasa, maleato en fumarato por fumarasa y adicionalmente fumarato en succinato, catalizadas por succinato deshidrogenasa. Después de eso, el succinato se convierte en succinil-CoA, que luego se convierte en metilmalonil-CoA. La metilmalonil-CoA se convierte en propionil-CoA por oxaloacetato transcarboxilasa. Al final del ciclo, la propionil-CoA se convierte en propionato junto con una reacción acoplada de succinato en succinil-CoA, catalizada por propionil-CoA: succinato transferasa. Después de que 1 mol de piruvato entra en el ciclo de Wood-Werkman, se generan 1 mol de propionato, 2 moles de NAD^+ y 1 mol de ATP. Además del ácido propiónico como producto de fermentación principal, producido en el ciclo de Wood-Werkman, en este ciclo también se

produce la regeneración de NAD^+ para la glucólisis.

En la ruta de la rama de acetato, el piruvato se convierte en acetil-CoA y CO_2 , catalizada por complejo de piruvato deshidrogenasa. La acetil-CoA se convierte en fosfato de acetilo por fosfotransacetilasa y adicionalmente el fosfato de acetilo en acetato, catalizada por acetato cinasa. En la ruta de la rama de acetato se obtiene 1 mol de acetato, CO_2 , NADH y ATP a partir de 1 mol de piruvato. La producción de ácido propiónico va normalmente acompañada de la formación de acetato como principal ruta de producción de ATP que suministra energía para el metabolismo celular.

10 Las siguientes ecuaciones representan una formulación teórica de la fermentación de ácido propiónico a partir de glucosa o lactato (P. Piveteau, Lait, 79, pág. 23, 1999):



15 Según estas ecuaciones, el máximo rendimiento teórico de la glucosa es 66,7% en moles de C o 54,8% en peso de ácido propiónico, 22,2% en moles de C o 22% en peso de ácido acético, 11,1% en moles de C o 17% en peso de CO_2 . La relación molar teórica de ácido propiónico con respecto a ácido acético (P/A) es 2:1.

20 Puede llevarse a cabo un desplazamiento en la ruta metabólica hacia la producción de ácido propiónico usando fuentes de carbono con mayor nivel reductor (desplazamiento de la producción de ácido heterofermentativa a homofermentativa). Un mayor nivel reductor de sustrato puede producir un aumento significativo de la relación de P/A debido al equilibrio de NADH/NAD^+

25 intracelular. Podría esperarse una mejor eficiencia de la producción de ácido

propiónico a partir de glicerol debido a su mayor nivel de reducción en comparación con sustratos convencionales. Eficazmente, un rendimiento de ácido propiónico del 84,4% en moles de C y una baja producción de ácido acético (relación molar de P/A que alcanza 37) han sido obtenidos a partir de glicerol con

- 5 *P. acidipropionici* (Barbirato, F., Chedaille, D. y Bories, A., "Propionic acid fermentation from glycerol: comparison with conventional substrates", Appl Microbiol Biotechnol, **47**, pág. 441-446, 1997). Esta cepa también produce algo de propanol a partir de glicerol, que indica que cuando el sustrato tiene un mayor nivel de reducción también pueden producirse productos con un mayor nivel de
- 10 reducción debido al mejor equilibrio de NADH/NAD⁺.

Glicerol $\rightarrow$ propionato + 1 H₂O

- Himmi y col. compararon la fermentación de glicerol y glucosa y la formación de productos para *P. acidipropionici* y *P. freudenreichii ssp. shermanii*. Los productos de fermentación finales fueron ácido propiónico como producto
- 15 principal, ácido acético como subproducto principal y dos metabolitos secundarios, n-propanol y ácido succínico. El rendimiento del ácido propiónico fue de hasta el 79% en moles de C (64% en peso) con glicerol como fuente de carbono (Himmi, E. H., Bories, A., Boussaid, A. y Hassani, L., "Propionic acid fermentation of glycerol and glucose by *Propionibacterium acidipropionici* and *Propionibacterium*
- 20 *freudenreichii ssp. Shermanii*", Appl Microbiol Biotechnol, 53, pág. 435-440, 2000).

- Los microorganismos de la panza que fermentan lactato mediante la ruta de ácido dicarboxílico producen más propionato con respecto a acetato cuando se añade hidrógeno (M. Schulmunda y D. Valentino, "Factors Influencing Rumen Fermentation: Effect of Hydrogen on formation of Propionate", Journal of Dairy
- 25 Science, vol. 59 (8), pág. 1444-1451, 1976). El ácido acético casi se eliminó

cuando se aplicó una alta presión de H₂ durante la fermentación con hidrogenasa que contenía *Propionispira arboris* (Thompson T.E, Conrad R, Zeikus J.G., "Regulation of carbon and electron flow in *Propionispira arboris*: Physiological function of hydrogenase and its role in homopropionate formation", FEMS Microbiol Lett 22, pág. 265-271, 1984 y patente de EE.UU. 4732855). Según el ciclo de Wood-Werkman, se libera CO₂ endógeno con la formación de ácido acético por propionibacterias de la fermentación de glucosa, lactosa o lactato (Deborde C, Boyaval P. 2000, Interactions between pyruvate and lactate metabolism in *Propionibacterium freudenreichii* subsp. *shermanii*: In vivo ¹³C nuclear magnetic resonance studies, Appl Environ Microbiol 66: 2012-2020). El CO₂ puede fijarse en propionibacterias para formar oxaloacetato a partir de PEP catalizado por PEP carboxilasa y luego conducir a la generación de succinato. Basándose en la ruta metabólica (ciclo de Wood-Werkman), se requiere CO₂ (HCO₃⁻) para convertir fosfoenolpiruvato (PEP) en oxaloacetato por la enzima fosfoenolpiruvato carboxilasa. Mediante varias reacciones secuenciales, la oxaloacetato se convierte finalmente en ácido propiónico. En el caso de glicerol como sustrato, casi no se produce acetato y de ahí CO₂. El aplicar una presión de CO₂ exógena durante la fermentación tiene un efecto positivo sobre la tasa de producción de metabolitos y en particular una mayor acumulación de succinato gracias a la mayor actividad de carboxilación de PEP ("Effect of carbon dioxide on propionic acid productivity from glycerol by *Propionibacterium acidipropionici*", An Zhang y Shang-Tian Yang, SIM annual meeting and Exhibition, San Diego, 2008).

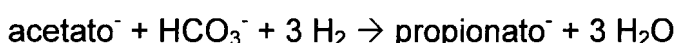
La mayoría de las bacterias productoras de ácido propiónico tienen las enzimas del ciclo de ácido tricarboxílico (TCA) que explican las relaciones de P/A variables para diferentes cepas. Algunas de las acetil-CoA pueden utilizarse en el

ciclo de TCA por condensación con piruvato en citrato (véase la Figura 2). El resultado final es que se produce más CO₂ en el ciclo de TCA mediante las descarboxilaciones y se secreta menos acetato. Se han informado relaciones de P/A de 2,1 a 14,7 y la relación de CO₂/acetato de 1,0 a 6,3 de la glucosa (Wood
 5 HG., "Metabolic cycles in the fermentation of propionic acid", en Current Topics in Cellular regulation, Estabrook y Sreer RW, eds., Nueva York: Academic Press, vol 18, pág. 225-287, 1981).

Se ha mostrado que *Pelobacter propionicus*, usando la ruta de ácido dicarboxílico, se cultiva sobre etanol como sustrato, a la vez que produce
 10 propionato en presencia de CO₂ (Schink, B., Kremer, D. y Hansen, T., "Pathway of propionate formation from ethanol in *Pelobacter propionicus*", Arch. Microbiol. 147, 321-327, 1987 y S. Seeliger, P. Janssen, B. Schink, "Energetics and kinetics of lactate fermentation to acetate and propionate via methylmalonyl-CoA or acrylyl-CoA", FEMS Microbiology Letters, 211, pág. 65-70, 2002). Si se alimenta etanol
 15 junto con CO₂ e hidrógeno, se producen cantidades significativas de propanol. El etanol se convierte en acetil-CoA (mediante acetaldehído), a la vez que produce electrones para la carboxilación de la acetil-CoA en piruvato, catalizada por piruvato sintasa. Combinado con la ruta de ácido dicarboxílico, el propionato se produce a partir de etanol y CO₂ (Schink y col., 1987).



Pelobacter propionicus no puede convertir reductoramente acetato y CO₂ en propionato, mientras que *Desulfobulbus propionicus* prepara propionato a partir de acetato y CO₂ (Schink y col., 1987).



25 **b. Ruta de acrilato**

Aunque muchas bacterias pueden fermentar una variedad de sustratos anaeróbicamente en lactato como producto final, algunas pueden reducir adicionalmente el lactato en propionato, como *Clostridium propionicum*, *Clostridium neopropionicum*, *Megasphaera elsdenii* y *Prevotella ruminicola* (P. Boyaval, C. Corre, "Production of propionic acid", Lait, 75, 453-461, 1995) usando la ruta de acrilil-CoA (véase la Figura 3). Varios sustratos (azúcares, etanol y algunos aminoácidos) que pueden convertirse en piruvato como producto intermedio pueden reducirse adicionalmente en propionato como producto principal con acetato y butirato como co-producto. La reacción clave es la deshidratación de lactil-CoA en acrilil-CoA, que posteriormente es reducida a propionil-CoA. Los electrones para esta reducción se proporcionan por la oxidación de piruvato/lactato en acetato y CO₂ (G. Gottstiza, "Bacterial Metabolism", 2ª ed., Springer, Nueva York, 1986).

Clostridium neopropionicum (cepa X4), usando la ruta de acrilato, puede convertir etanol y CO₂ en acetato, propionato y algo de propanol (J. Tholozan, J. Touzel, E. Samain, J. Grivet, G. Prensier y G. Albagnac, "Clostridium neopropionicum sp. Nov., a strict anaerobic bacterium fermenting ethanol to propionate through acrylate pathway", Arch. Microbiol., 157, pág. 249-257, 1992). Como para la ruta de ácido dicarboxílico, el producto intermedio acetil-CoA producido a partir del sustrato etanol se liga a la ruta de acrilato mediante la piruvato sintasa que convierte la acetil-CoA en piruvato por carboxilación con CO₂.

Recientemente, una ruta alternativa que conduce a acrilil-CoA consiste en la conversión de acetil-CoA en malonil-CoA por carboxilación con CO₂. La malonil-CoA se convierte adicionalmente en acrilil-CoA mediante cuatro etapas que implican malonato-semialdehído, hidroxipropanoato, hidroxipropanoil-CoA y

finalmente acrilil CoA. La acrilil-CoA producida por esta ruta se reduce posteriormente a propionil-CoA similarmente a las reacciones que conducen a acrilil-CoA por deshidratación de lactil-CoA (J. Zarzycki, "Identifying the missing steps of the autotrophic 3-hydroxypropionate CO₂ fixation cycle in *Chloroflexus aurantiacus*, PNAS, 106(50), pág. 21317, 2009; I. Berg, "A 3-hydroxypropionate/ 4-hydroxybutyrate autotrophic carbon dioxide assimilation pathway in archaea, Science, 318, pág.1782, 2007).

2. Producción de acetona/isopropanol

Miembros de la flora de *Clostridium*, *Butyrivibrio*, *Bacillus* y otra flora menos bien definida de los aparatos digestivos anaerobios producen ácido butírico, butanol, acetona, isopropanol o 2,3-butanodiol (A. Moat, J. Foster & M. Spector, "Microbial Physiology", 4ª ed., Wiley-Liss, 2002). Concomitantemente se producen hidrógeno, dióxido de carbono, acetato y etanol en cantidades menores durante la fermentación. *Clostridium acetobutylicum* utiliza la ruta de EMP para el catabolismo de la glucosa con formación de etanol, dióxido de carbono, hidrógeno, acetona, isopropanol, butirato y butanol a partir de piruvato mediante acetil-CoA como se muestra en la Figura 4 (D. Jones y D. Woods, "Acetone-Butanol Fermentation Revisited", Microbiological Reviews, pág. 484-524, 1986; R. Gheshlaghi, J. Scarbón animaler, M. Moo-Young, C. Chou, "Metabolic Pathways of *Clostridia* for producing butanol", Biotechnology Advances, 27, pág. 764, 2009).

Cepas tales como *Clostridium acetobutylicum*, *Clostridium beijerinckii*, *Clostridium sacchaributylicum* y *Clostridium sacchaributylicum* producen butanol en alta concentración con acetona (o isopropanol) y etanol. Durante el crecimiento, el organismo forma primero acetato y butirato (fase acidogénica), desechándose el exceso de electrones

reduciendo H^+ a H_2 . Como el pH disminuye, debido a la acumulación de ácidos, el cultivo está entrando en las fases estacionarias, hay un desplazamiento metabólico a la producción de disolvente (fase solventogénica). La acetil-CoA experimenta condensación para formar acetoacetato, que puede reducirse a

5 butirato y butanol o escindirse mediante descarboxilación en acetona con producción simultánea de CO_2 . La acetona puede reducirse adicionalmente a isopropanol. La acetil-CoA también puede reducirse a acetaldehído y etanol. Durante la fase solventogénica, los azúcares se fermentan directamente a disolventes, mientras que los productos ácidos presentes también se convierten

10 en disolventes. Se activan acetato y butirato en acetil-CoA y butiril-CoA mediante las reacciones catalizadas por acetoacetil-CoA:acetato-CoA transferasa o cinasa y fosfotransacetilasa. Estas acil-CoA se reducen a etanol y butanol por aldehído deshidrogenasa y alcohol deshidrogenasa. La acetona puede reducirse en algunas especies adicionalmente a isopropanol, como en *Clostridium beijerinckii*,

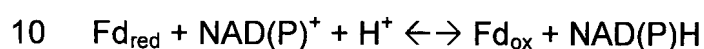
15 ya que la alcohol deshidrogenasa es receptora de diferentes sustratos, no solo para aldehídos, sino también para cetonas (A. Ismaierl, C. Zhu, G. Colby, J. Chen, "Purification and carbón animalacterization of a primary-secondary alcohol dehydrogenase from two strain of *Clostridium beijerinckii*", J. Bacterial., 175, pág. 5097, 1993; J. Chen y S. Hiu, "Acetone-butanol-isopropanol production by

20 *Clostridium Beijerinckii*" Biotechnology Letters, vol 8(5), pág. 371-376, 1986; H. George, J. Jonhson, W. Moore, L. Holdeman, J. Chen, "Acetone, Isopropanol, and Butanol Production by *Clostridium beijerinckii* (syn. *Clostridium butylicum*) and *Clostridium aurantibutyricum*", Applied and Environmental Microbiology, pág. 1160-1163, 1983). La descarboxilación de acetoacetato produce una brusca disminución

25 en el número de aceptores de electrones ($H^+ + e^-$) disponibles a medida que se

expulsa CO₂. La acetil-CoA y la acetona pueden actuar de aceptor de electrones, dando lugar a etanol o isopropanol. Para la aparición de solventogénesis, el flujo de electrones, además del flujo de carbono, es redirigido con el fin de preservar el equilibrio oxido-reductor. Se necesitan más electrones para producir moléculas de

5 disolvente (butanol, isopropanol y etanol) que para los ácidos correspondientes. El exceso de electrones que se usó para reducir H⁺ a H₂ durante la fase acidogénica es usado por aldehído deshidrogenasa y alcohol deshidrogenasa durante la fase solventogénica. En clostridios productores de disolvente, la NAD(P)⁺:ferredoxina oxidorreductasa es activa, intercambiando electrones entre NAD(P)⁺ y ferredoxina.

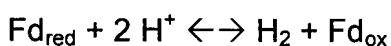


Durante la fase acidogénica, esta enzima es activa para reducir ferredoxina, a la vez que oxida NAD(P)H y cataliza la reacción inversa durante la solventogénesis. La regeneración de NAD(P)⁺ se requiere para la oxidación de gliceraldehído-3-fosfato y por tanto previene la acumulación de NAD(P)H. El H₂

15 producido durante la acidogénesis es recogido por las bacterias para la producción de disolvente. Los clostridios solventogénicos tienen una hidrogenasa productora de H₂, además de una hidrogenasa de captación.

En clostridios y otros anaerobios, el hidrógeno se forma mediante una piruvato:ferredoxina (Fd) oxidorreductasa sin la producción intermedia de formiato.

20 La ferredoxina reducida se convierte en hidrógeno por hidrogenasa:



La hidrogenasa compite con la ferredoxina: NAD(P) reductasa por oxidar la ferredoxina reducida. La tasa de desprendimiento de hidrógeno en la fase

25 acidogénica es significativamente mayor. El flujo de electrones de NADH a Fd_{red} y

25

a H₂ explica por qué estos organismos producen grandes cantidades de H₂. Durante la fase solventogénica, más NAD(P)H se desvía a la formación de alcoholes disolventes por la reducción de los ácidos correspondientes. Se han desarrollado varios procedimientos con el fin de reducir la actividad de

5 hidrogenasa basada en sulfuro de hierro y de ahí reducir la formación de hidrógeno para el beneficio de reducir adicionalmente los ácidos en alcoholes: adición de presión de hidrógeno externa, adición de monóxido de carbono que inhibe la actividad de hidrogenasa basada en hierro, crecimiento bajo condiciones limitantes de hierro y sustratos altamente reducidos co-fermentantes como glicerol

10 (véase Gheshlaghi y col. y Jones y col.). Bajo tales condiciones, el flujo de electrones de la ferredoxina reducida a hidrógeno molecular mediante el sistema de hidrogenasa se inhibe y se desplaza hacia la generación de NAD(P)H mediante la acción de la ferredoxina oxidorreductasa apropiada, produciendo un aumento en la producción de butanol y etanol. Frecuentemente, bajo actividad reducida de la

15 hidrogenasa, también se produce menos acetona/isopropanol a medida que la formación de acetona por descarboxilación de acetoacetato reduce la cantidad de aceptores de electrones disponibles.

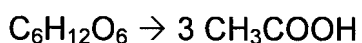
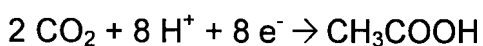
Recientemente se ha manipulado *E. coli* con una ruta específica para la producción de isopropanol a partir de glucosa introduciendo acetil-CoA

20 acetiltransferasa, acetoacetil-CoA transferasa, acetoacetato descarboxilasa y alcohol deshidrogenasa de las especies de *Clostridium* apropiadas (T. Hanai, "Engineered Synthetic Pathway for Isopropanol Production in *Escherichia coli*", Applied and Environmental Microbiology, 73(24), pág. 7814, 2007; T. Jojima, "Production of isopropanol by metabolically engineered *Escherichia coli*", Appl.

25 Microbiol. Biotechnol., 77, pág. 1219, 2008; documentos US 2009/0246842; PCT

2009/049274; EP2184354). Se obtienen rendimientos molares del 43,5%, que es próximo al rendimiento teórico del 50%. Un mol de glucosa se convierte en 2 moles de acetil-CoA y 2 moles de CO₂. Las 2 acetil-CoA se condensan con un acetoacetato que posteriormente se descarboxila para preparar 1 mol de isopropanol y 1 CO₂ adicional.

La producción de ácido acético a partir de glucosa por *C. thermoaceticum* es eficaz porque se producen 3 moléculas de acetato por molécula de glucosa. Se producen dos moléculas de acetato según la muy conocida glucólisis, descarboxilación de piruvato en acetil-CoA y CO₂, seguido de transformación de acetil-CoA en acetato. Durante estas reacciones se producen CO₂ y un exceso de electrones. Algunos microorganismos, tales como *C. thermoaceticum*, también poseen las enzimas de Wood-Ljungdahl que permiten convertir el CO₂ y el exceso de electrones en más acetato.



Esto es particular porque la mayoría de los heterótrofos solo pueden añadir dióxido de carbono a un compuesto preexistente y añadir un grupo carboxilo. Algunas bacterias que pueden formar un compuesto orgánico directamente a partir de dióxido de carbono e hidrógeno contienen hidrogenasa, una enzima que convierte hidrógeno en dos protones y dos electrones. Estos electrones proporcionan el potencial de reducción necesario para la transformación de dióxido de carbono. La reacción global implica participación de ferredoxina como portador de electrones reducido y las enzimas hidrogenasa, monóxido de carbono deshidrogenasa y metilentetrahidrofolato reductasa.

3. Degradación de treonina

Los aminoácidos serina y treonina proporcionan los precursores, directamente o indirectamente, de otros ocho aminoácidos. También pueden derivarse de otro mediante el producto intermedio común glicina. Tanto la serina
5 como la treonina pueden metabolizarse en una única etapa enzimática dando cetoácidos ricos en energía, que pueden catabolizarse para generar ATP por fosforilación a nivel de sustrato, produciendo ácidos carboxílicos. La treonina se deriva de oxaloacetato (véase la Figura 5) mediante transaminación para preparar productos intermedios de aspartato y serina. El oxaloacetato es un metabolito
10 importante del ciclo de TCA que está en el origen de muchos metabolitos esenciales y puede producirse como producto final que condensa de nuevo con acetil-CoA para formar citrato. El oxaloacetato también necesita producirse por condensación de PEP con CO₂ con la ayuda de PEP carboxilasa, particularmente cuando los productos intermedios del ciclo de CTA se agotan para preparar otros
15 metabolitos. Uno de estos metabolitos derivados es la treonina, que se produce directamente a partir de oxaloacetato con la ayuda de múltiples enzimas (véase la Figura 5).

La treonina, un aminoácido nativo, es conocida por ser fermentada por varios microorganismos como *Clostridium tetanomorphum*, *Escherichia coli*,
20 *Salmonella typhimurium* (G. Sawers, "The anaerobic degradation of L-serine and L-threonine in enterobacteria: networks of pathways and regulatory signals", Arch Microbiol, 171, pág. 1-5, 1998; H. Barker, "Amino acid degradation by anaerobic bacteria", Annual Review of Biochemistry 50, pág. 23-40, 1981). Los productos son normalmente acetato, propionato, butirato y 2-aminobutirato y combinaciones del
25 último. La explotación de productos intermedios de aminoácidos nativos como

productos finales se implementa frecuentemente fácilmente en microorganismos ya que no hay perturbación metabólica o hay perturbación metabólica secundaria debida a productos intermedios no tóxicos. La ruta básica de la fermentación de treonina, mediante 2-cetobutirato a propionato, es una ruta muy conocida (G. Gottstiza, "Bacterial Metabolism", 2ª ed. Springer, 1986). Las bacterias entéricas poseen dos tipos de treonina deshidratasa/desaminasa - una enzima catabólica y enzima biosintética que convierten ambas la treonina en 2-cetobutirato (Umbarger H., Brown B., "Threonine deamination in *Escherichia coli*. II. Evidence for two L-threonine deaminases", J. Bacteriol., 73(1), pág. 105-12, 1957). Mientras que la deshidratasa biosintética participa en la biosíntesis de L-isoleucina a partir de 2-cetobutirato, la enzima catabólica participa en la degradación de treonina a propionato, en una ruta que genera ATP, y permite que las bacterias utilicen treonina como la única fuente de carbono y energía, normalmente solo en condiciones anaerobias y cuando están disponibles bajos niveles de energía en la célula (véase la Figura 5). La 2-cetobutirato formiato-liasa solo se expresa en condiciones anaerobias (Sawers y col.). Una vez se forma propanoil-CoA, se procesa mediante propionil-P a propionato, en una secuencia de reacciones que produce ATP.

El uso de una ruta reductora para hacer que el n-propanol deseche electrones generados durante la fermentación de aminoácidos es inusual. El 2-cetobutirato se escinde dando fosfato de propionilo, CO₂ y H₂ (o formiato) por la enzima 2-cetobutirato formiato-liasa en presencia de ferredoxina, CoA-SH y fosfato inorgánico. La cepa 17cr1 de *Clostridium sp.* puede fermentar L-treonina a propionato y propanol (PH. Janssen, "Propanol as an end product of threonine fermentation", Arch Microbiol., 182, pág. 482-486, 2004). Los electrones que se

producen de la oxidación de 2-cetobutirato en propionil-CoA se usan en la ruta reductora que conduce a la formación de propanol. Si se elimina hidrógeno del medio, cesa la formación de propanol.

Las levaduras también tienen la capacidad para convertir aminoácidos en los alcoholes correspondientes (M. Lambrechts, "Yeast and its importance to Wine Aroma - A Review", S. Afr. J. Enol. Vitic, 21, pág. 97, 2000). Éstas se producen según la ruta de Ehrlich en la que los aminoácidos son desaminados al 2-cetoácido correspondiente, que posteriormente se descarboxila y adicionalmente reduce a alcoholes.

Recientemente, la 2-cetoácido descarboxilasa de *Lactococcus lactis* y la alcohol deshidrogenasa de *Saccharbón animalomyces cerevisiae* se han introducido en *E. coli* con el fin de convertir glucosa mediante 2-cetobutirato en n-propanol (C. Shen, "Metabolic engineering of *E. coli* for 1-butanol and 1-propanol production via the ceto-acid pathways", Metabolic Engineering, 10, pág. 312, 2008).

4. Ruta de citramaleato

Se ha informado de una ruta alternativa a 2-cetobutirato de piruvato y acetil-CoA mediante citramaleato sintasa en varios organismos como una ruta alternativa para la producción de isoleucina, en comparación con la desaminación de treonina en 2-cetobutirato; en arqueas como *Ignicoccus hospitalis* y *Methanococcus jannaschii*, en bacterias como *Leptospira interrogans*, *Geobacter sulfurreducens* y en cianobacterias como *Cyanothece* sp. ATCC 51 142 (B. Wu, "Alternative isoleucine synthesis pathway in cyanobacterial species", Microbiology, 156, 596-602, 2010; Howell D., "(R)-citramalate synthase in methanogenic archaea" J. Bacterid., 181 (1), pág. 331-3, 1999; J. Huber, "Insights into the

autotrophic CO₂ fixation pathway of the archaeon *Ignicoccus hospitalis*: comprehensive analysis of the central carbon metabolism", *J Bacteriol.*, 189(11), pág. 4108, 2007). Esta ruta, designada la ruta de citramaleato (véase la Figura 6), es la ruta más directa para sintetizar 2-cetobutirato y no implica transaminación seguida de desaminación. El (R)-citramaleato sintetizado a partir de piruvato y acetil-CoA se convierte entonces en 2-cetobutirato mediante una etapa de isomerasa y deshidrogenasa. Como se ha explicado para la degradación de treonina, el 2-cetobutirato es el precursor para el propionato y el propanol.

Recientemente, la citramaleato sintasa de *Metanococcus jannaschii* se ha introducido en *Escherichia coli* y mejorado por evolución dirigida para permitir la producción directa de n-propanol a partir de glucosa mediante la ruta de citramaleato (S. Atsumi, "Directed Evolution of Methanococcus jannaschii Citramalate Synthase for Biosynthesis of 1-Propanol and 1-Butanol by *Escherichia coli*", *Applied and Environmental Microbiology*, 74(24), pág. 7802-7808, 2008).

5. Ruta de reducción de propanodiol

Puede producirse 1,2-propanodiol por una amplia variedad de bacterias como *Clostridium thermobutyricum*, *Escherichia coli*, *Bacteroides ruminicola* y levaduras. Se conocen tres posibles rutas de biosíntesis: (i) en las que desoxi-azúcares se convierten en fosfato de dihidroxiacetona y lactaldehído que se reduce adicionalmente a propanodiol, (ii) en las que el producto intermedio de glucólisis, fosfato de dihidroxiacetona, de azúcares regulares se convierte en metilglioxal y adicionalmente se reduce a propanodiol (G. Bennet, "Microbial formation, biotechnological production and applications of 1,2-propanediol", *Appl. Microbiol. Biotechnol.*, 55, pág. 1, 2001; R. K. Saxena, "Microbial production and applications of 1,2-propanediol", *Indian Journal of Microbiology*, 50(1), pág. 2-11,

2010).

El 1,2-propanodiol es un producto final de fermentación de los azúcares de 6-desoxihexosa L-ramnosa y L-fucosa, que están abundantemente presentes en hemicelulosa de paredes de células vegetales (Badia, J., "Fermentation mechanisms of fucose and rhamnose in *Salmonella typhimurium* and *Klebsiella pneumonia*", J. Bacteriol., 161, pág. 435-437, 1985; Forsberg, "Metabolism of rhamnose and other sugars by strains of *Clostridium acetobutylicum* and other *Clostridium* species", Can. J. Microbiol., 33, pág. 21, 1987; Tran-Din K., "Formation of 1,2-propanediol and lactate from glucose by *Clostridium sphenoides* under phosphate limitation", Arch. Microbiol., 142, pág. 87, 1985). Estas metilpentosas son metabolizadas por rutas que implican una isomerasa, una cinasa y una aldolasa y forman cantidades equimolares de fosfato de dihidroxiacetona (DHAP) y lactaldehído (véase la Figura 7 para la fermentación de fucosa; existen rutas similares para ramnosa). El DHAP se convierte en ácido pirúvico y se incorpora en el principal metabolismo de los carbonos. Pero el destino del lactaldehído puede diferir según la especie y condiciones. En presencia de oxígeno, el lactaldehído se convierte en lactato. En condiciones anaerobias, el lactaldehído se reduce a 1,2-propanodiol por una propanodiol oxidorreductasa dependiente de NAD.

Varias bacterias, que incluyen *Salmonella*, *Klebsiella*, *Shigella*, *Yersinia*, *Listeria*, *Lactobacillus* y *Lactococcus*, incluyen especies que se cultivan sobre 1,2-propanodiol en una forma dependiente de la coenzima B12 (E. Sampson, "Microcompartments for B12-Dependent 1,2-Propanediol Degradation Provide Protection from DNA and Cellular Damage by a Reactive Metabolic Intermediate", J. of Bacteriology, 190(8), pág. 2966-2971, 2008). La etapa inicial se lleva a cabo por la propanodiol deshidratasa dependiente de coenzima B12, que deshidrata

1,2-propanodiol a propionaldehído. El propionaldehído es entonces desproporcionado a n-propanol y propionil-CoA. La propionil-CoA puede utilizarse en dos formas diferentes dependiendo de la disponibilidad del oxígeno. En condiciones anaerobias, la propionil-CoA se convierte en fosfato de propionilo por fosfotransacilasa y el fosfato de propionilo es escindido por propionil cinasa, produciendo ATP. Tanto el n-propanol como el propionato son normalmente secretados, de ahí que la fermentación de 1,2-propanodiol proporcione un disipador y una fuente de electrones para la producción de ATP y eventualmente un producto intermedio de tres carbonos (propionil-CoA) para el crecimiento por degradación en piruvato y succinato mediante la ruta de metilcitrato.

La segunda ruta es el metabolismo de azúcares comunes (por ejemplo, glucosa o xilosa) mediante la ruta de glucólisis, seguida de la ruta de metilglioxal. El fosfato de dihidroxiacetona se convierte en metilglioxal que puede reducirse tanto a lactaldehído como a acetol. Estos últimos compuestos pueden reducirse adicionalmente dando 1,2-propanodiol (véase la Figura 8, todavía no se han caracterizado muchas de las enzimas que participan). Esta ruta es usada por productores de cepas naturales de 1,2-propanodiol tales como *Clostridium sphenoides* (Tran Din K. y Gottstiza G., Arch. Microbiol. 142: 87-92, 1985) y *Thermoanaerobacter thermosaccharophilus animalolyticum* (Cameron D. y Cooney C., Bio/Technology, 4, pág. 651-654, 1986; Sanchez-Rivera F, Cameron D., Cooney C, Biotechnol. Lett. 9, pág. 449-454, 1987). Los documentos US 6.303.352 y US 2010/0261239 desvelan microorganismos manipulados metabólicos que convierten azúcares en 1,2-propanodiol mediante la ruta de metilglioxal. Hay dos enlaces posibles más directos entre la ruta de metilglioxal y el piruvato mediante la formación de lactato (véase la Figura 8): (i) mediante enzimas glioxalosas, el

metilglioxal (que es frecuentemente tóxico para microorganismos) se transforma en lactato y (ii) mediante lactaldehído deshidrogenasa, el lactaldehído se transforma en lactato (R. Saxena, "Microbial production and application of 1,2-propanediol", Ind. J. Microbiol., 50(1), pág. 2, 2010; J. Weber, "Metabolic flux analysis of Escherichia coli in glucose-limited continuous culture. II. Dynamic response to famine and feast, activation of the methylglyoxal pathway and oscillatory behavior", Microbiology, 151, pág. 707-716, 2005).

La técnica anterior para la producción de propanol que puede deshidratarse en propileno, descrita aquí anteriormente, tiene varias desventajas técnicas que abstienen la comercialización, en particular cuando se compara con la fermentación de etanol de azúcares, produciendo una eficiencia teórica del 66,7% de carbono y eficiencia de casi el 100% de hidrógeno en etanol útil ($C_6H_{12}O_6 \rightarrow 2 C_2H_6O + 2 CO_2$):

- a. Algunas de las rutas descritas implican una etapa de descarboxilación que se desecha el electrón como hidrógeno (o formiato). Esto es el caso para la ruta de acetona/isopropanol en la que el acetoacetato es descarboxilado y la ruta de treonina y citramaleato en la que el 2-cetobutirato es descarboxilado. A partir de azúcares como materia prima, esto reduce el posible rendimiento al 50%.
- b. Algunas de las rutas descritas tienen el potencial de un alto rendimiento de carbono (Wood-Werkman, ruta de acrilato, degradación de propanodiol y degradación de treonina), pero la ruta es principalmente capaz de proporcionar ácido propiónico como producto final, ya que hay un acortamiento de los equivalentes reductores. Frecuentemente, tales rutas conllevan la formación de subproductos como ácido acético que

consume menos equivalentes reductores y de ahí que queden más equivalentes reductores para preparar algo de propanol.

5 c. Como para las rutas metabólicas descritas la materia prima de partida siempre es un hidrato de carbono, el potencial de la valorización de la biomasa está limitado a la parte de hidrato de carbono de la biomasa. Además de esto, las rutas son la mayoría de las veces incapaces de usar azúcares C₅ como materia prima de partida mientras que la biomasa contenga cantidades significativas de hemicelulosas, constituidas principalmente por azúcares C₅.

10 El objetivo de la presente invención es proporcionar un procedimiento para la producción de propileno gasificando materia prima carbonosa, o reformando gas natural en gas de síntesis, convirtiendo el gas de síntesis en propanol (tanto n-propanol como isopropanol) por medio de un microorganismo, que posee las enzimas de Wood-Ljungdahl requeridas y las enzimas de al menos una de las rutas
15 para la producción de compuestos oxigenados C₃, y deshidratando el propanol en propileno y agua. Ventajosamente se deshidrata sobre un catalizador ácido.

El gas de síntesis es una mezcla de hidrógeno, monóxido de carbono y dióxido de carbono. Como los portadores de energía son hidrógeno y monóxido de carbono, se recomienda que el gas de síntesis contenga al menos hidrógeno o
20 monóxido de carbono próximo al dióxido de carbono.

Transformando la materia prima carbonosa en gas de síntesis, la mayor parte del carbono en la materia prima puede valorizarse, que incluye los constituyentes de lignina y de azúcares C₅ de biomasa, pero también puede transformarse en gas de síntesis materia prima carbonosa residual como plásticos
25 residuales, llantas y residuos municipales, carbón, coque de petróleo, residuos

líquidos de procedimientos petroquímicos como refino de crudo o residuos líquidos de la industrial del papel y gas natural, gas asociado o gas no convencional.

En las rutas metabólicas anteriormente descritas, la interconversión de PEP-piruvato-acetil-CoA (que implica las enzimas piruvato fosfato
5 dicinasa/piruvato cinasa y piruvato deshidrogenasa/piruvato sintasa) es el nodo de partida central de la biosíntesis de compuestos oxigenados C_3 . En lugar de producir estos productos intermedios del nodo central directamente a partir de hidratos de carbono, es ventajoso producir acetil-CoA, como parte de este nodo, sustancialmente a partir de gas de síntesis mediante la ruta de Wood-Ljungdahl (la
10 ruta de acetil-CoA reductora). Una vez se ha producido suficiente acetil-CoA en las células, mediante el nodo central, la PEP-piruvato-acetil-CoA, una de las posibles rutas descritas anteriormente, puede producir la formación de compuestos oxigenados C_3 . Para las rutas de biosíntesis que implican la descarboxilación para preparar CO_2 y eliminación de electrón por producción de hidrógeno o implican la
15 descarboxilación para preparar formiato, puede mejorarse con respecto al rendimiento del carbono ya que el CO_2/H_2 o formiato puede ser recirculado mediante las enzimas de la ruta de Wood-Ljungdahl.

Mediante la ruta de Wood-Ljungdahl, el hidrógeno también puede proporcionar electrones requeridos mediante actividad de hidrogenasa para la
20 posterior reducción de ácido propiónico, propanodiol, propanal o acetona.

Es objetivo de la presente invención mejorar el rendimiento de carbono para la producción de propanol a partir de materia prima carbonosa optimizando el flujo de electrón disponible para la producción de propanol combinando las rutas
25 descritas para preparar ácido propiónico, propanodiol, propanal, acetona o propanol con la ruta de Wood-Ljungdahl (la ruta de acetil-CoA reductora) de

manera que electrones desechados puedan ser recirculados mediante las enzimas de la ruta de Wood-Ljungdahl.

La técnica anterior proporciona una primera indicación de que la cepa P11 de *Clostridium* posee la enzima de la ruta de Wood-Ljungdahl, proporcionando la
5 formación de acetil-CoA y probablemente las enzimas de la ruta de acetona/isopropanol. Esta cepa convierte gas de síntesis en isopropanol (9,25 g/l), además de etanol (25,26 g/l), ácido acético (4,82 g/l) y 1-butanol (0,47 g/l) (D. Kundiya, J. of Bioscience and Bioengineering, 109(5), pág. 492, 2010). También se ha informado por E. Caldwell que CAT1 1 y CP19 de *Clostridium* pueden
10 producir acetato, etanol, propionato, propanol y butirato/butanol como productos finales de fermentación de gas de síntesis (M. Caldwell, T. Allen, P. Lawson y R. Tanner, Annual Meeting of the Missouri Valley Branch of the American Society of Microbiology, 27-28 de marzo de 2009, Universidad de Kansas, Lawrence, KS).

Es objetivo de la presente invención aislar de la naturaleza
15 microorganismos de cepas naturales que poseen la información de secuencias de ácidos nucleicos para expresar las enzimas de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida) y que poseen la información de secuencias de ácidos nucleicos de una de las rutas descritas para preparar compuestos oxigenados C₃ (ruta de acetona/isopropanol, ruta de dicarboxílico, ruta de acrilato, ruta de
20 degradación de treonina, ruta de citramaleato o la ruta de reducción de propanodiol).

Es un objetivo de la presente invención producir propileno por deshidratación de propanol que se produce por un microorganismo mejorado que optimiza el flujo de carbono hacia la producción de propanol.

25 El documento US 4.727.214 describe un procedimiento para convertir

etanol anhidro o acuoso en etileno por medio de un catalizador del tipo zeolita cristalina, teniendo dicho catalizador, por una parte, canales o poros formados por ciclos o anillos de átomos de oxígeno que tienen 8 y/o 10 elementos o miembros y, por otra parte, una relación atómica de Si/Al inferior a aproximadamente 20. En los ejemplos, la relación atómica de Si/Al del FER usado es de 5 a 20, la temperatura de 217 a 280 °C y la WHSV de 2,5 h⁻¹.

El documento JP 2009-215244 A publicado el 24 de septiembre de 2009 se refiere a un procedimiento para producir etileno poniendo en contacto etanol sobre un catalizador de H-FER que tiene una Si/Al atómica entre 3 y 20, más específicamente entre 4 y 10, contenidos de sodio y potasio ambos del 0,1% en peso o menos, más específicamente del 0,005% en peso o menos, oscilando la temperatura de 200 a 300 °C, presión de 10 a 100 bar (1,0 a 10,0 MPa) y WHSV de 0,1 a 10 h⁻¹. En los ejemplos, la valoración de la reacción de etanol se logra por el procedimiento de reacción por pulsos de gas usando cromatógrafo de gases. En los ejemplos, la temperatura es 260 °C o inferior.

El documento EP 379803 proporciona un nuevo procedimiento para la preparación de propileno que comprende deshidratar iso-propanol en presencia de un catalizador de alúmina gamma que tiene un diámetro de poro medio de 3-15 nm con una desviación estándar de 1-4 nm basándose en un cálculo estadístico del diámetro de poro y el volumen de poro. Específicamente, el catalizador de alúmina gamma tiene un volumen de poro de al menos 0,4 cm³/g en base seca; es una alúmina gamma de baja alcalinidad que comprende al menos el 90% en peso de alúmina gamma, menos del 10 % en peso de sílice y hasta el 0,5% en peso de óxido alcalino metálico; y es una alúmina gamma débilmente ácida que tiene un pKa de 3,3-6,8 medido por la ecuación de Hammett y una cantidad de ácido

integrada de hasta 0,5 micro-equivalentes por 1 g en base seca. El nuevo procedimiento proporciona propileno con alta selectividad y rendimiento usando un reactor no resistente a la corrosión simple a temperatura menor que para procedimientos convencionales.

- 5 El documento WO 2009-098262 (en una primera realización) se refiere a un procedimiento para la deshidratación de un alcohol que tiene al menos 2 átomos de carbono para preparar la olefina correspondiente, que comprende:

Introducir en un reactor una corriente (A) que comprende al menos un alcohol, opcionalmente agua, opcionalmente un componente inerte, poner en
10 contacto dicha corriente con un catalizador en dicho reactor en condiciones eficaces para deshidratar al menos una parte del alcohol para preparar una olefina,

recuperar de dicho reactor una corriente que contiene olefina (B),

en el que

- 15 el catalizador es:

un silicato cristalino que tiene una relación de Si/Al de al menos aproximadamente 100, o

un silicato cristalino desaluminado, o

una zeolita modificada con fósforo,

- 20 la WHSV de los alcoholes es al menos 2 h^{-1} ,

la temperatura oscila de 280 °C a 500 °C.

El documento WO 2009-098262 (en una segunda realización) se refiere a un procedimiento para la deshidratación de un alcohol que tiene al menos 2 átomos de carbono para preparar la olefina correspondiente, que comprende:

- 25 Introducir en un reactor una corriente (A) que comprende al menos un

alcohol, opcionalmente agua, opcionalmente un componente inerte, poner en contacto dicha corriente con un catalizador en dicho reactor en condiciones eficaces para deshidratar al menos una parte del alcohol para preparar una olefina,

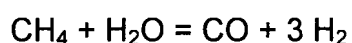
- 5 recuperar de dicho reactor una corriente que contiene olefina (B),
 en el que
 el catalizador es una zeolita modificada con fósforo,
 la temperatura oscila de 280 °C a 500 °C.

Producción de gas de síntesis

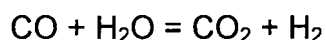
- 10 El gas de síntesis puede producirse a partir de hidrocarburos de bajo peso molecular mediante reformado o a partir de sólido de hidrocarburos líquidos de alto peso molecular mediante gasificación.

Los hidrocarburos de bajo peso molecular se transforman en presencia de vapor y/u oxígeno en gas de síntesis en el "reformado con vapor" (SMR), reformado con CO₂ o reformado autotérmico (ATR) (K. Aasberg-Petersen, "Technologies for large-scale gas conversion", Applied Catalysis A: General, 221, pág. 379-387, 2001)

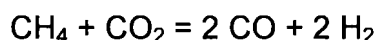
Reformado con vapor



- 20 $\text{C}_n\text{H}_m + n \text{H}_2\text{O} = n \text{CO} + n + (m/2) \text{H}_2$

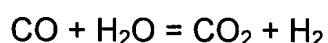
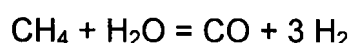


Reformado con CO₂



Reformado autotérmico (ATR)

- 25 $\text{CH}_4 + 1,5 \text{O}_2 = \text{CO} + 2 \text{H}_2\text{O}$



La gasificación es un procedimiento de combustión parcial que convierte materiales carbonosos en CO, CO₂ y H₂. En la reacción de gasificación, cantidades inferiores a las estequiométricas de oxígeno (en forma de aire, oxígeno puro o vapor) se alimentan al reactor a altas temperaturas (superiores a 700 °C) y por tanto los productos solo se reducen parcialmente (C. Higman y M. van der Burgt, "Gasification", Elsevier, 2003; Bridgwater A. "Renewable fuels and chemicals by thermal processing of biomass", Chemical Engineering Journal, 91, pág. 87-102, 2003).

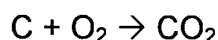
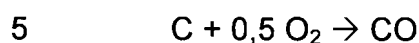
En un gasificador, el material carbonoso se somete a 4 procedimientos:

- (i) Calentamiento inicial para secar cualquier humedad incorporada en el sólido carbonoso
- (ii) El procedimiento de *pirólisis* (o desvolatilización) se produce a medida que se calienta la partícula carbonosa. Los volátiles son liberados y se produce carbón animal, produciendo una significativa pérdida de peso. El procedimiento depende de las propiedades del material carbonoso y determina la estructura y composición del carbón animal, que entonces experimentará reacciones de gasificación.
- (iii) El procedimiento de *combustión* se produce a medida que los productos volátiles y algo del carbón animal reaccionan con O₂ para formar CO₂ y CO, que proporciona calor para las posteriores reacciones de gasificación. La pirólisis y la combustión son procedimientos muy rápidos.
- (iv) El procedimiento de *gasificación* se produce a medida que el carbón animal reacciona con CO₂ y vapor para producir CO e H₂. El gas resultante se

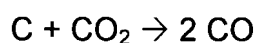
llama gas productor (H_2 , CO y cantidades considerables de CH_4) o gas de síntesis (principalmente H_2 y CO y poco CH_4 , luego "más limpio").

La química de la gasificación del carbón es bastante compleja:

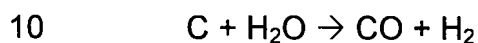
Gasificación con oxígeno:



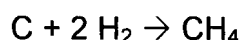
Gasificación con dióxido de carbono:



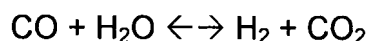
Gasificación con vapor:



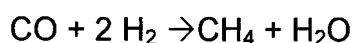
Gasificación con hidrógeno:



Desplazamiento de agua-gas:



15 Metanación:



La gasificación consiste en una serie de reacciones químicas controladas que tienen lugar a hasta 70 bar (7,0 MPa) o más y temperatura de hasta 1400 °C. A medida que la materia prima se expone a temperatura creciente en el gasificador, se produce la desvolatilización y rotura de enlaces químicos más débiles, dando alquitranes, aceites, fenoles y gases de hidrocarburo. Estos productos reaccionan generalmente adicionalmente para formar CO , H_2 y menores cantidades de CO_2 . El carbono fijo que queda después de la desvolatilización se gasifica mediante reacciones con O_2 , agua, CO_2 e H_2 y estos gases reaccionan adicionalmente para producir la mezcla de gases final. La reacción de

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desplazamiento de agua-gas altera la relación de H_2 con respecto a CO de la mezcla de gas final. Las reacciones de metanación son favorecidas por presiones altas y temperaturas bajas. Como no se consume O_2 , las reacciones de metanación exotérmicas (que liberan calor) aumentan la eficiencia de gasificación y el valor de calentamiento del gas de síntesis (gas productor) producido, pero reduce la relación de hidrógeno con respecto a monóxido de carbono, que es preferencialmente tan alta como sea posible para la síntesis química de secuencias.

Bajo condiciones de gasificación reductoras inferiores a las estequiométricas, la mayor parte de la materia prima de azufre se convierte en sulfuro de hidrógeno (H_2S) y algo se convierte en sulfuro de carbonilo (COS). El nitrógeno químicamente unido en la alimentación generalmente se convierte en nitrógeno gaseoso (N_2), formándose también algo de amoníaco (NH_3) y una pequeña cantidad de cianuro de hidrógeno (HCN). El cloro en la alimentación se convierte principalmente en cloruro de hidrógeno (HCl), apareciendo alguno como partículas que contienen cloruro. Los oligoelementos asociados a tanto componentes orgánicos como inorgánicos de la materia prima de carbón, tal como mercurio (Hg) y arsénico (As), aparecen en las diversas fracciones de ceniza, además de en emisiones gaseosas y necesitan eliminarse del gas de síntesis antes de su uso posterior.

[Breve resumen de la invención]

En una realización de la presente invención se proporciona:

Un procedimiento de preparación de propileno por deshidratación de propanol, que implica las siguientes etapas:

1. Gasificar materia prima sólida o líquida carbonosa o reformar materia

prima carbonosa gaseosa en gas de síntesis,

2. Eliminar los contaminantes del gas de síntesis
3. Fermentar el gas de síntesis por medio de un microorganismo en sustancialmente propanol en el que el microorganismo

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1. Es una cepa natural que tiene la capacidad natural para fermentar gas de síntesis en sustancialmente propanol o

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2. Es un microorganismo que posee la información de secuencias de ácidos nucleicos requerida para expresar las enzimas para la biosíntesis de compuestos oxigenados C_3 (ruta de acetona/isopropanol, ruta de dicarboxílico, ruta de acrilato, ruta de degradación de treonina, ruta de citramaleato o la ruta de reducción de propanodiol), modificado confiriéndole la información de secuencias de ácidos nucleicos requerida para expresar las enzimas de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida), o

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3. Es un microorganismo que posee la información de secuencias de ácidos nucleicos requerida para expresar las enzimas de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida), modificado confiriéndole la información de secuencias de ácidos nucleicos requerida para expresar las enzimas para la biosíntesis de compuestos oxigenados C_3 (ruta de acetona/isopropanol, ruta de dicarboxílico, ruta de acrilato, ruta de degradación de treonina, ruta de citramaleato o la ruta de reducción de propanodiol).

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4. Fraccionar y purificar la corriente que contiene predominantemente

propanol,

5. Deshidratar dicha corriente anterior en un reactor en condiciones eficaces para deshidratar al menos una parte del propanol para preparar propileno,

- 5 6. Recuperar de dicho reactor la corriente que contiene propileno.

Es otra realización de la presente invención proporcionar

Un procedimiento de preparación de propileno por deshidratación de propanol, que implica las siguientes etapas:

- 10 1. Gasificar materia prima sólida o líquida carbonosa o reformar materia prima carbonosa gaseosa en gas de síntesis,
2. Eliminar contaminantes del gas de síntesis,
3. Co-fermentar el gas de síntesis con al menos un compuesto oxigenado líquido, como hidratos de carbono, glicerol, propanodiol, lactaldehído, ácido láctico, acetol, metanol, etanol, ácido acético, 15 acetaldehído, ácido propiónico, propanal y acetona, por medio de un microorganismo en sustancialmente propanol en el que el microorganismo

1. Es una cepa natural que tiene la capacidad natural para fermentar gas de síntesis en sustancialmente propanol o
- 20 2. Es un microorganismo que posee la información de secuencias de ácidos nucleicos requerida para expresar las enzimas para la biosíntesis de compuestos oxigenados C₃ (ruta de acetona/isopropanol, ruta de dicarboxílico, ruta de acrilato, ruta de degradación de treonina, ruta de citramaleato o la ruta de reducción de propanodiol), 25

modificado confiriéndole la información de secuencias de ácidos nucleicos requerida para expresar las enzimas de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida), o

5 3. Es un microorganismo que posee la información de secuencias de ácidos nucleicos requerida para expresar las enzimas de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida), modificado confiriéndole la información de secuencias de ácidos nucleicos requerida para expresar las enzimas para la biosíntesis de compuestos oxigenados C₃ (ruta de acetona/isopropanol, ruta de dicarboxílico, ruta de acrilato, ruta de degradación de treonina, ruta de citramaleato o la ruta de reducción de propanodiol).

- 4. Fraccionar y purificar la corriente que contiene predominantemente propanol,
- 15 5. Deshidratar dicha corriente anterior en un reactor en condiciones eficaces para deshidratar al menos una parte del propanol para preparar propileno,
- 6. Recuperar de dicho reactor la corriente que contiene propileno.

20 La corriente que contiene predominantemente propanol puede contener otros alcoholes, por ejemplo, etanol, butanoles.

La etapa de fermentación o de co-fermentación (3) produce una mezcla de alcoholes que contiene al menos 50% en peso de propanol, entre 0 y 50% en peso de etanol y entre 0 y 50% en peso de butanol.

25 En una realización, la relación de peso de dichos alcoholes con respecto al propanol es inferior a 20/80.

En una realización, la relación de peso de dicho alcoholes con respecto al propanol es inferior a 10/90. En una realización, la relación de peso de dicho alcoholes con respecto al propanol es inferior a 5/95.

En una realización, la deshidratación se hace sobre un catalizador ácido a una temperatura de al menos 200 °C y una WHSV de al menos 1 h⁻¹. La deshidratación puede hacerse introduciendo en un reactor una corriente que comprende al menos propanol, opcionalmente agua, opcionalmente un componente inerte, poner en contacto dicha corriente con un catalizador en dicho reactor en condiciones eficaces para deshidratar al menos una parte del propanol para preparar propileno.

Por deshidratación se prepara una olefina que tiene el mismo número de carbonos que el alcohol a deshidratar. Por supuesto, si están presentes otros alcoholes, la deshidratación produce la olefina correspondiente (el etanol conduce a etileno y el butanol conduce a buteno).

15 **[Descripción detallada de la invención]**

Con respecto a la producción de gas de síntesis, puede producirse a partir de hidrocarburos de bajo peso molecular por reformado o a partir de hidrocarburos líquidos de alto peso molecular o sólidos carbonosos mediante gasificación.

20 Esencialmente, el gas de síntesis se forma mediante reacción de reformado con vapor y oxidación parcial de gas natural (Dybkjaer I., "Tubular reforming and autothermal reforming of natural gas - an overview of available processes", Fuel. Proc. Tech., 42, pág. 85, 1995; Moulijn J., "Chemical Process Technology", Wiley & Sons, 2001). En el reformador con vapor (SMR), un catalizador es empaquetado
25 en tubos dispuestos en un calentador a fuego directo. Los tubos del reformador se

calientan externamente por quemadores usando normalmente gas natural. La materia prima para el reformador es una mezcla de gas natural desulfurado y vapor (relación de vapor con respecto a carbono $>3:1$ con el fin de prevenir el coquizado del catalizador). El reformado de metano con vapor es endotérmico y la

5 relación molar de hidrógeno con respecto a monóxido de carbono producida por SMR es aproximadamente $3:1$. Si se añade CO_2 (reformado con CO_2 o reformado en seco) a la materia prima, la relación de H_2/CO puede reducirse. Si la materia prima contiene hidrocarburos pesados, se usa un pre-reformador para reformar primero los hidrocarburos pesados.

10 El reactor del reformador autotérmico (ATR) tiene un diseño compacto constituido por un quemador, cámara de combustión y lecho de catalizador dispuesto en un recipiente a presión revestido de material refractario. La materia prima de hidrocarburo se hace reaccionar con una mezcla de oxígeno y vapor en una llama inferior a la estequiométrica. La relación de vapor con respecto a

15 carbono puede ser de tan solo $0,6$. En el lecho de catalizador fijo, el gas de síntesis se equilibra adicionalmente. La composición del gas producto se determinará por el equilibrio termodinámico en la presión y temperatura de salida, que se determina por el equilibrio de calor adiabático basándose en la composición y flujos de la alimentación, vapor y oxígeno añadidos al reactor. El

20 gas de síntesis producido está completamente libre de hollín.

En el reformador combinado (o reformador de 2 etapas), el reformador tubular (SMR) se combina con un reformador secundario, que actúa de ATR, al que se añade oxígeno.

Hay cuatro tipos principales de gasificadores actualmente usados

25 comercialmente: lecho fijo en contracorriente, lecho fijo en paralelo, lecho fluido y

flujo arrastrado (C. Higman y M. van der Burgt, "Gasification", Elsevier, 2003).

Lecho fijo en contracorriente (corriente de aire ascendente, también llamados gasificadores de lecho móvil): Un lecho fijo de materia prima carbonosa (carbón, coque de petróleo, carbón animal o biomasa) que entra en la parte superior con un flujo en contracorriente de vapor, oxígeno y/o aire circula hacia arriba por el lecho de sólido. La materia prima se seca por el gas de síntesis que abandona la cámara, mientras que el gas que abandona el gasificador se enfría por la materia prima entrante. En el caso de materia prima que contiene oxígeno como biomasa, el carbón animal que se genera continúa bajando por el recipiente del gasificador en el que se reduce y empieza a reaccionar con el oxígeno y CO₂. La materia prima seca se desplaza hacia abajo por el recipiente en el que se produce la gasificación del carbón o carbón animal. Entonces, la ceniza se elimina seca o como una escoria. Las temperaturas de salida del gas son bajas, que es bueno para la eficiencia térmica, pero esto aumenta el alquitrán y las impurezas de metano en el gas de síntesis (McKendry P., "Energy production from biomass (part 1): overview of biomass", Bioresource Technology 83, 37-46, 2002; Bridgwater T., "Review Biomass for energy", Journal of the Science of Food and Agriculture 86, pág. 1755-1768, 2006). Las ventajas de gasificadores de corriente de aire ascendente son su simple construcción, bajo coste, capacidad para manipular contenido de alta humedad e inorgánico y su alta eficiencia energética debido a la menor temperatura del gas que abandona la cámara.

Lecho fijo en paralelo (corriente de aire descendente): Éste es similar al gasificador en contracorriente descrito anteriormente junto al que el vapor, oxígeno y/o aire circula en paralelo hacia abajo con el lecho de materia prima carbonosa sólida. Tiene que añadirse algo de calor al gasificador por combustión o

intercambio de calor. Debido a que el gas pasa por la zona caliente quemando los alquitranes y abandonando el reactor por la parte inferior, de ahí que el producto final tenga una mayor pureza. La temperatura de salida del gas es mayor, produciendo una eficiencia global menor. Tiene un diseño bastante simple y es de bajo coste, y produce un gas relativamente más limpio con muy baja formación de alquitrán. Algunas de las desventajas son que el sistema requiere baja humedad y materia prima de ceniza y tiene baja eficiencia debido a que el gas producto abandona el gasificador a mayores temperaturas, que requiere un sistema de enfriamiento adicional con respecto a un gasificador de corriente de aire ascendente.

Lecho fluido: La materia prima carbonosa se fluidiza en oxígeno/aire y vapor. El tamaño de la materia prima se reduce a un tamaño de partícula pequeño, y se mezcla eventualmente con el material fluidizante, que es normalmente arena de sílice, cerámica o alúmina. El oxidante y la materia prima triturada sólida entran en el reactor por la parte inferior, en la que se forma un lecho caliente, en el que se produce la mayor parte de la conversión en gas de síntesis. La ceniza se elimina como un producto seco ya que se desfluidiza. El rendimiento del combustible es superior al de para un lecho fijo, y tiene la ventaja de distribución de temperatura uniforme alcanzada en la zona de gasificación, produciendo reacciones más limpias. Sin embargo, la conversión global puede ser muy alta, la corriente gaseosa arrastra muchas partículas finas que tienen que separarse del gas y recircularse al gasificador (lecho fluidizado en circulación). Los lechos fluidizados trabajan particularmente bien para biomasa, ya que tiene mucha ceniza altamente corrosiva que dañaría los reactores de lecho fijo.

Flujo arrastrado: Un sólido pulverizado seco, un combustible líquido

atomizado o una suspensión de combustible se gasifica con O_2 (o aire) en flujo en paralelo. Las reacciones de gasificación tienen lugar en una densa nube de partículas muy finas. La mayoría de los carbones son adecuados para este tipo de gasificador debido a las altas temperaturas de operación (muy por encima del punto de fusión de las cenizas, para asegurar una alta conversión de carbono) y debido a que las partículas de carbón están muy separadas las unas de las otras. Las altas temperaturas y presiones también significan que puede lograrse un mayor rendimiento (tiempo de contacto corto); sin embargo, la eficiencia térmica es algo inferior ya que el gas debe enfriarse antes de poder limpiarse con la tecnología existente. Las altas temperaturas también significan que el alquitrán y el CH_4 no están presentes en el gas producto; sin embargo, el requisito de O_2 es mayor que para los otros tipos de gasificadores. Todos los gasificadores de flujo arrastrado eliminan la mayor parte de la ceniza como una escoria ya que la temperatura de operación está muy por encima de la temperatura de fusión de las cenizas. Algunos combustibles (en particular ciertos tipos de biomasa) pueden formar escoria que es corrosiva para las paredes internas cerámicas que sirven para proteger la pared externa del gasificador. Sin embargo, algún tipo de gasificadores de lecho arrastrado no tiene una pared interna cerámica, pero tiene una pared interna refrigerada con agua o vapor cubierta con escoria parcialmente solidificada y de ahí que no sufra escorias corrosivas.

Con respecto al microorganismo, pueden incluir especies microbianas procariotas y eucariotas de los dominios *Archaea*, *Bacteria* y *Eucarya* (incluyendo levadura y hongos filamentosos, protozoos, algas o protistas superiores). El término "procariotas" se refiere a células que no contienen núcleo u otros orgánulos celulares y se clasifican generalmente en uno de dos dominios, *Bacteria*

y *Archaea*. La última diferencia entre organismos de los dominios *Archaea* y *Bacterium* se basa en diferencias fundamentales en la secuencia base de nucleótidos en el ARN ribosómico 16S.

Los microorganismos de la presente invención son tanto microorganismos
5 que se producen naturalmente como microorganismos metabólicamente manipulados.

Los microorganismos que se producen naturalmente de la presente invención se aíslan de la naturaleza, las llamadas cepas naturales que tienen la capacidad para fermentar gas de síntesis en propanol. Tales cepas naturales
10 pueden mejorarse por cribado al azar y selección racionalizada. La mutagénesis al azar se basa en aplicaciones repetidas de tres etapas: (i) mutagénesis con productos químicos o radiación de una población para inducir variabilidad genética, (ii) cribado de la población superviviente para identificar una cepa mejorada bajo prueba de fermentación convencional a pequeña escala y (iii)
15 ensayo de prueba de fermentación para la confirmación de cepas mejoradas (S. Parekh, "Improvement of microbial strains and fermentation processes", Appl. Microbiol. Biotechnol., 54, pág. 287, 2000). Además, puede aplicarse evolución adaptiva para hacer el microorganismo más resistente a ciertas condiciones externas, como mayor tolerancia a disolventes, mayor temperatura de
20 fermentación, mayor presión del gas de síntesis, etc.

La mutagénesis al azar puede realizarse usando un agente mutagenizante físico o químico adecuado. Ejemplos de un agente mutagenizante físico o químico adecuado para el presente fin incluye, pero no se limita a, irradiación UV, irradiación ionizante tal como irradiación gamma, hidroxilamina, N-metil-N'-nitro-N-nitrosoguanidina (MNNG), O-metil-hidroxilamina, ácido nitroso, sulfonato de
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etilmetano (EMS), bisulfito de sodio y análogos de nucleótidos. Si se usan tales agentes, la mutagénesis se realiza normalmente incubando la célula que va a mutagenizarse en presencia del agente mutagenizante de elección bajo condiciones adecuadas, y seleccionando células que muestran una producción
5 significativamente elevada o reducida de la(s) molécula(s) elegidas como diana.

El término “metabólicamente manipulado” implica ruta diseñada racional y ensamblaje de genes biosintéticos, genes asociados a operones y elementos de control de tales secuencias de ácidos nucleicos, para la producción de un metabolito deseado, en particular como el producto final propanol (isopropanol o n-
10 propanol). Engloba adicionalmente la optimización del flujo metabólico por regulación y optimización de la transcripción, traducción, estabilidad de proteínas y funcionalidad de proteínas, funcional en un modo óptimo bajo las condiciones de fermentación aplicadas. Los genes biosintéticos pueden ser heterólogos para el microorganismo huésped, tanto en virtud de ser extraños al huésped como ser
15 modificados por mutagénesis, recombinación y/o asociación con una secuencia de control de la expresión heteróloga en una célula huésped endógena. Por consiguiente, los microorganismos “metabólicamente manipulados” son creados mediante la introducción de material genético en un microorganismo huésped de elección, adquiriendo así nuevas propiedades, por ejemplo, la capacidad para
20 producir un nuevo metabolito o mayor cantidad de metabolito. También engloba la activación de secuencias de ácidos nucleicos endógenas que codifican una enzima diana mediante modificación genética de, por ejemplo, una secuencia promotora en un microorganismo huésped y la introducción de secuencias de ácidos nucleicos exógenas que codifican una enzima diana en un microorganismo
25 huésped. La introducción de material genético (que contiene gene(s), o partes de

genes, que codifican una o más de las enzimas que participan en una ruta biosintética para la producción de isopropanol o n-propanol, y eventualmente que incluyen elementos adicionales para la expresión (o regulación) de estos genes, por ejemplo, secuencias promotoras) en un microorganismo huésped produce una
5 nueva capacidad o capacidad mejorada para producir isopropanol o n-propanol a partir de gas de síntesis.

Un "metabolito" se refiere a cualquier sustancia producida por metabolismo anabólico o catabólico. Un metabolito intermedio importante es acetil-CoA, producido a partir de gas de síntesis u otros compuestos oxigenados como
10 hidratos de carbono, etanol, ácido acético, requeridos para la síntesis del metabolito final deseado isopropanol o n-propanol.

Con el fin de expresar la ruta metabólica de interés y/o reorientar el flujo metabólico, la célula huésped de la invención podría modificarse genéticamente usando tecnologías convencionales conocidas para el experto en la técnica. Por
15 ejemplo, si los presentes inventores quieren eliminar una ruta para aumentar el flujo metabólico para la producción de propanol, las secuencias del gen responsables de la producción de enzimas de la ruta endógena pueden inactivarse o eliminarse parcialmente o totalmente. La inactivación podría obtenerse por modificación de las regiones estructurales o reguladoras respectivas (tal como
20 genes). Técnicas conocidas y útiles incluyen, pero no se limitan a, mutagénesis específica o al azar, mutagénesis generada por PCR, delección de ADN específica para sitio, inserción y/o sustitución, rotura de genes o sustitución de genes, técnicas antisentido, o una combinación de los mismos.

El término "heterólogo" o "exógeno" indica enzimas o ácidos nucleicos que
25 se expresan en un organismo distinto del organismo a partir del cual se originaron.

El término "endógeno" indica enzimas y ácidos nucleicos que se expresan en el organismo en el que se originaron.

El "microorganismo recombinante" resultante es un microorganismo huésped que ha sido modificado genéticamente para expresar o expresar en
5 exceso secuencias de ácidos nucleicos endógenas, o para expresar secuencias de ácidos nucleicos no endógenas tales como aquellas incluidas en un vector (que codifica una enzima diana o enzimas que participan en una ruta metabólica para producir un metabolito deseado). Se entiende que los términos "microorganismo recombinante" también se refieren a la progenie.

10 El término "microorganismo huésped" representa (i) una célula que se produce en la naturaleza, es decir, una célula "natural" que no se ha modificado genéticamente o (ii) una célula que se ha modificado genéticamente, pero que no expresa o expresa en exceso una enzima diana (tal microorganismo puede actuar de huésped en la generación de un microorganismo modificado para expresar o
15 expresar en exceso otra enzima diana).

Procedimientos de expresión en exceso en microorganismos son muy conocidos en la técnica, y cualquier de tal procedimiento se contempla para su uso en la construcción de los microorganismos de la presente invención.

Cualquier procedimiento puede usarse para introducir una molécula de
20 ácido nucleico exógena en microorganismos y muchos de tales procedimientos son muy conocidos para aquellos expertos en la técnica: por ejemplo, transformación, electroporación, conjugación y fusión de protoplastos son procedimientos comunes para introducir ácido nucleico en microorganismos (J. Dale, "Molecular Genetics of bacteria", 4 ed., Wiley, 2004; C. Smolke, "The
25 Metabolic Pathway Engineering Handbook", CRC Press, 2010).

La molécula de ácido nucleico exógena transportada a un microorganismo huésped puede mantenerse dentro de esa célula en cualquier forma: por ejemplo, moléculas de ácidos nucleicos exógenas pueden incorporarse en el genoma de la célula o mantenerse en un estado episómico (elementos genéticos extracromosómicos, como plásmidos) que puede pasarse establemente a células hijas. Los microorganismos descritos en el presente documento pueden contener una única copia, o múltiples copias de una molécula de ácido nucleico exógena particular.

Los procedimientos de expresión de enzimas de una molécula de ácido nucleico exógena son muy conocidos para aquellos expertos en la técnica, que incluyen, sin limitación, ensamblar una secuencia de ácidos nucleicos de forma que un elemento regulador promueva la expresión de una secuencia de ácidos nucleicos que codifica la enzima deseada. Normalmente, elementos reguladores (promotores, potenciadores, etc.) son secuencias de ácidos nucleicos que regulan la expresión de otras secuencias de ácidos nucleicos al nivel de transcripción.

Adicionalmente, cuando la expresión de cierta actividad enzimática va a reprimirse o eliminarse, el gen para la enzima, proteína o ARN relevante puede eliminarse por técnicas de delección conocidas. Estas técnicas de delección son útiles para mejorar más el rendimiento de isopropanol o n-propanol eliminando las rutas que conducen a subproductos, como compuestos oxigenados C_2 o C_4 o hidrógeno. Pueden usarse elementos de control heterólogos para reprimir la expresión de genes endógenos. Pueden usarse técnicas de delección para optimizar tanto la cepa natural como los microorganismos metabólicamente manipulados.

Es una realización de la presente invención que el gas de síntesis pueda

co-fermentarse con compuestos oxigenados como hidratos de carbono, glicerol, propanodiol, lactaldehído, ácido láctico, acetol, metanol, etanol, ácido acético, acetaldehído, ácido propiónico, propanal y acetona. Los hidratos de carbono son esencialmente “azúcares derivados de biomasa” e incluyen, pero no se limitan a, 5 moléculas tales como glucosa, manosa, fucosa, ramnosa, xilosa y arabinosa, lactosa, sorbosa, fructosa, idosa, galactosa, manosa y dímeros, oligómeros o polímeros de la última (almidón, celulosa, hemicelulosas y pectinas).

Con respecto a la fermentación, ésta es conocida por sí misma y está documentada en la bibliografía (P. Munasinghe, “Biomass-derived syngas 10 fermentation into biofuels: Opportunities and challenges”, Bioresource Technology, 101 (13), pág. 5013, 2010; Capítulo 11 y 13 de “Bioenergy and Biofuel from biowastes and biomass”, S. Khanal ed., ASCE, 2010; J. Williams, “Keys to bioreactor selection”, Chemical Engineering Progress, pág. 34, marzo de 2002). La fermentación de gas de síntesis puede llevarse a cabo en tanto biorreactores de 15 flujo discontinuo como continuo. En reactores discontinuos, el sustrato gaseoso se introduce en el biorreactor de sistema cerrado en el que el gas de síntesis se suministra continuamente, pero los productos quedan en el reactor o pueden extraerse en un momento seleccionado durante la fermentación. Los reactores de columna de burbujeo, reactores de biopelícula monolíticos, reactores de 20 membrana y reactores de lecho de goteo son algunos de los otros biorreactores comunes. En algunos de los biorreactores pueden implementarse burbujeadores de microburbujas que potencian la transferencia de masa de dos formas (M. Bredwell, P. Srivastava y R.M. Worden, “Reactor design issues for synthesis-gas fermentations”, Biotechnology Progress, 15, pág. 834-844, 1999). En primer lugar, 25 la disminución de los tamaños de burbuja produce aumentos de la presión interna,

conduciendo a un aumento en la fuerza conductora. En segundo lugar, el gradiente de concentración en fase líquida en estado estacionario en la superficie de la burbuja es inversamente proporcional al diámetro. Muchas otras técnicas esenciales (cinética de fermentación, preservación de microorganismo, medios
5 nutrientes, esterilización, desarrollo de inóculos y diseño del fermentador) para implementar la fermentación se describen en libros de referencia (P. Stanbury, "Principles of fermentation technology", Elsevier Science, 2003; B. McNeil, "Practical Fermentation Technology", John Wiley&Son, 2008)

- 10 a. El reactor de tanque agitado continuo es el biorreactor más común empleado en la fermentación de gas de síntesis. Un CSTR tiene un flujo continuo de gas a través de un volumen de líquido constante. El líquido consiste en una suspensión del microorganismo y una disolución diluida de nutrientes esenciales para que el microorganismo se cultive y sobreviva y una preparación de tal disolución de nutriente líquido y/o
15 suspensión de microorganismos es continuamente o intermitentemente añadida. El gas de síntesis es burbujeado a través de un burbujeador y se aplica una agitación para potenciar la transferencia de masa entre las dos fases. El producto de fermentación se extrae del sistema a la misma velocidad de flujo que la alimentación entra en el reactor. Pueden usarse
20 sistemas de recirculación de células conjuntamente con el CSTR para aumentar la densidad de células dentro del reactor. En un sistema tal, el caldo de fermentación es bombeado por un filtro de recirculación, decantador o centrífuga y el concentrado (que contiene las células del microorganismo) se separa del permeado (medio sin células) y se
25 recircula al biorreactor. Un grado de agitación o mezcla potenciado se

lleva a cabo por impulsores con deflectores para potenciar la transferencia de masa entre el sustrato y los microorganismos. Mayores velocidades de rotación de los impulsores tienden a romper las burbujas de gas en otras más finas, aumentando así el área interfacial entre el gas de síntesis y la suspensión acuosa de los microorganismos.

- b. Los reactores de columna de burbujeo son los más adecuados para aplicaciones industriales de volúmenes muy grandes. Estos reactores tienen una gran relación de altura con respecto a diámetro (relación de aspecto) en los que puede obtenerse una alta transferencia de masa incluso sin el uso de agitación adicional. Puede obtenerse tamaño de burbujas más pequeño y dispersión de gases mejorada usando dispositivos especiales para dispersar el gas de síntesis en el fondo del bioreactor, como discos fritados porosos o anillos de múltiples burbujeadores para dispersar el gas de síntesis. Los reactores de elevación de gas son como columnas de burbujeo que contienen un tubo de aspiración. El gas es dispersado en el fondo de un tubo de aspiración central que controla la circulación del gas y el medio de cultivo. El gas sube por el tubo formando burbujas que arrastran la suspensión líquida y el gas de escape se libera en la cabeza de la columna. Entonces, el líquido desgasificado circula hacia abajo externamente al tubo de aspiración y el producto es drenado del tanque. Como hay una dirección de flujo controlada de la suspensión líquida, el tubo puede diseñarse para servir de intercambiador de calor interno, o un intercambiador de calor puede añadirse a un circuito de circulación interna. Una variación en este reactor de elevación de gas es el reactor

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de elevación de gas externo constituido por una sección elevadora interna y un tubo de retorno descendente externo. Por el elevador, el gas sube e induce la suspensión líquida hacia arriba. En la parte superior, el gas es liberado y la suspensión líquida vuelve mediante el tubo de retorno descendente externo de nuevo al fondo del reactor. El tubo de retorno descendente puede servir de intercambiador de calor si se requiere.

- c. Los reactores de biopelícula monolíticos son como un haz de pequeños canales hechos de material poroso sólido sobre el que crece una biopelícula de microorganismos. Presenta una buena transferencia de masa de gas a la biopelícula y una baja caída de presión. Durante la operación, los microorganismos unidos en la biopelícula utilizan los sustratos gaseosos para producir productos de fermentación. Los reactores monolíticos tiene altas características de transferencia de masa, en particular en operación multifásica. El líquido (normalmente disolución acuosa que contiene los nutrientes y productos de fermentación) se mueve como una delgada película sobre la superficie del canal y el gas circula por el centro de los canales. Si la velocidad de flujo del líquido es alta o la velocidad de flujo del gas baja, gas y líquido se mueven por el canal como bolsas separadas (flujo de Taylor). La burbuja de gas llena casi completamente el diámetro del canal, dejando solo una película muy delgada de líquido próxima a la biopelícula estancada, por consiguiente es posible una alta transferencia de masa de gas-biopelícula estancada. La relación de líquido/gas apropiada con el fin de alcanzar flujo de Taylor puede instalarse recirculando el líquido

alrededor del monolito.

- 5 d. Los reactores de lecho de goteo son reactores continuos de lechos empaquetados en los que el cultivo líquido circula hacia abajo, goteando sobre la superficie del empaquetamiento, a la vez que se maximiza la superficie de separación gas-líquido. Se permite que el gas de síntesis se mueva tanto en dirección hacia abajo (paralelo) como hacia arriba (contracorriente). Como estos tipos de reactores no requieren agitación mecánica, el consumo de potencia de los reactores de lecho de goteo es inferior al de CSTR.
- 10 e. El biorreactor basado en membrana consiste en membranas (lámina plana (FS), fibra multitubular o hueca (HFM)) que permiten facilitar eficazmente la transferencia de masa en medios de cultivo acuosos (K. Lee y B. Rittmann, "Applying a novel autohydrogenotrophic hollow-fiber membrane biofilm reactor for denitrification of drinking water", Water Research, 36, pág. 2040-2052, 2001; S. Judd, "The MBR Book", Elsevier, 2006; patente de EE.UU. 2009/0215163). En el reactor de
- 15 membrana, el gas de síntesis difunde a través de las paredes de las membranas sin formar burbujas. La comunidad microbiana crece y se establece como una biopelícula sobre la pared de las membranas en las
- 20 que fermenta continuamente H_2 y CO en alcoholes. Estos biorreactores de membrana pueden operarse bajo alta presión con mayores tasas de transferencia de masa y volúmenes de reactor reducidos.

25 **Con respecto al fraccionamiento y purificación del propanol**, el propanol como producto principal de la fermentación se fracciona por cualquier medio convencional, como arrastre (evaporación por cambio brusco de presión) de

los gases disueltos en el caldo de fermentación, como filtración, decantación y/o centrifugación para eliminar sólidos (microorganismo y cualquier otro material precipitado) de la disolución acuosa, como destilación (arrastre y/o rectificación) con el fin de concentrar la corriente de propanol o como separación de membrana
5 con el fin de aislar el propanol de la disolución acuosa o caldo de fermentación.

Antes de ir al reactor de deshidratación, el propanol (y eventualmente el etanol y el butanol) pueden purificarse por el procedimiento del documento WO 2010/060981 cuyo contenido se incorpora en la presente solicitud. Describe un procedimiento para la purificación de un alcohol en el transcurso de un
10 procedimiento que comprende: (1) proporcionar una zona de reacción (C) que comprende un catalizador tipo ácido; (2) proporcionar una zona de reacción (B) que comprende un material adsorbente ácido; (3) proporcionar una corriente de alcohol que comprende impurezas; (4) introducir la corriente de alcohol de (3) en la zona de reacción (B) y poner dicha corriente en contacto con el material
15 adsorbente ácido a condiciones eficaces para reducir la cantidad de impurezas que tienen un efecto adverso sobre el catalizador tipo ácido de la zona de reacción (C); (5) recuperar de la etapa (4) una corriente de alcohol e introducirla en la zona de reacción (C); (6) opcionalmente introducir uno o más reactivos (R) en la zona de reacción (C); (7) operar dicha zona de reacción (C) a condiciones eficaces para
20 recuperar un efluente valioso.

Con respecto a la deshidratación del propanol, la deshidratación de alcoholes es por sí misma conocida. La deshidratación de alcoholes se ha descrito en los documentos WO-2009-098262 y WO-2009-098268 cuyo contenido se incorpora en la presente solicitud.

25 El documento WO-2009-098262 se refiere a un procedimiento para la

deshidratación de al menos un alcohol para preparar al menos una olefina, en el que el catalizador es:

- un silicato cristalino que tiene una relación de Si/Al de al menos aproximadamente 100, o

5 • un silicato cristalino desaluminado, o

- una zeolita modificada con fósforo, la WHSV de los alcoholes es al menos 2 h^{-1} , la temperatura oscila de 280°C a 500°C . También se refiere al mismo procedimiento que antes, pero en el que el catalizador es una zeolita modificada con fósforo y a cualquier WHSV.

10 El documento WO-2009-098268 se refiere a un procedimiento para la deshidratación de al menos un alcohol para preparar al menos una olefina, que comprende:

a) introducir en un reactor una corriente (A) que comprende al menos un alcohol opcionalmente en disolución acuosa y un componente inerte,

15 b) poner en contacto dicha corriente con un catalizador en dicho reactor en condiciones eficaces para deshidratar al menos una parte del alcohol para preparar una olefina,

c) recuperar de dicho reactor una corriente (B) que comprende: el componente inerte y al menos una olefina, agua y opcionalmente alcohol sin

20 convertir,

d) opcionalmente fraccionar la corriente (B) para recuperar el alcohol sin convertir y recircular dicho alcohol sin convertir al reactor de la etapa a),

e) opcionalmente fraccionar la corriente (B) para recuperar el componente inerte y la olefina y recircular dicho componente inerte al reactor de la etapa a), en

25 el que el componente inerte está seleccionado entre etano, teniendo los

hidrocarburos de 3 a 10 átomos de carbono, naftenos y CO_2 , la proporción del componente inerte es tal que el reactor opera esencialmente adiabáticamente.

También se refiere a un procedimiento similar al de antes, pero el catalizador es:

- un silicato cristalino que tiene una relación de Si/Al de al menos 100, o
- 5 • un silicato cristalino desaluminado, o
- una zeolita modificada con fósforo, la WHSV del alcohol es al menos 2 h^{-1} , en el que el catalizador es un silicato cristalino que tiene una relación de Si/Al de al menos 100 o un silicato cristalino desaluminado. Ventajosamente, la presión del reactor de deshidratación es suficientemente alta para ayudar en la recuperación
- 10 del componente inerte y recircular el mismo en el reactor de la etapa a) sin un compresor de gas, sino solo una bomba.

Con respecto a la corriente de propanol, el propanol puede someterse a deshidratación solo o en mezcla con un medio inerte. El componente inerte es cualquier componente, siempre que no haya efecto adverso sobre el catalizador.

- 15 Debido a que la deshidratación es endotérmica, el componente inerte puede usarse para aportar energía. El componente inerte puede seleccionarse entre los hidrocarburos saturados que tienen, hasta 10 átomos de carbono, naftenos, nitrógeno y CO_2 . Ventajosamente es un hidrocarburo saturado o una mezcla de hidrocarburos saturados que tiene de 3 a 7 átomos de carbono, más
- 20 ventajosamente que tiene de 4 a 6 átomos de carbono y es preferentemente pentano. Un ejemplo de componente inerte puede ser cualquier compuesto saturado individual, una mezcla sintética de compuestos saturados individuales, además de algunas corrientes de refinería equilibradas como nafta lineal, butanos, etc. Ventajosamente, el componente inerte es un hidrocarburo saturado que tiene
- 25 de 3 a 6 átomos de carbono y es preferentemente pentano. Las proporciones en

peso de respectivamente propanol, agua y componente inerte son, por ejemplo, 5-100/0-95/0-95 (siendo el total 100). La corriente de alcohol, que contiene sustancialmente propanol, también puede contener etanol y butanoles. La concentración de propanol dentro de los alcoholes debe ser al menos el 50% en peso; la concentración de etanol entre el 0 y el 50% en peso y el butanol entre el 0 y el 50% en peso.

Con respecto al reactor de deshidratación, puede ser un reactor de lecho fijo, un reactor de lecho móvil o un reactor de lecho fluidizado. Un reactor de lecho fluido típico es uno del tipo de FCC usado para el craqueo catalítico en lecho fluidizado en la refinería de petróleo. Un reactor de lecho móvil típico es del tipo de reformado catalítico continuo. La deshidratación puede realizarse continuamente en una configuración de reactor de lecho fijo usando un par de reactores de "balanceo" paralelos. Se ha encontrado que los diversos catalizadores preferidos de la presente invención presentan alta estabilidad. Esto permite que el procedimiento de deshidratación se realice continuamente en dos reactores de "balanceo" paralelos en los que cuando un reactor está operando, el otro reactor está sometiendo a regeneración el catalizador. El catalizador de la presente invención también puede regenerarse varias veces.

Con respecto a la presión de deshidratación, puede ser cualquier presión, pero es más fácil y económico operar a presión moderada. A modo de ejemplo, la presión del reactor oscila de 0,5 a 30 bares absolutos (50 kPa a 3 MPa), ventajosamente de 0,5 a 5 bares absolutos (50 kPa a 0,5 MPa), más ventajosamente de 1,2 a 5 bares absolutos (0,12 MPa a 0,5 MPa) y preferentemente de 1,2 a 4 bares absolutos (0,12 MPa a 0,4 MPa). Ventajosamente, la presión parcial de los alcoholes (propanol y eventualmente

etanol y butanol) es de 1,2 a 4 bares absolutos (0,12 MPa a 0,4 MPa), más ventajosamente de 1,2 a 3,5 bares absolutos (0,35 MPa).

Con respecto a la temperatura de deshidratación, y la primera realización, oscila de 200 °C a 600 °C, ventajosamente de 300 °C a 580 °C, más
5 ventajosamente de 350 °C a 580 °C. Con respecto a la temperatura y la segunda realización, oscila de 320 °C a 600 °C, ventajosamente de 320 °C a 580 °C, más ventajosamente de 350 °C a 580 °C.

Estas temperaturas de reacción se refieren sustancialmente a temperatura promedio del lecho de catalizador. La deshidratación del propanol es una reacción
10 endotérmica y requiere la entrada de calor de reacción con el fin de mantener la actividad del catalizador suficientemente alta y desplazar el equilibrio termodinámico a niveles de conversión suficientemente alta.

En el caso de reactores de lecho fluidizado: (i) para lechos fluidizados estacionarios sin circulación de catalizador, la temperatura de reacción es
15 sustancialmente homogénea en todo el lecho de catalizador; (ii) en el caso de lechos fluidizados en circulación en los que el catalizador circula entre una sección de reacción de conversión y una sección de regeneración de catalizador, dependiendo del grado de retromezcla del catalizador, la temperatura en el lecho de catalizador se aproxima a condiciones homogéneas (mucho retromezcla) o se
20 aproxima a condiciones de flujo pistón (casi no hay retromezcla) y, por tanto, se instalará un perfil de temperatura decreciente a medida que avanza la conversión.

En el caso de reactor de lecho fijo o de lecho móvil, se instalará un perfil de temperatura decreciente a medida que avanza la conversión de los alcoholes. Con el fin de compensar la caída de temperatura y, por consiguiente, disminuir la
25 actividad del catalizador o aproximarse al equilibrio termodinámico, el calor de

reacción puede introducirse usando varios lechos de catalizador en serie con calentamiento intermedio del efluente del reactor del primer lecho a mayores temperaturas e introducir el efluente calentado en un segundo lecho de catalizador, etc. Si se usan reactores de lecho fijo, puede usarse un reactor multitubular en el que el catalizador se carga en tubos de pequeño diámetro que están instalados en una vaina del reactor. En el lado de la vaina se introduce un medio de calentamiento que proporciona el calor de reacción requerido por transferencia de calor por la pared de los tubos del reactor al catalizador.

Con respecto a la WHSV de deshidratación de los alcoholes, oscila ventajosamente de 1 a 20 h^{-1} , preferentemente de 3 a 15 h^{-1} , más preferentemente de 4 a 10 h^{-1} .

Con respecto a la corriente de efluente de deshidratación, comprende esencialmente agua, olefina, el componente inerte (si lo hay) y alcoholes sin convertir. Se supone que dichos alcoholes sin convertir son tan pocos como sea posible. La olefina se recupera por medios de fraccionamiento usuales. Ventajosamente, el componente inerte, si lo hay, se recircula en la corriente (A), además de los alcoholes sin convertir, si los hay. Los alcoholes sin convertir, si lo hay, se recirculan al reactor en la corriente (A).

Con respecto al catalizador de deshidratación, es, a modo de ejemplo, un silicato cristalino del grupo FER (ferrierita, FU-9, ZSM-35), MWW (MCM-22, PSH-3, ITQ-1, MCM-49), EUO (ZSM-50, EU-1), MFS (ZSM-57), ZSM-48, MTT (ZSM-23), MFI (ZSM-5 o silicalita), MEL (ZSM-11) o TON (ZSM-22, Theta-1, NU-10),

o un silicato cristalino desaluminado del grupo FER (ferrierita, FU-9, ZSM-35), MWW (MCM-22, PSH-3, ITQ-1, MCM-49), EUO (ZSM-50, EU-1), MFS (ZSM-

57), ZSM-48, MTT (ZSM-23), MFI (ZSM-5 o silicalita), MEL (ZSM-11) o TON (ZSM-22, Theta-1, NU-10),

o un silicato cristalino modificado con fósforo del grupo FER (ferrierita, FU-9, ZSM-35), MWW (MCM-22, PSH-3, ITQ-1, MCM-49), EUO (ZSM-50, EU-1), MFS (ZSM-57), ZSM-48, MTT (ZSM-23), MFI (ZSM-5 o silicalita), MEL (ZSM-11) o TON (ZSM-22, Theta-1, NU-10),

o un tamiz molecular de silicoaluminofosfato del grupo AEI, CHA o AEL, o alúmina gamma, beta, eta o delta y alúmina amorfa

o sílice-alúmina

10 o una alúmina alcalinizada, silicatada, circonada o titanada o fluorada.

El silicato cristalino puede someterse a diversos tratamientos antes de uso en la deshidratación, que incluyen intercambio iónico, modificación con metales (en un modo no restrictivo elementos alcalinos, alcalinotérreos, de transición o de tierras raras), pasivación de superficies externas, modificación con compuestos de fósforo, vaporización, tratamiento con ácido u otros procedimientos de desaluminación, o combinación de los mismos.

Otro catalizador adecuado para el presente procedimiento es el tamiz molecular de silicoaluminofosfato del grupo AEI, CHA o AEL con ejemplo típico el tamiz molecular SAPO-18, SAPO-34 o SAPO-11. El tamiz molecular SAPO se basa en ALPO, que tiene esencialmente una relación de Al/P de 1 átomo/átomo. Durante la síntesis se añade precursor de silicio y la inserción del silicio en la región estructural de ALPO produce un sitio ácido en la superficie de los microporos del tamiz de anillo de 10 miembros. El contenido de silicio oscila del 0,1 al 10% de átomos (Al + P + Si es 100).

25 En otra realización específica, el silicato cristalino o tamiz molecular de

silicoaluminofosfato se mezcla con un aglutinante, preferentemente un aglutinante inorgánico, y se moldea a una forma deseada, por ejemplo, gránulos. El aglutinante se selecciona de manera que sea resistente a la temperatura y otras condiciones empleadas en el procedimiento de deshidratación de la invención. El

5 aglutinante es un material inorgánico seleccionado de arcillas, sílice, silicatos metálicos, óxidos metálicos tales como ZrO_2 y/o metales, o geles que incluyen mezclas de sílice y óxidos metálicos. Si el aglutinante que se usa conjuntamente con el silicato cristalino es por sí mismo catalíticamente activo, éste puede alterar la conversión y/o la selectividad del catalizador. Materiales inactivos para el

10 aglutinante pueden servir adecuadamente de diluyentes para controlar la cantidad de conversión de manera que puedan obtenerse productos económicamente y ordenadamente sin emplear otros medios para controlar la tasa de reacción. Se desea proporcionar un catalizador que tenga una buena resistencia al machacado. Esto es debido a que en uso comercial se desea prevenir la rotura del catalizador

15 en materiales tipo polvo. Tales aglutinantes de arcilla u óxido se han empleado normalmente solo con el fin de mejorar la resistencia al machacado del catalizador. Un aglutinante particularmente preferido para el catalizador de la presente invención comprende sílice. Las proporciones relativas del material de silicato cristalino finamente dividido y la matriz de óxido inorgánico del aglutinante

20 pueden variar ampliamente. Normalmente, el contenido de aglutinante oscila del 5 al 95% en peso, más normalmente del 20 al 75% en peso, basándose en el peso del catalizador de material compuesto. Una mezcla tal del silicato cristalino y un aglutinante de óxido inorgánico se denomina un silicato cristalino formulado. En la mezcla del catalizador con un aglutinante, el catalizador puede formularse en

25 gránulos, extruirse en otras formas o formarse en esferas o un polvo secado por

pulverización. Normalmente, el aglutinante y el silicato cristalino se mezclan juntos por un procedimiento de mezcla. En tal procedimiento, el aglutinante, por ejemplo sílice, en forma de un gel se mezcla con el material de silicato cristalino y la mezcla resultante se extruye en la forma deseada, por ejemplo, barras cilíndricas o multilobulares. Pueden prepararse formas esféricas en granuladores giratorios o por la técnica de gota de aceite. Las esferas pequeñas pueden prepararse adicionalmente secando por pulverización una suspensión de catalizador-aglutinante. Después, el silicato cristalino formulado se calcina en aire o un gas inerte, normalmente a una temperatura de 200 a 900 °C durante un periodo de 1 a 48 horas.

Otra familia de catalizadores adecuados para la deshidratación de propanol en propileno son alúminas (alúminas amorfas, gamma, beta, eta o delta) como tales o alúminas que han sido modificadas por tratamiento superficial con álcalis, silicio, circonio o titanio, y sílice-alúminas. Las alúminas se caracterizan generalmente por una distribución de resistencia a los ácidos bastante ancha y por tener, tanto sitios ácido tipo Lewis, como tipo Bronsted. La presencia de una distribución de resistencia a los ácidos bastante ancha hace posible la catálisis de varias reacciones, requiriendo cada una una resistencia a los ácidos diferente. Esto produce frecuentemente baja selectividad por el producto deseado. La deposición de álcalis, silicio, circonio o titanio sobre la superficie de la alúmina permite que el catalizador sea significativamente más selectivo. Para la preparación del catalizador basado en alúmina pueden usarse alúminas comerciales adecuadas, preferentemente alúmina eta o gamma, que tienen un área superficial de 10 a 500 m²/gramo. El catalizador según la presente invención se prepara añadiendo 0,05 al 10% de metal alcalino (alcalinotérreo), silicio,

circonio o titanio. La adición de estos metales puede hacerse durante la preparación de la alúmina o puede añadirse a la alúmina existente, eventualmente ya activada. La adición del metal durante la preparación de la alúmina puede hacerse disolviendo el precursor metálico junto con el precursor de aluminio antes de la precipitación de la alúmina final o mediante la adición del precursor metálico al gel de hidróxido de aluminio. Un procedimiento preferido es añadir precursores metálicos a la alúmina moldeada. Los precursores metálicos se disuelven en un disolvente adecuado, tanto acuoso como orgánico, y se ponen en contacto con la alúmina por impregnación de humedad incipiente o por impregnación húmeda o poniendo en contacto con un exceso de soluto durante un tiempo dado, seguido de eliminación del exceso de soluto. La alúmina también puede ponerse en contacto con vapor del precursor metálico. Precursores metálicos adecuados son haluros de metales alcalinos (alcalinotérreos), silicio, circonio o titanio, oxihaluros de circonio o titanio; alcóxidos de metales alcalinos (alcalinotérreos), silicio, circonio o titanio; acetatos, oxalatos o citratos de metales alcalinos (alcalinotérreos), circonio o titanio; nitratos, sulfatos o fosfatos de metales alcalinos (alcalinotérreos), o mezclas de los anteriores. El disolvente se selecciona según la solubilidad del precursor metálico. La puesta en contacto puede hacerse a temperatura de 0 °C a 500 °C, más preferida de 10 °C a 200 °C. Después de la puesta en contacto, la alúmina se lava eventualmente, se seca y finalmente se calcina, en otras cosas, para potenciar la reacción superficial entre los metales alcalinos (alcalinotérreos), silicio, circonio o titanio y la alúmina, y la eliminación de los ligandos del precursor metálico. El uso de alúminas alcalinizadas, silicatadas, circonada o titanadas o fluoradas para la deshidratación del propanol en propileno se hace preferentemente en presencia de agua. La relación de peso de agua con

respecto a propanol oscila de 1/25 a 3/1. La alúmina fluorada es conocida por sí misma y puede prepararse según la técnica anterior.

Un experto en la técnica también apreciará que el propileno preparado por el procedimiento de deshidratación de la presente invención puede, a modo de ejemplo, polimerizarse en polipropileno, copolímeros de etileno, hexano, octano o deceno y propileno, cauchos de etileno-propileno (EPR), polímeros de etileno-propileno-dieno (EPDM), puede usarse para alquilación de benceno para preparar cumeno, un precursor para la producción de fenol, puede oxidarse a ácido acrílico, puede amoxidarse en acrilonitrilo, puede oxidarse a óxido de propileno para preparar polioléteres y propanodiol y puede hidroformilarse a n-butiraldehído.

La presente invención también se refiere a dicho polipropileno, cauchos de etileno-propileno (EPR), polímeros de etileno-propileno-dieno (EPDM), cumeno, ácido acrílico, acrilonitrilo, óxido de propileno y n-butiraldehído.

REIVINDICACIONES

1. Un procedimiento de preparación de propileno por deshidratación de propanol, que implica las siguientes etapas:

- 5 1. Gasificar materia prima sólida o líquida carbonosa, o reformar materia prima carbonosa gaseosa, en gas de síntesis,
2. Eliminar contaminantes del gas de síntesis,
3. Fermentar el gas de síntesis por medio de un microorganismo en sustancialmente propanol, en el que el microorganismo
- 10 i. Es una cepa natural que tiene la capacidad natural para fermentar gas de síntesis en sustancialmente propanol, o
- ii. Es un microorganismo que posee la información de secuencias de ácidos nucleicos requerida, para expresar las enzimas para la biosíntesis de compuestos oxigenados C_3 ,
- 15 modificado confiriéndole la información de secuencias de ácidos nucleicos requerida, para expresar las enzimas de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida), o
- iii. Es un microorganismo que posee la información de secuencias de ácidos nucleicos requerida para expresar las
- 20 enzimas de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida), modificado confiriéndole la información de secuencias de ácidos nucleicos requerida, para expresar las
- enzimas para la biosíntesis de compuestos oxigenados C_3 .
- 25 4. Fraccionar y purificar la corriente que contiene predominantemente propanol,

5. Deshidratar dicha corriente anterior en un reactor en condiciones eficaces para deshidratar al menos una parte del propanol para preparar propileno,
6. Recuperar de dicho reactor la corriente que contiene propileno.

5

2. Un procedimiento de preparación de propileno por deshidratación de propanol, que implica las siguientes etapas:

1. Gasificar materia prima sólida o líquida carbonosa, o reformar materia prima carbonosa gaseosa, en gas de síntesis,
- 10 2. Eliminar contaminantes del gas de síntesis,
3. Co-fermentar el gas de síntesis con al menos un compuesto oxigenado líquido, como hidratos de carbono, glicerol, propanodiol, lactaldehído, ácido láctico, acetol, metanol, etanol, ácido acético, ácido propiónico, propanal y acetona, por medio de un microorganismo en
15 sustancialmente propanol, en el que el microorganismo
 - i. Es una cepa natural que tiene la capacidad natural para fermentar gas de síntesis en sustancialmente propanol, o
 - ii. Es un microorganismo que posee la información de secuencias de ácidos nucleicos requerida para expresar las
20 enzimas para la biosíntesis de compuestos oxigenados C₃, modificado confiriéndole la información de secuencias de ácidos nucleicos requerida para expresar las enzimas de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida), o
 - iii. Es un microorganismo que posee la información de
25 secuencias de ácidos nucleicos requerida para expresar las

enzimas de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida), modificado confiriéndole la información de secuencias de ácidos nucleicos requerida para expresar las enzimas para la biosíntesis de compuestos oxigenados C₃.

- 5 4. Fraccionar y purificar la corriente que contiene predominantemente propanol,
5. Deshidratar dicha corriente anterior en un reactor en condiciones eficaces para deshidratar al menos una parte del propanol para preparar propileno,
- 10 6. Recuperar de dicho reactor la corriente que contiene propileno.

3. Un procedimiento según la reivindicación 1 ó 2, en el que la etapa de fermentación o de co-fermentación (3), produce una mezcla de alcoholes que contiene al menos 50% en peso de propanol, entre 0 y 50% en peso de etanol y
15 entre 0 y 50% en peso de butanol, y en el que en la etapa de fraccionamiento y de purificación (4) el propanol, etanol y butanol se fraccionan y se purifican, y en el que en la etapa de deshidratación (5), el propanol en presencia de etanol y butanol, si están presentes, se deshidratan simultáneamente, y en el que en la etapa de recuperación (6), el propileno y etileno y los butenos, si están presentes,
20 se recuperan de dicho reactor.

4. Un procedimiento según la reivindicación 1 ó 2, en el que los compuestos oxigenados C₃ se producen mediante una combinación de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida) y la ruta de acetona/isopropanol, que
25 implican al menos una acetil-CoA sintasa, una piruvato sintasa y acetoacetato

descarboxilasa, y que producen principalmente isopropanol.

5. Un procedimiento según la reivindicación 1 ó 2, en el que los compuestos oxigenados C_3 se producen mediante una combinación de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida) y la ruta de dicarboxílico, que implica al menos una acetil-CoA sintasa, una piruvato sintasa y una metilmalonil-CoA carboxitransferasa o una propionil-CoA succinato transferasa, y que produce principalmente n-propanol.
- 10 6. Un procedimiento según la reivindicación 1 ó 2, en el que los compuestos oxigenados C_3 se producen mediante una combinación de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida) y la ruta de acrilato, que implica al menos una acetil-CoA sintasa, una piruvato sintasa y una acriloil-CoA reductasa, y que produce principalmente n-propanol.
- 15 7. Un procedimiento según la reivindicación 1 ó 2, en el que los compuestos oxigenados C_3 se producen mediante una combinación de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida) y la ruta de degradación de treonina, que implica al menos una acetil-CoA sintasa, una piruvato sintasa y una 2-cetobutirato
- 20 formiato liasa y que produce principalmente n-propanol.
8. Un procedimiento según la reivindicación 1 ó 2, en el que los compuestos oxigenados C_3 se producen mediante una combinación de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida) y la ruta de citramaleato, que implica al menos una acetil-CoA sintasa, una piruvato sintasa y una citramaleato sintasa, y
- 25

que produce principalmente n-propanol.

9. Un procedimiento según la reivindicación 1 ó 2, en el que los compuestos oxigenados C_3 se producen mediante una combinación de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida) y la ruta de reducción de propanodiol, que implica al menos una acetil-CoA sintasa, una piruvato sintasa, una propanodiol oxidorreductasa y una propanodiol deshidratasa, y que produce principalmente n-propanol.
10. Procedimiento según una cualquiera de las reivindicaciones precedentes, en el que la deshidratación se realiza sobre un catalizador ácido a una temperatura de al menos 200 °C y una WHSV de al menos 1 h⁻¹.
11. Procedimiento según la reivindicación 10, en el que la deshidratación se realiza introduciendo en un reactor una corriente que comprende al menos propanol, opcionalmente agua, opcionalmente un componente inerte, poner en contacto dicha corriente con un catalizador en dicho reactor en condiciones eficaces para deshidratar al menos una parte del propanol para preparar propileno.
12. Uso del propileno preparado según una cualquiera de las reivindicaciones precedentes para preparar polipropileno.
13. Uso del propileno preparado según una cualquiera de las reivindicaciones 1 a 11 para preparar copolímeros de propileno, que contienen etileno, hexeno, octeno

o deceno como comonomero.

14. Uso del propileno preparado según una cualquiera de las reivindicaciones 1 a 11 para preparar cauchos de etileno-propileno (EPR).

5

15. Uso del propileno preparado según una cualquiera de las reivindicaciones 1 a 11 para preparar copolímeros de etileno-propileno-dieno (EPDM).

10 16. Uso del propileno preparado según una cualquiera de las reivindicaciones 1 a 11 para preparar acrilonitrilo.

17. Uso del propileno preparado según una cualquiera de las reivindicaciones 1 a 11 para preparar ácido acrílico.

15 18. Uso del propileno preparado según una cualquiera de las reivindicaciones 1 a 11 para preparar cumeno.

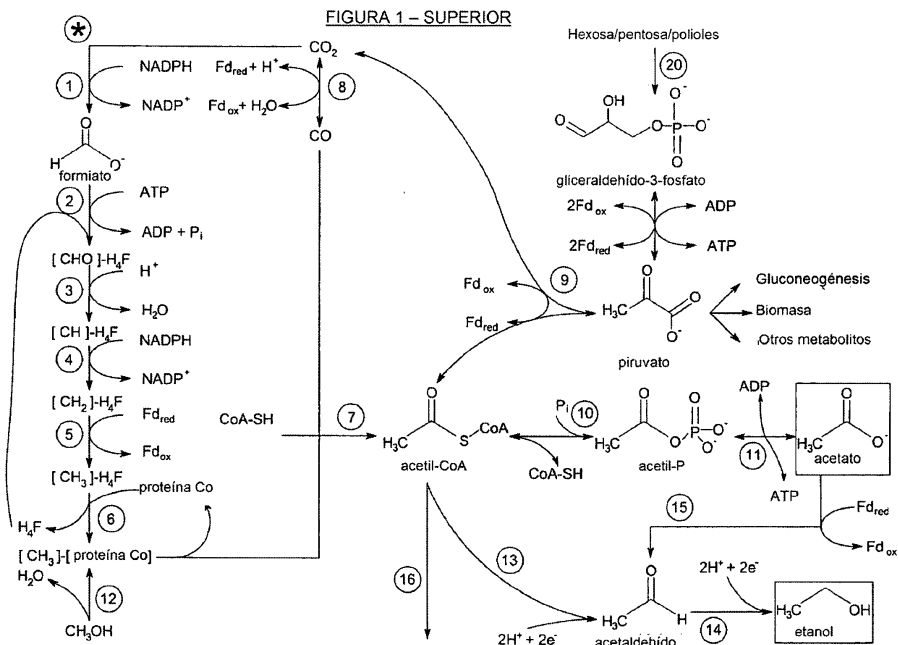
19. Uso del propileno preparado según una cualquiera de las reivindicaciones 1 a 11 para preparar óxido de propileno.

20

20. Uso del propileno preparado según una cualquiera de las reivindicaciones 1 a 11 para preparar n-butiraldehído.

RESUMEN

Procedimiento de preparación de propileno por deshidratación de propanol, que implica las siguientes etapas: o Gasificar materia prima sólida o líquida carbonosa o reformar materia prima carbonosa gaseosa en gas de síntesis, o Fermentar el gas de síntesis por medio de un microorganismo en sustancialmente propanol o o Co-fermentar el gas de síntesis con al menos un compuesto oxigenado líquido por medio de un microorganismo en sustancialmente propanol en el que el microorganismo • Es una cepa natural que tiene la capacidad natural para fermentar gas de síntesis en sustancialmente propanol o • Es un microorganismo que posee la información de secuencias de ácidos nucleicos requerida para expresar las enzimas para la biosíntesis de compuestos oxigenados C_3 , modificado confiriéndole la información de secuencias de ácidos nucleicos requerida para expresar las enzimas de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida), o • Es un microorganismo que posee la información de secuencias de ácidos nucleicos requerida para expresar las enzimas de la ruta de Wood-Ljungdahl (ruta de acetil-CoA reducida), modificado confiriéndole la información de secuencias de ácidos nucleicos requerida para expresar las enzimas para la biosíntesis de compuestos oxigenados C_3 o Deshidratar dicha corriente anterior en un reactor en condiciones eficaces para deshidratar al menos una parte del propanol para preparar propileno.



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FIGURA 1 – INFERIOR

- Fd: ferredoxina
Proteína Co: proteína Fe-S corrinoide
- 1: Formiato deshidrogenasa
 - 2: Formiltetrahydrofolato sintasa
 - 3: Meteniltetrahydrofolato ciclohidrolasa
 - 4: Metilentratahydrofolato deshidrogenasa
 - 5: Metilentratahydrofolato reductasa
 - 6: Metiltransferasa
 - 7: Acetil-CoA sintasa
 - 8: CO deshidrogenasa (CODH)
 - 9: Piruvato sintasa (PFOR)
 - 10: Fosfostrasacetilasa/butirilasa
 - 11: Acetato/butirato cinasa
 - 12: Metanol metiltransferasa
 - 13: Acetaldehido/butiraldehido deshidrogenasa
 - 14: Alcohol deshidrogenasa
 - 15: Aldehido oxidoreductasa
 - 16: Acetil transferasa
 - 17: 3-hidroxi-butilil-CoA deshidrogenasa
 - 18: Crotonasa
 - 19: Butiril-CoA deshidrogenasa
 - 20: Ruta de glicólisis (EMO) y de pentosa fosfato

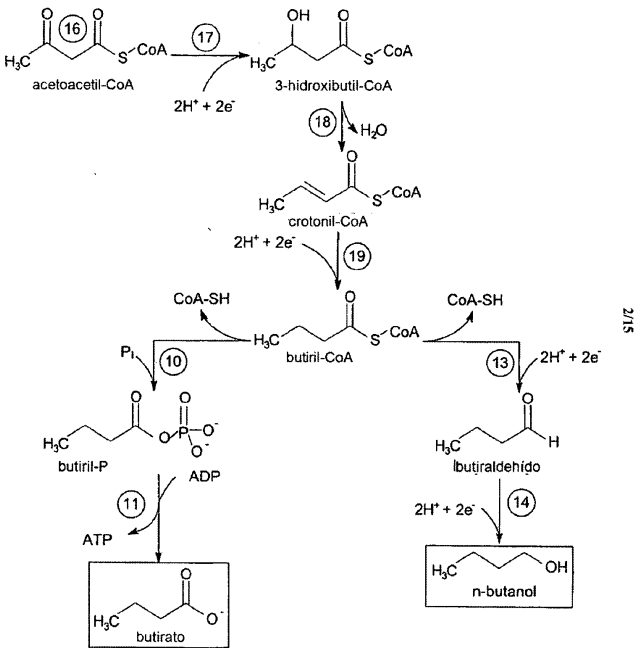
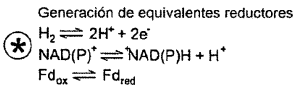


FIGURA 2 – SUPERIOR

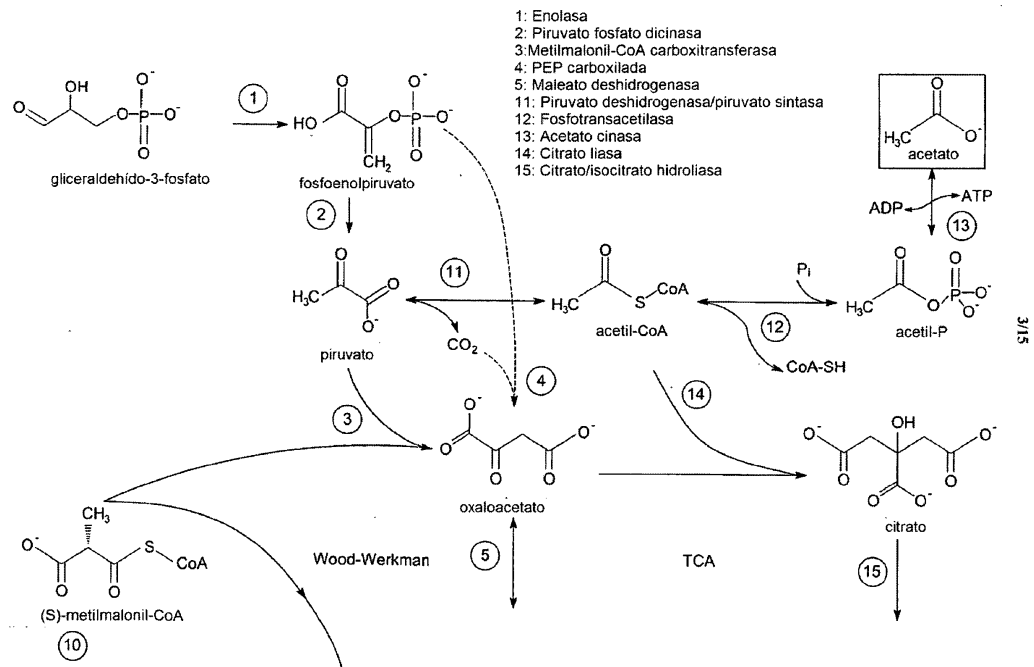
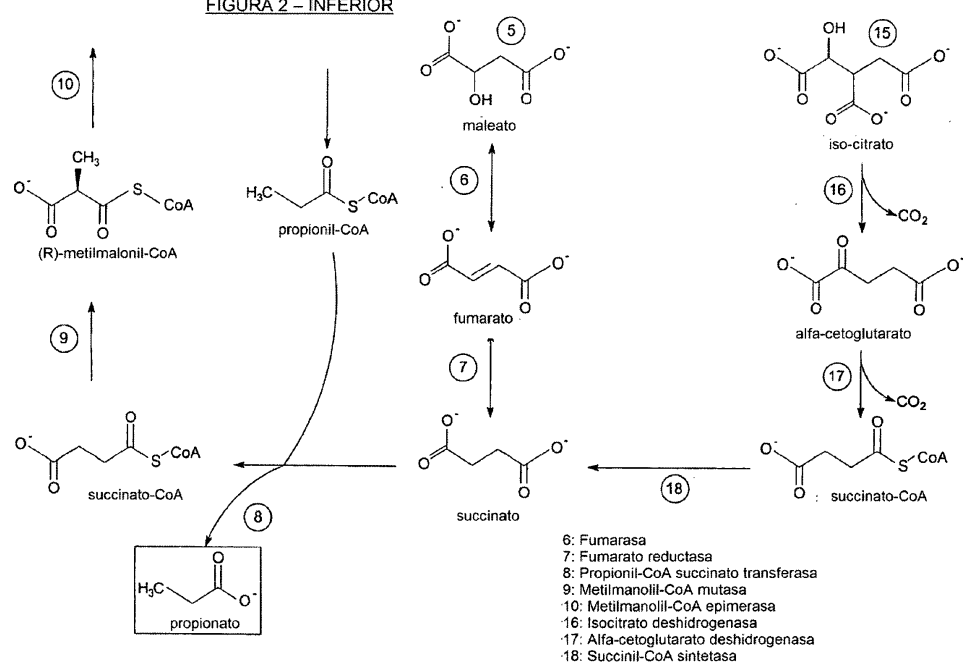


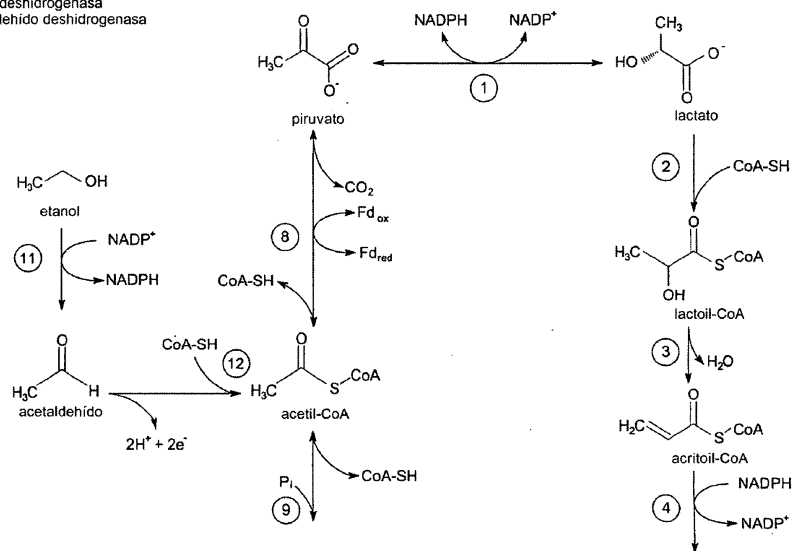
FIGURA 2 – INFERIOR



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- 1: Lactato deshidrogenasa
- 2: Lactato CoA transferasa
- 3: Lactoil-CoA deshidratasa
- 4: Acrilil-CoA reductasa
- 8: Piruvato deshidrogenasa/piruvato sintasa
- 9: Fosfoacetilasa
- 11: Etanol deshidrogenasa
- 12: Acetaldehido deshidrogenasa

FIGURA 3 – SUPERIOR



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FIGURA 3 INFERIOR

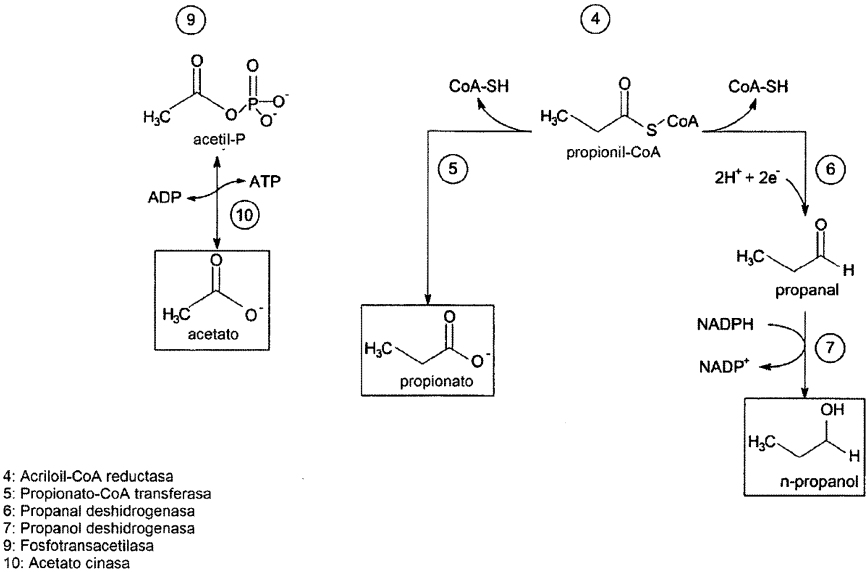
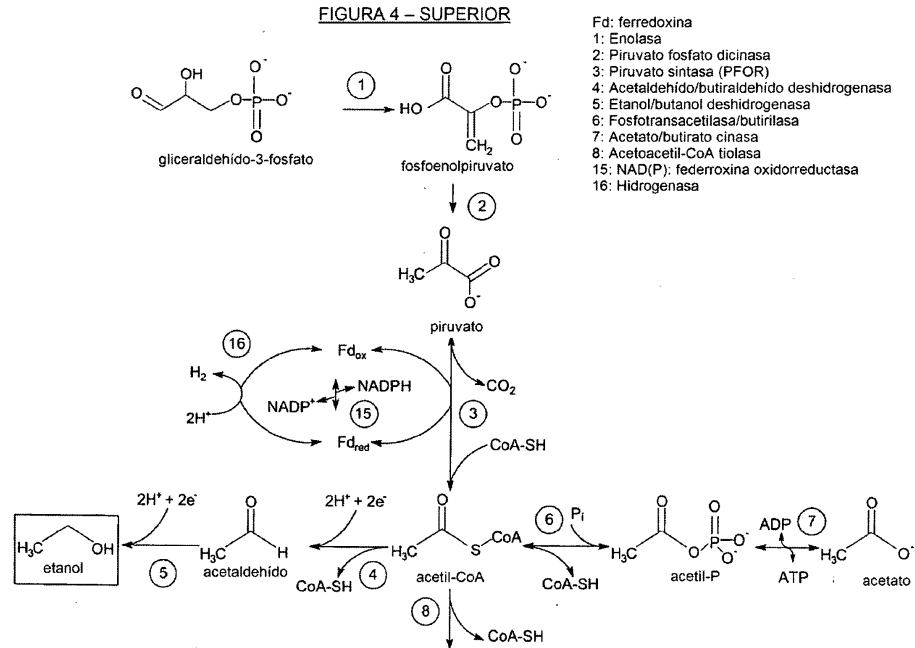
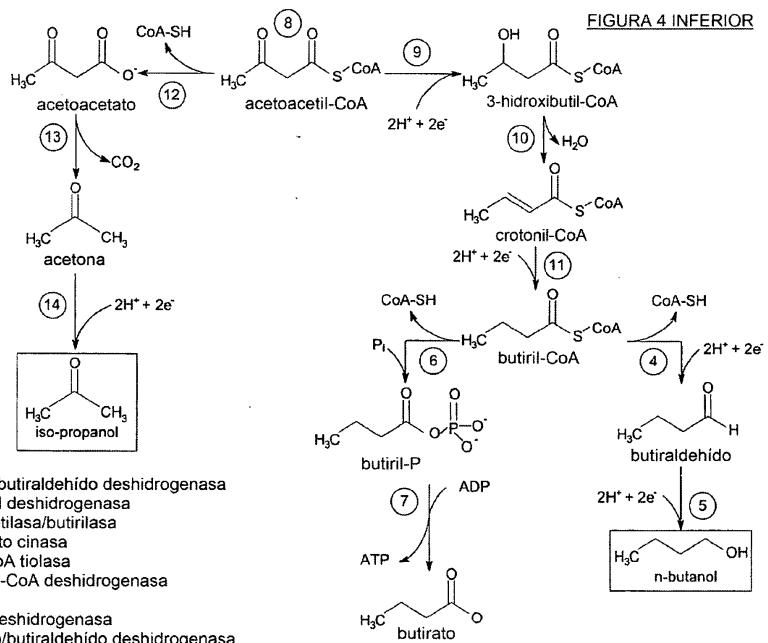


FIGURA 4 – SUPERIOR



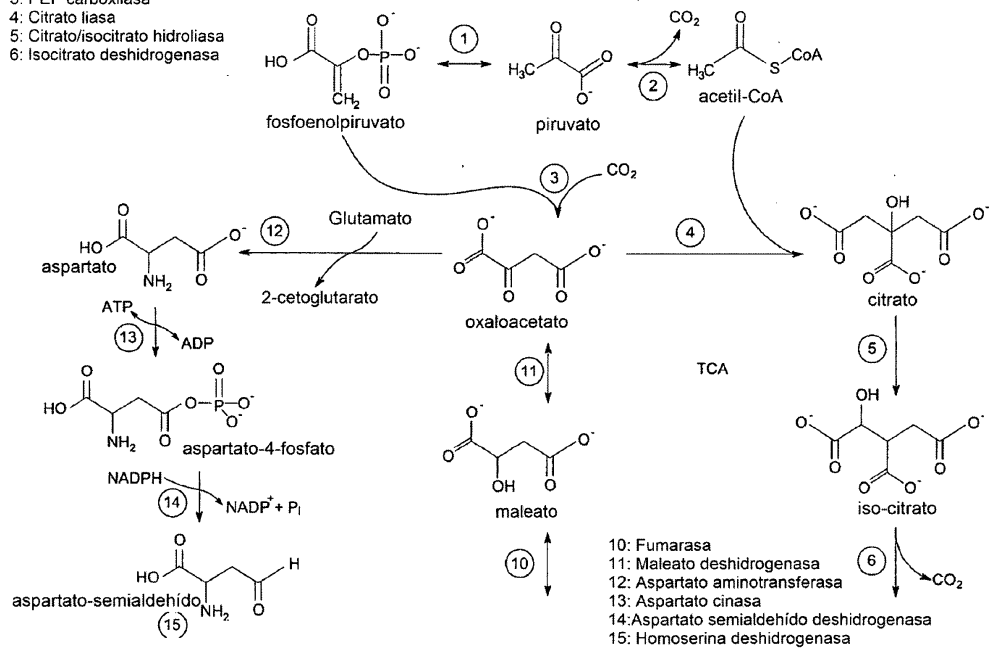
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- Fd: ferredoxina
 4: Acetaldehído/butiraldehído deshidrogenasa
 5: Etanol/butanol deshidrogenasa
 6: Fosfotransacetilasa/butirilasa
 7: Acetato/butirato cinasa
 8: Acetoacetyl-CoA tiolasa
 9: 3-hidroxiutil-CoA deshidrogenasa
 10: Crotonasa
 11: Butiril-CoA deshidrogenasa
 12: Acetaldehído/butiraldehído deshidrogenasa
 13: Acetoacetato descarboxilasa
 14: Isopropanol deshidrogenasa

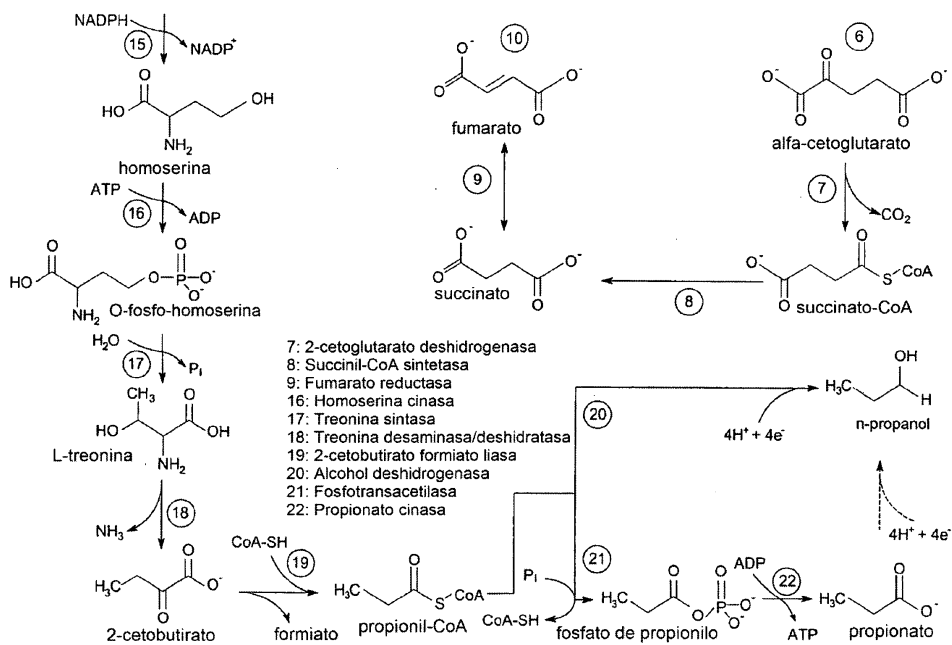
- 1: Piruvato fosfato dicinasa
- 2: Piruvato sintasa
- 3: PEP carboxilasa
- 4: Citrato liasa
- 5: Citrato/isocitrato hidrolasa
- 6: Isocitrato deshidrogenasa

FIGURA 5 – SUPERIOR



- 10: Fumarasa
- 11: Maleato deshidrogenasa
- 12: Aspartato aminotransferasa
- 13: Aspartato cinasa
- 14: Aspartato semialdehído deshidrogenasa
- 15: Homoserina deshidrogenasa

FIGURA 5 – INFERIOR



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FIGURA 6 – SUPERIOR

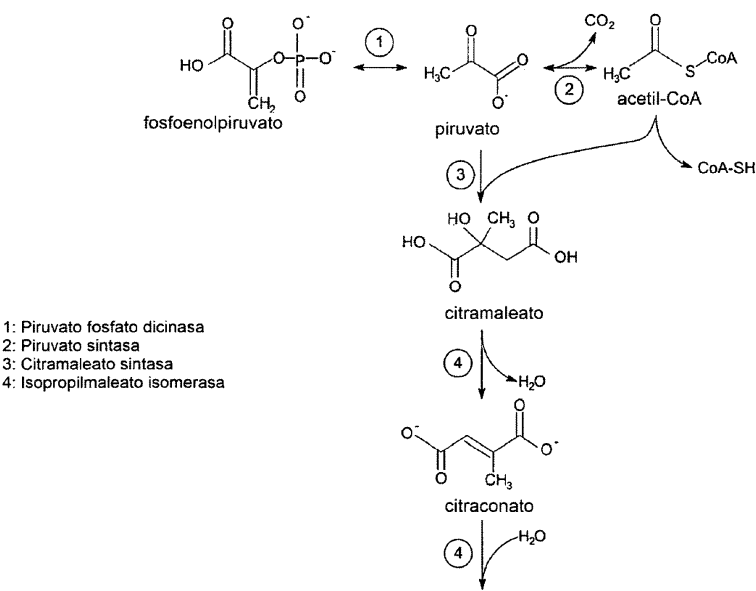


FIGURA 6 – INFERIOR

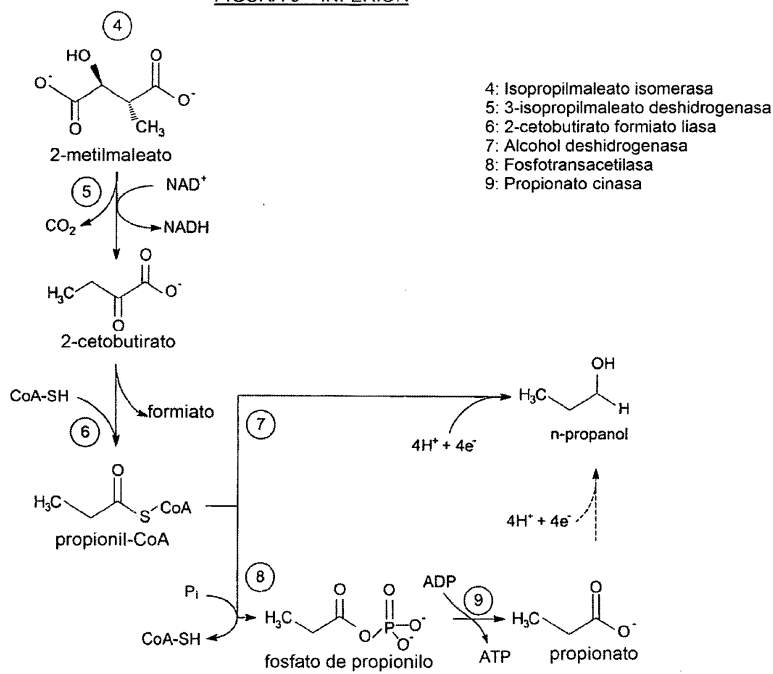


FIGURA 7 – SUPERIOR

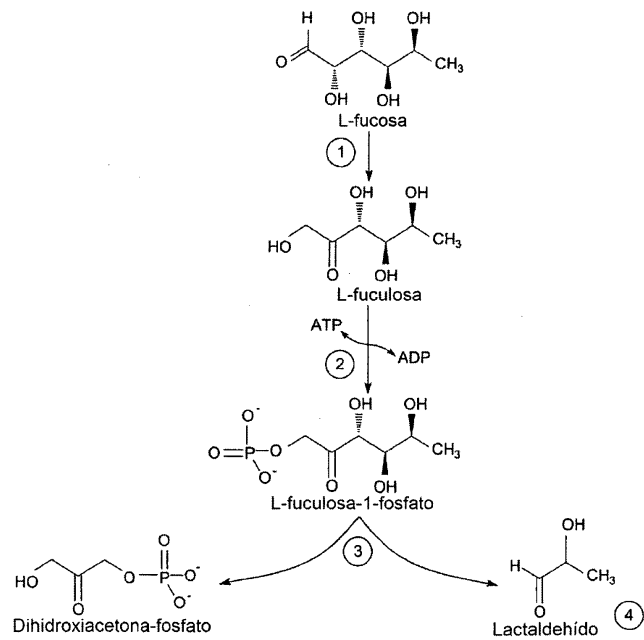
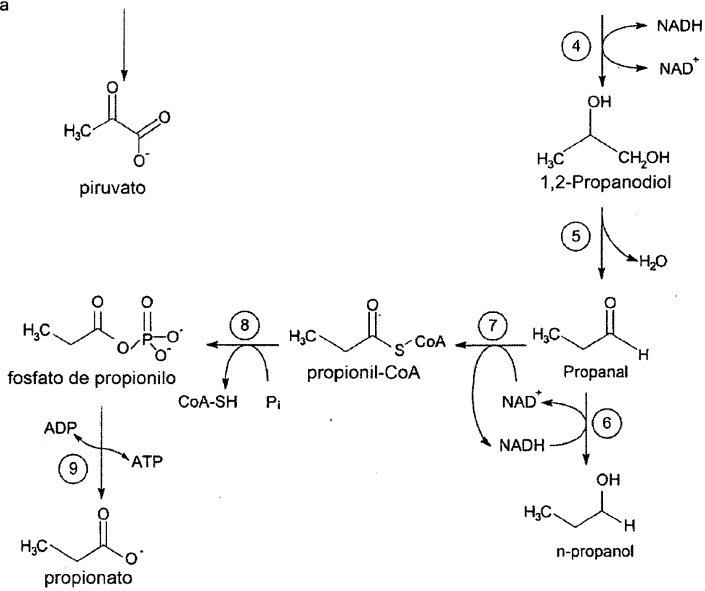


FIGURA 7 – INFERIOR

- 4: Propanodiol oxidorreductasa
- 5: Propanodiol deshidratasa
- 6: Propanol deshidrogenasa
- 7: Propanal deshidrogenasa
- 8: Fosfotransacetilasa
- 9: Propionato cinasa

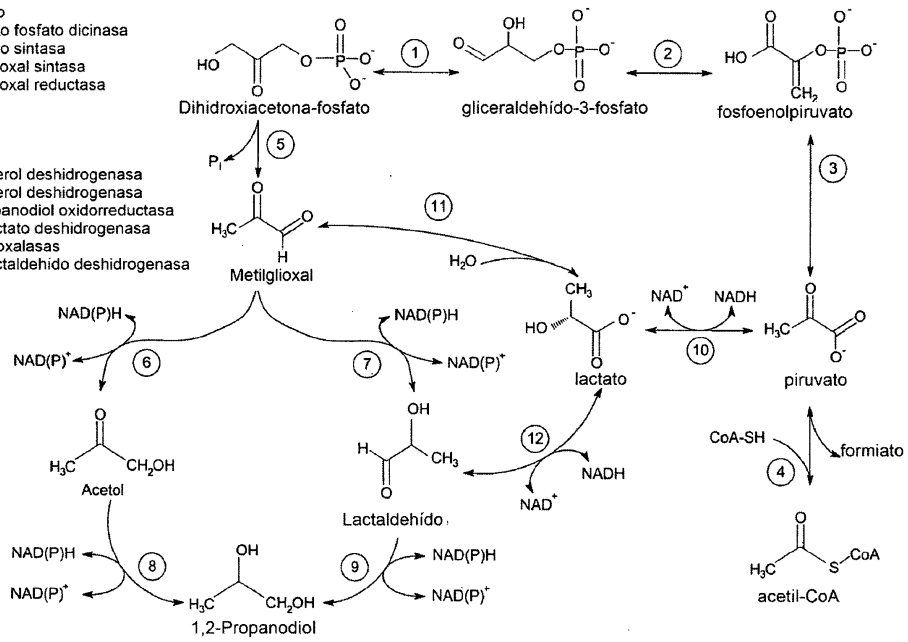


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FIGURA 8

- 1: Triosa fosfato isomerasa
- 2: Enolato
- 3: Piruvato fosfato dicinasa
- 4: Piruvato sintasa
- 5: Metilglioxal sintasa
- 6: Metilglioxal reductasa

- 7: Glicerol deshidrogenasa
- 8: Glicerol deshidrogenasa
- 9: Propanodiol oxidoreductasa
- 10: Lactato deshidrogenasa
- 11: Glioxalasa
- 12: Lactaldehido deshidrogenasa



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Date of mailing (day/month/year) 20 January 2012 (20.01.2012)	IMPORTANT NOTIFICATION	
Applicant's or agent's file reference F-1267-WO		
International application No. PCT/EP2011/072958		International filing date (day/month/year) 15 December 2011 (15.12.2011)
International publication date (day/month/year) Not yet published		Priority date (day/month/year) 17 December 2010 (17.12.2010)
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17 December 2010 (17.12.2010)	10195765.2	EP	09 January 2012 (09.01.2012)

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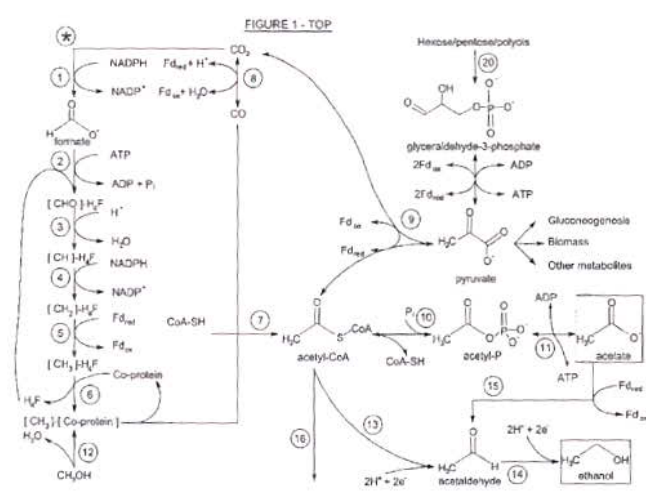
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(54) Title: PROCESS FOR PRODUCING PROPYLENE FROM SYNGAS VIA FERMENTATIVE PROPANOL PRODUCTION AND DEHYDRATION



(57) Abstract: Process for making propylene by dehydration of propanol, involving the following steps: o Gasifying carbonaceous solid or liquid feedstock or reforming gaseous carbonaceous feedstock into synthesis gas, o Fermenting the synthesis gas by means of a microorganism into substantially propanol or o Co-Fermenting the synthesis gas with at least one liquid oxygenate by means of a microorganism into substantially propanol in which the microorganism • Is a wild strain having the natural capability to ferment synthesis gas into substantially propanol or • Is a microorganism, possessing the required nucleic acid sequence information to express the enzymes for the biosynthesis of C₃-oxygenates, modified by conferring it with the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway), or • Is a microorganism, possessing the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes for the biosynthesis of C₃-oxygenates. o Dehydrating said above stream in a reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene.

WO 2012/080421 A1

**PROCESS FOR PRODUCING PROPYLENE FROM SYNGAS VIA
FERMENTATIVE PROPANOL PRODUCTION AND DEHYDRATION**

5 [Field of the invention]

The present invention relates to the production of propylene via dehydration of propanol on advantageously an acidic catalyst, whereby the propanol is obtained by metabolic conversion of synthesis gas, that is produced
10 by gasification of biomass, waste, coal, liquid residu's, effluent gases from steel furnaces or by reforming of natural gas, into propanol (either n-propanol or isopropanol). The limited supply and increasing cost of crude oil has prompted the search for alternative processes for producing hydrocarbon products such as propylene. Propanol can be obtained by metabolic conversion of synthesis
15 gas by microorganisms. Made up of organic matter from living organisms, biomass is the world's leading renewable energy source.

[Background of the invention]

20 The production of propanols is limited compared to the production of propylene. In 2005, the world production of n-propanol was about 140 kta and of isopropanol more than 2000 kta whereas the world production of propylene exceeds 65000 kta.

Historically, isopropanol has been produced by hydration of propylene over
25 acidic catalyst (Kirk-Othmer Encyclopedia of Chemical Technology, 2007 & Ullmann's Encyclopedia of Industrial Chemistry, 2002). In the indirect hydration, isopropyl sulfate esters are formed out of propylene and sulfuric acid, which is subsequently hydrolyzed into isopropanol. In the direct hydration, propylene is converted at high pressure and low temperature over an acid fixed bed catalyst.

30 n-Propanol can be obtained by hydroformylation of ethylene via the intermediate propanal that is hydrogenated further in n-propanol. Typical

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conditions employed in the low pressure rhodium-substituted phosphine catalysed oxo process are 90-130°C and less than 28 bars whereas the conditions for the cobalt catalysed oxo process are 110-180°C and above 200 bars. The propanal is subsequently hydrogenated into n-propanol in the presence of excess hydrogen over a metallic catalyst, typically composed of copper, zinc, nickel and chromium compounds.

Propanol is also been produced by hydrogenation of acetone over metallic catalyst into isopropanol. Acetone is the byproduct in the phenol production from cumene. (Ullmann's Encyclopedia of Industrial Chemistry, 2002).

- 10 Aceton, as one of the three products in the ABE (Aceton-butanol-ethanol) fermentation technology based on sugars, could also be hydrogenated into isopropanol ("Bacterial acetone and butanol production by industrial fermentation in the Soviet Union: use of hydrolyzed agricultural waste for biorefinery", Appl. Microbiol. Biotechnol., 71, p. 587-597, 2006; "History of the
- 15 Acetone-Butanol-Ethanol Fermentation Industry in China: Development of Continuous Production Technology", J. Mol. Microbiol Biotechnol., 13, p. 12-14, 2007; "Acetone-butanol fermentation revisited", Microbiol. Rev. 50, p. 484-524, 1986 and Jones, D., "Applied acetone-butanol fermentation", In "Clostridia. Biotechnology and medical application", p. 125-168, 2001, Wiley-VCH Verlag
- 20 GmbH, Weinheim, Germany). The acetone-butanol-ethanol (ABE) fermentation by *Clostridium acetobutylicum* is one of the oldest known industrial fermentations. It was ranked second only to ethanol fermentation by yeast in its scale of production, and is one of the largest biotechnological processes ever known. However, since the 1950's industrial ABE fermentation has declined
- 25 gradually, and almost all acetone is now produced via petrochemical routes. More recently, biochemical metabolic routes are being developed for making propanol from carbohydrates. US Patent 2009/0246842 describes a process for the fermentation of sugars into isopropanol.

- Propanol can also be produced from synthesis gas (mixture of CO, H₂ and CO₂)
- 30 by a catalytic process similar to Fischer-Tropsch, resulting in a mixture of higher alcohols, although often a preferential formation of propanol occurs (Applied

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Catalysis A, general, 186, p. 407, 1999 and Chemiker Zeitung, 106, p. 249, 1982). Still another route to obtain propanol, is the base-catalysed Guerbet condensation of methanol with ethanol (J. of Molecular Catalysis A: Chemical 200, 137, 2003, J. Chem. Soc. Chem. Commun., 22, p. 1558, 1990 and 5 Applied Biochemistry and Biotechnology, 113-116, p. 913, 2004).

Whereas in the past, propanols have been produced mostly out of ethylene or propylene, being high added value chemicals, there is a need to produce propanols from other carbon sources so that the respective propanols can be dehydrated into propylene.

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Since many centuries, simple sugars are being fermented into ethanol with the help of *sacharomycis cerevisae*. The last decade's new routes starting from cellulose and hemicelluloses have been developed to ferment more complex carbohydrates into ethanol. Hereto, the carbohydrates need to be unlocked 15 from the lignocellulosic biomass. Biomass consists approximately of 30% cellulose, 35% hemicelluloses and 25% lignin. The lignin fraction cannot be valorised as ethanol, as of its aromatic nature but can only be used as energy source which present in many cases an excess for running an industrial plant.

Recently, more efficient routes that produce synthesis gas from carbon- 20 containing materials and that subsequently is fermented into ethanol are being developed ("Bioconversion of synthesis gas into liquid or gaseous fuels", K. Klasson, M. Ackerson, E. Clausen, J. Gaddy, Enzyme and Microbial Technology, 14(8), p.602, 1992; "Fermentation of Biomass-Generated Producer Gas to Ethanol", R. Datar, R. Shenkman, B. Cateni, R. Huhnke, R. Lewis, 25 Biotechnology and Bioengineering, 86 (5), p.587, 2004; "Microbiology of synthesis gas fermentation for biofuel production", A. Hemstra, J. Sipma, A. Rinzema, A. Stams, Current Opinion in Biotechnology, 18, p. 200, 2007; "Old Acetogens, New Light", H. Drake, A. Gößner, S. Daniel, Ann. N.Y. Acad. Sci. 1125: 100-128, 2008). Synthesis gas can be produced by gasification of the 30 whole biomass without need to unlock certain fractions. Synthesis gas can also be produced from other feedstock via gasification: (i) coal, (ii) municipal waste

(iii) plastic waste, (iv) petcoke and (v) liquid residue's from refineries or from the paper industry (black liquor). Synthesis gas can also be produced from natural gas via steamreforming or autothermal reforming (partial oxidation). For conventional methanol synthesis, higher alcohol synthesis or Fischer-Tropsch a ratio of hydrogen to carbonmonoxide of about 2 is required. In case of gasification of hydrogen-poor feedstock this ratio will be below 1 and hence a watergas shift ($\text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + \text{H}_2$) is required to adjust the ratio. The biochemical pathway to transform synthesis gas into ethanol is much less stringent regarding the hydrogen to carbonmonoxide ratio.

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The biochemical pathway of synthesis gas conversion is described by the Wood-Ljungdahl Pathway. Fermentation of syngas offers several advantages such as high specificity of biocatalysts, lower energy costs (because of low pressure and low temperature bioconversion conditions), greater resistance to biocatalyst poisoning and nearly no constraint for a preset H_2 to CO ratio ("Reactor design issues for synthesis-gas fermentations" M. Bredwell, P. Srivastava, R. Worden, Biotechnology Progress 15, 834–844, 1999; "Biological conversion of synthesis gas into fuels", K. Klasson, C. Ackerson, E. Clausen, J. Gaddy, International Journal of Hydrogen Energy 17, p.281, 1992). Acetogens are a group of anaerobic bacteria able to convert syngas components, like CO , CO_2 and H_2 to acetate via the reductive acetyl-CoA or the Wood-Ljungdahl pathway.

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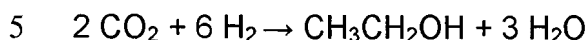
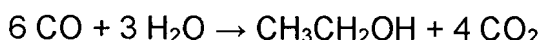
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Several anaerobic bacteria have been isolated that have the ability to ferment syngas to ethanol, acetic acid and other useful end products. *Clostridium ljungdahlii* and *Clostridium autoethanogenum*, were two of the first known organisms to convert CO , CO_2 and H_2 to ethanol and acetic acid. Commonly known as acetogens, these microorganisms have the ability to reduce CO_2 to acetate in order to produce required energy and to produce cell mass. The overall stoichiometry for the synthesis of ethanol using three different combinations of syngas components is as follows (J. Vega, S. Prieto, B. Elmore,

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E. Clausen, J. Gaddy, "The Biological Production of Ethanol from Synthesis Gas", Applied Biochemistry and Biotechnology, 20-1, p. 781, 1989):



Acetogenic bacteria are obligate anaerobes that utilize the acetyl-CoA pathway as their predominant mechanism for the reductive synthesis of acetyl-CoA from
10 CO_2 (Drake, H. L. (1994). Acetogenesis. New York: Chapman & Hall). This group of microorganisms is even more versatile in the sense that they can use simple gases like CO_2/H_2 and CO as well as sugars, carboxylic acids, alcohols and aminoacids.

Clostridium ljungdahlii, one of the first autotrophic microorganism known to
15 ferment synthesis gas to ethanol was isolated in 1987, as an acetogen favours the production of acetate during its active growth phase (acetogenesis)) while ethanol is produced primarily as a non-growth-related product (solventogenesis) ("Biological conversion of synthesis gas into fuels", K. Klasson, C. Ackerson, E. Clausen, J. Gaddy, International Journal of Hydrogen Energy 17, p.281, 1992).

20 Eubacterium limosum is an acetogen, isolated from habitats like the human intestine, rumen, sewage and soil, exhibits high growth rate under high CO concentrations producing acetate, ethanol, butyrate and isobutyrate (I. Chang, B. Kim, R. Lovitt, J. Bang, "Effect of CO partial pressure on cell-recycled continuous CO fermentation by Eubacterium limosum KIST612", Process Biochemistry, 37(4),
25 p. 411, 2001).

Peptostreptococcus productus is a mesophilic, gram-positive anaerobic coccus, found in the human bowel and is capable of metabolizing CO_2/H_2 or CO to produce acetate (W. Lorowitz, M. Bryant, "Peptostreptococcus productus Strain That Grows Rapidly with CO as the Energy-Source", Applied and Environmental
30 Microbiology, 47(5), p. 961, 1984).

Clostridium autoethanogenum is a strictly anaerobic, gram-positive, spore-forming, rod-like, motile bacterium which metabolizes CO to form ethanol,

acetate and CO₂ as end products, beside its ability to use CO₂ and H₂, pyruvate, xylose, arabinose, fructose, rhamnose and L-glutamate as substrates (J. Abrini, H. Naveau, E. Nyns.), "Clostridium autoethanogenum, Sp-Nov, an Anaerobic Bacterium That Produces Ethanol from Carbon-Monoxide", Archives of Microbiology, 161(4), p. 345, 1994).

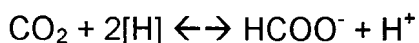
Clostridium carboxidivorans P7 is a solvent-producing anaerobe, which was isolated from the sediment of an agricultural settling lagoon. It is motile, gram-positive, spore-forming and primarily acetogenic, forming acetate, ethanol, butyrate, and butanol as end-products. (J. Liou, D. Balkwill, G. Drake, R. Tanner, "Clostridium carboxidivorans sp. nov., a solvent-producing clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen Clostridium scatologenes strain SL1 as Clostridium drakei sp. nov.", International Journal of Systematic and Evolutionary Microbiology, 55(5), p. 2085, 2005).

Acetogens are obligate anaerobic bacteria that use the reductive acetyl-CoA pathway as their predominant (i) mechanism for the reductive synthesis of acetyl-CoA from CO₂, (ii) terminal electron-accepting, energy-conserving process, and (iii) mechanism for the synthesis of cell carbon from CO₂" (Drake, H. L. (1994). Acetogenesis. New York: Chapman & Hall). Like other anaerobes, acetogens require a terminal electron acceptor different from oxygen. In the acetyl-CoA pathway, CO₂ serves as an electron acceptor and H₂ serves as the electron donor. The synthesis of acetyl-CoA from CO₂ and H₂ requires an 8-electron reduction of CO₂ involving the following three steps:

- Formation of the carbonyl precursor of acetyl-CoA
- Formation of the methyl precursor of acetyl-CoA
- 25 Condensation of the above two precursors to form acetyl-CoA.

The Methyl Branch of the Acetyl-CoA Pathway (see figure 1)

This part of the pathway results in the formation of the methyl-corrinoid protein that combines with the product of the carbonyl branch, to form acetyl-CoA. In the first step of this branch, CO₂ is reduced to formate (HCOO⁻) as shown in the following equation:

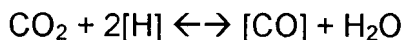


This reversible reaction is catalyzed by the enzyme formate dehydrogenase (FDH). Ferredoxin is the most commonly employed electron acceptor, among acetogens, NADH often acts as the electron donor. For acetogens grown on CO, Ljungdahl suggested that CO must first be oxidised to CO₂ by the enzyme carbon monoxide dehydrogenase (CODH) and subsequently reduced to formate by FDH (L. Ljungdahl, "The autotrophic pathway of acetate synthesis in acetogenic bacteria", Annual Review of Microbiology, 40, 415, 1986).

Formate is activated with tetrahydrofolate (THF) to form 10-formyl-THF by the enzyme formyl-THF synthetase in an ATP-dependent condensation. This bound formyl group is then reduced by a series of 3 enzymes to a bound methyl group (methyl-THF). In the final step of this branch, the methyl group is transferred to a corrinoid-containing protein [Co]-protein.

15 The Carbonyl Branch (see figure 1)

This branch of the pathway results in the formation of a bound carbonyl group which is then merged with the bound methyl group formed in the methyl branch to form acetyl-CoA. Carbon monoxide dehydrogenase (CODH) plays a very essential role with a double functionality. First, it catalyzes the oxidation of CO to CO₂, the reduction of CO₂ to bound carbonyl, finally mediating the synthesis of acetyl-CoA from the methyl and carbonyl groups. For the latter reason, CODH is also known as acetyl-CoA synthase. In the carbonyl branch, CO₂ is first reduced to [CO] ([] indicates that carbon monoxide is enzyme-bound) as follows:

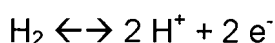


The bound carbonyl moiety is condensed with the bound methyl moiety from the methyl branch to form a bound acetyl-CODH moiety. In the final step, CODH condenses the bound acetyl with free coenzyme A to form acetyl-CoA, as follows:



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Hydrogenase enzymes are used by microorganisms either to dispose of electrons accumulated during fermentation via hydrogen formation, or hydrogen uptake and oxidation to produce energy. The reaction involving hydrogen is a reversible reaction catalysed by hydrogenase:



The CODH enzyme works in combination with hydrogenase to form the carbonyl precursor of acetyl-CoA (L. Ljungdahl, "The autotrophic pathway of acetate synthesis in acetogenic bacteria", Annual Review of Microbiology, 40, 415, 1986).

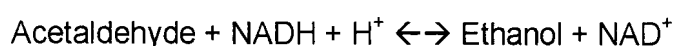
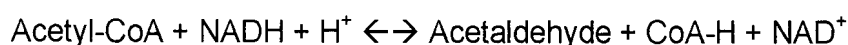
Acetyl-CoA is a central intermediate in the metabolic pathway of acetogens as it is a versatile precursor of alcohols, carboxylic acids, diacids, hydroxyacids, diols, lipids, amino acids, nucleotides and carbohydrates (Ljungdahl, L., "The autotrophic pathway of acetate synthesis in acetogenic bacteria", Annual Review of Microbiology, 40, p. 415-450, 1986.): (i) for cellular material, formed via the anabolic pathway, in which acetyl-CoA is reductively carboxylated into pyruvate by the enzyme pyruvate synthase (Diekert, G., "Metabolism of Homoacetogens", Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 66(1-3), p. 209-221, 1994) that is subsequently converted to phosphoenolpyruvate (PEP) which is an intermediate in the conversion to biomass and (ii) for energy conservation, acetyl-CoA goes through the catabolic pathway in order to make ATP. Beside the essential intermediate PEP that is at the basis of many biochemical pathways, acetyl-CoA can condense further to longer chain hydrocarbyl moieties.

In case of homoacetogens the acetyl-CoA is converted to acetate catalysed by Phosphotransacetylase and acetate kinase while producing ATP by substrate-level phosphorylation:



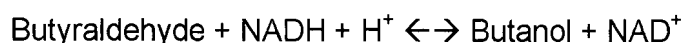
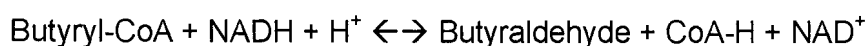
In the first reaction, the CoA unit is removed from the acetyl-CoA and a phosphate group is added by the enzyme phosphotransacetylase, resulting in the formation of acetyl-phosphate. In the second reaction, the acetyl phosphate

is converted to acetate while a molecule of adenosine diphosphate (ADP) is phosphorylated to form ATP. This part of energy-conservation pathway is usually favoured over the alcohol forming pathway during its exponential growth phase of the microorganism as it provides the cell with energy in the form of ATP, known as the acidogenic phase of the metabolism, which also results in a decrease in pH of the medium due to acid production. The second phase of the fermentation is the solventogenic phase, in which mainly ethanol is produced:



In the solventogenic branch of the pathway, the microorganism utilizes NADH as the reducing potential to first form acetaldehyde by the enzyme acetaldehyde dehydrogenase, followed by further reduction to ethanol by the enzyme alcohol dehydrogenase.

Many acetogens (*Clostridium acetobutylicum*) have the ability to produce 4-carbon products like butanol and butyric acid by condensation of 2 molecules of acetyl-CoA to form acetoacetyl-CoA that is further isomerised into butyryl-CoA. Analogously to acetyl-CoA, subsequent transformation into butyric acid produces ATP while the formation of butanol results in the consumption of reducing equivalents:



Pathways for the production of oxygenates having three carbons:

1. Propionic acid production

Propionibacterium species (*Propionibacterium acidipropionici*, *Propionibacterium acnes*, *Propionibacterium cyclohexanicum*, *Propionibacterium freudenreichii*, *Propionibacterium freudenreichii shermanii*)

and several other anaerobic bacteria such as *Desulfobulbus propionicus*, *Pectinatus frisingensis*, *Pelobacter propionicus*, *Veillonella*, *Selenomonas*, *Fusobacterium* and *Clostridium*, in particular *Clostridium propionicum*, produce propionic acid as a main fermentation product (Playne M., "Propionic and butyric acids", In: Moo-Young M, editor. Comprehensive biotechnology, New York: Pergamon Press, vol 3, p 731-759, 1985; Seshadri N, Mukhopadhyay S., "Influence of environmental parameters on propionic acid upstream bioprocessing by *Propionibacterium acidipropionici*", J. Biotechnology 29, p.321-328, 1993). In swiss-type cheeses, propionibacteria consume lactate and produce propionic acid, acetic acid, and CO₂. In general, a broad range of substrates can be converted into propionic acid, like glucose, lactose, sucrose, xylose, glycerol and lactate. Propionibacteria are Gram-positive, non-motile, non-sporulating, short-rodshaped, mesophilic anaerobes. The genus of *Propionibacterium*, belonging to the class of high G+C actinobacteria is divided into two groups: the "cutaneous" and the "dairy" *Propionibacteria*, based on their habitat (Stackebrandt, E., Cummins, C., Johnson, J., "The Genus *Propionibacterium*", in The Prokaryotes, E. Balows, H. Truper, M. Dworkin, W. Harder, K. Scheifer, eds., 2006).

20 **a. Dicarboxylic pathway**

Propionibacteria convert carbon sources to produce propionic acid as a main product via the mainly dicarboxylic acid pathway (also called the Wood-Werkman cycle or the methyl-malonyl-CoA pathway), as shown in Figure 2.

25 Glycolysis pathway catabolyses glucose into phosphoenolpyruvate (PEP), an energy-rich metabolite. Two alternative glycolysis pathways exist: Embden-Meyerhorf-Parnaz (EMP) pathway and Hexose Monophosphate (HMP) pathway. In the EMP pathway, 1 mole of glucose is converted into 2 moles of PEP and 2 moles of NADH, while in the HMP pathway 1 mole of glucose

30 provides 5/3 moles of PEP and 11/3 moles of NADH. PEP is further converted into two possible intermediates, pyruvate and oxaloacetate. The majority of PEP

is converted into pyruvate whereas the remaining PEP is converted into oxaloacetate. For pyruvate production, 1 mole of PEP is converted into 1 mole of pyruvate and 1 mole of ATP obtained from a transfer of one phosphoryl moiety from PEP to ADP. The total ATP obtained from the EMP and HMP pathways per mole of glucose is 2 and 5/3 moles, respectively. Glycolysis via the EMP pathway provides a lower amount of NADH (EMP: HMP = 2: 11/3) but a higher amount of ATP (EMP: HMP = 2: 5/3). The ratio of EMP to HMP pathway contribution in glycolysis is dependent on propionibacterium species, substrates and fermentation conditions. At the pyruvate node, pyruvate is directed toward three main pathways. Most of pyruvate is converted into propionic acid via the Wood-Werkman cycle. Some of pyruvate converts into acetate while some is incorporated into biomass. In the propionate formation pathway, pyruvate enters the Wood-Werkman cycle, via a transcarboxylation of a carboxyl-moiety from methylmalonyl-CoA to pyruvate, catalysed by oxaloacetate transcarboxylase in a coupled reaction of pyruvate to oxaloacetate and methylmalonyl CoA to propionyl CoA. In this coupled reaction, the carboxyl group transferred from methylmalonyl CoA to pyruvate to form propionyl CoA and oxaloacetate is never released from the reaction or no exchange between this carboxyl group with the dissolved CO₂ in the fermentation broth is observed (Wood HG., "Metabolic cycles in the fermentation of propionic acid", in Current Topics in Cellular regulation, Estabrook and Srera RW, eds., New York: Academic Press. vol 18, p 225-287, 1981). Because of this transcarboxylation reaction, CO₂ fixation is minimal and only used to produce catalytic amounts of oxaloacetate to reinitiate the cycle when for instance succinate accumulates as end-product. Under such circumstances, oxaloacetate is generated by condensation of CO₂ with phosphoenolpyruvate catalysed by a PEP carboxylase. Susequently, oxaloacetate is converted into malate by malate dehydrogenase, malate into fumarate by fumarase and further fumarate to succinate, catalyzed by succinate dehydrogenase. After that succinate is converted into succinyl-CoA, which is then converted into methylmalonyl-CoA. Methylmalonyl-CoA is converted into propionyl-CoA by oxaloacetate

transcarboxylase. At the end of the cycle, propionyl-CoA is converted into propionate along with a coupled reaction of succinate to succinyl-CoA, catalysed by propionyl-CoA: succinate transferase. After 1 mole of pyruvate enters the Wood-Werkman cycle, 1 mole of propionate, 2 moles of NAD⁺, and 1 mole of ATP are generated. Beside propionic acid as main fermentation product, produced in the Wood-Werkman cycle, also NAD⁺ regeneration for glycolysis occurs in this cycle.

In acetate branch pathway, pyruvate converts to acetyl-CoA and CO₂, catalyzed by pyruvate dehydrogenase complex. Acetyl-CoA is converted into acetyl-phosphate by phosphotransacetylase and further acetyl-phosphate to acetate, catalyzed by acetate kinase. In the acetate branch pathway, 1 mole of acetate, CO₂, NADH, and ATP are obtained from 1 mole of pyruvate. Propionic acid production is usually accompanied by the acetate formation as a major ATP production route supplying energy for cellular metabolism.

The following equations represent a theoretical formulation of propionic acid fermentation from glucose or lactate (P. Piveteau, Lait, 79, p. 23, 1999):



According to these equations, the theoretical maximum yield from glucose is 66.7 C-mole% or 54.8 wt% of propionic acid, 22.2 C-mole% or 22 wt% of acetic acid, 11.1 C-mole% or 17 wt% of CO₂. The theoretically propionic acid to acetic acid (P/A) molar ratio is 2:1.

A shift in the metabolic pathway towards the production of propionic acid can be accomplished by using carbon sources with higher reductive level (shift from heterofermentative to homofermentative acid production). A higher reductive level of substrate can cause significant increase in the P/A ratio due to the intracellular NADH/NAD⁺ balance. A better efficiency of propionic acid production from glycerol could be expected because of its higher reduction level compared to conventional substrates. Effectively, a propionic acid yield of 84.4

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C-mole% and a low acetic acid production (P/A molar ratio reaching 37) have been obtained from glycerol with *P. acidipropionici* (Barbirato, F., Chedaille, D. and Bories, A., "Propionic acid fermentation from glycerol: comparison with conventional substrates", *Appl Microbiol Biotechnol*, **47**, p. 441-446, 1997). This strain also produces some propanol from glycerol, indicating that when the substrate has a higher reduction level also products with a higher reduction level can be produced because of the better NADH/NAD⁺ balance.

Glycerol → propionate + 1 H₂O

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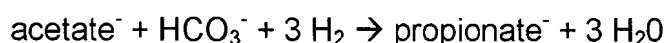
Himmi et. al. compared the fermentation of glycerol and glucose and product formation for *P. acidipropionici* and *P. freudenreichii* ssp. *shermanii*. Fermentation end-products were propionic acid as the major product, acetic acid as the main byproduct and two minor metabolites, n-propanol and succinic acid. The yield of propionic acid was up to 79 C-mole% (64 wt%) with glycerol as the carbon source (Himmi, E. H., Bories, A., Boussaid, A. and Hassani, L., "Propionic acid fermentation of glycerol and glucose by *Propionibacterium acidipropionici* and *Propionibacterium freudenreichii* ssp. *Shermanii*", *Appl Microbiol Biotechnol*, **53**, p. 435-440, 2000). Rumen microorganisms that ferment lactate via the dicarboxylic acid pathway, produce more propionate relative to acetate when hydrogen is added (M. Schulmunda and D. Valentino, "Factors Influencing Rumen Fermentation: Effect of Hydrogen on formation of Propionate", *Journal of Dairy Science*, vol. 59 (8), p. 1444-1451, 1976). Acetic acid was almost eliminated when a high H₂ pressure was applied during the fermentation with *Propionispira arboris* containing hydrogenase (Thompson T.E., Conrad R, Zeikus J.G., "Regulation of carbon and electron flow in *Propionispira arboris*: Physiological function of hydrogenase and its role in homopropionate formation", *FEMS Microbiol Lett* 22, p. 265-271, 1984 and US Patent 4732855). According to the Wood-Werkman cycle, endogenous CO₂ is released with acetic acid formation by *Propionibacteria* from glucose, lactose, or lactate fermentation (Deborde C., Boyaval P. 2000, Interactions between pyruvate and

- lactate metabolism in *Propionibacterium freudenreichii* subsp. *shermanii*: In vivo ^{13}C nuclear magnetic resonance studies, *Appl Environ Microbiol* 66: 2012-2020). CO_2 can be fixed in *Propionibacteria* to form oxaloacetate from PEP catalyzed by PEP carboxylase and then lead to succinate generation. Based on
- 5 the metabolic pathway (Wood-Werkman cycle), CO_2 (HCO_3^-) is required to convert phosphoenolpyruvate (PEP) into oxaloacetate by the enzyme phosphoenolpyruvate carboxylase. Through several sequential reactions, oxaloacetate is finally converted to propionic acid. In case of glycerol as substrate, nearly no acetate and hence CO_2 is produced. Applying an
- 10 exogenous CO_2 pressure during fermentation has an positive effect on metabolite production rate and in particular a higher succinate accumulation thanks to the higher PEP carboxylation activity ("Effect of carbon dioxide on propionic acid productivity from glycerol by *Propionibacterium acidipropionici*", An Zhang and Shang-Tian Yang, SIM annual meeting and Exhibition, San
- 15 Diego, 2008).
- Most propionic acid producing bacteria have the enzymes of the tricarboxylic acid cycle (TCA) which explain the variable P/A ratios for different strains. Some of the acetyl-CoA can be utilized in the TCA cycle by condensation with pyruvate into citrate (see figure 2). The end result is that more CO_2 is produced
- 20 in the TCA cycle through the decarboxylations and less acetate is secreted. P/A ratios from 2.1 to 14.7 and CO_2 /acetate ratio from 1.0 to 6.3 have been reported from glucose (Wood HG., "Metabolic cycles in the fermentation of propionic acid", in *Current Topics in Cellular regulation*, Estabrook and Sreer RW, eds., New York: Academic Press. vol 18, p 225-287, 1981).
- 25 *Pelobacter propionicus*, using the dicarboxylic acid pathway, has been show to grow on ethanol as substrate while producing propionate in presence of CO_2 (Schink, B., Kremer, D. and Hansen, T., "Pathway of propionate formation from ethanol in *Pelobacter propionicus*", *Arch. Microbiol.* 147, 321-327, 1987 and S. Seeliger, P. Janssen, B. Schink, "Energetics and kinetics of lactate fermentation
- 30 to acetate and propionate via methylmalonyl-CoA or acrylyl-CoA", *FEMS Microbiology Letters*, 211, pp. 65-70, 2002). When ethanol is fed together with

CO₂ and hydrogen, significant amounts of propanol are produced. Ethanol is converted into acetyl-CoA (via acetaldehyde) while producing electrons for the carboxylation of acetyl-CoA into pyruvate, catalysed by pyruvate synthase. Combined with the dicarboxylic acid pathway propionate is produced from ethanol and CO₂ (Schink et al., 1987).



Pelobacter propionicus is not able to reductively convert acetate and CO₂ into propionate whereas Desulfobulbus propionicus does make propionate from acetate and CO₂ (Schink et al., 1987).



15 **b. Acrylate pathway**

Though many bacteria can ferment a variety of substrates anaerobically into lactate as end product, some can further reduce the lactate into propionate, like Clostridium propionicum, Clostridium neopropionicum, Megasphaera elsdenii and Prevotella ruminicola (P. Boyaval, C. Corre, "Production of propionic acid", Lait, 75, 453-461, 1995) by using the acryloyl-CoA pathway (see figure 3). Several substrates (sugars, ethanol and some aminoacids) that can be converted into pyruvate as intermediate can be further reduced into propionate as main product with acetate and butyrate as co-product. The key reaction is the lactoyl-CoA dehydration into acryloyl-CoA that is subsequently reduced to propionyl-CoA. The electrons for this reduction are provided by the oxidation of pyruvate/lactate into acetate and CO₂ (G. Gottschalk, "Bacterial Metabolism", 2nd ed., Springer, New York, 1986).

Clostridium neopropionicum (strain X4), using the acrylate pathway, is able to convert ethanol and CO₂ into acetate, propionate and some propanol (J. Tholozan, J. Touzel, E. Samain, J. Grivet, G. Prensier and G. Albagnac, "Clostridium neopropionicum sp. Nov., a strict anaerobic bacterium fermenting

ethanol to propionate through acrylate pathway", Arch. Microbiol., 157, p. 249-257, 1992). As for the dicarboxylic acid pathway, the intermediate acetyl-CoA produced from the substrate ethanol is linked to the acrylate pathway via the pyruvate synthase that converts acetyl-CoA into pyruvate by carboxylation with CO₂.

Recently, an alternative route leading to acryloyl-CoA consists in the conversion of acetyl-CoA into malonyl-CoA by carboxylation with CO₂. The malonyl-CoA is further converted into acryloyl-CoA via four steps implicating malonate-semialdehyde, hydroxypropanoate, hydroxypropanoyl-CoA and finally acryloyl-CoA. Acryloyl-CoA produced by this pathway is subsequently reduced to propionyl-CoA similarly to the reactions leading to acryloyl-CoA by dehydration of lactoyl-CoA (J. Zarzycki, "Identifying the missing steps of the autotrophic 3-hydroxypropionate CO₂ fixation cycle in *Chloroflexus aurantiacus*, PNAS, 106(50), p. 21317, 2009; I. Berg, "A 3-hydroxypropionate/ 4-hydroxybutyrate autotrophic carbon dioxide assimilation pathway in archaea, Science, 318, p.1782, 2007).

2. Aceton/Isopropanol production

Members of the *Clostridium*, *Butyrivibrio*, *Bacillus*, and other less-well defined flora of anaerobic digestion systems produce butyric acid, butanol, acetone, isopropanol, or 2,3-butanediol (A. Moat, J. Foster & M. Spector, "Microbial Physiology", 4th Ed., Wiley-Liss, 2002). Hydrogen, carbon dioxide, acetate, and ethanol are concomitantly produced in minor amounts during the fermentation. *Clostridium acetobutylicum* utilizes the EMP pathway for glucose catabolism with the formation of ethanol, carbon dioxide, hydrogen, acetone, isopropanol, butyrate, and butanol from pyruvate via acetyl-CoA as shown in Figure 4 (D. Jones and D. Woods, "Acetone-Butanol Fermentation Revisited", Microbiological Reviews, , p. 484-524, 1986; R. Gheshlaghi, J. Scharer, M. Moo-Young, C. Chou, "Metabolic Pathways of Clostridia for producing butanol", Biotechnology Advances, 27, p. 764, 2009).

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Strains such as *Clostridium acetobutylicum*, *Clostridium beijerinckii*, *Clostridium saccharobutylicum*, and *Clostridium saccharoperbutylacetonicum* produce butanol in high concentration with acetone (or isopropanol) and ethanol. During growth, the organism first forms acetate and butyrate (acidogenic phase),

5 disposing the excess electrons by reducing H^+ to H_2 . As the pH drops, due to the accumulation of acids, the culture is entering the stationary phase, there is a metabolic shift to solvent production (solventogenic phase). Acetyl-CoA undergoes condensation to form acetoacetate, which may be reduced to butyrate and butanol or cleaved via decarboxylation to acetone with

10 concomitant production of CO_2 . Acetone may be further reduced to isopropanol. Acetyl-CoA may also be reduced to acetaldehyde and ethanol. During the solventogenic phase, sugars are fermented directly to solvents, while the present acidic products are also converted to solvents. Acetate and butyrate are activated to acetyl-CoA and butyryl-CoA through the reactions catalyzed by

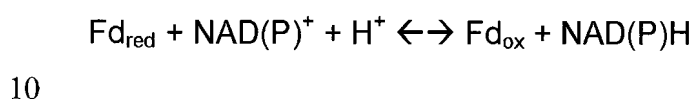
15 acetoacetyl-CoA:acetate-CoA transferase or kinase and phosphotransacetylase. These acyl-CoA's are reduced to ethanol and butanol by aldehyde dehydrogenase and alcohol dehydrogenase. Acetone can in some species further be reduced to isopropanol, like in *Clostridium beijerinckii*, since the alcohol dehydrogenase is receptive for different substrates, not only for

20 aldehydes but also for ketones (A. Ismaierl, C. Zhu, G. Colby, J. Chen, "Purification and characterization of a primary-secondary alcohol dehydrogenase from two strain of *Clostridium beijerinckii*", J. Bacterial., 175, p. 5097, 1993; J. Chen and S. Hiu, "Acetone-butanol-isopropanol production by *Clostridium Beijerinckii*" Biotechnology Letters, Vol 8(5), p. 371-376, 1986; H. George, J.

25 Jonhson, W. Moore, L. Holdeman, J. Chen, "Acetone, Isopropanol, and Butanol Production by *Clostridium beijerinckii* (syn. *Clostridium butylicum*) and *Clostridium aurantibutyricum*", Applied and Environmental Microbiology, p. 1160-1163, 1983). The decarboxylation of acetoacetate results in a sharp decrease in the number of electron ($H^+ + e^-$) acceptors available as CO_2 is

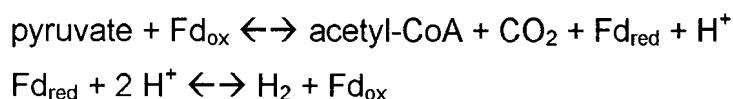
30 expelled. Acetyl-CoA and acetone can act as electron acceptor, giving rise to ethanol or isopropanol. For the onset of solventogenesis, electron flux as well

as carbon flux is redirected in order to preserve the oxido-reductive balance. More electrons are needed to produce solvents molecules (butanol, isopropanol and ethanol) than for the corresponding acids. The excess electrons that were used to reduce H^+ to H_2 during the acidogenic phase are used by aldehyde
 5 dehydrogenase and alcohol dehydrogenase during the solventogenic phase. In the solvent-producing clostridia, $NAD(P)^+$:ferredoxin oxidoreductase is active, exchanging electrons between $NAD(P)^+$ and ferredoxin.



During the acidogenic phase this enzyme is active to reduce ferredoxin while oxidizing $NAD(P)H$, and catalyzes the reverse reaction during solventogenesis. The regeneration of $NAD(P)^+$ is required for the oxidation of glyceraldehydes-3-phosphate and prevents hence the accumulation of $NAD(P)H$. H_2 produced
 15 during acidogenesis is taken up by the bacteria for solvent production. Solventogenic clostridia have a H_2 -producing hydrogenase as well as an uptake hydrogenase.

In clostridia and other anaerobes, hydrogen is formed through a pyruvate:ferredoxin (Fd) oxidoreductase without the intermediary production of
 20 formate. The reduced ferredoxin is converted to hydrogen by hydrogenase:



25 Hydrogenase competes with ferredoxin: $NAD(P)$ reductase to oxidize the reduced ferredoxin. The hydrogen evolution rate in the acidogenic phase is significantly higher. The flow of electrons from $NADH$ to Fd_{red} and to H_2 explains why these organisms produce large quantities of H_2 . During the Solventogenic phase more $NAD(P)H$ is diverted to the formation of solvent alcohols by
 30 reduction of the corresponding acids. Several methods have been developed in order to reduce the iron-sulfide based hydrogenase activity and hence reducing the formation of hydrogen for the benefit of further reducing the acids into

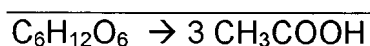
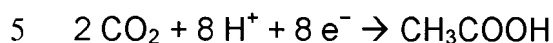
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alcohols: external hydrogen pressure addition, carbonmonoxide addition that inhibits the iron-based hydrogenase activity, growth under iron-limiting conditions and co-fermenting highly reduced substrates like glycerol (see Gheshlaghi et al. and Jones et al.). Under such conditions the flow of electrons from reduced ferredoxin to molecular hydrogen via the hydrogenase system is inhibited and shifted to the generation of NAD(P)H via the action of the appropriate ferredoxin oxidoreductase, resulting in an increase in the production of butanol and ethanol. Often under reduced activity of the hydrogenase, also less acetone/isopropanol is produced as the formation of acetone by decarboxylation of acetoacetate lowers the amount of available electron acceptors.

Recently, *E. Coli* has been engineered with a specific pathway for the production of isopropanol from glucose by introducing acetyl-CoA acetyltransferase, acetoacetyl-CoA transferase, acetoacetate decarboxylase and alcohol dehydrogenase from the appropriate *Clostridium* species (T. Hanai, "Engineered Synthetic Pathway for Isopropanol Production in *Escherichia coli*", Applied and Environmental Microbiology, 73(24), p. 7814, 2007; T. Jojima, "Production of isopropanol by metabolically engineered *Escherichia coli*", Appl. Microbiol. Biotechnol., 77, p. 1219, 2008; US 2009/0246842; PCT 2009/049274; EP2184354). Molar yields of 43.5% are obtained, which is close to the theoretical yield of 50%. One mole of glucose is converted to 2 moles of acetyl-CoA and 2 mole of CO₂. The 2 acetyl-CoA's are condensed to one acetoacetate that is subsequently decarboxylated to make 1 mole of isopropanol and 1 additional CO₂.

Production of acetic acid from glucose by *C. thermoaceticum* is efficient in that 3 molecules of acetate are produced per molecule of glucose. Two acetate molecules are produced according to the well-known glycolysis, decarboxylation of pyruvate into acetyl-CoA and CO₂, followed by transformation of acetyl-CoA into acetate. During these reactions CO₂ and an excess of electrons are produced. Some microorganisms, such as *C. thermoaceticum* possess also the

Wood- Ljungdahl enzymes that allow converting the CO₂ and excess electrons into more acetate.



This is particular in that most heterotrophs can only add carbon dioxide to a pre-existing compound and add a carboxyl group. Some bacteria that can form an organic compound directly from carbon dioxide and hydrogen contain hydrogenase, an enzyme that converts hydrogen to two protons and two electrons. These electrons provide the necessary reductive potential for the transformation of carbon dioxide. The overall reaction involves participation of ferredoxin as a reduced electron carrier and the enzymes hydrogenase, carbon monoxide dehydrogenase, and methylenetetrahydrofolate reductase.

3. Threonine degradation

The amino-acids serine and threonine provide the precursors, directly or indirectly, of eight other amino-acids. They also can be derived from one another through the common intermediate glycine. Both serine and threonine can be metabolized in a single enzymic step to energy-rich keto-acids, which can be catabolized to generate ATP by substrate-level phosphorylation, resulting in carboxylic acids. Threonine is derived from oxaloacetate (see figure 5) via transamination to make aspartate and serine intermediates. Oxaloacetate is a central metabolite of the TCA cycle that is at the origin of many essential metabolites and can be produced as end-product that condenses again with acetyl-CoA to form citrate. Oxaloacetate needs also to be produced by condensation of PEP with CO₂ with the help of PEP carboxylase, particularly when intermediates of the TCA cycle gets depleted to make other metabolites. One of these derivative metabolites is threonine that is directly produced from oxaloacetate with the help of multiple enzymes (see figure 5).

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Threonine, a native amino-acid, is known to be fermented by a number of microorganisms like, *Clostridium tetanomorphum*, *Escherichia coli*, *Salmonella Typhimurium* (G. Sawers, "The anaerobic degradation of L-serine and L-threonine in enterobacteria: networks of pathways and regulatory signals", Arch Microbiol, 171, p. 1–5, 1998; H. Barker, "Amino acid degradation by anaerobic bacteria", Annual Review of Biochemistry 50, p.23-40, 1981). The products are typically acetate, propionate, butyrate and 2-aminobutyrate and combinations of the latter. The exploitation of native amino-acid intermediates as final products is often easily implemented in micro-organism as there is no or minor metabolic perturbation because of non toxic intermediates. The basic pathway of threonine fermentation, via 2-ketobutyrate to propionate, is a well-known pathway (G. Gottschalk, "Bacterial Metabolism", 2nd ed. Springer, 1986). Enteric bacteria possess two types of threonine dehydratases/deaminases - a catabolic enzyme and biosynthetic enzyme that both convert threonine to 2-ketobutyrate (Umbarger H., Brown B., "Threonine deamination in *Escherichia coli*. II. Evidence for two L-threonine deaminases", J. Bacteriol., 73(1), p.105-12, 1957). While the biosynthetic dehydratase is involved in L-isoleucine biosynthesis out of 2-ketobutyrate, the catabolic enzyme participates in the degradation of threonine to propionate, in a pathway that generates ATP, and enables the bacteria to utilize threonine as the sole source of carbon and energy, typically only under anaerobic conditions and when low levels of energy in the cell are available (see figure 5). 2-ketobutyrate formate-lyase, is only expressed under anaerobic conditions (Sawers et al.). Once propanoyl-CoA is formed, it is processed via propionyl-P to propionate, in a reaction sequence that produces ATP.

The use of a reductive pathway to make n-propanol to dispose of electrons generated during amino-acid fermentation is unusual. 2-Ketobutyrate is cleaved to yield propionyl-phosphate, CO₂ and H₂ (or formate) by the enzyme, 2-ketobutyrate formate-lyase, in the presence of ferredoxin, CoA-SH, and inorganic phosphate. *Clostridium* sp. strain 17cr1 is able to ferment L-threonine to propionate and propanol (PH. Janssen, "Propanol as an end product of

threonine fermentation", Arch Microbiol., 182, p. 482–486, 2004). Electrons arising from the oxidation of 2-ketobutyrate to propionyl-CoA are used in reductive pathway leading to propanol formation. When hydrogen is removed from the medium, the formation of propanol ceases.

- 5 Also yeasts have the ability to convert amino-acids into the corresponding alcohols (M. Lambrechts, "Yeast and its importance to Wine Aroma – A Review", S. Afr. J. Enol. Vitic., 21, p. 97, 2000). This occurs according to the Ehrlich pathway where the amino-acids is deaminated to the corresponding 2-ketoacid that is subsequently decarboxylated and further reduced to alcohols.
- 10 Recently, the 2-ketoacid decarboxylase from *Lactococcus Lactis* and alcohol dehydrogenase from *Saccharomyces Cerevisiae* have been introduced into *E. Coli* in order to convert glucose via 2-ketobutyrate into n-propanol (C. Shen, "Metabolic engineering of *E. Coli* for 1-butanol and 1-propanol production via the keto-acid pathways", Metabolic Engineering, 10, p. 312, 2008).

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4. Citramalate pathway

- An alternative route to 2-ketobutyrate from pyruvate and acetyl-CoA via citramalate synthase has been reported in several organisms as an alternative
- 20 pathway for the production of isoleucine, compared to the threonine deamination into 2-ketobutyrate; in archea like *Ignicoccus hospitalis* and *Methanococcus jannaschii*, in bacteria like *Leptospira interrogans*, *Geobacter sulfurreducens* and in cyanobacteria like *Cyanothece* sp. ATCC 51142 (B. Wu, "Alternative isoleucine synthesis pathway in cyanobacterial species",
- 25 Microbiology, 156, 596–602, 2010; Howell D., "(R)-citramalate synthase in methanogenic archaea." J. Bacteriol., 181(1), p.331-3, 1999; J. Huber, "Insights into the autotrophic CO₂ fixation pathway of the archaeon *Ignicoccus hospitalis*: comprehensive analysis of the central carbon metabolism", J Bacteriol., 189(11), p.4108, 2007). This pathway, designated the citramalate pathway (see
- 30 figure 6) is the most direct route to synthesize 2-ketobutyrate and does not involve transamination followed by deamination. (R)-Citramalate synthesized

from pyruvate and acetyl-CoA is then converted to 2-ketobutyrate via an isomerase and dehydrogenase step. As explained for the threonine degradation, the 2-ketobutyrate is the precursor for propionate and propanol.

Recently, citramalate synthase from *Methanococcus jannaschii* has been introduced in *Escherichia coli* and improved by directed evolution to allow the direct production of n-propanol from glucose via the citramalate pathway (S. Atsumi, "Directed Evolution of *Methanococcus jannaschii* Citramalate Synthase for Biosynthesis of 1-Propanol and 1-Butanol by *Escherichia coli*", *Applied and Environmental Microbiology*, 74(24), p. 7802–7808, 2008).

5. Propanediol reduction pathway

1,2-propanediol can be produced by a wide variety of bacteria like, *Clostridium thermobutyricum*, *Escherichia coli*, *Bacteroides rumenicola* and yeasts. Three possible biosynthesis routes are known: (i) in which deoxy-sugars are converted in dihydroxyacetone-phosphate and lactaldehyde that is further reduced to propanediol, (ii) in which the glycolysis intermediate, dihydroxyacetone-phosphate, of regular sugars is converted into methylglyoxal and further reduced to propanediol (G. Bennet, "Microbial formation, biotechnological production and applications of 1,2-propanediol", *Appl. Microbiol. Biotechnol.*, 55, p. 1, 2001; R. K. Saxena, "Microbial production and applications of 1,2-propanediol", *Indian Journal of Microbiology*, 50(1), p. 2-11, 2010).

1,2-Propanediol is a fermentation end product of the 6-deoxyhexose sugars L-rhamnose and L-fucose, which are abundantly present in hemicellulose of plant cell walls (Badia, J., "Fermentation mechanisms of fucose and rhamnose in *Salmonella typhimurium* and *Klebsiella pneumonia*", *J. Bacteriol.*, 161, p. 435-437, 1985; Forsberg, "Metabolism of rhamnose and other sugars by strains of *Clostridium acetobutylicum* and other clostridium species", *Can. J. Microbiol.*, 33, p. 21, 1987; Tran-Din K., "Formation of 1,2-propanediol and lactate from glucose by *Clostridium sperioides* under phosphate limitation", *Arch. Microbiol.*, 142, p. 87, 1985). These methylpentoses are metabolized by pathways that

involve an isomerase, a kinase and an aldolase and form equimolar amounts of dihydroxyacetone phosphate (DHAP) and lactaldehyde (see figure 7 for fucose fermentation; similar pathway exist for rhamnose). DHAP is converted to pyruvic acid and incorporated into central carbon metabolism. But the fate of the lactaldehyde can differ according to the species and conditions. In presence of oxygen, lactaldehyde is converted to lactate. Under anaerobic conditions, lactaldehyde is reduced to 1,2-propanediol by an NAD-dependent propanediol oxidoreductase.

Several bacteria, including *Salmonella*, *Klebsiella*, *Shigella*, *Yersinia*, *Listeria*, *Lactobacillus* and *Lactococcus*, include species that grow on 1,2-propanediol in a coenzyme B12-dependent way (E. Sampson, "Microcompartments for B12-Dependent 1,2-Propanediol Degradation Provide Protection from DNA and Cellular Damage by a Reactive Metabolic Intermediate", *J. of Bacteriology*, 190(8), p. 2966–2971, 2008). The initial step is carried out by coenzyme B12-dependent propanediol dehydratase, which dehydrates 1,2-propanediol to propionaldehyde. Propionaldehyde is then disproportionated to n-propanol and propionyl-CoA. Propionyl-CoA can be utilized in two different ways depending on the availability of oxygen. Under anaerobic conditions, propionyl-CoA is converted to propionyl phosphate by phosphotransacylase and the propionyl-phosphate is cleaved by propionyl kinase, producing ATP. Both n-propanol and propionate are typically excreted, hence 1,2-propanediol fermentation provides an electron sink and a source for the production of ATP and eventually a three-carbon intermediate (propionyl-CoA) for growth by degradation to pyruvate and succinate via the methylcitrate pathway.

The second route is the metabolism of common sugars (e.g. glucose or xylose) through the glycolysis pathway followed by the methylglyoxal pathway. Dihydroxyacetone phosphate is converted to methylglyoxal that can be reduced either to lactaldehyde or to acetol. These latter compounds can further be reduced yielding 1,2-propanediol (see figure 8, many of the involved enzymes have not yet been characterized). This route is used by wild strain producers of 1,2-propanediol, such as *Clostridium sphenoides* (Tran Din K. and Gottschalk

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G., Arch. Microbiol. 142: 87-92, 1985) and Thermoanaerobacter
thermosaccharolyticum (Cameron D. and Cooney C., Bio/Technology, 4, p.
651-654, 1986; Sanchez-Rivera F, Cameron D., Cooney C., Biotechnol. Lett. 9,
p. 449-454, 1987). US 6,303,352 and US 2010/0261239 disclose metabolic
5 engineered microorganism that convert sugars into 1,2 propanediol via the
methylglyoxal pathway. There are two possible more direct links between de
methylglyoxal pathway and pyruvate via the formation of lactate (see figure 8):
(i) via glyoxalases enzymes methylglyoxal (which is often toxic for
microorganisms) is transformed into lactate and (ii) via lactaldehyde
10 dehydrogenase, lactaldehyde is transformed into lactate (R. Saxena, "Microbial
production and application of 1,2-propanediol", Ind. J. Microbiol., 50(1), p. 2,
2010; J. Weber, "Metabolic flux analysis of Escherichia coli in glucose-limited
continuous culture. II. Dynamic response to famine and feast, activation of the
methylglyoxal pathway and oscillatory behavior", Microbiology, 151, p. 707-716,
15 2005).

The prior art for the production of propanol that can be dehydrated into
propylene, described here above, has several technical disadvantages that
refrain commercialization, in particular when compared to the ethanol
20 fermentation of sugars, resulting in a theoretical 66.7% carbon efficiency and
nearly 100% hydrogen efficiency into useful ethanol ($C_6H_{12}O_6 \rightarrow 2 C_2H_6O + 2$
 CO_2):

- a. Some of the described pathways involve a decarboxylation step
that dispose electron as hydrogen (or formate). This is the case
25 for the acetone/isopropanol pathway where acetoacetate is
decarboxylated and the threonine and citramalate pathway where
2-ketobutyrate is decarboxylated. Starting from sugars as
feedstock, this reduces the potential yield down to 50%.
- b. Some of the described pathways have the potential of a high
30 carbon yield (Wood-Werkman, acrylate pathway, propanediol
degradation and threonine degradation), but the pathway is mainly

able to provide propionic acid as end product as there is a shortage of reducing equivalents. Often such pathways entail formation of byproducts as acetic acid that consumes less reducing equivalents and hence leave more reducing equivalents to make some propanol.

- c. As for the described metabolic pathways the starting feedstock is always a carbohydrate, the potential of biomass valorization is limited to the carbohydrate part of biomass. On top of that the pathways are most of the time not able to use C₅ sugars as starting feedstock whereas biomass contains significant amounts of hemicelluloses, consisting of mainly C₅-sugars.

It is the object of the present invention to provide a process for the production of propylene by gasifying carbonaceous feedstock or reforming natural gas into synthesis gas, converting the synthesis gas into propanol (either n-propanol or isopropanol) by means of a microorganism, possessing the required Wood-ljundahl enzymes and the enzymes of at least one of the pathways for the production of C₃-oxygenates and dehydrating the propanol into propylene and water. Advantageously it is dehydrated over an acidic catalyst.

Synthesis gas is a mixture of hydrogen, carbonmonoxide and carbondioxide. As the energy carriers are hydrogen and carbonmonoxide, it is recommended that the synthesis gas contains at least hydrogen or carbonmonoxide next to carbondioxide.

By transforming the carbonaceous feedstock into synthesis gas, most of the carbon in the feedstock can be valorized, including the lignin and C₅-sugar constituents of biomass, but also waste carbonaceous feedstock like waste plastics, tires and municipal waste, coal, petcoke, liquid residu's from petrochemical processes like crude refining or liquid residu's from the paper industry and natural gas, associated gas or unconventional gas can be transformed into synthesis gas.

In the above described metabolic pathways, the PEP-pyruvate-acetyl-CoA interconversion (involving the enzymes pyruvate phosphate dikinase/pyruvate kinase and pyruvate dehydrogenase/pyruvate synthase) is the central starting node of the biosynthesis of C₃-oxygenates. Instead of producing these central node intermediates directly from carbohydrates, it is advantageous to produce acetyl-CoA, as part of this node, substantially from synthesis gas via the Wood-Ljungdahl pathway (the reductive acetyl-CoA pathway). Once sufficient acetyl-CoA is produced in the cells, via the central node, PEP-pyruvate-acetyl-CoA, one of the possible pathways described above can result in C₃-oxygenates formation. For biosynthesis pathways that involve decarboxylation to make CO₂ and disposal of electron by hydrogen production or involve decarboxylation to make formate, can be improved with respect to carbon yield as the CO₂/H₂ or formate can be recycled by the Wood-Ljungdahl pathway enzymes.

Via the Wood-Ljungdahl pathway, hydrogen can also provide required electrons via hydrogenase activity for the further reduction of propionic acid, propanediol, propanal or acetone.

It is the object of the present invention to improve the carbon yield for propanol production from carbonaceous feedstock's by optimizing the flow of available electron to the production of propanol by combining the described pathways for making propionic acid, propanediol, propanal, acetone or propanol with the Wood-Ljungdahl pathway (the reductive acetyl-CoA pathway) so that disposed electrons can be recycled via the Wood-Ljungdahl pathway enzymes.

The prior art provides a first indication that *Clostridium* strain P11 possesses the Wood-Ljungdahl pathway enzyme, to provide the formation of acetyl-CoA and probably the enzymes of the acetone/isopropanol pathway. This strain converts synthesis gas in isopropanol (9.25 g/l) beside ethanol (25.26 g/l), acetic acid (4.82 g/l) and 1-butanol (0.47 g/l) (D. Kundiyana, J. of Bioscience and Bioengineering, 109(5), p. 492, 2010). It has also been reported by E. Caldwell that *Clostridium* CAT11 and CP19 are able to produce acetate, ethanol,

propionate, propanol and butyrate/butanol as end products of fermentation of synthesis gas (M. Caldwell, T. Allen, P. Lawson and R. Tanner, Annual Meeting of the Missouri Valley Branch of the American Society of Microbiology, March 27-28, 2009, University of Kansas, Lawrence, KS).

5

It is the object of the present invention to isolate from nature, wild strain microorganisms that possess the nucleic acid sequence information, to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway) and that possess the nucleic acid sequence information of one of the described pathways to make C₃-oxygenates (acetone/isopropanol pathway, dicarboxylic pathway, acrylate pathway, threonine degradation pathway, citramalate pathway or the propanediol reduction pathway).

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It is one object of the present invention to produce propylene by dehydration of propanol that is produced by an improved microorganism that optimizes the carbon flux toward to production of propanol.

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US 4,727,214 describes a process for converting anhydrous or aqueous ethanol into ethylene by means of a catalyst of the crystalline zeolite type, said catalyst having, on the one hand, channels or pores formed by cycles or rings of oxygen atoms having 8 and/or 10 elements or members, and on the other hand, an atomic Si/Al ratio of less than about 20. In the examples, the atomic ratio Si/Al of the FER used is from 5 to 20, the temperature from 217 to 280°C, and the WHSV of 2.5 h⁻¹.

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JP 2009-215244 A published on 24 sept 2009 relates to a method to produce ethylene by contacting ethanol on a H-FER catalyst, having an atomic Si/Al between 3 and 20, more specifically between 4 and 10, sodium and potassium contents both of 0.1%wt or less, more specifically of 0.005%wt or less, the temperature ranging from 200 to 300°C, pressure from 10 to 100 bara, and WHSV from 0.1 to 10 h⁻¹. In examples, the appraisal of the ethanol reaction is

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achieved by method of gas pulse reaction using gas chromatograph. In the examples the temperature is 260°C or under.

EP 379803 provides a new process for propylene preparation comprises
5 dehydrating iso-propanol in the presence of a gamma-alumina catalyst having mean pore diameter of 3-15 nm with a standard deviation of 1-4 nm based on a statistical calculation from pore diameter and pore volume. Specifically, the gamma-alumina catalyst has pore volume of at least 0.4 cc/g on dry basis; is a low alkali gamma-alumina comprising at least 90 weight% of gamma-alumina,
10 less than 10 weight% of silica and up to 0.5 weight% of alkali metal oxide; and is a weakly acidic gamma-alumina having pKa of 3.3-6.8 measured by Hammett's equation and an integrated acid quantity of up to 0.5 micro-equivalent 1g on dry basis. The new process gives propylene in high selectivity and yield using a simple non-corrosion resistant reactor at lower temperature
15 than for conventional processes.

WO 2009-098262 (in a first embodiment) relates to a process for the dehydration of an alcohol having at least 2 carbon atoms to make the corresponding olefin, comprising:
20 introducing in a reactor a stream (A) comprising at least an alcohol, optionally water, optionally an inert component, contacting said stream with a catalyst in said reactor at conditions effective to dehydrate at least a portion of the alcohol to make an olefin,
recovering from said reactor an olefin containing stream (B),
25 Wherein
the catalyst is :
a crystalline silicate having a ratio Si/Al of at least about 100, or
a dealuminated crystalline silicate, or
a phosphorus modified zeolite,
30 the WHSV of the alcohols is at least 2 h⁻¹,
the temperature ranges from 280°C to 500°C.

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WO 2009-098262 (in a second embodiment) relates to a process for the dehydration of an alcohol having at least 2 carbon atoms to make the corresponding olefin, comprising:

- 5 introducing in a reactor a stream (A) comprising at least an alcohol, optionally water, optionally an inert component, contacting said stream with a catalyst in said reactor at conditions effective to dehydrate at least a portion of the alcohol to make an olefin,
- recovering from said reactor an olefin containing stream (B),
- 10 Wherein
- the catalyst is a phosphorus modified zeolite,
- the temperature ranges from 280°C to 500°C.

Synthesis gas Production

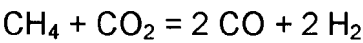
- 15 Synthesis gas can be produced from low-molecular weight hydrocarbons via reforming or from solid or high-molecular weight liquid hydrocarbons via gasification.

Low-molecular weight hydrocarbons are transformed in presence of steam and/or oxygen into synthesis gas in the "steam reforming" (SMR), CO₂ reforming or autothermal reforming (ATR) (K. Aasberg-Petersen, "Technologies for large-scale gas conversion", Applied Catalysis A: General, 221, p. 379–387, 2001)

Steam reforming

- $$\text{CH}_4 + \text{H}_2\text{O} = \text{CO} + 3 \text{H}_2$$
- 25
$$\text{C}_n\text{H}_m + n \text{H}_2\text{O} = n \text{CO} + n + (m/2) \text{H}_2$$
- $$\text{CO} + \text{H}_2\text{O} = \text{CO}_2 + \text{H}_2$$

CO₂ reforming



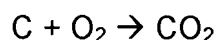
Autothermal reforming (ATR)

- 30
$$\text{CH}_4 + 1.5 \text{O}_2 = \text{CO} + 2 \text{H}_2\text{O}$$
- $$\text{CH}_4 + \text{H}_2\text{O} = \text{CO} + 3 \text{H}_2$$
- $$\text{CO} + \text{H}_2\text{O} = \text{CO}_2 + \text{H}_2$$

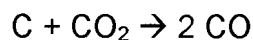
- Gasification is a partial combustion process that converts carbonaceous materials into CO, CO₂ and H₂. In the gasification reaction, lower than stoichiometric amounts of oxygen (in the form of air, pure oxygen or steam) are fed to the reactor at high temperatures (greater than 700° C) and therefore the products are only partially reduced (C. Higman and M. van der Burgt, "Gasification", Elsevier, 2003; Bridgwater A. "Renewable fuels and chemicals by thermal processing of biomass", Chemical Engineering Journal, 91, p.87-102, 2003).
- 10 In a gasifier, the carbonaceous material is subjected to 4 processes:
- (i) Initial heating to dry out any moisture embedded in the carbonaceous solid
 - (ii) The *pyrolysis* (or devolatilization) process occurs as the carbonaceous particle heats up. Volatiles are released and char is produced, resulting in significant weight loss. The process is dependent on the properties of the carbonaceous material and determines the structure and composition of the char, which will then undergo gasification reactions.
 - (iii) The *combustion* process occurs as the volatile products and some of the char react with O₂ to form CO₂ and CO, which provides heat for the subsequent gasification reactions. Pyrolysis and combustion are very rapid processes.
 - 20 (iv) The *gasification* process occurs as the char reacts with CO₂ and steam to produce CO and H₂. The resulting gas is called producer gas (H₂, CO and considerable amounts of CH₄) or syngas (mainly H₂ and CO and little CH₄, so "cleaner").

The chemistry of coal gasification is quite complex:

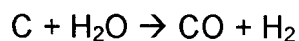
- 25 Gasification with oxygen:



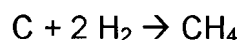
Gasification with carbondioxide:



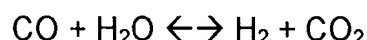
- 30 Gasification with steam:



Gasification with hydrogen:



Water-gas shift:



5 Methanation:



Gasification consists of a series of controlled chemical reactions taking place at up to 70 bar or more and temperature as high as 1400°C. As the feedstock is exposed to rising temperature in the gasifier, devolatilization and breaking of weaker chemical bonds occur, yielding tars, oils, phenols, and hydrocarbon gases. These products generally react further to form CO, H₂ and lesser quantities of CO₂. The fixed carbon that remains after devolatilization is gasified through reactions with O₂, water, CO₂ and H₂ and these gases react further to produce the final gas mixture. The water-gas shift reaction alters the H₂ to CO ratio of the final gas mixture. Methanation reactions are favored by high pressures and low temperatures. Since no O₂ is consumed, exothermic (heat releasing) methanation reactions increase the efficiency of gasification and the heating value of the syngas (producer gas) produced but reduced the hydrogen to carbonmonoxide ratio which is preferentially as high as possible for sequence chemical synthesis.

Under the substoichiometric reducing conditions of gasification, most of the feedstock sulfur converts to hydrogen sulfide (H₂S) and some converts to carbonyl sulfide (COS). Nitrogen chemically bound in the feed generally converts to gaseous nitrogen (N₂), with some ammonia (NH₃) and a small amount of hydrogen cyanide (HCN) also being formed. Chlorine in the feed is primarily converted to hydrogen chloride (HCl), with some appearing as chloride-containing particulates. Trace elements associated with both organic and inorganic components of coal feedstock, such as mercury (Hg) and arsenic (As), appear in the various ash fractions as well as in gaseous emissions and need to be removed from the syngas prior to further use.

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[Brief summary of the invention]

In one embodiment of the present invention is provided:

A process for making propylene by dehydration of propanol, involving the
5 following steps:

1. Gasifying carbonaceous solid or liquid feedstock or reforming gaseous carbonaceous feedstock into synthesis gas,
2. Removing contaminants from the synthesis gas
3. Fermenting the synthesis gas by means of a microorganism into
10 substantially propanol in which the microorganism
 1. Is a wild strain having the natural capability to ferment synthesis gas into substantially propanol or
 2. Is a microorganism, possessing the required nucleic acid
15 sequence information to express the enzymes for the biosynthesis of C₃-oxygenates (acetone/isopropanol pathway, dicarboxylic pathway, acrylate pathway, threonine degradation pathway, citramalate pathway or the propanediol reduction pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl
20 pathway (reduced Acetyl-CoA pathway), or
 3. Is a microorganism, possessing the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway), modified by conferring it with the required nucleic acid sequence information to
25 express the enzymes for the biosynthesis of C₃-oxygenates (acetone/isopropanol pathway, dicarboxylic pathway, acrylate pathway, threonine degradation pathway, citramalate pathway or the propanediol reduction pathway).
4. Fractionating and purifying the stream containing predominantly propanol,
- 30 5. Dehydrating said above stream in a reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene,

6. recovering from said reactor the propylene containing stream

It is another embodiment of the present invention to provide
 A process for making propylene by dehydration of propanol, involving the
 5 following steps:

1. Gasifying carbonaceous solid or liquid feedstock or reforming gaseous carbonaceous feedstock into synthesis gas,
2. Removing contaminants from the synthesis gas
3. Co-Fermenting the synthesis gas with at least one liquid oxygenate, like
 10 carbohydrates, glycerol, propanediol, lactaldehyde, lactic acid, acetol, methanol, ethanol, acetic acid, acetaldehyde, propionic acid, propanal and acetone, by means of a microorganism into substantially propanol in which the microorganism
 1. Is a wild strain having the natural capability to ferment synthesis
 15 gas into substantially propanol or
 2. Is a microorganism, possessing the required nucleic acid sequence information to express the enzymes for the biosynthesis of C₃-oxygenates (acetone/isopropanol pathway, dicarboxylic pathway, acrylate pathway, threonine degradation pathway, citramalate pathway or the propanediol reduction pathway),
 20 modified by conferring it with the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway), or
 3. Is a microorganism, possessing the required nucleic acid
 25 sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes for the biosynthesis of C₃-oxygenates (acetone/isopropanol pathway, dicarboxylic pathway, acrylate
 30 pathway, threonine degradation pathway, citramalate pathway or the propanediol reduction pathway).
4. Fractionating and purifying the stream containing predominantly propanol,

5. Dehydrating said above stream in a reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene,
6. recovering from said reactor the propylene containing stream

- 5 The stream containing predominantly propanol can contain other alcohols e.g. ethanol, butanols.

The fermentation or the co-fermentation step (3), produces a mixture of alcohols, containing at least 50 wt% of propanol, between 0 and 50 wt% of ethanol and between 0 and 50 wt% of butanol.

- 10 In an embodiment the weight ratio of said alcohols to the propanol is less than 20/80.

In an embodiment the weight ratio of said alcohols to the propanol is less than 10/90. In an embodiment the weight ratio of said alcohols to the propanol is less than 5/95.

- 15 In an embodiment dehydration is made over an acidic catalyst at a temperature of at least 200°C and a WHSV of at least 1 h⁻¹. Dehydration can be made by introducing in a reactor a stream comprising at least propanol, optionally water, optionally an inert component, contacting said stream with a catalyst in said reactor at conditions effective to dehydrate at least a portion of the propanol to
20 make propylene.

By dehydration is made an olefin having the same number of carbons as the alcohol to be dehydrated. Of course if other alcohols are present the dehydration produces the corresponding olefin (ethanol leads to ethylene and butanol leads to butene).

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[Detailed description of the invention]

- As regards the production of synthesis gas**, it can be produced from
30 low-molecular hydrocarbons by reforming or from high-molecular weight liquid hydrocarbons or solid carbonaceous solid via gasification.

Essentially, synthesis gas is formed via steam reforming reaction and partial oxidation of natural gas (Dybkaer I., "Tubular reforming and autothermal reforming of natural gas – an overview of available processes", Fuel. Proc. Tech., 42, p. 85, 1995; Moulijn J., "Chemical Process Technology", Wiley & Sons, 2001). In steam reformer (SMR) a catalyst is packed in tubes placed in a fired heater. The reformer tubes are heated externally by burners using typically natural gas. The feedstock to the reformer is a mixture of desulfurised natural gas and steam (>3:1 steam to carbon ratio in order to prevent coking of the catalyst). Steam methane reforming is endothermic and the molar ratio of hydrogen to carbon monoxide produced by SMR is approximately 3:1. If CO₂ (CO₂ reforming or dry-reforming) is added to the feedstock the H₂/CO ratio can be reduced. When the feedstock contains heavy hydrocarbons, a prereformer is used to first reform the heavy hydrocarbons.

The autothermal reformer (ATR) reactor has a compact design consisting of a burner, combustion chamber and catalyst bed placed in a refractory lined pressure vessel as. The hydrocarbon feedstock is reacted with a mixture of oxygen and steam in a sub-stoichiometric flame. The steam to carbon ratio can be as low as 0.6. In the fixed catalyst bed, the synthesis gas is further equilibrated. The composition of the product gas will be determined by the thermodynamic equilibrium at the exit pressure and temperature, which is determined through the adiabatic heat balance based on the composition and flows of the feed, steam and oxygen added to the reactor. The produced synthesis gas is completely soot-free.

In the combined reformer (or 2-step reformer), the tubular reformer (SMR) is combined with a secondary reformer, acting as an ATR, to which oxygen is added.

There are four main types of gasifiers currently used commercially: counter-current fixed bed, co-current fixed bed, fluid bed and entrained flow (C. Higman and M. van der Burgt, "Gasification", Elsevier, 2003).

Counter-current fixed bed (Updraft, also called moving bed gasifiers): A fixed bed of carbonaceous feedstock (coal, petcoke, char or biomass) entering

at the top with a counter current flow of steam, oxygen and/or air flows up through the solid bed. The feedstock is dried by the syngas leaving the chamber while the gas leaving the gasifier is cooled by the entering feedstock. In case of oxygen-containing feedstock like biomass, char that is generated continues moving down the gasifier vessel where it is reduced and starts reacting with the oxygen and CO₂. The dried feedstock travels down the vessel where gasification of coal or char occurs. The ash is then removed dry or as slag. Gas exit temperatures are low, which is good for thermal efficiency, but this increases the tar and methane impurities in the synthesis gas (McKendry P., "Energy production from biomass (part 1): overview of biomass", Bioresource Technology 83, 37-46, 2002; Bridgwater T., "Review Biomass for energy", Journal of the Science of Food and Agriculture 86, p.1755-1768, 2006). The advantages of updraft gasifiers are their simple construction, low cost, ability to handle high moisture and inorganic content, and their high energy efficiency due to the lower temperature of the gas leaving the chamber.

Co-current fixed bed (Downdraft): This is similar to the counter-current gasifier described above beside that the steam, oxygen and/or air flows co-currently down with the solid carbonaceous feedstock bed. Some heat has to be added to the top of the gasifier by combustion or heat exchange. Because the gas passes through the hot zone combusting the tars and leaving the reactor from the bottom hence the final product has a higher purity. The exit temperature of the gas is higher, resulting in a lower overall efficiency. It has a fairly simple design and is low cost, and it produces a relatively cleaner gas with very low tar make. Some of the disadvantages are that the system requires low moisture and ash feedstock and it has low efficiency because the product gas leaves the gasifier at higher temperatures, which requires an additional cooling system as compared to an updraft gasifier.

Fluid bed: The carbonaceous feedstock is fluidized in oxygen/air and steam. The feedstock size is reduced to a small particle size, and is mixed eventually with the fluidizing material, which is usually silica sand, ceramic, or alumina. The oxidizer and the solid crushed feedstock enter the reactor from the bottom,

where a hot bed forms, where most of the conversion to synthesis gas occurs. The ash is removed as a dry product as it becomes defluidized. Fuel throughput is higher than for a fixed bed, and has the advantage of uniform temperature distribution achieved in the gasification zone resulting in cleaner reactions.

- 5 However, overall conversion can be very high, the gas stream entrains a lot of fine particulates which has be separated from the gas and recycled back to the gasifier (circulating fluidized bed). Fluidized beds work particularly well for biomass, as it has a lot of highly corrosive ash that would harm the fixed bed reactors.
- 10 **Entrained flow:** a dry pulverized solid, an atomized liquid fuel or a fuel slurry is gasified with O₂ (or air) in co-current flow. The gasification reactions take place in a dense cloud of very fine particles. Most coals are suitable for this type of gasifier because of the high operating temperatures (well above ash melting point, to assure high carbon conversion) and because the coal particles are well
- 15 separated from one another. The high temperatures and pressures also mean that a higher throughput can be achieved (short contact time), however thermal efficiency is somewhat lower as the gas must be cooled before it can be cleaned with existing technology. The high temperatures also mean that tar and CH₄ are not present in the product gas; however the O₂ requirement is higher
- 20 than for the other types of gasifiers. All entrained flow gasifiers remove the major part of the ash as a slag as the operating temperature is well above the ash fusion temperature. Some fuels (in particular certain types of biomass) can form slag that is corrosive for the ceramic inner walls that serve to protect the gasifier outer wall. However some entrained bed type of gasifiers do not have a
- 25 ceramic inner wall but have an inner water or steam cooled wall covered with partially solidified slag and hence do not suffer from corrosive slags.

- 30 **As regards the microorganism,** they can include prokaryotic and eukaryotic microbial species from the Domains Archaea, Bacteria and Eucarya (including yeast and filamentous fungi, protozoa, algae, or higher Protista). The term "prokaryotes" refers to cells which contain no nucleus or other cell

organelles and are generally classified in one of two domains, the Bacteria and the Archaea. The ultimate difference between organisms of the Archaea and Bacteria domains is based on fundamental differences in the nucleotide base sequence in the 16S ribosomal RNA.

- 5 The microorganisms of the present invention are either naturally occurring microorganisms or metabolically engineered microorganism.

Naturally occurring microorganism of the present invention are isolated from nature, so called wild strains that have the ability to ferment synthesis gas into propanol. Such wild strains can be improved by random screening and
10 rationalized selection. Random mutagenesis is based on repeated applications of three steps: (i) mutagenesis with chemicals or radiation of a population to induce genetic variability, (ii) screening from the surviving population to identify an improved strain under small-scale standard fermentation testing and (iii) assay of fermentation test for confirmation of improved strains (S. Parekh,
15 "Improvement of microbial strains and fermentation processes", Appl. Microbiol. Biotechnol., 54, p.287, 2000). Furthermore, adaptive evolution can be applied to make the microorganism more resistance to certain external conditions, like higher solvent tolerance, higher fermentation temperature, higher syngas pressure etc..

- 20 Random mutagenesis may be performed using a suitable physical or chemical mutagenising agent. Examples of a physical or chemical mutagenising agent suitable for the present purpose include, but are not limited to, UV irradiation, ionizing irradiation such as gamma irradiation, hydroxylamine, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), O-methyl hydroxylamine, nitrous acid, ethyl
25 methane sulphonate (EMS), sodium bisulfite, and nucleotide analogues. When such agents are used the mutagenesis is typically performed by incubating the cell to be mutagenised in the presence of the mutagenising agent of choice under suitable conditions, and selecting for cells showing a significantly increased or decreased production of the targeted molecule(s).

The term "metabolically engineered" entails rational designed pathway and assembly of biosynthetic genes, genes associated with operons, and control elements of such nucleic acid sequences, for the production of a desired metabolite, in particular as end-product propanol (isopropanol or n-propanol). It further embraces optimization of metabolic flux by regulation and optimization of transcription, translation, protein stability and protein functionality, functional in an optimal manner under the applied fermentation conditions. The biosynthetic genes can be heterologous to the host microorganism, either by virtue of being foreign to the host, or being modified by mutagenesis, recombination, and/or association with a heterologous expression control sequence in an endogenous host cell. Accordingly, "metabolically engineered" microorganisms are created via the introduction of genetic material into a host microorganism of choice thereby acquiring new properties, e.g. the ability to produce a new or greater quantity of metabolite. It also encompasses the activation of endogenous nucleic acid sequences encoding a target enzyme through genetic modification of e.g., a promoter sequence in a host microorganism and the introduction of exogenous nucleic acid sequences encoding a target enzyme into a host microorganism. The introduction of genetic material (containing gene(s), or parts of genes, coding for one or more of the enzymes involved in a biosynthetic pathway for the production of isopropanol or n-propanol and eventually including additional elements for the expression (or regulation) of these genes, e.g. promoter sequences) into a host microorganism results in a new or improved ability to produce isopropanol or n-propanol from synthesis gas.

A "metabolite" refers to any substance produced by anabolic or catabolic metabolism. An important intermediate metabolite is Acetyl-CoA, produced from synthesis gas or other oxygenated compounds like carbohydrates, ethanol, acetic acid, required for the synthesis of the desired end-metabolite isopropanol or n-propanol.

In order to express the metabolic pathway of interest and/or reorienting metabolic flux, the host cell of the invention could be genetically modified by using standard technologies known to the person skilled in the art. For example,

- if we want to delete a pathway to increase metabolic flux for the production of propanol, the gene sequences responsible for production of enzymes of endogenous pathway may be inactivated or partially or entirely eliminated. The inactivation could be obtained by modification of the respective structural or regulatory regions (such as genes). Known and useful techniques include, but are not limited to, specific or random mutagenesis, PCR generated mutagenesis, site specific DNA deletion, insertion and/or substitution, gene disruption or gene replacement, anti-sense techniques, or a combination thereof.
- 10 The term "heterologous" or "exogenous" indicates enzymes or nucleic acids that are expressed in an organism other than the organism from which they originated. The term "endogenous" indicates enzymes and nucleic acids that are expressed in the organism in which they originated.
- The resulting "recombinant microorganism" is a host microorganism that has been genetically modified to express or over-express endogenous nucleic acid sequences, or to express non-endogenous nucleic acid sequences, such as those included in a vector (encoding a target enzyme or enzymes involved in a metabolic pathway for producing a desired metabolite). It is understood that the terms "recombinant microorganism" refer also to the progeny.
- 15 The term "host microorganism" represents (i) a cell that occurs in nature, i.e. a "wild-type" cell that has not been genetically modified or (ii) a cell that has been genetically modified but which does not express or over-express a target enzyme (such microorganism can act as a host in the generation of a microorganism modified to express or over-express another target enzyme).
- 20 Methods of over-expressing in microorganisms are well known in the art, and any such method is contemplated for use in the construction of the microorganisms of the present invention.
- Any method can be used to introduce an exogenous nucleic acid molecule into microorganisms and many such methods are well known to those skilled in the art: for example, transformation, electroporation, conjugation, and fusion of protoplasts are common methods for introducing nucleic acid into
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microorganisms (J. Dale, "Molecular Genetics of bacteria", 4th ed., Wiley, 2004; C. Smolke, "The Metabolic Pathway Engineering Handbook", CRC Press, 2010).

The exogenous nucleic acid molecule transposed to a host microorganism can
5 be maintained within that cell in any form: for example, exogenous nucleic acid molecules can be incorporated into the genome of the cell or maintained in an episomal state (extra-chromosomal genetic elements, like plasmids) that can be passed stably on to daughter cells. The microorganisms described herein can contain a single copy, or multiple copies of a particular exogenous nucleic acid
10 molecule.

Methods for expressing enzymes from an exogenous nucleic acid molecule are well known to those skilled in the art, including, without limitation, assembling a nucleic acid sequence such that a regulatory element promotes the expression of a nucleic acid sequence that encodes the desired enzyme. Typically,
15 regulatory elements (promoters, enhancers, etc) are nucleic acid sequences that regulate the expression of other nucleic acid sequences at the level of transcription.

Additionally, when expression of certain enzymatic activity is to be repressed or eliminated, the gene for the relevant enzyme, protein or RNA can be eliminated
20 by known deletion techniques. These deletion techniques are useful to further improve the yield of isopropanol or n-propanol by eliminated the pathways leading to byproducts, like C₂ or C₄ oxygenates or hydrogen. Heterologous control elements can be used to repress expression of endogenous genes. Deletion techniques can be used for optimized both the wild strain and the
25 metabolically engineered microorganisms.

It is an embodiment of the present invention, that synthesis gas can be co-fermented with oxygenated compounds, like carbohydrates, glycerol, propanediol, lactaldehyde, lactic acid, acetol, methanol, ethanol, acetic acid,
30 acetaldehyde, propionic acid, propanal and acetone. The carbohydrates are essentially "biomass derived sugars" and includes, but is not limited to,

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molecules such as glucose, mannose, fucose, rhamnose, xylose, and arabinose, lactose, sorbose, fructose, idose, galactose, mannose and dimers, oligomers or polymers of the latter (starch, cellulose, hemicelluloses and pectins).

5

As regards the fermentation, this is known per se and documented in literature (P. Munasinghe, "Biomass-derived syngas fermentation into biofuels: Opportunities and challenges", *Bioresource Technology*, 101(13), p. 5013, 2010; Chapter 11 & 13 of "Bioenergy and Biofuel from biowastes and biomass", S. Khanal ed., ASCE, 2010; J. Williams, "Keys to bioreactor selection", *Chemical Engineering Progress*, p. 34, March 2002). Syngas fermentation can be carried out in both batch and continuous-flow bioreactors. In batch reactors, the gaseous substrate is introduced in closed system bioreactor where the syngas is supplied continuously but the products remain in the reactor or can be withdrawn at a selected time during fermentation. Bubble column reactors, monolithic biofilm reactors, membrane bioreactors and trickling bed reactors are some of the other common bioreactors. In some of the bioreactors microbubble spargers can be implemented which enhances the mass transfer in two ways (M. Bredwell, P. Srivastava and R.M. Worden, "Reactor design issues for synthesis-gas fermentations", *Biotechnology Progress*, 15, p. 834–844, 1999). Firstly, decreasing bubble sizes cause internal pressure increases, leading to an increase in the driving force. Secondly, the steady state liquid phase concentration gradient at the surface of the bubble is inversely proportional to the diameter. Many other essential techniques (fermentation kinetics, preservation of microorganism, nutrients media, sterilization, inocula development and fermentor design) to implement fermentation are described in reference books (P. Stanbury, "Principles of fermentation technology", Elsevier Science, 2003; B. McNeil, "Practical Fermentation Technology", John Wiley&Son, 2008)

30 a. The continuous stirred-tank reactor is the most common bioreactor employed in syngas fermentation. A CSTR has a continuous flow of gas

through a constant liquid volume. The liquid consists of a suspension of the microorganism and a dilute solution of essential nutrients for the microorganism to grow and survive and a make-up of such liquid nutrient solution and/or microorganism suspension is continuously or intermittently added. Syngas is bubbled through a sparger and an agitation is applied for enhanced mass transfer between the two phases. The fermentation product is withdrawn from the system at the same flow rate as the feed entering the reactor. Cell-recycle systems can be used in conjunction with the CSTR to increase cell-density within the reactor. In such a system, the fermentation broth is pumped through a recycle filter, decanter or centrifuge and the retentate (containing the microorganism cells) is separated from the permeate (cell-free media) and recycled to the bioreactor. An enhanced degree of agitation or mixing is accomplished by baffled impellers to enhance the mass transfer between the substrate and the microorganisms. Higher rotational speeds of the impellers tend to break the gas bubbles into finer ones thereby increasing the interfacial area between the syngas and the aqueous suspension of the microorganisms.

b. Bubble column reactors are most suitable for very large volume industrial applications. These reactors have a large height-to-diameter ratio (aspect ratio) in which high mass transfer can be obtained even without the use of additional agitation. Smaller bubble size and improved gas dispersion can be obtained by using special devices to disperse the syngas in the bottom of the bioreactor, like porous fritted discs or multiple sparger rings to disperse the syngas. Gas-lift reactors are like bubble columns containing a draught tube. Gas is dispersed into the bottom of a central draught tube that controls the circulation of gas and the culture medium. Gas rises through the tube, forming bubbles, entraining the liquid suspension and exhaust gas disengages at the top of the column. The degassed liquid then flows downward external to the draught tube and the product is drained from the tank. As there is an induced controlled flow direction of the liquid suspension, the tube can be designed to serve as an internal heat exchanger, or a heat exchanger

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can be added to an internal circulation loop. A variation on this gaslift reactor is the external gaslift reactor consisting of an internal riser section and an external downcomer. Through the riser the gas rises and induces the liquid suspension upwards. At the top, the gas disengages and the liquid suspension returns via the external downcomer back to the bottom of the reactor. The downcomer can serve as a heat exchanger if required.

c. Monolithic biofilm reactors, is like a bundle of small channels made out of solid porous material on which a biofilm of microorganisms grows. It exhibits a good mass-transfer of gas to the biofilm and a low pressure drop. During the operation, attached microorganisms in the biofilm utilize the gaseous substrates to produce fermentation products. Monolith reactors have high mass-transfer characteristics in particular in multiphase operation. The liquid (typically aqueous solution containing the nutrients and fermentation products) moves as a thin film over the channel surface and the gas flow through the core of the channels. When liquid flow rate are high or gas flow rate low, the gas and liquid move through the channel as separate slugs (Taylor flow). The gas bubble nearly completely fills the diameter of the channel, leaving only a very thin film of liquid near the stagnant biofilm, consequently a high gas-stagnant biofilm mass-transfer is possible. The appropriate liquid/gas ratio in order to reach Taylor flow can be installed by recirculating the liquid around the monolith.

d. Trickle-bed reactors are packed beds, continuous reactor in which the liquid culture flows down, trickling over the surface of the packing, while maximizing the gas-liquid interface. The syngas is allowed to move either downward (co-current) or upward (counter-current) direction. Since these types of reactors do not require mechanical agitation, the power consumption of trickle-bed reactors is lower than the CSTR.

e. Membrane-based bioreactor consists of membranes (flat sheet (FS), multitubular or hollow fiber (HFM)) that allow effectively facilitating the

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mass transfer in aqueous culture media (K. Lee and B. Rittmann, "Applying a novel autohydrogenotrophic hollow-fiber membrane biofilm reactor for denitrification of drinking water", Water Research, 36, p. 2040–2052, 2001; S. Judd, "The MBR Book", Elsevier, 2006; US patent 2009/0215163). In the membrane reactor, syngas is diffused through the walls of membranes without forming bubbles. The microbial community grows and settles as a biofilm on the wall of the membranes where it continuously ferments H_2 and CO to alcohols. These membrane bioreactors can be operated under high pressure with higher mass transfer rates and reduced reactor volumes.

As regards the propanol fractionation and purification, the propanol as the main product of the fermentation is fractionated by any conventional means, like stripping (flashing) of the dissolved gases in the fermentation broth, like filtration, decantation and/or centrifugation to remove solids (microorganism and any other precipitated material) from the aqueous solution, like distillation (stripping and/or rectification) in order to concentrate the propanol stream or like membrane separation in order to isolate the propanol from the aqueous solution or fermentation broth.

Before going to the dehydration reactor, the propanol (and eventually ethanol and butanol) can be purified by the process of WO 2010/060981 the content of which is incorporated in the present application. It describes a process for the purification of an alcohol in the course of a process comprising :

- (1) providing a reaction zone (C) comprising an acid type catalyst;
- (2) providing a reaction zone (B) comprising an acid adsorbent material;
- (3) providing an alcohol stream comprising impurities;
- (4) introducing the alcohol stream of (3) into the reaction zone (B) and bringing said stream into contact with the acid adsorbent material at conditions effective to reduce the amount of impurities having an adverse effect on the acid type catalyst of the reaction zone (C);
- (5) recovering from step (4) an alcohol stream and introducing it into the reaction zone (C);
- (6) optionally introducing one or more reactants (R) into the reaction

zone (C); (7) operating said reaction zone (C) at conditions effective to recover a valuable effluent.

As regards the propanol dehydration, dehydration of alcohols is known per se. Alcohols dehydration has been described in WO-2009-098262 and WO-2009-098268 the content of which are incorporated in the present application.

WO-2009-098262 relates to a process for the dehydration of at least an alcohol to make at least an olefin, wherein the catalyst is:

- a crystalline silicate having a ratio Si/Al of at least about 100, or
- a dealuminated crystalline silicate, or
- a phosphorus modified zeolite, the WHSV of the alcohols is at least 2 h⁻¹, the temperature ranges from 280°C to 500°C. It relates also to the same process as above but wherein the catalyst is a phosphorus modified zeolite and at any WHSV.

WO-2009-098268 relates to a process for the dehydration of at least an alcohol to make at least an olefin, comprising:

- a) introducing in a reactor a stream (A) comprising at least an alcohol optionally in aqueous solution and an inert component,
- b) contacting said stream with a catalyst in said reactor at conditions effective to dehydrate at least a portion of the alcohol to make an olefin,
- c) recovering from said reactor a stream (B) comprising: the inert component and at least an olefin, water and optionally unconverted alcohol,
- d) optionally fractionating the stream (B) to recover the unconverted alcohol and recycling said unconverted alcohol to the reactor of step a),
- e) optionally fractionating the stream (B) to recover the inert component and the olefin and recycling said inert component to the reactor of step a), wherein, the inert component is selected among ethane, the hydrocarbons having from 3 to 10 carbon atoms, naphthenes and CO₂, the proportion of the inert component is

such as the reactor operates essentially adiabatically. It also relates to a similar process as above but the catalyst is :

- a crystalline silicate having a ratio Si/Al of at least 100, or
 - a dealuminated crystalline silicate, or
 - 5 • a phosphorus modified zeolite, the WHSV of the alcohol is at least 2 h⁻¹ wherein the catalyst is a crystalline silicate having a ratio Si/Al of at least 100 or a dealuminated crystalline silicate. Advantageously the pressure of the dehydration reactor is high enough to help the recovery of the inert component and recycling thereof in the reactor of step a) without a gas compressor but only
- 10 a pump.

As regards the propanol stream, the propanol may be subjected to dehydration alone or in mixture with an inert medium. The inert component is any component provided there is no adverse effect on the catalyst. Because the

15 dehydration is endothermic the inert component can be used to bring energy. The inert component may be selected among the saturated hydrocarbons having up to 10 carbon atoms, naphtenes, nitrogen and CO₂. Advantageously it is a saturated hydrocarbon or a mixture of saturated hydrocarbons having from 3 to 7 carbon atoms, more advantageously having from 4 to 6 carbon atoms

20 and is preferably pentane. An example of inert component can be any individual saturated compound, a synthetic mixture of the individual saturated compounds as well as some equilibrated refinery streams like straight naphtha, butanes etc. Advantageously the inert component is a saturated hydrocarbon having from 3 to 6 carbon atoms and is preferably pentane. The weight proportions of

25 respectively propanol, water and inert component are, for example, 5-100/0-95/0-95 (the total being 100). The alcohol stream, containing substantially propanol may also contain ethanol and butanols. The propanol concentration within the alcohols should be at least 50 wt%; the ethanol concentration between 0 and 50 wt% and the butanol between 0 and 50 wt%.

As regards the dehydration reactor, it can be a fixed bed reactor, a moving bed reactor or a fluidized bed reactor. A typical fluid bed reactor is one of the FCC type used for fluidized-bed catalytic cracking in the oil refinery. A typical moving bed reactor is of the continuous catalytic reforming type. The dehydration may be performed continuously in a fixed bed reactor configuration using a pair of parallel "swing" reactors. The various preferred catalysts of the present invention have been found to exhibit high stability. This enables the dehydration process to be performed continuously in two parallel "swing" reactors wherein when one reactor is operating, the other reactor is undergoing catalyst regeneration. The catalyst of the present invention also can be regenerated several times.

As regards the dehydration pressure, it can be any pressure but it is more easy and economical to operate at moderate pressure. By way of example the pressure of the reactor ranges from 0.5 to 30 bars absolute (50 kPa to 3 MPa), advantageously from 0.5 to 5 bars absolute (50 kPa to 0.5 MPa), more advantageously from 1.2 to 5 bars absolute (0.12 MPa to 0.5 MPa) and preferably from 1.2 to 4 bars absolute (0.12 MPa to 0.4 MPa). Advantageously the partial pressure of the alcohols (propanol and eventually ethanol and butanol) is from 1.2 to 4 bars absolute (0.12 MPa to 0.4 MPa), more advantageously from 1.2 to 3.5 bars absolute (0.35 MPa).

As regards the dehydration temperature, and the first embodiment it ranges from 200°C to 600°C, advantageously from 300°C to 580°C, more advantageously from 350°C to 580°C. As regards the temperature and the second embodiment it ranges from 320°C to 600°C, advantageously from 320°C to 580°C, more advantageously from 350°C to 580°C.

These reaction temperatures refer substantially to average catalyst bed temperature. The propanol dehydration is an endothermic reaction and requires the input of reaction heat in order to maintain catalyst activity sufficiently high and shift the thermodynamic equilibrium to sufficiently high conversion levels.

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In case of fluidised bed reactors: (i) for stationary fluidised beds without catalyst circulation, the reaction temperature is substantially homogeneous throughout the catalyst bed; (ii) in case of circulating fluidised beds where catalyst circulates between a converting reaction section and a catalyst regeneration section, depending on the degree of catalyst backmixing the temperature in the catalyst bed approaches homogeneous conditions (a lot of backmixing) or approaches plug flow conditions (nearly no backmixing) and hence a decreasing temperature profile will install as the conversion proceeds.

10 In case of fixed bed or moving bed reactors, a decreasing temperature profile will install as the conversion of the alcohols proceeds. In order to compensate for temperature drop and consequently decreasing catalyst activity or approach to thermodynamic equilibrium, reaction heat can be introduced by using several catalyst beds in series with interheating of the reactor effluent from the first bed to higher temperatures and introducing the heated effluent in a second catalyst
15 bed, etc. When fixed bed reactors are used, a multi-tubular reactor can be used where the catalyst is loaded in small-diameter tubes that are installed in a reactor shell. At the shell side, a heating medium is introduced that provides the required reaction heat by heat-transfer through the wall of the reactor tubes to the catalyst.

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As regards the dehydration WHSV of the alcohols, it ranges advantageously from 1 to 20 h⁻¹, preferably from 3 to 15 h⁻¹, more preferably from 4 to 10 h⁻¹.

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As regards the dehydration effluent stream, it comprises essentially water, olefin, the inert component (if any) and unconverted alcohols. Said unconverted alcohols is supposed to be as less as possible. The olefin is recovered by usual fractionation means. Advantageously the inert component, if any, is recycled in the stream (A) as well as the unconverted alcohols, if any.

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Unconverted alcohols, if any, is recycled to the reactor in the stream (A).

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- As regards the dehydration catalyst,** it is by way of example,
- a crystalline silicate of the group FER (ferrierite, FU-9, ZSM-35), MWW (MCM-22, PSH-3, ITQ-1, MCM-49), EUO (ZSM-50, EU-1), MFS (ZSM-57), ZSM-48, MTT (ZSM-23), MFI (ZSM-5 or silicalite), MEL (ZSM-11) or TON (ZSM-22, Theta-1, NU-10),
- 5 or a dealuminated crystalline silicate of the group FER (ferrierite, FU-9, ZSM-35), MWW (MCM-22, PSH-3, ITQ-1, MCM-49), EUO (ZSM-50, EU-1), MFS (ZSM-57), ZSM-48, MTT (ZSM-23), MFI (ZSM-5 or silicalite), MEL (ZSM-11) or TON (ZSM-22, Theta-1, NU-10),
- 10 or a phosphorus modified crystalline silicate of the group FER (ferrierite, FU-9, ZSM-35), MWW (MCM-22, PSH-3, ITQ-1, MCM-49), EUO (ZSM-50, EU-1), MFS (ZSM-57), ZSM-48, MTT (ZSM-23), MFI (ZSM-5 or silicalite), MEL (ZSM-11) or TON (ZSM-22, Theta-1, NU-10),
- or a silicoaluminophosphate molecular sieve of the group AEI, CHA or AEL,
- 15 or gamma-, beta-, eta- or delta-alumina and amorphous alumina
- or silica-alumina
- or a alkalized, silicated, zirconated or titanated or fluorinated alumina.

The crystalline silicate can be subjected to various treatments before use in the

20 dehydration including, ion exchange, modification with metals (in a not restrictive manner alkali, alkali-earth, transition, or rare earth elements), external surface passivation, modification with phosphorus-compounds, steaming, acid treatment or other dealumination methods, or combination thereof.

25 Another suitable catalyst for the present process is the silicoaluminophosphate molecular sieves of the AEI, CHA or AEL group with typical example the SAPO-18, SAPO-34 or SAPO-11 molecular sieve. The SAPO molecular sieve is based on the ALPO, having essentially an Al/P ratio of 1 atom/atom. During the synthesis silicon precursor is added and insertion of

30 silicon in the ALPO framework results in an acid site at the surface of the

micropores of the 10-membered ring sieve. The silicon content ranges from 0.1 to 10 atom% (Al + P + Si is 100).

In another specific embodiment the crystalline silicate or silicoaluminophosphate molecular sieve is mixed with a binder, preferably an inorganic binder, and shaped to a desired shape, e.g. pellets. The binder is selected so as to be resistant to the temperature and other conditions employed in the dehydration process of the invention. The binder is an inorganic material selected from clays, silica, metal silicates, metal oxides such as ZrO_2 and/or metals, or gels including mixtures of silica and metal oxides. If the binder which is used in conjunction with the crystalline silicate is itself catalytically active, this may alter the conversion and/or the selectivity of the catalyst. Inactive materials for the binder may suitably serve as diluents to control the amount of conversion so that products can be obtained economically and orderly without employing other means for controlling the reaction rate. It is desirable to provide a catalyst having a good crush strength. This is because in commercial use, it is desirable to prevent the catalyst from breaking down into powder-like materials. Such clay or oxide binders have been employed normally only for the purpose of improving the crush strength of the catalyst. A particularly preferred binder for the catalyst of the present invention comprises silica. The relative proportions of the finely divided crystalline silicate material and the inorganic oxide matrix of the binder can vary widely. Typically, the binder content ranges from 5 to 95% by weight, more typically from 20 to 75% by weight, based on the weight of the composite catalyst. Such a mixture of the crystalline silicate and an inorganic oxide binder is referred to as a formulated crystalline silicate. In mixing the catalyst with a binder, the catalyst may be formulated into pellets, extruded into other shapes, or formed into spheres or a spray-dried powder. Typically, the binder and the crystalline silicate are mixed together by a mixing process. In such a process, the binder, for example silica, in the form of a gel is mixed with the crystalline silicate material and the resultant mixture is extruded into the desired shape, for example cylindrical or multi-lobe bars. Spherical shapes can

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be made in rotating granulators or by oil-drop technique. Small spheres can further be made by spray-drying a catalyst-binder suspension. Thereafter, the formulated crystalline silicate is calcined in air or an inert gas, typically at a temperature of from 200 to 900°C for a period of from 1 to 48 hours.

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Another family of suitable catalysts for the dehydration of propanol into propylene are alumina's (amorphous, gamma-, beta-, eta- or delta-alumina's) as such or alumina's that have been modified by surface treatment with alkali's, silicon, zirconium or titanium and silica-alumina's. Alumina's are generally characterised by a rather broad acid strength distribution and having both Lewis-type and Bronsted-type acid sites. The presence of a broad acid strength distribution makes the catalysis of several reactions, requiring each a different acid strength, possible. This often results in low selectivity for the desired product. Deposition of alkali's, silicon, zirconium or titanium on the surface of alumina allows rendering the catalyst significantly more selective. For the preparation of the alumina based catalyst, suitable commercial alumina's can be used, preferably eta or gamma alumina, having a surface area of 10 to 500 m²/gram. The catalyst according to the present invention is prepared by adding 0.05 to 10% of alkali (earth) metal, silicon, zirconium or titanium. The addition of these metals can be done during the preparation of the alumina or can be added to the existing alumina, eventually already activated. Addition of the metal during the preparation of the alumina can be done by dissolving the metal precursor together with the aluminium precursor before precipitation of the final alumina or by addition of the metal precursor to the aluminium hydroxide gel. A preferred method is adding metal precursors to the shaped alumina. Metal precursors are dissolved in a suitable solvent, either aqueous or organic, and contacted with the alumina by incipient wetness impregnation or by wet impregnation or by contacting with an excess of solute during a given time, followed by removing the excess solute. The alumina can also be contacted with vapour of the metal precursor. Suitable metal precursors are halides of alkali (earth) metals, silicon, zirconium or titanium, oxyhalides of zirconium or

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titanium; alcoxides of alkali (earth) metals, silicon, zirconium or titanium; acetates, oxalates or citrates of alkali (earth) metals, zirconium or titanium; nitrates, sulfates or phosphates of alkali (earth) metals, or mixtures of the above. The solvent is selected according to the solubility of the metal precursor.

- 5 The contacting can be done at temperature of 0°C to 500°C, most preferred from 10°C to 200°C. After the contacting, the alumina is eventually washed, dried and finally calcined in order to enhance the surface reaction between the alkali (earth) metals, silicon, zirconium or titanium and the alumina and the removal of the metal precursor ligands. The use of alkalized, silicated, zirconated or titanated or fluorinated alumina's for the dehydration of propanol into propylene is preferably done in the presence of water. The weight ratio of water to propanol ranges from 1/25 to 3/1. Fluorinated alumina is known in itself and can be made according to the prior art.

- 15 One skilled in the art will also appreciate that the propylene made by the dehydration process of the present invention can be, by way of example, polymerized into polypropylene, copolymers of ethylene, hexane, octane or decene and propylene, ethylene-propylene rubbers (EPR), ethylene-propylene-diene polymers (EPDM), can be used for alkylation of benzene to make cumene, a precursor for phenol production, can be oxidized to acrylic acid, can be ammoxidised into acrylonitrile, can be oxidized to propylene oxide for making polyether polyols and propanediol and can be hydroformylated to n-butylaldehyde.

- 20 The present invention relates also to said polypropylene, ethylene-propylene rubbers (EPR), ethylene-propylene-diene polymers (EPDM), cumene, acrylic acid, acrylonitrile, propyleneoxide and n-butylaldehyde.

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CLAIMS

1. A process for making propylene by dehydration of propanol, involving the
5 following steps:
1. Gasifying carbonaceous solid or liquid feedstock or reforming
gaseous carbonaceous feedstock into synthesis gas,
 2. Removing contaminants from the synthesis gas
 3. Fermenting the synthesis gas by means of a microorganism into
10 substantially propanol in which the microorganism
 - i. Is a wild strain having the natural capability to ferment
synthesis gas into substantially propanol or
 - ii. Is a microorganism, possessing the required nucleic acid
sequence information to express the enzymes for the
15 biosynthesis of C₃-oxygenates, modified by conferring it
with the required nucleic acid sequence information to
express the enzymes of the Wood-Ljungdahl pathway
(reduced Acetyl-CoA pathway), or
 - iii. Is a microorganism, possessing the required nucleic acid
20 sequence information to express the enzymes of the Wood-
Ljungdahl pathway (reduced Acetyl-CoA pathway),
modified by conferring it with the required nucleic acid
sequence information to express the enzymes for the
biosynthesis of C₃-oxygenates.
 4. Fractionating and purifying the stream containing predominantly
25 propanol
 5. Dehydrating said above stream in a reactor at conditions effective
to dehydrate at least a portion of the propanol to make propylene,
 6. recovering from said reactor the propylene containing stream.
- 30 2. A process for making propylene by dehydration of propanol, involving the
following steps:

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1. Gasifying carbonaceous solid or liquid feedstock or reforming gaseous carbonaceous feedstock into synthesis gas,
 2. Removing contaminants from the synthesis gas
 3. Co-Fermenting the synthesis gas with at least one liquid oxygenate, like carbohydrates, glycerol, propanediol, lactaldehyde, lactic acid, acetol, methanol, ethanol, acetic acid, propionic acid, propanal and acetone, by means of a microorganism into substantially propanol in which the microorganism
 - i. Is a wild strain having the natural capability to ferment synthesis gas into substantially propanol or
 - ii. Is a microorganism, possessing the required nucleic acid sequence information to express the enzymes for the biosynthesis of C₃-oxygenates, modified by conferring it with the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway) or
 - iii. Is a microorganism, possessing the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes for the biosynthesis of C₃-oxygenates.
 4. Fractionating and purifying the stream containing predominantly propanol
 5. Dehydrating said above stream in a reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene,
 6. recovering from said reactor the propylene containing stream
3. A process according to claim 1 or 2, wherein the fermentation or the co-fermentation step (3), produces a mixture of alcohols, containing at least 50

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wt% of propanol, between 0 and 50 wt% of ethanol and between 0 and 50 wt% of butanol and where in the fractionation and purification step (4) the propanol, ethanol and butanol are fractionated and purified and where in the dehydration step (5) propanol in presence of ethanol and butanol, if any, are dehydrated simultaneously and where in the recovery step (6) the propylene and ethylene and butenes, if any, are recovered from said reactor.

4. A process according to claim 1 or 2, wherein the C₃-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway) and the acetone/isopropanol pathway, involving at least an acetyl-CoA synthase, a pyruvate synthase and acetoacetate decarboxylase, and producing mainly isopropanol.

5. A process according to claim 1 or 2, wherein the C₃-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway) and the dicarboxylic pathway, involving at least an acetyl-CoA synthase, a pyruvate synthase and an methylmalonyl-CoA carboxytransferase or an propionyl-CoA succinate transferase, and producing mainly n-propanol.

6. A process according to claim 1 or 2, wherein the C₃-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway) and the acrylate pathway, involving at least an acetyl-CoA synthase, a pyruvate synthase and an acryloyl-CoA reductase and producing mainly n-propanol.

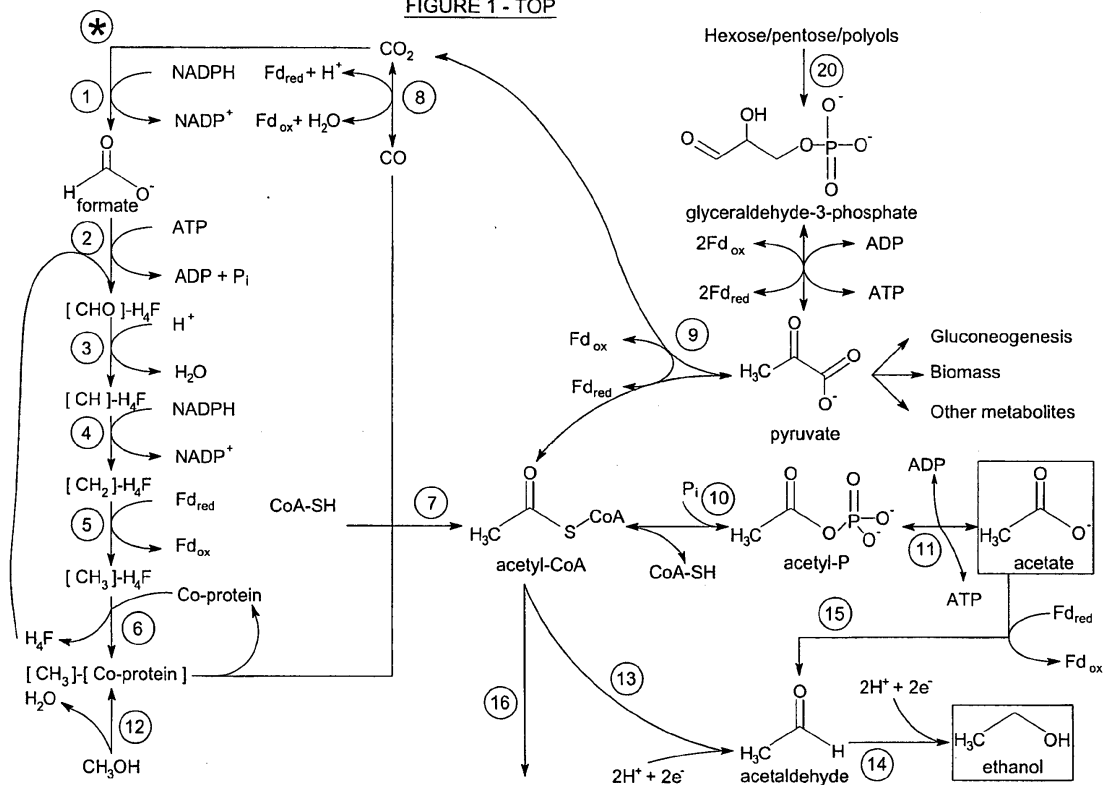
7. A process according to claim 1 or 2, wherein the C₃-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway) and the threonine degradation pathway, involving at least an acetyl-CoA synthase, a pyruvate synthase and a 2-ketobutyrate formate lyase and producing mainly n-propanol.

8. A process according to claim 1 or 2, wherein the C₃-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway) and the citramalate pathway, involving at least an acetyl-CoA synthase, a pyruvate synthase and a citramalate synthase and producing mainly n-propanol.
9. A process according to claim 1 or 2, wherein the C₃-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway) and the propanediol reduction pathway, involving at least an acetyl-CoA synthase, a pyruvate synthase, a propanediol oxidoreductase and a propanediol dehydratase and producing mainly n-propanol.
10. Process according to any one of the preceding claims wherein the dehydration is made over an acidic catalyst at a temperature of at least 200°C and a WHSV of at least 1 h⁻¹.
11. Process according to claim 10 wherein dehydration is be made by introducing in a reactor a stream comprising at least propanol, optionally water, optionally an inert component, contacting said stream with a catalyst in said reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene.
12. Use of the propylene made according any one of the preceding claims to make polypropylene.
13. Use of the propylene made according any one of the claims 1 to 11 to make propylene copolymers, containing ethylene, hexene, octene or decene as comonomer.

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14. Use of the propylene made according any one of claims 1 to 11 to make ethylene-propylene rubbers (EPR).
15. Use of the propylene made according any one of claims 1 to 11 to make ethylene-propylene-diene copolymers (EPDM).
16. Use of the propylene made according any one of claims 1 to 11 to make acrylonitrile.
17. Use of the propylene made according any one of claims 1 to 11 to make acrylic acid.
18. Use of the propylene made according any one of claims 1 to 11 to make cumene.
19. Use of the propylene made according any one of claims 1 to 11 to make propylene-oxide.
20. Use of the propylene made according any one of claims 1 to 11 to make n-butyraldehyde.

FIGURE 1 - TOP



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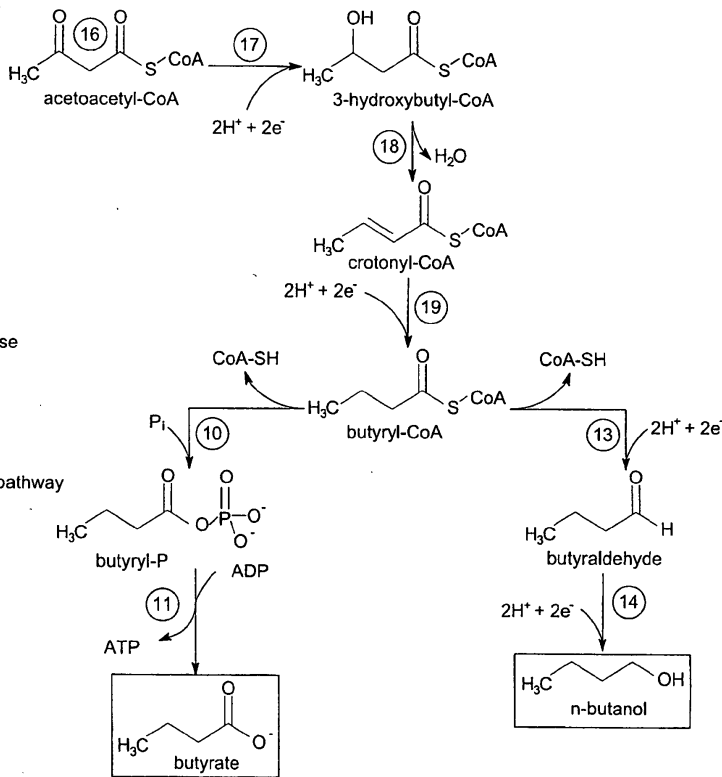
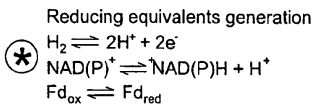
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FIGURE 1 - BOTTOM

- Fd : ferredoxine
Co-protein : corrinoid Fe-S protein
- 1 : Formate dehydrogenase
 - 2 : Formyltetrahydrofolate synthase
 - 3 : Methenyltetrahydrofolate cyclohydrolase
 - 4 : Methylene tetrahydrofolate dehydrogenase
 - 5 : Methylene tetrahydrofolate reductase
 - 6 : Methyltransferase
 - 7 : Acetyl-CoA synthase
 - 8 : CO dehydrogenase (CODH)
 - 9 : Pyruvate synthase (PFOR)
 - 10 : Phosphotransacetylase/butyrylase
 - 11 : Acetate/butyrate kinase
 - 12 : Methanol methyltransferase
 - 13 : Acetaldehyde/butyraldehyde dehydrogenase
 - 14 : Alcohol dehydrogenase
 - 15 : Aldehyde oxidoreductase
 - 16 : Acetyl transferase
 - 17 : 3-hydroxybutyryl-CoA dehydrogenase
 - 18 : Crotonase
 - 19 : Butyryl-CoA dehydrogenase
 - 20 : Glycolyse (EMP) and Pentose phosphate pathway



15x

FIGURE 2 - TOP

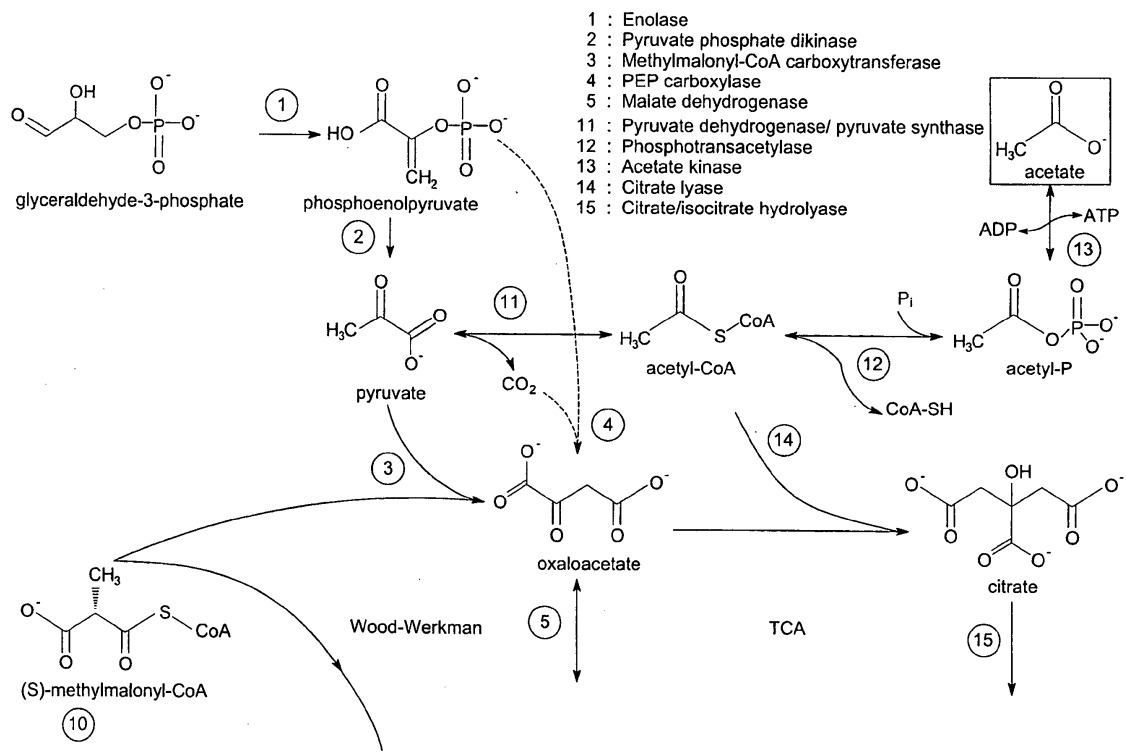
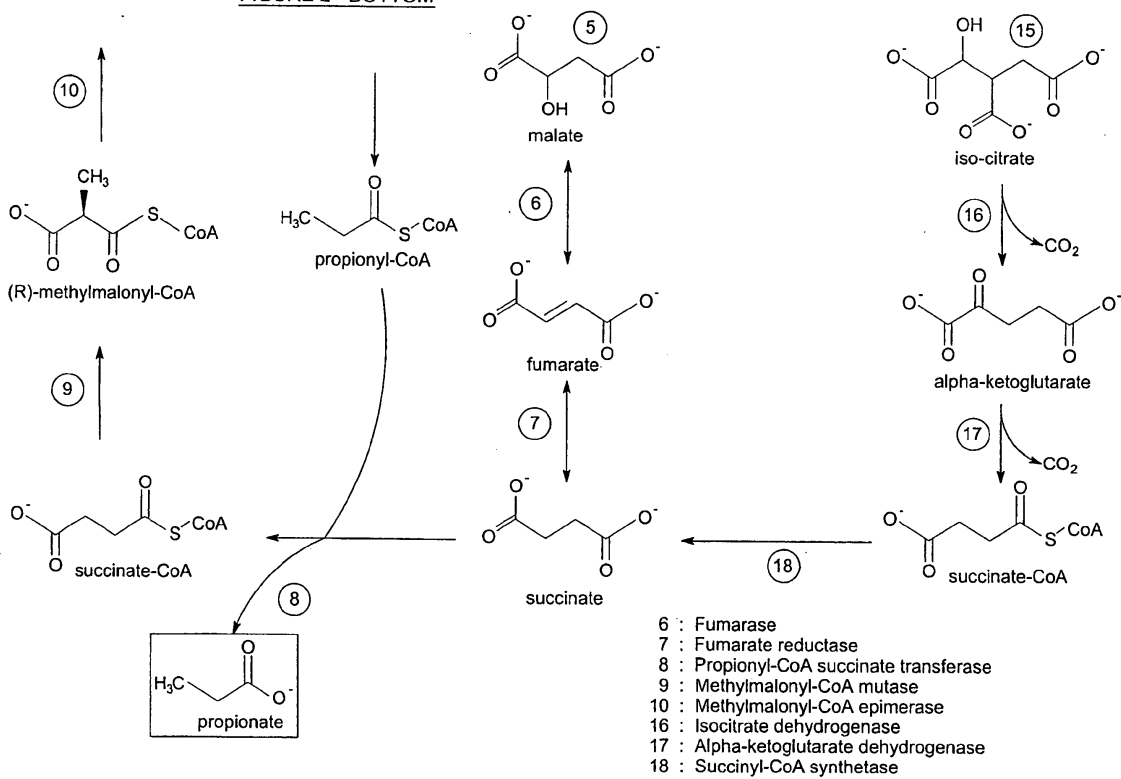


FIGURE 2 - BOTTOM



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- 1 : Lactate dehydrogenase
- 2 : Lactate CoA transferase
- 3 : Lactoyl-CoA dehydratase
- 4 : Acryloyl-CoA reductase
- 8 : Pyruvate dehydrogenase/pyruvate synthase
- 9 : Phosphotransacetylase
- 11 : Ethanol dehydrogenase
- 12 : Acetaldehyde dehydrogenase

FIGURE 3 - TOP

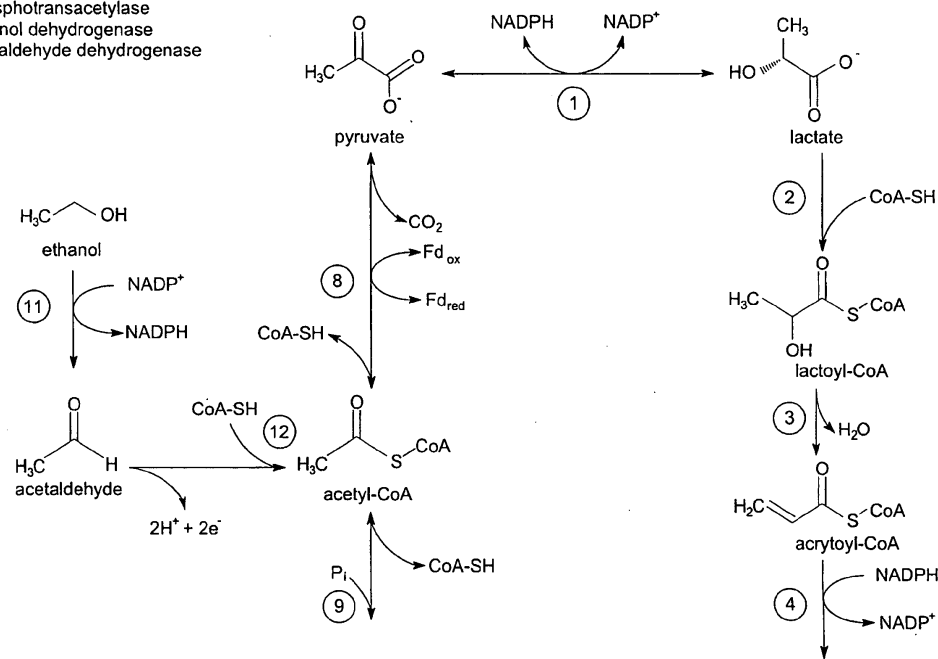
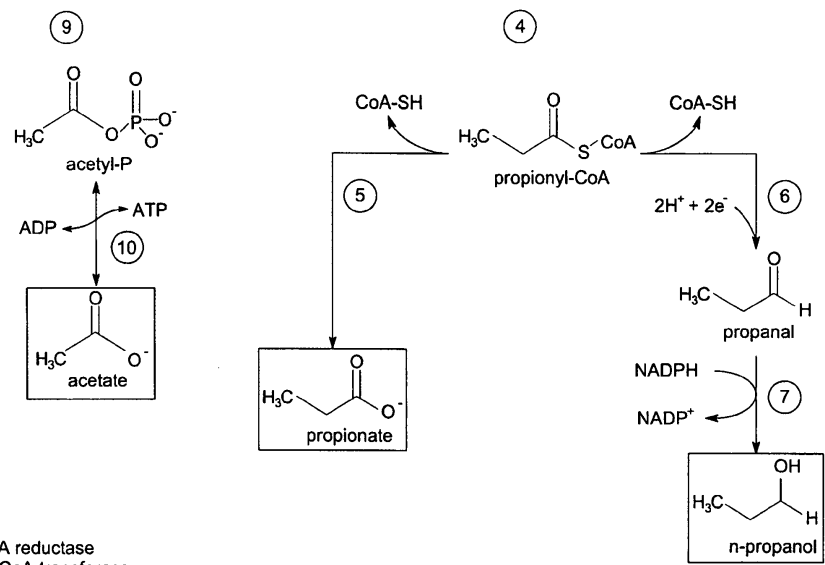
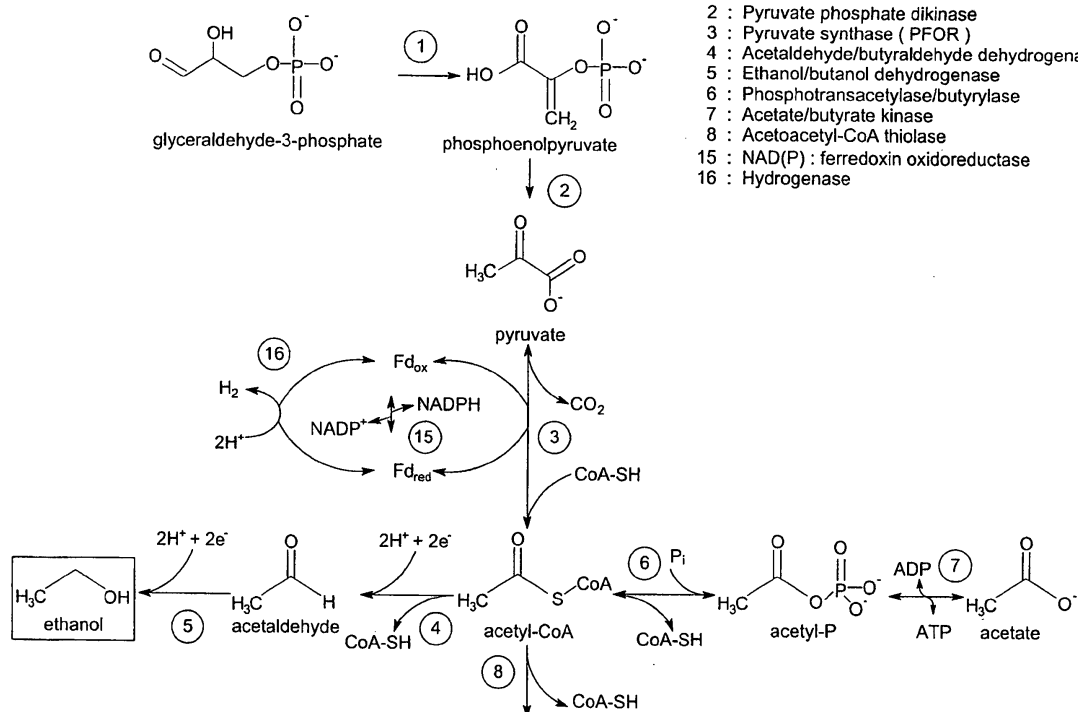


FIGURE 3 - BOTTOM



- 4 : Acryloyl-CoA reductase
- 5 : Propionate-CoA transferase
- 6 : Propanal dehydrogenase
- 7 : Propanol dehydrogenase
- 9 : Phosphotransacetylase
- 10 : Acetate kinase

Fd : ferredoxine
1 : Enolase
2 : Pyruvate phosphate dikinase
3 : Pyruvate synthase (PFOR)
4 : Acetaldehyde/butyraldehyde dehydrogenase
5 : Ethanol/butanol dehydrogenase
6 : Phosphotransacetylase/butyrylase
7 : Acetate/butyrate kinase
8 : Acetoacetyl-CoA thiolase
15 : NAD(P) : ferredoxin oxidoreductase
16 : Hydrogenase



SUBSTITUTE SHEET (RULE 26)

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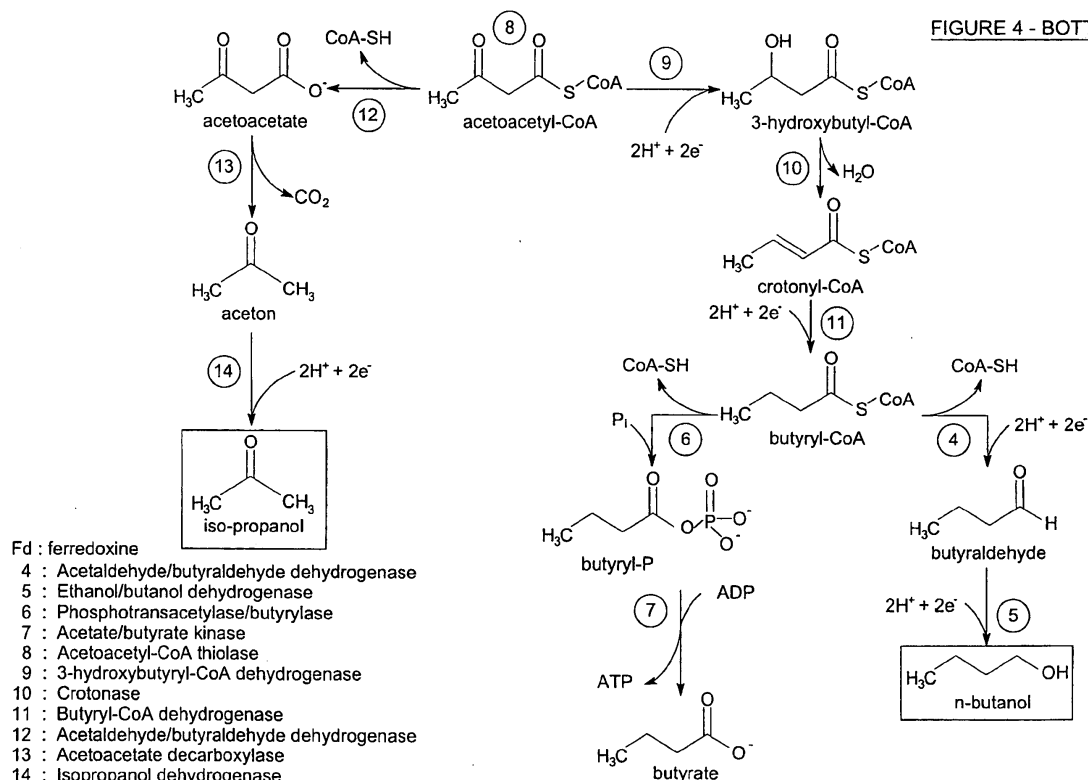
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FIGURE 4 - BOTTOM

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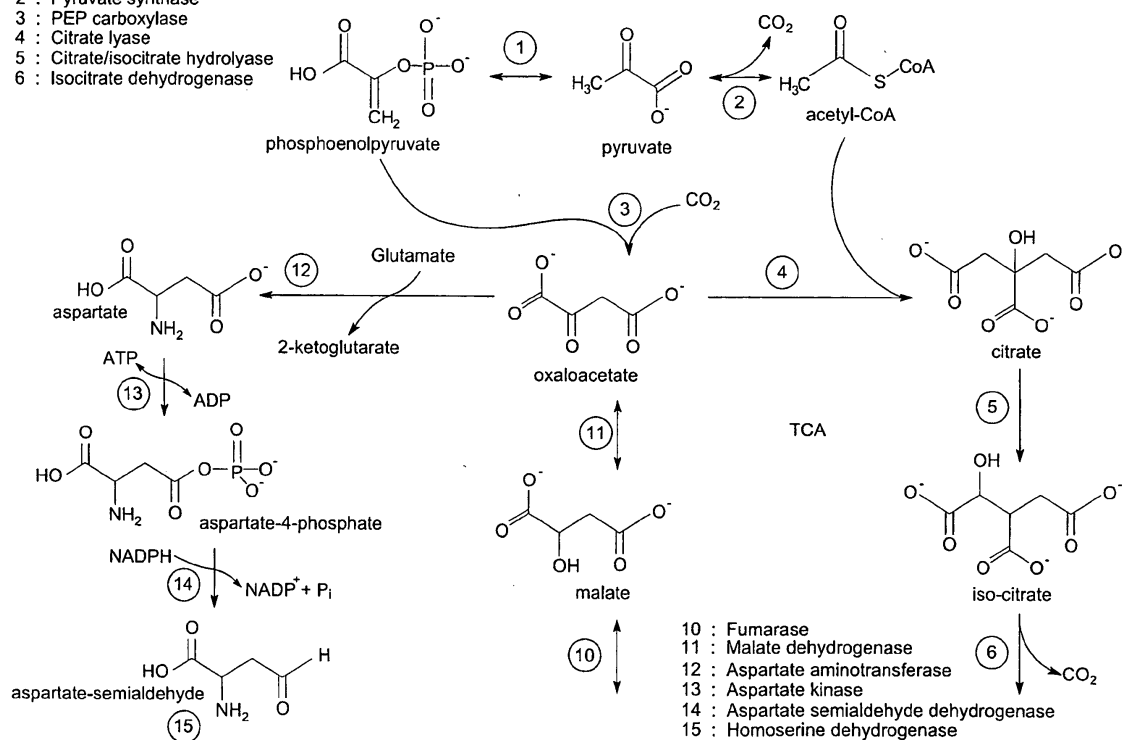
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- 1 : Pyruvate phosphate dikinase
- 2 : Pyruvate synthase
- 3 : PEP carboxylase
- 4 : Citrate lyase
- 5 : Citrate/isocitrate hydrolyase
- 6 : Isocitrate dehydrogenase

FIGURE 5 - TOP



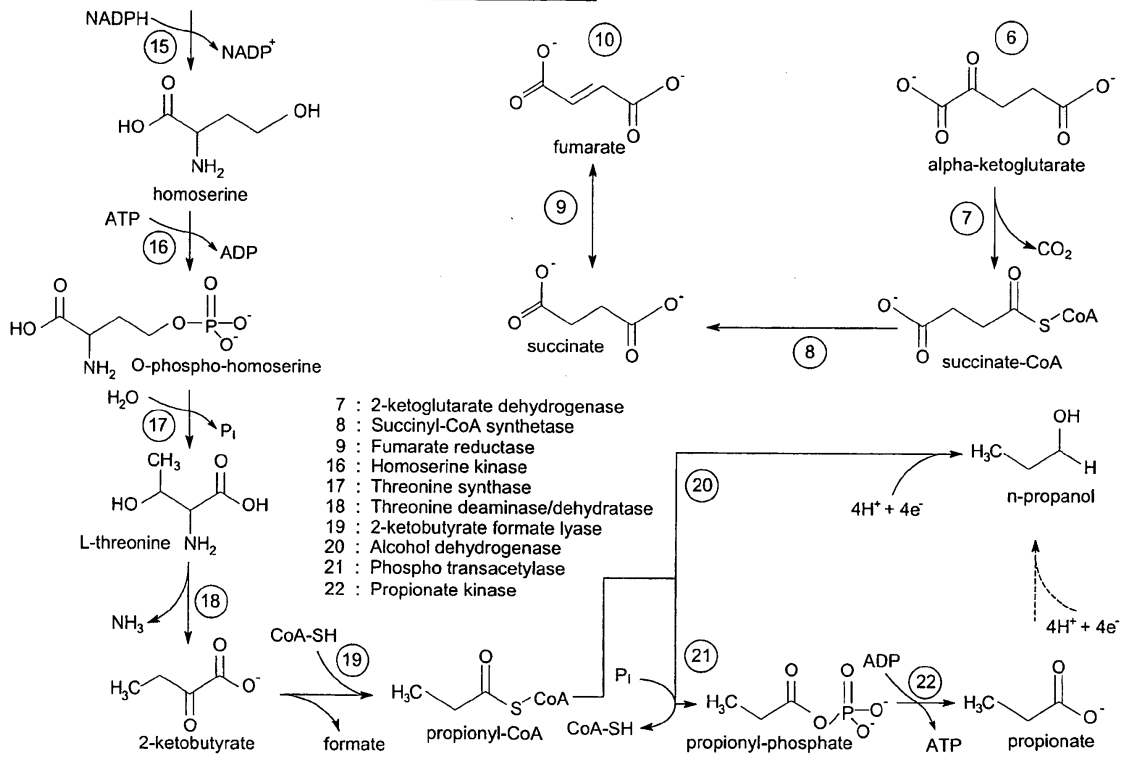
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FIGURE 5 - BOTTOM



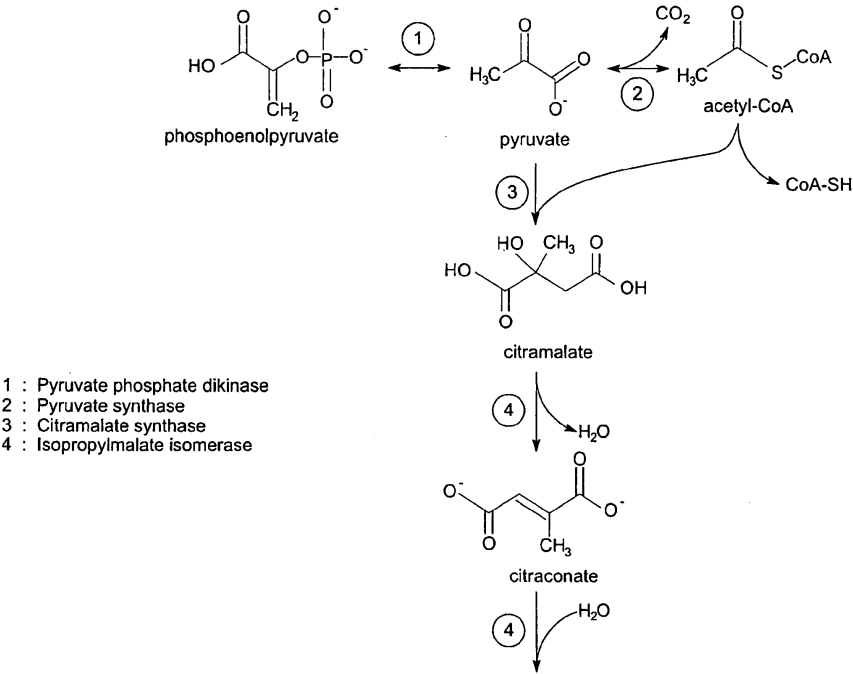
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FIGURE 6 - TOP



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FIGURE 6 - BOTTOM

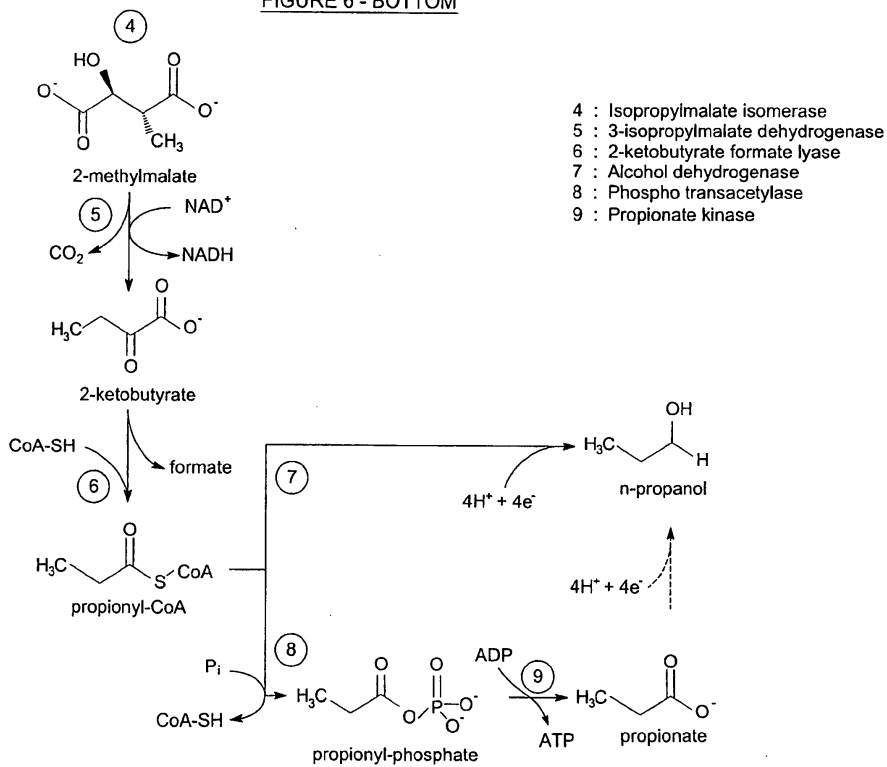
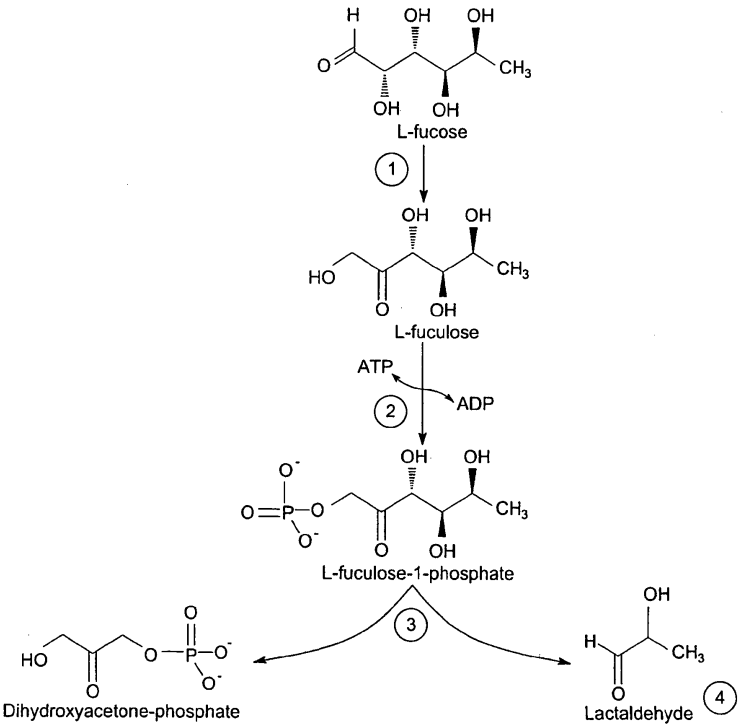


FIGURE 7 - TOP



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FIGURE 7 - BOTTOM

- 4 : Propanediol Oxidoreductase
- 5 : Propanediol dehydratase
- 6 : Propanol dehydrogenase
- 7 : Propanal dehydrogenase
- 8 : Phospho transacetylase
- 9 : Propionate kinase

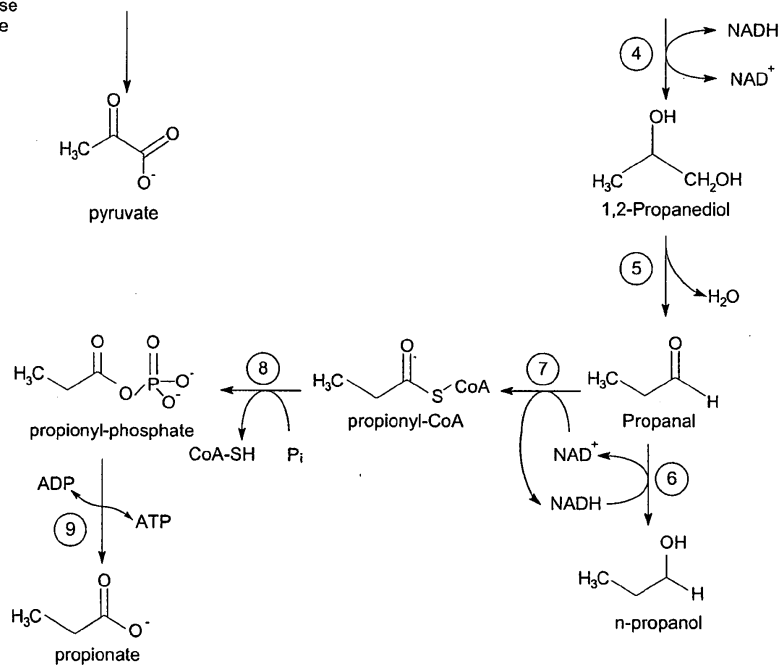
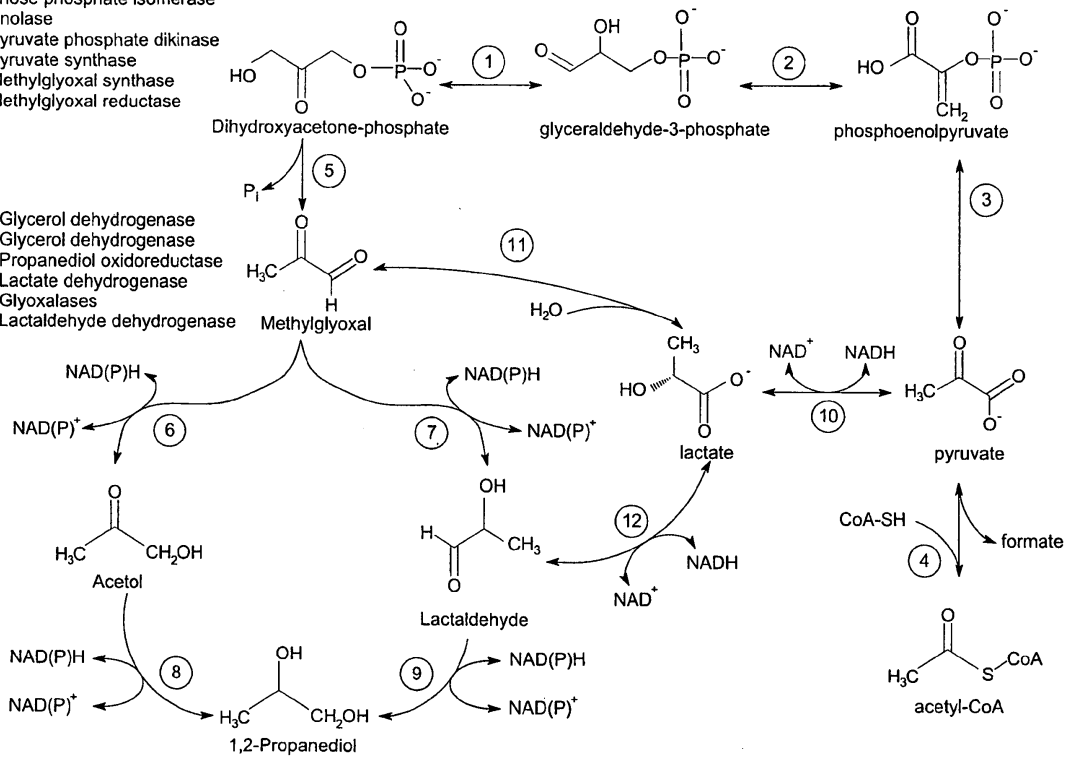


FIGURE 8

- 1 : Triose phosphate isomerase
- 2 : Enolase
- 3 : Pyruvate phosphate dikinase
- 4 : Pyruvate synthase
- 5 : Methylglyoxal synthase
- 6 : Methylglyoxal reductase

- 7 : Glycerol dehydrogenase
- 8 : Glycerol dehydrogenase
- 9 : Propanediol oxidoreductase
- 10 : Lactate dehydrogenase
- 11 : Glyoxalases
- 12 : Lactaldehyde dehydrogenase



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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/072958

A. CLASSIFICATION OF SUBJECT MATTER INV. C12P7/04 C07C11/06 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C12P C07C		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, COMPENDEX, FSTA, BIOSIS, EMBASE		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/103026 A1 (GEVO, INC.) 20 August 2009 (2009-08-20) cited in the application	1-4,8, 10,11
A	page 5, paragraph 14 - page 10, paragraph 29 page 23, paragraph 92 - paragraph 93 page 24, paragraph 95 - page 27, paragraph 105 page 39 - page 43; examples 3-9 page 55 - page 59; claims 1-3, 10-12, 17-19, 25-30, 36-38 ----- -/--	5-7,9
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
16 March 2012		07/05/2012
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Fuchs, Ulrike

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/072958

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KUNDIYANA, D.K. ET AL.: "Syngas fermentation in a 100-L pilot scale fermentor: Design and process considerations", JOURNAL OF BIOSCIENCE AND BIOENGINEERING, vol. 109, no. 5, May 2010 (2010-05), pages 492-498, XP002656397, cited in the application abstract page 494, column 2, line 19 - page 495, column 1, line 46 page 496, column 1, line 13 - column 2, line 10; figure 3; table 4 -----	1-4,10, 11
A	WO 2010/071697 A1 (GENOMATICA, INC.) 24 June 2010 (2010-06-24) page 7, line 25 - page 10, line 12 page 16, line 6 - page 18, line 19 page 30, line 4 - page 31, line 12; table 15 page 31, line 23 - page 33, line 3; tables 16-18 page 43, line 22 - page 44, line 7 page 61, line 12 - line 15 page 81; claims 91-94 -----	1-4,10, 11
X	WO 2009/098268 A1 (TOTAL PETROCHEMICALS RESEARCH FELUY) 13 August 2009 (2009-08-13) cited in the application	1-3,5-7, 9-11
A	page 6, line 8 - page 8, line 21 page 9, line 27 - page 10, line 27 page 11, line 14 - line 18 page 24, line 16 - line 18 page 28 - page 30; claims 1, 3-7, 12 -----	4,8
X,P	WO 2011/031897 A1 (GENOMATICA, INC.) 17 March 2011 (2011-03-17)	1,4
A,P	page 1, line 13 - line 15 page 3, line 4 - page 6, line 22 page 11, line 21 - page 12, line 12 page 12, line 13 - line 17 page 21, line 18 - line 25 page 22, line 3 - line 6 page 22, line 27 - page 23, line 2 page 24, line 4 - page 25, line 10 page 72, line 15 - line 33 page 94, line 16 - page 95, line 32 page 121, line 1 - line 21 page 121 - page 133; table 29 page 131, line 17 - page 132, line 2; table 43 figures 1, 4 ----- -/--	2,3,5-7, 10,11

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/072958

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CALDWELL, M.E. ET AL.: "Expanding Biofuels Potential: Microbial Propanol and Propionate Production from Synthesis Gas", May 2009 (2009-05), XP002671574, Retrieved from the Internet: URL:http://tinyurl.com/CALDWELLETTAL cited in the application abstract	1-3,5-11
A	----- ATSUMI, S. & LIAO, J.C.: "Directed Evolution of Methanococcus jannaschii Citramalate Synthase for Biosynthesis of 1-Propanol and 1-Butanol by Escherichia coli", APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 74, no. 24, December 2008 (2008-12), pages 7802-7808, XP002671575, cited in the application abstract page 7802, column 2, line 5 - line 18 page 7803; figure 1 page 7806, column 1, line 7 - page 7807, column 2, line 17; figures 5, 6B; table 3	1-3,8, 10,11
A,P	----- JAIN, R. & YAN, Y.: "Dehydratase mediated 1-propanol production in metabolically engineered Escherichia coli", MICROBIAL CELL FACTORIES, vol. 10, 97, 10 November 2011 (2011-11-10) , XP002671576, abstract page 2, column 1, line 29 - column 2, line 33 page 3; figure 1 page 5, column 1, line 1 - page 6, column 2, line 21; table 3 -----	1-3,9-11

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2011/072958

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 2009103026	A1	20-08-2009	NONE	

WO 2010071697	A1	24-06-2010	AU 2009327490 A1	28-07-2011
			CA 2746952 A1	24-06-2010
			CN 102307986 A	04-01-2012
			EP 2373781 A1	12-10-2011
			KR 20110097951 A	31-08-2011
			US 2010304453 A1	02-12-2010
			WO 2010071697 A1	24-06-2010

WO 2009098268	A1	13-08-2009	AU 2009211370 A1	13-08-2009
			CA 2713172 A1	13-08-2009
			CN 101939275 A	05-01-2011
			EA 201001093 A1	28-02-2011
			EP 2238093 A1	13-10-2010
			JP 2011511037 A	07-04-2011
			KR 20100102707 A	24-09-2010
			US 2011098518 A1	28-04-2011
			WO 2009098268 A1	13-08-2009

WO 2011031897	A1	17-03-2011	US 2011201068 A1	18-08-2011
			WO 2011031897 A1	17-03-2011

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2011/072958

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

- ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
- ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
- ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

- ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
- ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
- ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-11
- ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☒ No protest accompanied the payment of additional search fees.

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 4(completely); 1-3, 10, 11(partially)

process for producing propylene by dehydration of propanol, involving the steps:

1. gasifying carbonaceous solid or liquid feedstock or reforming gaseous carbonaceous feedstock into synthesis gas
 2. removing contaminants from the synthesis gas
 3. fermenting the synthesis gas by means of a microorganism into substantially propanol or co-fermenting the synthesis gas with at least one liquid oxygenate, like carbohydrates, glycerol, propanediol, lactaldehyde, lactic acid, acetol, methanol, ethanol, acetic acid, propionic acid, propanal and acetone, by means of a microorganism into substantially propanol in which the microorganism:
 - i. is a wild strain having the natural capability to ferment synthesis gas into substantially propanol, or
 - ii. is a microorganism, possessing the required nucleic acid sequence information to express the enzymes for the biosynthesis of C3-oxygenates, modified by conferring it with the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway), or
 - iii. is a microorganism, possessing the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes for the biosynthesis of C3-oxygenates
 4. fractionating and purifying the stream containing predominantly propanol
 5. dehydrating said above stream in a reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene
 6. recovering from said reactor the propylene containing stream,
- wherein the C3-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway) and the acetone/isopropanol pathway, involving at least an acetyl-CoA synthase, a pyruvate synthase and acetoacetate decarboxylase, and producing mainly isopropanol

2. claims: 5(completely); 1-3, 10, 11(partially)

process for producing propylene by dehydration of propanol, involving the steps:

1. gasifying carbonaceous solid or liquid feedstock or reforming gaseous carbonaceous feedstock into synthesis gas
2. removing contaminants from the synthesis gas
3. fermenting the synthesis gas by means of a microorganism into substantially propanol or co-fermenting the synthesis gas with at least one liquid oxygenate, like carbohydrates,

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glycerol, propanediol, lactaldehyde, lactic acid, acetol, methanol, ethanol, acetic acid, propionic acid, propanal and acetone, by means of a microorganism into substantially propanol in which the microorganism:

- i. is a wild strain having the natural capability to ferment synthesis gas into substantially propanol, or
- ii. is a microorganism, possessing the required nucleic acid sequence information to express the enzymes for the biosynthesis of C3-oxygenates, modified by conferring it with the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway), or
- iii. is a microorganism, possessing the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes for the biosynthesis of C3-oxygenates

4. fractionating and purifying the stream containing predominantly propanol

5. dehydrating said above stream in a reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene

6. recovering from said reactor the propylene containing stream,

wherein the C3-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway) and the dicarboxylic pathway, involving at least an acetyl-CoA synthase, a pyruvate synthase and an methylmalonyl-CoA carboxytransferase or an propionyl-CoA succinate transferase, and producing mainly n-propanol

3. claims: 6(completely); 1-3, 10, 11(partially)

process for producing propylene by dehydration of propanol, involving the steps:

- 1. gasifying carbonaceous solid or liquid feedstock or reforming gaseous carbonaceous feedstock into synthesis gas
- 2. removing contaminants from the synthesis gas
- 3. fermenting the synthesis gas by means of a microorganism into substantially propanol or co-fermenting the synthesis gas with at least one liquid oxygenate, like carbohydrates, glycerol, propanediol, lactaldehyde, lactic acid, acetol, methanol, ethanol, acetic acid, propionic acid, propanal and acetone, by means of a microorganism into substantially propanol in which the microorganism:
 - i. is a wild strain having the natural capability to ferment synthesis gas into substantially propanol or
 - ii. is a microorganism, possessing the required nucleic acid sequence information to express the enzymes for the biosynthesis of C3-oxygenates, modified by conferring it with the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway), or

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

iii. is a microorganism, possessing the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes for the biosynthesis of C3-oxygenates
4. fractionating and purifying the stream containing predominantly propanol
5. dehydrating said above stream in a reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene
6. recovering from said reactor the propylene containing stream,
wherein the C3-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway) and the acrylate pathway, involving at least an acetyl-CoA synthase, a pyruvate synthase and an acryloyl-CoA reductase and producing mainly n-propanol

4. claims: 7(completely); 1-3, 10, 11(partially)

process for producing propylene by dehydration of propanol, involving the steps:
1. gasifying carbonaceous solid or liquid feedstock or reforming gaseous carbonaceous feedstock into synthesis gas
2. removing contaminants from the synthesis gas
3. fermenting the synthesis gas by means of a microorganism into substantially propanol or co-fermenting the synthesis gas with at least one liquid oxygenate, like carbohydrates, glycerol, propanediol, lactaldehyde, lactic acid, acetol, methanol, ethanol, acetic acid, propionic acid, propanal and acetone, by means of a microorganism into substantially propanol in which the microorganism:
i. is a wild strain having the natural capability to ferment synthesis gas into substantially propanol or
ii. is a microorganism, possessing the required nucleic acid sequence information to express the enzymes for the biosynthesis of C3-oxygenates, modified by conferring it with the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway), or
iii. is a microorganism, possessing the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes for the biosynthesis of C3-oxygenates
4. fractionating and purifying the stream containing predominantly propanol
5. dehydrating said above stream in a reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene
6. recovering from said reactor the propylene containing stream,

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

wherein the C3-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway) and the threonine degradation pathway, involving at least an acetyl-CoA synthase, a pyruvate synthase and a 2-ketobutyrate formate lyase and producing mainly n-propanol

5. claims: 8(completely); 1-3, 10, 11(partially)

process for producing propylene by dehydration of propanol, involving the steps:
1. gasifying carbonaceous solid or liquid feedstock or reforming gaseous carbonaceous feedstock into synthesis gas
2. removing contaminants from the synthesis gas
3. fermenting the synthesis gas by means of a microorganism into substantially propanol or co-fermenting the synthesis gas with at least one liquid oxygenate, like carbohydrates, glycerol, propanediol, lactaldehyde, lactic acid, acetol, methanol, ethanol, acetic acid, propionic acid, propanal and acetone, by means of a microorganism into substantially propanol in which the microorganism:
i. is a wild strain having the natural capability to ferment synthesis gas into substantially propanol or
ii. is a microorganism, possessing the required nucleic acid sequence information to express the enzymes for the biosynthesis of C3-oxygenates, modified by conferring it with the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway), or
iii. is a microorganism, possessing the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes for the biosynthesis of C3-oxygenates
4. fractionating and purifying the stream containing predominantly propanol
5. dehydrating said above stream in a reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene
6. recovering from said reactor the propylene containing stream,
wherein the C3-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway) and the citramalate pathway, involving at least an acetyl-CoA synthase, a pyruvate synthase and a citramalate synthase and producing mainly n-propanol

6. claims: 9(completely); 1-3, 10, 11(partially)

process for producing propylene by dehydration of propanol, involving the steps:
1. gasifying carbonaceous solid or liquid feedstock or reforming gaseous carbonaceous feedstock into synthesis gas

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2. removing contaminants from the synthesis gas
 3. fermenting the synthesis gas by means of a microorganism into substantially propanol or co-fermenting the synthesis gas with at least one liquid oxygenate, like carbohydrates, glycerol, propanediol, lactaldehyde, lactic acid, acetol, methanol, ethanol, acetic acid, propionic acid, propanal and acetone, by means of a microorganism into substantially propanol in which the microorganism:
 - i. is a wild strain having the natural capability to ferment synthesis gas into substantially propanol or
 - ii. is a microorganism, possessing the required nucleic acid sequence information to express the enzymes for the biosynthesis of C3-oxygenates, modified by conferring it with the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway), or
 - iii. is a microorganism, possessing the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes for the biosynthesis of C3-oxygenates
 4. fractionating and purifying the stream containing predominantly propanol
 5. dehydrating said above stream in a reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene
 6. recovering from said reactor the propylene containing stream,
- wherein the C3-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced acetyl-CoA pathway) and the propanediol reduction pathway, involving at least an acetyl-CoA synthase, a pyruvate synthase, a propanediol oxidoreductase and a propanediol dehydratase and producing mainly n-propanol

7. claim: 12

use of the propylene produced according any one of claims 1-11 to prepare polypropylene

8. claim: 13

use of the propylene produced according any one of claims 1-11 to prepare propylene copolymers, containing ethylene, hexene, octene or decene as comonomer

9. claim: 14

use of the propylene produced according any one of claims 1-11 to prepare ethylene-propylene rubbers (EPR)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

10. claim: 15

use of the propylene produced according any one of claims
1-11 to prepare ethylene-propylene-diene copolymers (EPDM)

11. claim: 16

use of the propylene produced according any one of claims
1-11 to prepare acrylonitrile

12. claim: 17

use of the propylene produced according any one of claims
1-11 to prepare acrylic acid

13. claim: 18

use of the propylene produced according any one of claims
1-11 to prepare cumene

14. claim: 19

use of the propylene produced according any one of claims
1-11 to prepare propylene-oxide

15. claim: 20

use of the propylene produced according any one of claims
1-11 to prepare n-butyraldehyde

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DOCUMENT MADE AVAILABLE UNDER THE PATENT COOPERATION TREATY (PCT)

International application number:	PCT/EP2011/072958
International filing date:	15 December 2011 (15.12.2011)
Document type:	Certified copy of priority document
Document details:	Country/Office: EP
	Number: 10195765.2
	Filing date: 17 December 2010 (17.12.2010)
Date of receipt at the International Bureau:	09 January 2012 (09.01.2012)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a),(b) or (b-bis)

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Bescheinigung

Certificate

Attestation

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geltenden Fassung der auf
dem nächsten Blatt
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Patentanmeldung überein.

The attached documents are
exact copies of the text in
which the European patent
application described on the
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Patentanmeldung Nr.

Patent application No.

Demande de brevet n°

10195765.2 / EP10195765

The organization code and number of your priority application, to be used for filing abroad under the Paris Convention, is EP10195765.

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R.C. van Dijk

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Production of propylene from synthesis gas via metabolic propanol production and dehydration of the propanol

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PRODUCTION OF PROPYLENE FROM SYNTHESIS GAS VIA METABOLIC PROPANOL PRODUCTION AND DEHYDRATION OF THE PROPANOL

5 [Field of the invention]

The present invention relates to the production of propylene via dehydration of propanol on advantageously an acidic catalyst, whereby the propanol is obtained by metabolic conversion of synthesis gas, that is produced
10 by gasification of biomass, waste, coal, liquid residu's, effluent gases from steel furnaces or by reforming of natural gas, into propanol (either n-propanol or isopropanol). The limited supply and increasing cost of crude oil has prompted the search for alternative processes for producing hydrocarbon products such as propylene. Propanol can be obtained by metabolic conversion of synthesis
15 gas by microorganisms. Made up of organic matter from living organisms, biomass is the world's leading renewable energy source.

[Background of the invention]

20 The production of propanols is limited compared to the production of propylene. In 2005, the world production of n-propanol was about 140 kta and of isopropanol more than 2000 kta whereas the world production of propylene exceeds 65000 kta.

Historically, isopropanol has been produced by hydration of propylene over
25 acidic catalyst (Kirk-Othmer Encyclopedia of Chemical Technology, 2007 & Ullmann's Encyclopedia of Industrial Chemistry, 2002). In the indirect hydration, isopropyl sulfate esters are formed out of propylene and sulfuric acid, which is subsequently hydrolyzed into isopropanol. In the direct hydration, propylene is converted at high pressure and low temperature over an acid fixed bed catalyst.
30 n-Propanol can be obtained by hydroformylation of ethylene via the intermediate propanal that is hydrogenated further in n-propanol. Typical

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conditions employed in the low pressure rhodium-substituted phosphine catalysed oxo process are 90-130°C and less than 28 bars whereas the conditions for the cobalt catalysed oxo process are 110-180°C and above 200 bars. The propanal is subsequently hydrogenated into n-propanol in the presence of excess hydrogen over a metallic catalyst, typically composed of copper, zinc, nickel and chromium compounds.

Propanol is also been produced by hydrogenation of acetone over metallic catalyst into isopropanol. Acetone is the byproduct in the phenol production from cumene. (Ullmann's Encyclopedia of Industrial Chemistry, 2002).

- 10 Aceton, as one of the three products in the ABE (Aceton-butanol-ethanol) fermentation technology based on sugars, could also be hydrogenated into isopropanol ("Bacterial acetone and butanol production by industrial fermentation in the Soviet Union: use of hydrolyzed agricultural waste for biorefinery", Appl. Microbiol. Biotechnol., 71, p. 587-597, 2006; "History of the
- 15 Acetone-Butanol-Ethanol Fermentation Industry in China: Development of Continuous Production Technology", J. Mol. Microbiol Biotechnol., 13, p. 12-14, 2007; "Acetone-butanol fermentation revisited", Microbiol. Rev. 50, p. 484-524, 1986 and Jones, D., "Applied acetone-butanol fermentation", In "Clostridia. Biotechnology and medical application", p. 125-168, 2001, Wiley-VCH Verlag
- 20 GmbH, Weinheim, Germany). The acetone-butanol-ethanol (ABE) fermentation by *Clostridium acetobutylicum* is one of the oldest known industrial fermentations. It was ranked second only to ethanol fermentation by yeast in its scale of production, and is one of the largest biotechnological processes ever known. However, since the 1950's industrial ABE fermentation has declined
- 25 gradually, and almost all acetone is now produced via petrochemical routes. More recently, biochemical metabolic routes are being developed for making propanol from carbohydrates. US Patent 2009/0246842 describes a process for the fermentation of sugars into isopropanol.

- Propanol can also be produced from synthesis gas (mixture of CO, H₂ and CO₂)
- 30 by a catalytic process similar to Fischer-Tropsch, resulting in a mixture of higher alcohols, although often a preferential formation of propanol occurs (Applied

Catalysis A, general, 186, p. 407, 1999 and Chemiker Zeitung, 106, p. 249, 1982). Still another route to obtain propanol, is the base-catalysed Guerbet condensation of methanol with ethanol (J. of Molecular Catalysis A: Chemical 200, 137, 2003, J. Chem. Soc. Chem. Commun., 22, p. 1558, 1990 and
5 Applied Biochemistry and Biotechnology, 113-116, p. 913, 2004).

Whereas in the past, propanols have been produced mostly out of ethylene or propylene, being high added value chemicals, there is a need to produce propanols from other carbon sources so that the respective propanols can be dehydrated into propylene.

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Since many centuries, simple sugars are being fermented into ethanol with the help of *sacharomycis cerevisae*. The last decade's new routes starting from cellulose and hemicelluloses have been developed to ferment more complex carbohydrates into ethanol. Hereto, the carbohydrates need to be unlocked
15 from the lignocellulosic biomass. Biomass consists approximately of 30% cellulose, 35% hemicelluloses and 25% lignin. The lignin fraction cannot be valorised as ethanol, as of its aromatic nature but can only be used as energy source which present in many cases an excess for running an industrial plant.

Recently, more efficient routes that produce synthesis gas from carbon-containing materials and that subsequently is fermented into ethanol are being developed ("Bioconversion of synthesis gas into liquid or gaseous fuels", K. Klasson, M. Ackerson, E. Clausen, J. Gaddy, Enzyme and Microbial Technology, 14(8), p.602, 1992; "Fermentation of Biomass-Generated Producer Gas to Ethanol", R. Datar, R. Shenkman, B. Cateni, R. Huhnke, R. Lewis,
20 Biotechnology and Bioengineering, 86 (5), p.587, 2004; "Microbiology of synthesis gas fermentation for biofuel production", A. Hemstra, J. Sipma, A. Rinzema, A. Stams, Current Opinion in Biotechnology, 18, p. 200, 2007; "Old Acetogens, New Light", H. Drake, A. Gößner, S. Daniel, Ann. N.Y. Acad. Sci. 1125: 100–128, 2008). Synthesis gas can be produced by gasification of the
25 whole biomass without need to unlock certain fractions. Synthesis gas can also be produced from other feedstock via gasification: (i) coal, (ii) municipal waste
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(iii) plastic waste, (iv) petcoke and (v) liquid residu's from refineries or from the paper industry (black liquor). Synthesis gas can also be produced from natural gas via steamreforming or autothermal reforming (partial oxidation). For conventional methanol synthesis, higher alcohol synthesis or Fischer-Tropsch a ratio of hydrogen to carbonmonoxide of about 2 is required. In case of gasification of hydrogen-poor feedstock this ratio will be below 1 and hence a watergas shift ($\text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + \text{H}_2$) is required to adjust the ratio. The biochemical pathway to transform synthesis gas into ethanol is much less stringent regarding the hydrogen to carbonmonoxide ratio.

10

The biochemical pathway of synthesis gas conversion is described by the Wood-Ljungdahl Pathway. Fermentation of syngas offers several advantages such as high specificity of biocatalysts, lower energy costs (because of low pressure and low temperature bioconversion conditions), greater resistance to biocatalyst poisoning and nearly no constraint for a preset H_2 to CO ratio ("Reactor design issues for synthesis-gas fermentations" M. Bredwell, P. Srivastava, R. Worden, Biotechnology Progress 15, 834–844, 1999; "Biological conversion of synthesis gas into fuels", K. Klasson, C. Ackerson, E. Clausen, J. Gaddy, International Journal of Hydrogen Energy 17, p.281, 1992). Acetogens are a group of anaerobic bacteria able to convert syngas components, like CO , CO_2 and H_2 to acetate via the reductive acetyl-CoA or the Wood-Ljungdahl pathway.

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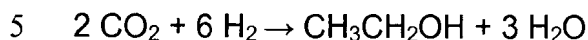
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Several anaerobic bacteria have been isolated that have the ability to ferment syngas to ethanol, acetic acid and other useful end products. *Clostridium ljungdahlii* and *Clostridium autoethanogenum*, were two of the first known organisms to convert CO , CO_2 and H_2 to ethanol and acetic acid. Commonly known as acetogens, these microorganisms have the ability to reduce CO_2 to acetate in order to produce required energy and to produce cell mass. The overall stoichiometry for the synthesis of ethanol using three different combinations of syngas components is as follows (J. Vega, S. Prieto, B. Elmore,

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E. Clausen, J. Gaddy, "The Biological Production of Ethanol from Synthesis Gas", Applied Biochemistry and Biotechnology, 20-1, p. 781, 1989):



Acetogenic bacteria are obligate anaerobes that utilize the acetyl-CoA pathway as their predominant mechanism for the reductive synthesis of acetyl-CoA from CO₂ (Drake, H. L. (1994). Acetogenesis. New York: Chapman & Hall). This group of microorganisms is even more versatile in the sense that they can use simple gases like CO₂/H₂ and CO as well as sugars, carboxylic acids, alcohols and aminoacids.

10 Clostridium ljungdahlii, one of the first autotrophic microorganism known to ferment synthesis gas to ethanol was isolated in 1987, as an acetogen favours the production of acetate during its active growth phase (acetogenesis)) while ethanol is produced primarily as a non-growth-related product (solventogenesis) ("Biological conversion of synthesis gas into fuels", K. Klasson, C. Ackerson, E. Clausen, J. Gaddy, International Journal of Hydrogen Energy 17, p.281, 1992).

20 Eubacterium limosum is an acetogen, isolated from habitats like the human intestine, rumen, sewage and soil, exhibits high growth rate under high CO concentrations producing acetate, ethanol, butyrate and isobutyrate (I. Chang, B. Kim, R. Lovitt, J. Bang, "Effect of CO partial pressure on cell-recycled continuous CO fermentation by Eubacterium limosum KIST612", Process Biochemistry, 37(4), p. 411, 2001).

25 Peptostreptococcus productus is a mesophilic, gram-positive anaerobic coccus, found in the human bowel and is capable of metabolizing CO₂/H₂ or CO to produce acetate (W. Lorowitz, M. Bryant, "Peptostreptococcus productus Strain That Grows Rapidly with CO as the Energy-Source", Applied and Environmental Microbiology, 47(5), p. 961, 1984).

30 Clostridium autoethanogenum is a strictly anaerobic, gram-positive, spore-forming, rod-like, motile bacterium which metabolizes CO to form ethanol,

acetate and CO₂ as end products, beside its ability to use CO₂ and H₂, pyruvate, xylose, arabinose, fructose, rhamnose and L-glutamate as substrates (J. Abrini, H. Naveau, E. Nyns,), "Clostridium autoethanogenum, Sp-Nov, an Anaerobic Bacterium That Produces Ethanol from Carbon-Monoxide", Archives of Microbiology, 161(4), p. 345, 1994).

Clostridium carboxidivorans P7 is a solvent-producing anaerobe, which was isolated from the sediment of an agricultural settling lagoon. It is motile, gram-positive, spore-forming and primarily acetogenic, forming acetate, ethanol, butyrate, and butanol as end-products. (J. Liou, D. Balkwill, G. Drake, R. Tanner, "Clostridium carboxidivorans sp. nov., a solvent-producing clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen Clostridium scatologenes strain SL1 as Clostridium drakei sp. nov.", International Journal of Systematic and Evolutionary Microbiology, 55(5), p. 2085, 2005).

Acetogens are obligate anaerobic bacteria that use the reductive acetyl-CoA pathway as their predominant (i) mechanism for the reductive synthesis of acetyl-CoA from CO₂, (ii) terminal electron-accepting, energy-conserving process, and (iii) mechanism for the synthesis of cell carbon from CO₂" (Drake, H. L. (1994). Acetogenesis. New York: Chapman & Hall). Like other anaerobes, acetogens require a terminal electron acceptor different from oxygen. In the acetyl-CoA pathway, CO₂ serves as an electron acceptor and H₂ serves as the electron donor. The synthesis of acetyl-CoA from CO₂ and H₂ requires an 8-electron reduction of CO₂ involving the following three steps:

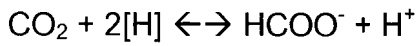
Formation of the carbonyl precursor of acetyl-CoA

Formation of the methyl precursor of acetyl-CoA

Condensation of the above two precursors to form acetyl-CoA.

The Methyl Branch of the Acetyl-CoA Pathway (see figure 1)

This part of the pathway results in the formation of the methyl-corrinoid protein that combines with the product of the carbonyl branch, to form acetyl-CoA. In the first step of this branch, CO₂ is reduced to formate (HCOO⁻) as shown in the following equation:

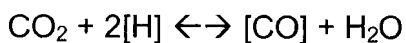


This reversible reaction is catalyzed by the enzyme formate dehydrogenase (FDH). Ferredoxin is the most commonly employed electron acceptor, among acetogens, NADH often acts as the electron donor. For acetogens grown on CO, Ljungdahl suggested that CO must first be oxidised to CO₂ by the enzyme carbon monoxide dehydrogenase (CODH) and subsequently reduced to formate by FDH (L. Ljungdahl, "The autotrophic pathway of acetate synthesis in acetogenic bacteria", Annual Review of Microbiology, 40, 415, 1986).

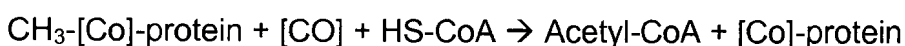
Formate is activated with tetrahydrofolate (THF) to form 10-formyl-THF by the enzyme formyl-THF synthetase in an ATP-dependent condensation. This bound formyl group is then reduced by a series of 3 enzymes to a bound methyl group (methyl-THF). In the final step of this branch, the methyl group is transferred to a corrinoid-containing protein [Co]-protein.

15 The Carbonyl Branch (see figure 1)

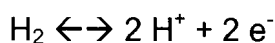
This branch of the pathway results in the formation of a bound carbonyl group which is then merged with the bound methyl group formed in the methyl branch to form acetyl-CoA. Carbon monoxide dehydrogenase (CODH) plays a very essential role with a double functionality. First, it catalyzes the oxidation of CO to CO₂, the reduction of CO₂ to bound carbonyl, finally mediating the synthesis of acetyl-CoA from the methyl and carbonyl groups. For the latter reason, CODH is also known as acetyl-CoA synthase. In the carbonyl branch, CO₂ is first reduced to [CO] ([] indicates that carbon monoxide is enzyme-bound) as follows:



The bound carbonyl moiety is condensed with the bound methyl moiety from the methyl branch to form a bound acetyl-CODH moiety. In the final step, CODH condenses the bound acetyl with free coenzyme A to form acetyl-CoA, as follows:



Hydrogenase enzymes are used by microorganisms either to dispose of electrons accumulated during fermentation via hydrogen formation, or hydrogen uptake and oxidation to produce energy. The reaction involving hydrogen is a reversible reaction catalysed by hydrogenase:



The CODH enzyme works in combination with hydrogenase to form the carbonyl precursor of acetyl-CoA (L. Ljungdahl, "The autotrophic pathway of acetate synthesis in acetogenic bacteria", Annual Review of Microbiology, 40, 415, 1986).

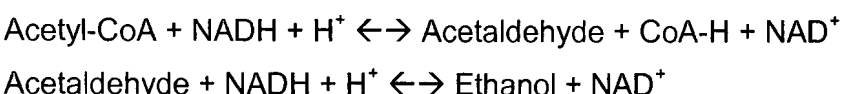
Acetyl-CoA is a central intermediate in the metabolic pathway of acetogens as it is a versatile precursor of alcohols, carboxylic acids, diacids, hydroxyacids, diols, lipids, amino acids, nucleotides and carbohydrates (Ljungdahl, L., "The autotrophic pathway of acetate synthesis in acetogenic bacteria", Annual Review of Microbiology, 40, p. 415-450, 1986.): (i) for cellular material, formed via the anabolic pathway, in which acetyl-CoA is reductively carboxylated into pyruvate by the enzyme pyruvate synthase (Diekert, G., "Metabolism of Homoacetogens", Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 66(1-3), p. 209-221, 1994) that is subsequently converted to phosphoenolpyruvate (PEP) which is an intermediate in the conversion to biomass and (ii) for energy conservation, acetyl-CoA goes through the catabolic pathway in order to make ATP. Beside the essential intermediate PEP that is at the basis of many biochemical pathways, acetyl-CoA can condense further to longer chain hydrocarbyl moieties.

In case of homoacetogens the acetyl-CoA is converted to acetate catalysed by Phosphotransacetylase and acetate kinase while producing ATP by substrate-level phosphorylation:



In the first reaction, the CoA unit is removed from the acetyl-CoA and a phosphate group is added by the enzyme phosphotransacetylase, resulting in the formation of acetyl-phosphate. In the second reaction, the acetyl phosphate

is converted to acetate while a molecule of adenosine diphosphate (ADP) is phosphorylated to form ATP. This part of energy-conservation pathway is usually favoured over the alcohol forming pathway during its exponential growth phase of the microorganism as it provides the cell with energy in the form of ATP, known as the acidogenic phase of the metabolism, which also results in a decrease in pH of the medium due to acid production. The second phase of the fermentation is the solventogenic phase, in which mainly ethanol is produced:



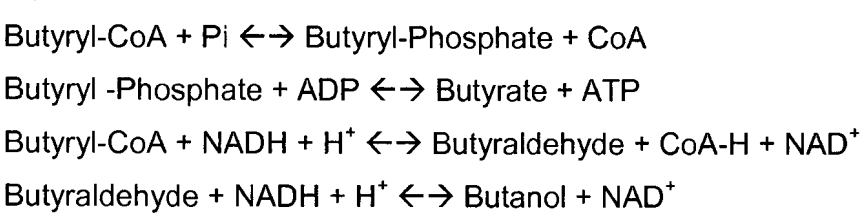
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In the solventogenic branch of the pathway, the microorganism utilizes NADH as the reducing potential to first form acetaldehyde by the enzyme acetaldehyde dehydrogenase, followed by further reduction to ethanol by the enzyme alcohol dehydrogenase.

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Many acetogens (*Clostridium acetobutylicum*) have the ability to produce 4-carbon products like butanol and butyric acid by condensation of 2 molecules of acetyl-CoA to form acetoacetyl-CoA that is further isomerised into butyryl-CoA. Analogously to acetyl-CoA, subsequent transformation into butyric acid produces ATP while the formation of butanol results in the consumption of reducing equivalents:

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Pathways for the production of oxygenates having three carbons:

1. Propionic acid production

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Propionibacterium species (*Propionibacterium acidipropionici*, *Propionibacterium acnes*, *Propionibacterium cyclohexanicum*, *Propionibacterium freudenreichii*, *Propionibacterium freudenreichii shermanii*)

and several other anaerobic bacteria such as *Desulfobulbus propionicus*, *Pectinatus frisingensis*, *Pelobacter propionicus*, *Veillonella*, *Selenomonas*, *Fusobacterium* and *Clostridium*, in particular *Clostridium propionicum*, produce propionic acid as a main fermentation product (Playne M., "Propionic and butyric acids", In: Moo-Young M, editor. Comprehensive biotechnology, New York: Pergamon Press, vol 3, p 731-759, 1985; Seshadri N, Mukhopadhyay S., "Influence of environmental parameters on propionic acid upstream bioprocessing by *Propionibacterium acidipropionici*", J. Biotechnology 29, p.321-328, 1993). In swiss-type cheeses, propionibacteria consume lactate and produce propionic acid, acetic acid, and CO₂. In general, a broad range of substrates can be converted into propionic acid, like glucose, lactose, sucrose, xylose, glycerol and lactate. Propionibacteria are Gram-positive, non-motile, non-sporulating, short-rodshaped, mesophilic anaerobes. The genus of *Propionibacterium*, belonging to the class of high G+C actinobacteria is divided into two groups: the "cutaneous" and the "dairy" *Propionibacteria*, based on their habitat (Stackebrandt, E., Cummins, C., Johnson, J., "The Genus *Propionibacterium*", in The Prokaryotes, E. Balows, H. Truper, M. Dworkin, W. Harder, K. Scheifer, eds., 2006).

20 a. Dicarboxylic pathway

Propionibacteria convert carbon sources to produce propionic acid as a main product via the mainly dicarboxylic acid pathway (also called the Wood-Werkman cycle or the methyl-malonyl-CoA pathway), as shown in Figure 2.

25 Glycolysis pathway catabolyses glucose into phosphoenolpyruvate (PEP), an energy-rich metabolite. Two alternative glycolysis pathways exist: Embden-Meyerhorf-Parnaz (EMP) pathway and Hexose Monophosphate (HMP) pathway. In the EMP pathway, 1 mole of glucose is converted into 2 moles of PEP and 2 moles of NADH, while in the HMP pathway 1 mole of glucose provides 5/3 moles of PEP and 11/3 moles of NADH. PEP is further converted

30 into two possible intermediates, pyruvate and oxaloacetate. The majority of PEP

is converted into pyruvate whereas the remaining PEP is converted into oxaloacetate. For pyruvate production, 1 mole of PEP is converted into 1 mole of pyruvate and 1 mole of ATP obtained from a transfer of one phosphoryl moiety from PEP to ADP. The total ATP obtained from the EMP and HMP pathways per mole of glucose is 2 and 5/3 moles, respectively. Glycolysis via the EMP pathway provides a lower amount of NADH (EMP: HMP = 2: 11/3) but a higher amount of ATP (EMP: HMP = 2: 5/3). The ratio of EMP to HMP pathway contribution in glycolysis is dependent on propionibacterium species, substrates and fermentation conditions. At the pyruvate node, pyruvate is directed toward three main pathways. Most of pyruvate is converted into propionic acid via the Wood-Werkman cycle. Some of pyruvate converts into acetate while some is incorporated into biomass. In the propionate formation pathway, pyruvate enters the Wood-Werkman cycle, via a transcarboxylation of a carboxyl-moiety from methylmalonyl-CoA to pyruvate, catalysed by oxaloacetate transcarboxylase in a coupled reaction of pyruvate to oxaloacetate and methylmalonyl CoA to propionyl CoA. In this coupled reaction, the carboxyl group transferred from methylmalonyl CoA to pyruvate to form propionyl CoA and oxaloacetate is never released from the reaction or no exchange between this carboxyl group with the dissolved CO₂ in the fermentation broth is observed (Wood HG., "Metabolic cycles in the fermentation of propionic acid", in Current Topics in Cellular regulation, Estabrook and Srera RW, eds., New York: Academic Press. vol 18, p 225-287, 1981). Because of this transcarboxylation reaction, CO₂ fixation is minimal and only used to produce catalytic amounts of oxaloacetate to reinitiate the cycle when for instance succinate accumulates as end-product. Under such circumstances, oxaloacetate is generated by condensation of CO₂ with phosphoenolpyruvate catalysed by a PEP carboxylase. Subsequently, oxaloacetate is converted into malate by malate dehydrogenase, malate into fumarate by fumarase and further fumarate to succinate, catalyzed by succinate dehydrogenase. After that succinate is converted into succinyl-CoA, which is then converted into methylmalonyl-CoA. Methylmalonyl-CoA is converted into propionyl-CoA by oxaloacetate

transcarboxylase. At the end of the cycle, propionyl-CoA is converted into propionate along with a coupled reaction of succinate to succinyl-CoA, catalysed by propionyl-CoA: succinate transferase. After 1 mole of pyruvate enters the Wood-Werkman cycle, 1 mole of propionate, 2 moles of NAD^+ , and 1 mole of ATP are generated. Beside propionic acid as main fermentation product, produced in the Wood-Werkman cycle, also NAD^+ regeneration for glycolysis occurs in this cycle.

In acetate branch pathway, pyruvate converts to acetyl-CoA and CO_2 , catalyzed by pyruvate dehydrogenase complex. Acetyl-CoA is converted into acetyl-phosphate by phosphotransacetylase and further acetyl-phosphate to acetate, catalyzed by acetate kinase. In the acetate branch pathway, 1 mole of acetate, CO_2 , NADH, and ATP are obtained from 1 mole of pyruvate. Propionic acid production is usually accompanied by the acetate formation as a major ATP production route supplying energy for cellular metabolism.

The following equations represent a theoretical formulation of propionic acid fermentation from glucose or lactate (P. Piveteau, Lait, 79, p. 23, 1999):



According to these equations, the theoretical maximum yield from glucose is 66.7 C-mole% or 54.8 wt% of propionic acid, 22.2 C-mole% or 22 wt% of acetic acid, 11.1 C-mole% or 17 wt% of CO_2 . The theoretically propionic acid to acetic acid (P/A) molar ratio is 2:1.

A shift in the metabolic pathway towards the production of propionic acid can be accomplished by using carbon sources with higher reductive level (shift from heterofermentative to homofermentative acid production). A higher reductive level of substrate can cause significant increase in the P/A ratio due to the intracellular NADH/NAD^+ balance. A better efficiency of propionic acid production from glycerol could be expected because of its higher reduction level compared to conventional substrates. Effectively, a propionic acid yield of 84.4

C-mole% and a low acetic acid production (P/A molar ratio reaching 37) have been obtained from glycerol with *P. acidipropionici* (Barbirato, F., Chedaille, D. and Bories, A., "Propionic acid fermentation from glycerol: comparison with conventional substrates", *Appl Microbiol Biotechnol*, **47**, p. 441-446, 1997). This strain also produces some propanol from glycerol, indicating that when the substrate has a higher reduction level also products with a higher reduction level can be produced because of the better NADH/NAD⁺ balance.

Glycerol → propionate + 1 H₂O

10

Himmi et. al. compared the fermentation of glycerol and glucose and product formation for *P. acidipropionici* and *P. freudenreichii* ssp. *shermanii*. Fermentation end-products were propionic acid as the major product, acetic acid as the main byproduct and two minor metabolites, n-propanol and succinic acid. The yield of propionic acid was up to 79 C-mole% (64 wt%) with glycerol as the carbon source (Himmi, E. H., Bories, A., Boussaid, A. and Hassani, L., "Propionic acid fermentation of glycerol and glucose by *Propionibacterium acidipropionici* and *Propionibacterium freudenreichii* ssp. *Shermanii*", *Appl Microbiol Biotechnol*, **53**, p. 435-440, 2000). Rumen microorganisms that ferment lactate via the dicarboxylic acid pathway, produce more propionate relative to acetate when hydrogen is added (M. Schulmunda and D. Valentino, "Factors Influencing Rumen Fermentation: Effect of Hydrogen on formation of Propionate", *Journal of Dairy Science*, vol. 59 (8), p. 1444-1451, 1976). Acetic acid was almost eliminated when a high H₂ pressure was applied during the fermentation with *Propionispira arboris* containing hydrogenase (Thompson T.E, Conrad R, Zeikus J.G., "Regulation of carbon and electron flow in *Propionispira arboris*: Physiological function of hydrogenase and its role in homopropionate formation", *FEMS Microbiol Lett* 22, p. 265-271, 1984 and US Patent 4732855). According to the Wood-Werkman cycle, endogenous CO₂ is released with acetic acid formation by *Propionibacteria* from glucose, lactose, or lactate fermentation (Deborde C., Boyaval P. 2000, Interactions between pyruvate and

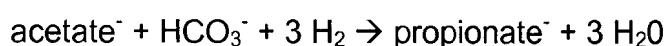
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- lactate metabolism in *Propionibacterium freudenreichii* subsp. *shermanii*: In vivo ^{13}C nuclear magnetic resonance studies, *Appl Environ Microbiol* 66: 2012-2020). CO_2 can be fixed in *Propionibacteria* to form oxaloacetate from PEP catalyzed by PEP carboxylase and then lead to succinate generation. Based on the metabolic pathway (Wood-Werkman cycle), CO_2 (HCO_3^-) is required to convert phosphoenolpyruvate (PEP) into oxaloacetate by the enzyme phosphoenolpyruvate carboxylase. Through several sequential reactions, oxaloacetate is finally converted to propionic acid. In case of glycerol as substrate, nearly no acetate and hence CO_2 is produced. Applying an exogenous CO_2 pressure during fermentation has an positive effect on metabolite production rate and in particular a higher succinate accumulation thanks to the higher PEP carboxylation activity ("Effect of carbon dioxide on propionic acid productivity from glycerol by *Propionibacterium acidipropionici*", An Zhang and Shang-Tian Yang, SIM annual meeting and Exhibition, San Diego, 2008).
- Most propionic acid producing bacteria have the enzymes of the tricarboxylic acid cycle (TCA) which explain the variable P/A ratios for different strains. Some of the acetyl-CoA can be utilized in the TCA cycle by condensation with pyruvate into citrate (see figure 2). The end result is that more CO_2 is produced in the TCA cycle through the decarboxylations and less acetate is secreted. P/A ratios from 2.1 to 14.7 and CO_2 /acetate ratio from 1.0 to 6.3 have been reported from glucose (Wood HG., "Metabolic cycles in the fermentation of propionic acid", in *Current Topics in Cellular regulation*, Estabrook and Sreer RW, eds., New York: Academic Press. vol 18, p 225-287, 1981).
- Pelobacter propionicus*, using the dicarboxylic acid pathway, has been show to grow on ethanol as substrate while producing propionate in presence of CO_2 (Schink, B., Kremer, D. and Hansen, T., "Pathway of propionate formation from ethanol in *Pelobacter propionicus*", *Arch. Microbiol.* 147, 321-327, 1987 and S. Seeliger, P. Janssen, B. Schink, "Energetics and kinetics of lactate fermentation to acetate and propionate via methylmalonyl-CoA or acrylyl-CoA", *FEMS Microbiology Letters*, 211, pp. 65-70, 2002). When ethanol is fed together with

CO₂ and hydrogen, significant amounts of propanol are produced. Ethanol is converted into acetyl-CoA (via acetaldehyde) while producing electrons for the carboxylation of acetyl-CoA into pyruvate, catalysed by pyruvate synthase. Combined with the dicarboxylic acid pathway propionate is produced from ethanol and CO₂ (Schink et al., 1987).



Pelobacter propionicus is not able to reductively convert acetate and CO₂ into propionate whereas Desulfobulbus propionicus does make propionate from acetate and CO₂ (Schink et al., 1987).



15 **b. Acrylate pathway**

Though many bacteria can ferment a variety of substrates anaerobically into lactate as end product, some can further reduce the lactate into propionate, like Clostridium propionicum, Clostridium neopropionicum, Megasphaera elsdenii and Prevotella ruminicola (P. Boyaval, C. Corre, "Production of propionic acid", Lait, 75, 453-461, 1995) by using the acryloyl-CoA pathway (see figure 3). Several substrates (sugars, ethanol and some aminoacids) that can be converted into pyruvate as intermediate can be further reduced into propionate as main product with acetate and butyrate as co-product. The key reaction is the lactoyl-CoA dehydration into acryloyl-CoA that is subsequently reduced to propionyl-CoA. The electrons for this reduction are provided by the oxidation of pyruvate/lactate into acetate and CO₂ (G. Gottschalk, "Bacterial Metabolism", 2nd ed., Springer, New York, 1986).

Clostridium neopropionicum (strain X4), using the acrylate pathway, is able to convert ethanol and CO₂ into acetate, propionate and some propanol (J. Tholozan, J. Touzel, E. Samain, J. Grivet, G. Prensier and G. Albagnac, "Clostridium neopropionicum sp. Nov., a strict anaerobic bacterium fermenting

ethanol to propionate through acrylate pathway", Arch. Microbiol., 157, p. 249-257, 1992). As for the dicarboxylic acid pathway, the intermediate acetyl-CoA produced from the substrate ethanol is linked to the acrylate pathway via the pyruvate synthase that converts acetyl-CoA into pyruvate by carboxylation with CO₂.

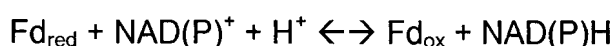
Recently, an alternative route leading to acryloyl-CoA consists in the conversion of acetyl-CoA into malonyl-CoA by carboxylation with CO₂. The malonyl-CoA is further converted into acryloyl-CoA via four steps implicating malonate-semialdehyde, hydroxypropanoate, hydroxypropanoyl-CoA and finally acryloyl-CoA. Acryloyl-CoA produced by this pathway is subsequently reduced to propionyl-CoA similarly to the reactions leading to acryloyl-CoA by dehydration of lactoyl-CoA (J. Zarzycki, "Identifying the missing steps of the autotrophic 3-hydroxypropionate CO₂ fixation cycle in *Chloroflexus aurantiacus*, PNAS, 106(50), p. 21317, 2009; I. Berg, "A 3-hydroxypropionate/ 4-hydroxybutyrate autotrophic carbon dioxide assimilation pathway in archaea, Science, 318, p.1782, 2007).

2. Aceton/Isopropanol production

Members of the *Clostridium*, *Butyrivibrio*, *Bacillus*, and other less-well defined flora of anaerobic digestion systems produce butyric acid, butanol, acetone, isopropanol, or 2,3-butanediol (A. Moat, J. Foster & M. Spector, "Microbial Physiology", 4th Ed., Wiley-Liss, 2002). Hydrogen, carbon dioxide, acetate, and ethanol are concomitantly produced in minor amounts during the fermentation. *Clostridium acetobutylicum* utilizes the EMP pathway for glucose catabolism with the formation of ethanol, carbon dioxide, hydrogen, acetone, isopropanol, butyrate, and butanol from pyruvate via acetyl-CoA as shown in Figure 4 (D. Jones and D. Woods, "Acetone-Butanol Fermentation Revisited", Microbiological Reviews, , p. 484-524, 1986; R. Gheshlaghi, J. Scharer, M. Moo-Young, C. Chou, "Metabolic Pathways of *Clostridia* for producing butanol", Biotechnology Advances, 27, p. 764, 2009).

Strains such as *Clostridium acetobutylicum*, *Clostridium beijerinckii*, *Clostridium saccharobutylicum*, and *Clostridium saccharoperbutylacetonicum* produce butanol in high concentration with acetone (or isopropanol) and ethanol. During growth, the organism first forms acetate and butyrate (acidogenic phase), disposing the excess electrons by reducing H^+ to H_2 . As the pH drops, due to the accumulation of acids, the culture is entering the stationary phase, there is a metabolic shift to solvent production (solventogenic phase). Acetyl-CoA undergoes condensation to form acetoacetate, which may be reduced to butyrate and butanol or cleaved via decarboxylation to acetone with concomitant production of CO_2 . Acetone may be further reduced to isopropanol. Acetyl-CoA may also be reduced to acetaldehyde and ethanol. During the solventogenic phase, sugars are fermented directly to solvents, while the present acidic products are also converted to solvents. Acetate and butyrate are activated to acetyl-CoA and butyryl-CoA through the reactions catalyzed by acetoacetyl-CoA:acetate-CoA transferase or kinase and phosphotransacetylase. These acyl-CoA's are reduced to ethanol and butanol by aldehyde dehydrogenase and alcohol dehydrogenase. Acetone can in some species further be reduced to isopropanol, like in *Clostridium beijerinckii*, since the alcohol dehydrogenase is receptive for different substrates, not only for aldehydes but also for ketones (A. Ismaierl, C. Zhu, G. Colby, J. Chen, "Purification and characterization of a primary-secondary alcohol dehydrogenase from two strain of *Clostridium beijerinckii*", J. Bacterial., 175, p. 5097, 1993; J. Chen and S. Hiu, "Acetone-butanol-isopropanol production by *Clostridium Beijerinckii*" Biotechnology Letters, Vol 8(5), p. 371-376, 1986; H. George, J. Jonhson, W. Moore, L. Holdeman, J. Chen, "Acetone, Isopropanol, and Butanol Production by *Clostridium beijerinckii* (syn. *Clostridium butylicum*) and *Clostridium aurantibutyricum*", Applied and Environmental Microbiology, p. 1160-1163, 1983). The decarboxylation of acetoacetate results in a sharp decrease in the number of electron ($H^+ + e^-$) acceptors available as CO_2 is expelled. Acetyl-CoA and acetone can act as electron acceptor, giving rise to ethanol or isopropanol. For the onset of solventogenesis, electron flux as well

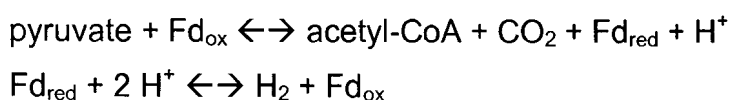
as carbon flux is redirected in order to preserve the oxido-reductive balance. More electrons are needed to produce solvents molecules (butanol, isopropanol and ethanol) than for the corresponding acids. The excess electrons that were used to reduce H^+ to H_2 during the acidogenic phase are used by aldehyde
 5 dehydrogenase and alcohol dehydrogenase during the solventogenic phase. In the solvent-producing clostridia, $NAD(P)^+$:ferredoxin oxidoreductase is active, exchanging electrons between $NAD(P)^+$ and ferredoxin.



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During the acidogenic phase this enzyme is active to reduce ferredoxin while oxidizing $NAD(P)H$, and catalyzes the reverse reaction during solventogenesis. The regeneration of $NAD(P)^+$ is required for the oxidation of glyceraldehydes-3-phosphate and prevents hence the accumulation of $NAD(P)H$. H_2 produced
 15 during acidogenesis is taken up by the bacteria for solvent production. Solventogenic clostridia have a H_2 -producing hydrogenase as well as an uptake hydrogenase.

In clostridia and other anaerobes, hydrogen is formed through a pyruvate:ferredoxin (Fd) oxidoreductase without the intermediary production of
 20 formate. The reduced ferredoxin is converted to hydrogen by hydrogenase:



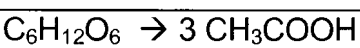
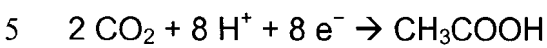
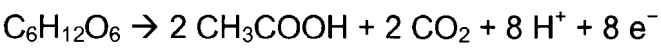
25 Hydrogenase competes with ferredoxin: $NAD(P)$ reductase to oxidize the reduced ferredoxin. The hydrogen evolution rate in the acidogenic phase is significantly higher. The flow of electrons from $NADH$ to Fd_{red} and to H_2 explains why these organisms produce large quantities of H_2 . During the Solventogenic phase more $NAD(P)H$ is diverted to the formation of solvent alcohols by
 30 reduction of the corresponding acids. Several methods have been developed in order to reduce the iron-sulfide based hydrogenase activity and hence reducing the formation of hydrogen for the benefit of further reducing the acids into

alcohols: external hydrogen pressure addition, carbonmonoxide addition that inhibits the iron-based hydrogenase activity, growth under iron-limiting conditions and co-fermenting highly reduced substrates like glycerol (see Gheshlaghi et al. and Jones et al.). Under such conditions the flow of electrons from reduced ferredoxin to molecular hydrogen via the hydrogenase system is inhibited and shifted to the generation of NAD(P)H via the action of the appropriate ferredoxin oxidoreductase, resulting in an increase in the production of butanol and ethanol. Often under reduced activity of the hydrogenase, also less acetone/isopropanol is produced as the formation of acetone by decarboxylation of acetoacetate lowers the amount of available electron acceptors.

Recently, *E. coli* has been engineered with a specific pathway for the production of isopropanol from glucose by introducing acetyl-CoA acetyltransferase, acetoacetyl-CoA transferase, acetoacetate decarboxylase and alcohol dehydrogenase from the appropriate *Clostridium* species (T. Hanai, "Engineered Synthetic Pathway for Isopropanol Production in *Escherichia coli*", *Applied and Environmental Microbiology*, 73(24), p. 7814, 2007; T. Jojima, "Production of isopropanol by metabolically engineered *Escherichia coli*", *Appl. Microbiol. Biotechnol.*, 77, p. 1219, 2008; US 2009/0246842; PCT 2009/049274; EP2184354). Molar yields of 43.5% are obtained, which is close to the theoretical yield of 50%. One mole of glucose is converted to 2 moles of acetyl-CoA and 2 mole of CO₂. The 2 acetyl-CoA's are condensed to one acetoacetate that is subsequently decarboxylated to make 1 mole of isopropanol and 1 additional CO₂.

Production of acetic acid from glucose by *C. thermoaceticum* is efficient in that 3 molecules of acetate are produced per molecule of glucose. Two acetate molecules are produced according to the well-known glycolysis, decarboxylation of pyruvate into acetyl-CoA and CO₂, followed by transformation of acetyl-CoA into acetate. During these reactions CO₂ and an excess of electrons are produced. Some microorganisms, such as *C. thermoaceticum* possess also the

Wood- Ljungdahl enzymes that allow converting the CO₂ and excess electrons into more acetate.



This is particular in that most heterotrophs can only add carbon dioxide to a pre-existing compound and add a carboxyl group. Some bacteria that can form an organic compound directly from carbon dioxide and hydrogen contain hydrogenase, an enzyme that converts hydrogen to two protons and two electrons. These electrons provide the necessary reductive potential for the transformation of carbon dioxide. The overall reaction involves participation of ferredoxin as a reduced electron carrier and the enzymes hydrogenase, carbon monoxide dehydrogenase, and methylenetetrahydrofolate reductase.

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3. Threonine degradation

The amino-acids serine and threonine provide the precursors, directly or indirectly, of eight other amino-acids. They also can be derived from one another through the common intermediate glycine. Both serine and threonine can be metabolized in a single enzymic step to energy-rich keto-acids, which can be catabolized to generate ATP by substrate-level phosphorylation, resulting in carboxylic acids. Threonine is derived from oxaloacetate (see figure 5) via transamination to make aspartate and serine intermediates. Oxaloacetate is a central metabolite of the TCA cycle that is at the origin of many essential metabolites and can be produced as end-product that condenses again with acetyl-CoA to form citrate. Oxaloacetate needs also to be produced by condensation of PEP with CO₂ with the help of PEP carboxylase, particularly when intermediates of the TCA cycle gets depleted to make other metabolites. One of these derivative metabolites is threonine that is directly produced from oxaloacetate with the help of multiple enzymes (see figure 5).

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Threonine, a native amino-acid, is known to be fermented by a number of microorganisms like, *Clostridium tetanomorphum*, *Escherichia coli*, *Salmonella Typhimurium* (G. Sawers, "The anaerobic degradation of L-serine and L-threonine in enterobacteria: networks of pathways and regulatory signals", *Arch Microbiol*, 171, p. 1–5, 1998; H. Barker, "Amino acid degradation by anaerobic bacteria", *Annual Review of Biochemistry* 50, p.23-40, 1981). The products are typically acetate, propionate, butyrate and 2-aminobutyrate and combinations of the latter. The exploitation of native amino-acid intermediates as final products is often easily implemented in micro-organism as there is no or minor metabolic perturbation because of non toxic intermediates. The basic pathway of threonine fermentation, via 2-ketobutyrate to propionate, is a well-known pathway (G. Gottschalk, "Bacterial Metabolism", 2nd ed. Springer, 1986). Enteric bacteria possess two types of threonine dehydratases/deaminases - a catabolic enzyme and biosynthetic enzyme that both convert threonine to 2-ketobutyrate (Umbarger H., Brown B., "Threonine deamination in *Escherichia coli*. II. Evidence for two L-threonine deaminases", *J. Bacteriol.*, 73(1), p.105-12, 1957). While the biosynthetic dehydratase is involved in L-isoleucine biosynthesis out of 2-ketobutyrate, the catabolic enzyme participates in the degradation of threonine to propionate, in a pathway that generates ATP, and enables the bacteria to utilize threonine as the sole source of carbon and energy, typically only under anaerobic conditions and when low levels of energy in the cell are available (see figure 5). 2-ketobutyrate formate-lyase, is only expressed under anaerobic conditions (Sawers et al.). Once propanoyl-CoA is formed, it is processed via propionyl-P to propionate, in a reaction sequence that produces ATP.

The use of a reductive pathway to make n-propanol to dispose of electrons generated during amino-acid fermentation is unusual. 2-Ketobutyrate is cleaved to yield propionyl-phosphate, CO₂ and H₂ (or formate) by the enzyme, 2-ketobutyrate formate-lyase, in the presence of ferredoxin, CoA-SH, and inorganic phosphate. *Clostridium* sp. strain 17cr1 is able to ferment L-threonine to propionate and propanol (PH. Janssen, "Propanol as an end product of

threonine fermentation", Arch Microbiol., 182, p. 482–486, 2004). Electrons arising from the oxidation of 2-ketobutyrate to propionyl-CoA are used in reductive pathway leading to propanol formation. When hydrogen is removed from the medium, the formation of propanol ceases.

- 5 Also yeasts have the ability to convert amino-acids into the corresponding alcohols (M. Lambrechts, "Yeast and its importance to Wine Aroma – A Review", S. Afr. J. Enol. Vitic., 21, p. 97, 2000). This occurs according to the Ehrlich pathway where the amino-acids is deaminated to the corresponding 2-ketoacid that is subsequently decarboxylated and further reduced to alcohols.
- 10 Recently, the 2-ketoacid decarboxylase from *Lactococcus Lactis* and alcohol dehydrogenase from *Saccharomyces Cerevisiae* have been introduced into *E. Coli* in order to convert glucose via 2-ketobutyrate into n-propanol (C. Shen, "Metabolic engineering of *E. Coli* for 1-butanol and 1-propanol production via the keto-acid pathways", Metabolic Engineering, 10, p. 312, 2008).

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4. Citramalate pathway

- An alternative route to 2-ketobutyrate from pyruvate and acetyl-CoA via citramalate synthase has been reported in several organisms as an alternative
- 20 pathway for the production of isoleucine, compared to the threonine deamination into 2-ketobutyrate; in archea like *Ignicoccus hospitalis* and *Methanococcus jannaschii*, in bacteria like *Leptospira interrogans*, *Geobacter sulfurreducens* and in cyanobacteria like *Cyanothece* sp. ATCC 51142 (B. Wu, "Alternative isoleucine synthesis pathway in cyanobacterial species",
- 25 Microbiology, 156, 596–602, 2010; Howell D., "(R)-citramalate synthase in methanogenic archaea." J. Bacteriol., 181(1), p.331-3, 1999; J. Huber, "Insights into the autotrophic CO₂ fixation pathway of the archaeon *Ignicoccus hospitalis*: comprehensive analysis of the central carbon metabolism", J Bacteriol., 189(11), p.4108, 2007). This pathway, designated the citramalate pathway (see
- 30 figure 6) is the most direct route to synthesize 2-ketobutyrate and does not involve transamination followed by deamination. (R)-Citramalate synthesized

from pyruvate and acetyl-CoA is then converted to 2-ketobutyrate via an isomerase and dehydrogenase step. As explained for the threonine degradation, the 2-ketobutyrate is the precursor for propionate and propanol.

Recently, citramalate synthase from *Methanococcus jannaschii* has been introduced in *Escherichia coli* and improved by directed evolution to allow the direct production of n-propanol from glucose via the citramalate pathway (S. Atsumi, "Directed Evolution of *Methanococcus jannaschii* Citramalate Synthase for Biosynthesis of 1-Propanol and 1-Butanol by *Escherichia coli*", *Applied and Environmental Microbiology*, 74(24), p. 7802–7808, 2008).

5. Propanediol reduction pathway

1,2-propanediol can be produced by a wide variety of bacteria like, *Clostridium thermobutyricum*, *Escherichia coli*, *Bacteroides ruminicola* and yeasts. Three possible biosynthesis routes are known: (i) in which deoxy-sugars are converted in dihydroxyacetone-phosphate and lactaldehyde that is further reduced to propanediol, (ii) in which the glycolysis intermediate, dihydroxyacetone-phosphate, of regular sugars is converted into methylglyoxal and further reduced to propanediol (G. Bennet, "Microbial formation, biotechnological production and applications of 1,2-propanediol", *Appl. Microbiol. Biotechnol.*, 55, p. 1, 2001; R. K. Saxena, "Microbial production and applications of 1,2-propanediol", *Indian Journal of Microbiology*, 50(1), p. 2-11, 2010).

1,2-Propanediol is a fermentation end product of the 6-deoxyhexose sugars L-rhamnose and L-fucose, which are abundantly present in hemicellulose of plant cell walls (Badia, J., "Fermentation mechanisms of fucose and rhamnose in *Salmonella typhimurium* and *Klebsiella pneumonia*", *J. Bacteriol.*, 161, p. 435-437, 1985; Forsberg, "Metabolism of rhamnose and other sugars by strains of *Clostridium acetobutylicum* and other clostridium species", *Can. J. Microbiol.*, 33, p. 21, 1987; Tran-Din K., "Formation of 1,2-propanediol and lactate from glucose by *Clostridium sperioides* under phosphate limitation", *Arch. Microbiol.*, 142, p. 87, 1985). These methylpentoses are metabolized by pathways that

involve an isomerase, a kinase and an aldolase and form equimolar amounts of dihydroxyacetone phosphate (DHAP) and lactaldehyde (see figure 7 for fucose fermentation; similar pathway exist for rhamnose). DHAP is converted to pyruvic acid and incorporated into central carbon metabolism. But the fate of the
5 lactaldehyde can differ according to the species and conditions. In presence of oxygen, lactaldehyde is converted to lactate. Under anaerobic conditions, lactaldehyde is reduced to 1,2-propanediol by an NAD-dependent propanediol oxidoreductase.

Several bacteria, including Salmonella, Klebsiella, Shigella, Yersinia, Listeria,
10 Lactobacillus and Lactococcus, include species that grow on 1,2-propanediol in a coenzyme B12-dependent way (E. Sampson, "Microcompartments for B12-Dependent 1,2-Propanediol Degradation Provide Protection from DNA and Cellular Damage by a Reactive Metabolic Intermediate", J. of Bacteriology, 190(8), p. 2966–2971, 2008). The initial step is carried out by coenzyme B12-
15 dependent propanediol dehydratase, which dehydrates 1,2-propanediol to propionaldehyde. Propionaldehyde is then disproportionated to n-propanol and propionyl-CoA. Propionyl-CoA can be utilized in two different ways depending on the availability of oxygen. Under anaerobic conditions, propionyl-CoA is converted to propionyl phosphate by phosphotransacylase and the propionyl-
20 phosphate is cleaved by propionyl kinase, producing ATP. Both n-propanol and propionate are typically excreted, hence 1,2-propanediol fermentation provides an electron sink and a source for the production of ATP and eventually a three-carbon intermediate (propionyl-CoA) for growth by degradation to pyruvate and succinate via the methylcitrate pathway.

25 The second route is the metabolism of common sugars (e.g. glucose or xylose) through the glycolysis pathway followed by the methylglyoxal pathway. Dihydroxyacetone phosphate is converted to methylglyoxal that can be reduced either to lactaldehyde or to acetol. These latter compounds can further be reduced yielding 1,2-propanediol (see figure 8, many of the involved enzymes
30 have not yet been characterized). This route is used by wild strain producers of 1,2-propanediol, such as Clostridium sphenoides (Tran Din K. and Gottschalk

G., Arch. Microbiol. 142: 87-92, 1985) and Thermoanaerobacter thermosaccharolyticum (Cameron D. and Cooney C., Bio/Technology, 4, p. 651-654, 1986; Sanchez-Rivera F, Cameron D., Cooney C., Biotechnol. Lett. 9, p. 449-454, 1987). US 6,303,352 and US 2010/0261239 disclose metabolic
5 engineered microorganism that convert sugars into 1,2 propanediol via the methylglyoxal pathway. There are two possible more direct links between de methylglyoxal pathway and pyruvate via the formation of lactate (see figure 8): (i) via glyoxalases enzymes methylglyoxal (which is often toxic for microorganisms) is transformed into lactate and (ii) via lactaldehyde
10 dehydrogenase, lactaldehyde is transformed into lactate (R. Saxena, "Microbial production and application of 1,2-propanediol", Ind. J. Microbiol., 50(1), p. 2, 2010; J. Weber, "Metabolic flux analysis of Escherichia coli in glucose-limited continuous culture. II. Dynamic response to famine and feast, activation of the methylglyoxal pathway and oscillatory behavior", Microbiology, 151, p. 707-716,
15 2005).

The prior art for the production of propanol that can be dehydrated into propylene, described here above, has several technical disadvantages that refrain commercialization, in particular when compared to the ethanol
20 fermentation of sugars, resulting in a theoretical 66.7% carbon efficiency and nearly 100% hydrogen efficiency into useful ethanol ($C_6H_{12}O_6 \rightarrow 2 C_2H_6O + 2 CO_2$):

- a. Some of the described pathways involve a decarboxylation step that dispose electron as hydrogen (or formate). This is the case
25 for the acetone/isopropanol pathway where acetoacetate is decarboxylated and the threonine and citramalate pathway where 2-ketobutyrate is decarboxylated. Starting from sugars as feedstock, this reduces the potential yield down to 50%.
- b. Some of the described pathways have the potential of a high
30 carbon yield (Wood-Werkman, acrylate pathway, propanediol degradation and threonine degradation), but the pathway is mainly

able to provide propionic acid as end product as there is a shortage of reducing equivalents. Often such pathways entail formation of byproducts as acetic acid that consumes less reducing equivalents and hence leave more reducing equivalents to make some propanol.

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- c. As for the described metabolic pathways the starting feedstock is always a carbohydrate, the potential of biomass valorization is limited to the carbohydrate part of biomass. On top of that the pathways are most of the time not able to use C₅ sugars as starting feedstock whereas biomass contains significant amounts of hemicelluloses, consisting of mainly C₅-sugars.

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It is the object of the present invention to provide a process for the production of propylene by gasifying carbonaceous feedstock or reforming natural gas into synthesis gas, converting the synthesis gas into propanol (either n-propanol or isopropanol) by means of a microorganism, possessing the required Wood-

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ljundahl enzymes and the enzymes of at least one of the pathways for the production of C₃-oxygenates and dehydrating the propanol into propylene and water. Advantageously it is dehydrated over an acidic catalyst.

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Synthesis gas is a mixture of hydrogen, carbonmonoxide and carbondioxide. As the energy carriers are hydrogen and carbonmonoxide, it is recommended that the synthesis gas contains at least hydrogen or carbonmonoxide next to carbondioxide.

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By transforming the carbonaceous feedstock into synthesis gas, most of the carbon in the feedstock can be valorized, including the lignin and C₅-sugar constituents of biomass, but also waste carbonaceous feedstock like waste plastics, tires and municipal waste, coal, petcoke, liquid residu's from petrochemical processes like crude refining or liquid residu's from the paper industry and natural gas, associated gas or unconventional gas can be transformed into synthesis gas.

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In the above described metabolic pathways, the PEP-pyruvate-acetyl-CoA interconversion (involving the enzymes pyruvate phosphate dikinase/pyruvate kinase and pyruvate dehydrogenase/pyruvate synthase) is the central starting node of the biosynthesis of C₃-oxygenates. Instead of producing these central node intermediates directly from carbohydrates, it is advantageous to produce acetyl-CoA, as part of this node, substantially from synthesis gas via the Wood-Ljungdahl pathway (the reductive acetyl-CoA pathway). Once sufficient acetyl-CoA is produced in the cells, via the central node, PEP-pyruvate-acetyl-CoA, one of the possible pathways described above can result in C₃-oxygenates formation. For biosynthesis pathways that involve decarboxylation to make CO₂ and disposal of electron by hydrogen production or involve decarboxylation to make formate, can be improved with respect to carbon yield as the CO₂/H₂ or formate can be recycled by the Wood-Ljungdahl pathway enzymes.

Via the Wood-Ljungdahl pathway, hydrogen can also provide required electrons via hydrogenase activity for the further reduction of propionic acid, propanediol, propanal or acetone.

It is the object of the present invention to improve the carbon yield for propanol production from carbonaceous feedstock's by optimizing the flow of available electron to the production of propanol by combining the described pathways for making propionic acid, propanediol, propanal, acetone or propanol with the Wood-Ljungdahl pathway (the reductive acetyl-CoA pathway) so that disposed electrons can be recycled via the Wood-Ljungdahl pathway enzymes.

The prior art provides a first indication that *Clostridium* strain P11 possesses the Wood-Ljungdahl pathway enzyme, to provide the formation of acetyl-CoA and probably the enzymes of the acetone/isopropanol pathway. This strain converts synthesis gas in isopropanol (9.25 g/l) beside ethanol (25.26 g/l), acetic acid (4.82 g/l) and 1-butanol (0.47 g/l) (D. Kundiyana, J. of Bioscience and Bioengineering, 109(5), p. 492, 2010). It has also been reported by E. Caldwell that *Clostridium* CAT11 and CP19 are able to produce acetate, ethanol,

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Z/H

propionate, propanol and butyrate/butanol as end products of fermentation of synthesis gas (M. Caldwell, T. Allen, P. Lawson and R. Tanner, Annual Meeting of the Missouri Valley Branch of the American Society of Microbiology, March 27-28, 2009, University of Kansas, Lawrence, KS).

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It is the object of the present invention to isolate from nature, wild strain microorganisms that possess the nucleic acid sequence information, to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway) and that possess the nucleic acid sequence information of one of the described pathways to make C₃-oxygenates (acetone/isopropanol pathway, dicarboxylic pathway, acrylate pathway, threonine degradation pathway, citramalate pathway or the propanediol reduction pathway).

It is one object of the present invention to produce propylene by dehydration of propanol that is produced by an improved microorganism that optimizes the carbon flux toward to production of propanol.

US 4,727,214 describes a process for converting anhydrous or aqueous ethanol into ethylene by means of a catalyst of the crystalline zeolite type, said catalyst having, on the one hand, channels or pores formed by cycles or rings of oxygen atoms having 8 and/or 10 elements or members, and on the other hand, an atomic Si/Al ratio of less than about 20. In the examples, the atomic ratio Si/Al of the FER used is from 5 to 20, the temperature from 217 to 280°C, and the WHSV of 2.5 h⁻¹.

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JP 2009-215244 A published on 24 sept 2009 relates to a method to produce ethylene by contacting ethanol on a H-FER catalyst, having an atomic Si/Al between 3 and 20, more specifically between 4 and 10, sodium and potassium contents both of 0.1%wt or less, more specifically of 0.005%wt or less, the temperature ranging from 200 to 300°C, pressure from 10 to 100 bara, and WHSV from 0.1 to 10 h⁻¹. In examples, the appraisal of the ethanol reaction is

achieved by method of gas pulse reaction using gas chromatograph. In the examples the temperature is 260°C or under.

EP 379803 provides a new process for propylene preparation comprises
5 dehydrating iso-propanol in the presence of a gamma-alumina catalyst having mean pore diameter of 3-15 nm with a standard deviation of 1-4 nm based on a statistical calculation from pore diameter and pore volume. Specifically, the gamma-alumina catalyst has pore volume of at least 0.4 cc/g on dry basis; is a low alkali gamma-alumina comprising at least 90 weight% of gamma-alumina,
10 less than 10 weight% of silica and up to 0.5 weight% of alkali metal oxide; and is a weakly acidic gamma-alumina having pKa of 3.3-6.8 measured by Hammett's equation and an integrated acid quantity of up to 0.5 micro-equivalent 1g on dry basis. The new process gives propylene in high selectivity and yield using a simple non-corrosion resistant reactor at lower temperature
15 than for conventional processes.

WO 2009-098262 (in a first embodiment) relates to a process for the dehydration of an alcohol having at least 2 carbon atoms to make the corresponding olefin, comprising:
20 introducing in a reactor a stream (A) comprising at least an alcohol, optionally water, optionally an inert component, contacting said stream with a catalyst in said reactor at conditions effective to dehydrate at least a portion of the alcohol to make an olefin,
recovering from said reactor an olefin containing stream (B),
25 Wherein
the catalyst is :
a crystalline silicate having a ratio Si/Al of at least about 100, or
a dealuminated crystalline silicate, or
a phosphorus modified zeolite,
30 the WHSV of the alcohols is at least 2 h⁻¹,
the temperature ranges from 280°C to 500°C.

WO 2009-098262 (in a second embodiment) relates to a process for the dehydration of an alcohol having at least 2 carbon atoms to make the corresponding olefin, comprising:

- 5 introducing in a reactor a stream (A) comprising at least an alcohol, optionally water, optionally an inert component, contacting said stream with a catalyst in said reactor at conditions effective to dehydrate at least a portion of the alcohol to make an olefin,
recovering from said reactor an olefin containing stream (B),
10 Wherein
the catalyst is a phosphorus modified zeolite,
the temperature ranges from 280°C to 500°C.

Synthesis gas Production

- 15 Synthesis gas can be produced from low-molecular weight hydrocarbons via reforming or from solid or high-molecular weight liquid hydrocarbons via gasification.

Low-molecular weight hydrocarbons are transformed in presence of steam and/or oxygen into synthesis gas in the “steam reforming” (SMR), CO₂ reforming or autothermal reforming (ATR) (K. Aasberg-Petersen, “Technologies for large-scale gas conversion”, Applied Catalysis A: General, 221, p. 379–387, 2001)

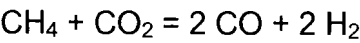
Steam reforming

- $$\text{CH}_4 + \text{H}_2\text{O} = \text{CO} + 3 \text{H}_2$$

25
$$\text{C}_n\text{H}_m + n \text{H}_2\text{O} = n \text{CO} + n + (m/2) \text{H}_2$$

$$\text{CO} + \text{H}_2\text{O} = \text{CO}_2 + \text{H}_2$$

CO₂ reforming



Autothermal reforming (ATR)

- 30
$$\text{CH}_4 + 1.5 \text{O}_2 = \text{CO} + 2 \text{H}_2\text{O}$$

$$\text{CH}_4 + \text{H}_2\text{O} = \text{CO} + 3 \text{H}_2$$

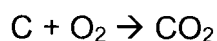
$$\text{CO} + \text{H}_2\text{O} = \text{CO}_2 + \text{H}_2$$

Gasification is a partial combustion process that converts carbonaceous materials into CO, CO₂ and H₂. In the gasification reaction, lower than stoichiometric amounts of oxygen (in the form of air, pure oxygen or steam) are fed to the reactor at high temperatures (greater than 700° C) and therefore the products are only partially reduced (C. Higman and M. van der Burgt, "Gasification", Elsevier, 2003; Bridgwater A. "Renewable fuels and chemicals by thermal processing of biomass", Chemical Engineering Journal, 91, p.87-102, 2003).

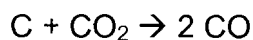
- 10 In a gasifier, the carbonaceous material is subjected to 4 processes:
 - (i) Initial heating to dry out any moisture embedded in the carbonaceous solid
 - (ii) The *pyrolysis* (or devolatilization) process occurs as the carbonaceous particle heats up. Volatiles are released and char is produced, resulting in significant weight loss. The process is dependent on the properties of the carbonaceous material and determines the structure and composition of the char, which will then undergo gasification reactions.
 - (iii) The *combustion* process occurs as the volatile products and some of the char react with O₂ to form CO₂ and CO, which provides heat for the subsequent gasification reactions. Pyrolysis and combustion are very rapid processes.
 - 20 (iv) The *gasification* process occurs as the char reacts with CO₂ and steam to produce CO and H₂. The resulting gas is called producer gas (H₂, CO and considerable amounts of CH₄) or syngas (mainly H₂ and CO and little CH₄, so "cleaner").

The chemistry of coal gasification is quite complex:

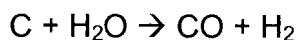
- 25 Gasification with oxygen:



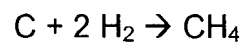
Gasification with carbondioxide:



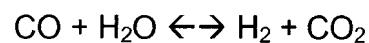
- 30 Gasification with steam:



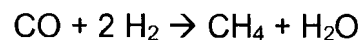
Gasification with hydrogen:



Water-gas shift:



5 Methanation:



Gasification consists of a series of controlled chemical reactions taking place at up to 70 bar or more and temperature as high as 1400°C. As the feedstock is exposed to rising temperature in the gasifier, devolatilization and breaking of weaker chemical bonds occur, yielding tars, oils, phenols, and hydrocarbon gases. These products generally react further to form CO, H₂ and lesser quantities of CO₂. The fixed carbon that remains after devolatilization is gasified through reactions with O₂, water, CO₂ and H₂ and these gases react further to produce the final gas mixture. The water-gas shift reaction alters the H₂ to CO ratio of the final gas mixture. Methanation reactions are favored by high pressures and low temperatures. Since no O₂ is consumed, exothermic (heat releasing) methanation reactions increase the efficiency of gasification and the heating value of the syngas (producer gas) produced but reduced the hydrogen to carbonmonoxide ratio which is preferentially as high as possible for sequence chemical synthesis.

Under the substoichiometric reducing conditions of gasification, most of the feedstock sulfur converts to hydrogen sulfide (H₂S) and some converts to carbonyl sulfide (COS). Nitrogen chemically bound in the feed generally converts to gaseous nitrogen (N₂), with some ammonia (NH₃) and a small amount of hydrogen cyanide (HCN) also being formed. Chlorine in the feed is primarily converted to hydrogen chloride (HCl), with some appearing as chloride-containing particulates. Trace elements associated with both organic and inorganic components of coal feedstock, such as mercury (Hg) and arsenic (As), appear in the various ash fractions as well as in gaseous emissions and need to be removed from the syngas prior to further use.

[Brief summary of the invention]

In one embodiment of the present invention is provided:

A process for making propylene by dehydration of propanol, involving the following steps:

1. Gasifying carbonaceous solid or liquid feedstock or reforming gaseous carbonaceous feedstock into synthesis gas,
2. Removing contaminants from the synthesis gas
3. Fermenting the synthesis gas by means of a microorganism into substantially propanol in which the microorganism
 1. Is a wild strain having the natural capability to ferment synthesis gas into substantially propanol or
 2. Is a microorganism, possessing the required nucleic acid sequence information to express the enzymes for the biosynthesis of C₃-oxygenates (acetone/isopropanol pathway, dicarboxylic pathway, acrylate pathway, threonine degradation pathway, citramalate pathway or the propanediol reduction pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway), or
 3. Is a microorganism, possessing the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes for the biosynthesis of C₃-oxygenates (acetone/isopropanol pathway, dicarboxylic pathway, acrylate pathway, threonine degradation pathway, citramalate pathway or the propanediol reduction pathway).
4. Fractionating and purifying the stream containing predominantly propanol,
5. Dehydrating said above stream in a reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene,

6. recovering from said reactor the propylene containing stream

It is another embodiment of the present invention to provide

A process for making propylene by dehydration of propanol, involving the following steps:

1. Gasifying carbonaceous solid or liquid feedstock or reforming gaseous carbonaceous feedstock into synthesis gas,
2. Removing contaminants from the synthesis gas
3. Co-Fermenting the synthesis gas with at least one liquid oxygenate, like carbohydrates, glycerol, propanediol, lactaldehyde, lactic acid, acetol, methanol, ethanol, acetic acid, acetaldehyde, propionic acid, propanal and acetone, by means of a microorganism into substantially propanol in which the microorganism
 1. Is a wild strain having the natural capability to ferment synthesis gas into substantially propanol or
 2. Is a microorganism, possessing the required nucleic acid sequence information to express the enzymes for the biosynthesis of C₃-oxygenates (acetone/isopropanol pathway, dicarboxylic pathway, acrylate pathway, threonine degradation pathway, citramalate pathway or the propanediol reduction pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway), or
 3. Is a microorganism, possessing the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes for the biosynthesis of C₃-oxygenates (acetone/isopropanol pathway, dicarboxylic pathway, acrylate pathway, threonine degradation pathway, citramalate pathway or the propanediol reduction pathway).
4. Fractionating and purifying the stream containing predominantly propanol,

5. Dehydrating said above stream in a reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene,
6. recovering from said reactor the propylene containing stream

- 5 The stream containing predominantly propanol can contain other alcohols e.g. ethanol, butanols.

The fermentation or the co-fermentation step (3), produces a mixture of alcohols, containing at least 50 wt% of propanol, between 0 and 50 wt% of ethanol and between 0 and 50 wt% of butanol.

- 10 In an embodiment the weight ratio of said alcohols to the propanol is less than 20/80.

In an embodiment the weight ratio of said alcohols to the propanol is less than 10/90. In an embodiment the weight ratio of said alcohols to the propanol is less than 5/95.

- 15 In an embodiment dehydration is made over an acidic catalyst at a temperature of at least 200°C and a WHSV of at least 1 h⁻¹. Dehydration can be made by introducing in a reactor a stream comprising at least propanol, optionally water, optionally an inert component, contacting said stream with a catalyst in said reactor at conditions effective to dehydrate at least a portion of the propanol to
- 20 make propylene.

By dehydration is made an olefin having the same number of carbons as the alcohol to be dehydrated. Of course if other alcohols are present the dehydration produces the corresponding olefin (ethanol leads to ethylene and butanol leads to butene).

25

[Detailed description of the invention]

- 30 **As regards the production of synthesis gas**, it can be produced from low-molecular hydrocarbons by reforming or from high-molecular weight liquid hydrocarbons or solid carbonaceous solid via gasification.

Essentially, synthesis gas is formed via steam reforming reaction and partial oxidation of natural gas (Dybkaer I., "Tubular reforming and autothermal reforming of natural gas – an overview of available processes", Fuel. Proc. Tech., 42, p. 85, 1995; Moulijn J., "Chemical Process Technology", Wiley & Sons, 2001). In steam reformer (SMR) a catalyst is packed in tubes placed in a fired heater. The reformer tubes are heated externally by burners using typically natural gas. The feedstock to the reformer is a mixture of desulfurised natural gas and steam (>3:1 steam to carbon ratio in order to prevent coking of the catalyst). Steam methane reforming is endothermic and the molar ratio of hydrogen to carbon monoxide produced by SMR is approximately 3:1. If CO₂ (CO₂ reforming or dry-reforming) is added to the feedstock the H₂/CO ratio can be reduced. When the feedstock contains heavy hydrocarbons, a prereformer is used to first reform the heavy hydrocarbons.

The autothermal reformer (ATR) reactor has a compact design consisting of a burner, combustion chamber and catalyst bed placed in a refractory lined pressure vessel as. The hydrocarbon feedstock is reacted with a mixture of oxygen and steam in a sub-stoichiometric flame. The steam to carbon ratio can be as low as 0.6. In the fixed catalyst bed, the synthesis gas is further equilibrated. The composition of the product gas will be determined by the thermodynamic equilibrium at the exit pressure and temperature, which is determined through the adiabatic heat balance based on the composition and flows of the feed, steam and oxygen added to the reactor. The produced synthesis gas is completely soot-free.

In the combined reformer (or 2-step reformer), the tubular reformer (SMR) is combined with a secondary reformer, acting as an ATR, to which oxygen is added.

There are four main types of gasifiers currently used commercially: counter-current fixed bed, co-current fixed bed, fluid bed and entrained flow (C. Higman and M. van der Burgt, "Gasification", Elsevier, 2003).

Counter-current fixed bed (Updraft, also called moving bed gasifiers): A fixed bed of carbonaceous feedstock (coal, petcoke, char or biomass) entering

at the top with a counter current flow of steam, oxygen and/or air flows up through the solid bed. The feedstock is dried by the syngas leaving the chamber while the gas leaving the gasifier is cooled by the entering feedstock. In case of oxygen-containing feedstock like biomass, char that is generated continues moving down the gasifier vessel where it is reduced and starts reacting with the oxygen and CO₂. The dried feedstock travels down the vessel where gasification of coal or char occurs. The ash is then removed dry or as slag. Gas exit temperatures are low, which is good for thermal efficiency, but this increases the tar and methane impurities in the synthesis gas (McKendry P., "Energy production from biomass (part 1): overview of biomass", Bioresource Technology 83, 37-46, 2002; Bridgwater T., "Review Biomass for energy", Journal of the Science of Food and Agriculture 86, p.1755-1768, 2006). The advantages of updraft gasifiers are their simple construction, low cost, ability to handle high moisture and inorganic content, and their high energy efficiency due to the lower temperature of the gas leaving the chamber.

Co-current fixed bed (Downdraft): This is similar to the counter-current gasifier described above beside that the steam, oxygen and/or air flows co-currently down with the solid carbonaceous feedstock bed. Some heat has to be added to the top of the gasifier by combustion or heat exchange. Because the gas passes through the hot zone combusting the tars and leaving the reactor from the bottom hence the final product has a higher purity. The exit temperature of the gas is higher, resulting in a lower overall efficiency. It has a fairly simple design and is low cost, and it produces a relatively cleaner gas with very low tar make. Some of the disadvantages are that the system requires low moisture and ash feedstock and it has low efficiency because the product gas leaves the gasifier at higher temperatures, which requires an additional cooling system as compared to an updraft gasifier.

Fluid bed: The carbonaceous feedstock is fluidized in oxygen/air and steam. The feedstock size is reduced to a small particle size, and is mixed eventually with the fluidizing material, which is usually silica sand, ceramic, or alumina. The oxidizer and the solid crushed feedstock enter the reactor from the bottom,

where a hot bed forms, where most of the conversion to synthesis gas occurs. The ash is removed as a dry product as it becomes defluidized. Fuel throughput is higher than for a fixed bed, and has the advantage of uniform temperature distribution achieved in the gasification zone resulting in cleaner reactions.

- 5 However, overall conversion can be very high, the gas stream entrains a lot of fine particulates which has be separated from the gas and recycled back to the gasifier (circulating fluidized bed). Fluidized beds work particularly well for biomass, as it has a lot of highly corrosive ash that would harm the fixed bed reactors.

- 10 **Entrained flow:** a dry pulverized solid, an atomized liquid fuel or a fuel slurry is gasified with O_2 (or air) in co-current flow. The gasification reactions take place in a dense cloud of very fine particles. Most coals are suitable for this type of gasifier because of the high operating temperatures (well above ash melting point, to assure high carbon conversion) and because the coal particles are well
- 15 separated from one another. The high temperatures and pressures also mean that a higher throughput can be achieved (short contact time), however thermal efficiency is somewhat lower as the gas must be cooled before it can be cleaned with existing technology. The high temperatures also mean that tar and CH_4 are not present in the product gas; however the O_2 requirement is higher
- 20 than for the other types of gasifiers. All entrained flow gasifiers remove the major part of the ash as a slag as the operating temperature is well above the ash fusion temperature. Some fuels (in particular certain types of biomass) can form slag that is corrosive for the ceramic inner walls that serve to protect the gasifier outer wall. However some entrained bed type of gasifiers do not have a
- 25 ceramic inner wall but have an inner water or steam cooled wall covered with partially solidified slag and hence do not suffer from corrosive slags.

- As regards the microorganism,** they can include prokaryotic and eukaryotic microbial species from the Domains Archaea, Bacteria and Eucarya
- 30 (including yeast and filamentous fungi, protozoa, algae, or higher Protista). The term "prokaryotes" refers to cells which contain no nucleus or other cell

organelles and are generally classified in one of two domains, the Bacteria and the Archaea. The ultimate difference between organisms of the Archaea and Bacteria domains is based on fundamental differences in the nucleotide base sequence in the 16S ribosomal RNA.

- 5 The microorganisms of the present invention are either naturally occurring microorganisms or metabolically engineered microorganism.

Naturally occurring microorganism of the present invention are isolated from nature, so called wild strains that have the ability to ferment synthesis gas into propanol. Such wild strains can be improved by random screening and
10 rationalized selection. Random mutagenesis is based on repeated applications of three steps: (i) mutagenesis with chemicals or radiation of a population to induce genetic variability, (ii) screening from the surviving population to identify an improved strain under small-scale standard fermentation testing and (iii) assay of fermentation test for confirmation of improved strains (S. Parekh,
15 "Improvement of microbial strains and fermentation processes", Appl. Microbiol. Biotechnol., 54, p.287, 2000). Furthermore, adaptive evolution can be applied to make the microorganism more resistance to certain external conditions, like higher solvent tolerance, higher fermentation temperature, higher syngas pressure etc..

- 20 Random mutagenesis may be performed using a suitable physical or chemical mutagenising agent. Examples of a physical or chemical mutagenising agent suitable for the present purpose include, but are not limited to, UV irradiation, ionizing irradiation such as gamma irradiation, hydroxylamine, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), O-methyl hydroxylamine, nitrous acid, ethyl
25 methane sulphonate (EMS), sodium bisulfite, and nucleotide analogues. When such agents are used the mutagenesis is typically performed by incubating the cell to be mutagenised in the presence of the mutagenising agent of choice under suitable conditions, and selecting for cells showing a significantly increased or decreased production of the targeted molecule(s).

The term "metabolically engineered" entails rational designed pathway and assembly of biosynthetic genes, genes associated with operons, and control elements of such nucleic acid sequences, for the production of a desired metabolite, in particular as end-product propanol (isopropanol or n-propanol). It further embraces optimization of metabolic flux by regulation and optimization of transcription, translation, protein stability and protein functionality, functional in an optimal manner under the applied fermentation conditions. The biosynthetic genes can be heterologous to the host microorganism, either by virtue of being foreign to the host, or being modified by mutagenesis, recombination, and/or association with a heterologous expression control sequence in an endogenous host cell. Accordingly, "metabolically engineered" microorganisms are created via the introduction of genetic material into a host microorganism of choice thereby acquiring new properties, e.g. the ability to produce a new or greater quantity of metabolite. It also encompasses the activation of endogenous nucleic acid sequences encoding a target enzyme through genetic modification of e.g., a promoter sequence in a host microorganism and the introduction of exogenous nucleic acid sequences encoding a target enzyme into a host microorganism. The introduction of genetic material (containing gene(s), or parts of genes, coding for one or more of the enzymes involved in a biosynthetic pathway for the production of isopropanol or n-propanol and eventually including additional elements for the expression (or regulation) of these genes, e.g. promoter sequences) into a host microorganism results in a new or improved ability to produce isopropanol or n-propanol from synthesis gas.

A "metabolite" refers to any substance produced by anabolic or catabolic metabolism. An important intermediate metabolite is Acetyl-CoA, produced from synthesis gas or other oxygenated compounds like carbohydrates, ethanol, acetic acid, required for the synthesis of the desired end-metabolite isopropanol or n-propanol.

In order to express the metabolic pathway of interest and/or reorienting metabolic flux, the host cell of the invention could be genetically modified by using standard technologies known to the person skilled in the art. For example,

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if we want to delete a pathway to increase metabolic flux for the production of propanol, the gene sequences responsible for production of enzymes of endogenous pathway may be inactivated or partially or entirely eliminated. The inactivation could be obtained by modification of the respective structural or regulatory regions (such as genes). Known and useful techniques include, but are not limited to, specific or random mutagenesis, PCR generated mutagenesis, site specific DNA deletion, insertion and/or substitution, gene disruption or gene replacement, anti-sense techniques, or a combination thereof.

10 The term "heterologous" or "exogenous" indicates enzymes or nucleic acids that are expressed in an organism other than the organism from which they originated. The term "endogenous" indicates enzymes and nucleic acids that are expressed in the organism in which they originated.

The resulting "recombinant microorganism" is a host microorganism that has been genetically modified to express or over-express endogenous nucleic acid sequences, or to express non-endogenous nucleic acid sequences, such as those included in a vector (encoding a target enzyme or enzymes involved in a metabolic pathway for producing a desired metabolite). It is understood that the terms "recombinant microorganism" refer also to the progeny.

20 The term "host microorganism" represents (i) a cell that occurs in nature, i.e. a "wild-type" cell that has not been genetically modified or (ii) a cell that has been genetically modified but which does not express or over-express a target enzyme (such microorganism can act as a host in the generation of a microorganism modified to express or over-express another target enzyme).

25 Methods of over-expressing in microorganisms are well known in the art, and any such method is contemplated for use in the construction of the microorganisms of the present invention.

Any method can be used to introduce an exogenous nucleic acid molecule into microorganisms and many such methods are well known to those skilled in the art: for example, transformation, electroporation, conjugation, and fusion of protoplasts are common methods for introducing nucleic acid into

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microorganisms (J. Dale, "Molecular Genetics of bacteria", 4th ed., Wiley, 2004; C. Smolke, "The Metabolic Pathway Engineering Handbook", CRC Press, 2010).

5 The exogenous nucleic acid molecule transposed to a host microorganism can be maintained within that cell in any form: for example, exogenous nucleic acid molecules can be incorporated into the genome of the cell or maintained in an episomal state (extra-chromosomal genetic elements, like plasmids) that can be passed stably on to daughter cells. The microorganisms described herein can contain a single copy, or multiple copies of a particular exogenous nucleic acid
10 molecule.

Methods for expressing enzymes from an exogenous nucleic acid molecule are well known to those skilled in the art, including, without limitation, assembling a nucleic acid sequence such that a regulatory element promotes the expression of a nucleic acid sequence that encodes the desired enzyme. Typically,
15 regulatory elements (promoters, enhancers, etc) are nucleic acid sequences that regulate the expression of other nucleic acid sequences at the level of transcription.

Additionally, when expression of certain enzymatic activity is to be repressed or eliminated, the gene for the relevant enzyme, protein or RNA can be eliminated
20 by known deletion techniques. These deletion techniques are useful to further improve the yield of isopropanol or n-propanol by eliminated the pathways leading to byproducts, like C₂ or C₄ oxygenates or hydrogen. Heterologous control elements can be used to repress expression of endogenous genes. Deletion techniques can be used for optimized both the wild strain and the
25 metabolically engineered microorganisms.

It is an embodiment of the present invention, that synthesis gas can be co-fermented with oxygenated compounds, like carbohydrates, glycerol, propanediol, lactaldehyde, lactic acid, acetol, methanol, ethanol, acetic acid,
30 acetaldehyde, propionic acid, propanal and acetone. The carbohydrates are essentially "biomass derived sugars" and includes, but is not limited to,

molecules such as glucose, mannose, fucose, rhamnose, xylose, and arabinose, lactose, sorbose, fructose, idose, galactose, mannose and dimers, oligomers or polymers of the latter (starch, cellulose, hemicelluloses and pectins).

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- As regards the fermentation**, this is known per se and documented in literature (P. Munasinghe, "Biomass-derived syngas fermentation into biofuels: Opportunities and challenges", *Bioresource Technology*, 101(13), p. 5013, 2010; Chapter 11 & 13 of "Bioenergy and Biofuel from biowastes and biomass", S. Khanal ed., ASCE, 2010; J. Williams, "Keys to bioreactor selection", *Chemical Engineering Progress*, p. 34, March 2002). Syngas fermentation can be carried out in both batch and continuous-flow bioreactors. In batch reactors, the gaseous substrate is introduced in closed system bioreactor where the syngas is supplied continuously but the products remain in the reactor or can be withdrawn at a selected time during fermentation. Bubble column reactors, monolithic biofilm reactors, membrane bioreactors and trickling bed reactors are some of the other common bioreactors. In some of the bioreactors microbubble spargers can be implemented which enhances the mass transfer in two ways (M. Bredwell, P. Srivastava and R.M. Worden, "Reactor design issues for synthesis-gas fermentations", *Biotechnology Progress*, 15, p. 834–844, 1999). Firstly, decreasing bubble sizes cause internal pressure increases, leading to an increase in the driving force. Secondly, the steady state liquid phase concentration gradient at the surface of the bubble is inversely proportional to the diameter. Many other essential techniques (fermentation kinetics, preservation of microorganism, nutrients media, sterilization, inocula development and fermentor design) to implement fermentation are described in reference books (P. Stanbury, "Principles of fermentation technology", Elsevier Science, 2003; B. McNeil, "Practical Fermentation Technology", John Wiley&Son, 2008)
- a. The continuous stirred-tank reactor is the most common bioreactor employed in syngas fermentation. A CSTR has a continuous flow of gas

through a constant liquid volume. The liquid consists of a suspension of the microorganism and a dilute solution of essential nutrients for the microorganism to grow and survive and a make-up of such liquid nutrient solution and/or microorganism suspension is continuously or intermittently added. Syngas is bubbled through a sparger and an agitation is applied for enhanced mass transfer between the two phases. The fermentation product is withdrawn from the system at the same flow rate as the feed entering the reactor. Cell-recycle systems can be used in conjunction with the CSTR to increase cell-density within the reactor. In such a system, the fermentation broth is pumped through a recycle filter, decanter or centrifuge and the retentate (containing the microorganism cells) is separated from the permeate (cell-free media) and recycled to the bioreactor. An enhanced degree of agitation or mixing is accomplished by baffled impellers to enhance the mass transfer between the substrate and the microorganisms. Higher rotational speeds of the impellers tend to break the gas bubbles into finer ones thereby increasing the interfacial area between the syngas and the aqueous suspension of the microorganisms.

- b. Bubble column reactors are most suitable for very large volume industrial applications. These reactors have a large height-to-diameter ratio (aspect ratio) in which high mass transfer can be obtained even without the use of additional agitation. Smaller bubble size and improved gas dispersion can be obtained by using special devices to disperse the syngas in the bottom of the bioreactor, like porous fritted discs or multiple sparger rings to disperse the syngas. Gas-lift reactors are like bubble columns containing a draught tube. Gas is dispersed into the bottom of a central draught tube that controls the circulation of gas and the culture medium. Gas rises through the tube, forming bubbles, entraining the liquid suspension and exhaust gas disengages at the top of the column. The degassed liquid then flows downward external to the draught tube and the product is drained from the tank. As there is an induced controlled flow direction of the liquid suspension, the tube can be designed to serve as an internal heat exchanger, or a heat exchanger

can be added to an internal circulation loop. A variation on this gaslift reactor is the external gaslift reactor consisting of an internal riser section and an external downcomer. Through the riser the gas rises and induces the liquid suspension upwards. At the top, the gas disengages and the liquid suspension returns via the external downcomer back to the bottom of the reactor. The downcomer can serve as a heat exchanger if required.

c. Monolithic biofilm reactors, is like a bundle of small channels made out of solid porous material on which a biofilm of microorganisms grows. It exhibits a good mass-transfer of gas to the biofilm and a low pressure drop. During the operation, attached microorganisms in the biofilm utilize the gaseous substrates to produce fermentation products. Monolith reactors have high mass-transfer characteristics in particular in multiphase operation. The liquid (typically aqueous solution containing the nutrients and fermentation products) moves as a thin film over the channel surface and the gas flow through the core of the channels. When liquid flow rate are high or gas flow rate low, the gas and liquid move through the channel as separate slugs (Taylor flow). The gas bubble nearly completely fills the diameter of the channel, leaving only a very thin film of liquid near the stagnant biofilm, consequently a high gas-stagnant biofilm mass-transfer is possible. The appropriate liquid/gas ratio in order to reach Taylor flow can be installed by recirculating the liquid around the monolith.

d. Trickle-bed reactors are packed beds, continuous reactor in which the liquid culture flows down, trickling over the surface of the packing, while maximizing the gas-liquid interface. The syngas is allowed to move either downward (co-current) or upward (counter-current) direction. Since these types of reactors do not require mechanical agitation, the power consumption of trickle-bed reactors is lower than the CSTR.

e. Membrane-based bioreactor consists of membranes (flat sheet (FS), multitubular or hollow fiber (HFM)) that allow effectively facilitating the

mass transfer in aqueous culture media (K. Lee and B. Rittmann, "Applying a novel autohydrogenotrophic hollow-fiber membrane biofilm reactor for denitrification of drinking water", Water Research, 36, p. 2040–2052, 2001; S. Judd, "The MBR Book", Elsevier, 2006; US patent 2009/0215163). In the membrane reactor, syngas is diffused through the walls of membranes without forming bubbles. The microbial community grows and settles as a biofilm on the wall of the membranes where it continuously ferments H_2 and CO to alcohols. These membrane bioreactors can be operated under high pressure with higher mass transfer rates and reduced reactor volumes.

As regards the propanol fractionation and purification, the propanol as the main product of the fermentation is fractionated by any conventional means, like stripping (flashing) of the dissolved gases in the fermentation broth, like filtration, decantation and/or centrifugation to remove solids (microorganism and any other precipitated material) from the aqueous solution, like distillation (stripping and/or rectification) in order to concentrate the propanol stream or like membrane separation in order to isolate the propanol from the aqueous solution or fermentation broth.

Before going to the dehydration reactor, the propanol (and eventually ethanol and butanol) can be purified by the process of WO 2010/060981 the content of which is incorporated in the present application. It describes a process for the purification of an alcohol in the course of a process comprising :

- (1) providing a reaction zone (C) comprising an acid type catalyst;
- (2) providing a reaction zone (B) comprising an acid adsorbent material;
- (3) providing an alcohol stream comprising impurities;
- (4) introducing the alcohol stream of (3) into the reaction zone (B) and bringing said stream into contact with the acid adsorbent material at conditions effective to reduce the amount of impurities having an adverse effect on the acid type catalyst of the reaction zone (C);
- (5) recovering from step (4) an alcohol stream and introducing it into the reaction zone (C);
- (6) optionally introducing one or more reactants (R) into the reaction

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zone (C); (7) operating said reaction zone (C) at conditions effective to recover a valuable effluent.

As regards the propanol dehydration, dehydration of alcohols is known per se. Alcohols dehydration has been described in WO-2009-098262 and WO-2009-098268 the content of which are incorporated in the present application.

WO-2009-098262 relates to a process for the dehydration of at least an alcohol to make at least an olefin, wherein the catalyst is:

- a crystalline silicate having a ratio Si/Al of at least about 100, or
- a dealuminated crystalline silicate, or
- a phosphorus modified zeolite, the WHSV of the alcohols is at least 2 h^{-1} , the temperature ranges from 280°C to 500°C . It relates also to the same process as above but wherein the catalyst is a phosphorus modified zeolite and at any WHSV.

WO-2009-098268 relates to a process for the dehydration of at least an alcohol to make at least an olefin, comprising:

- a) introducing in a reactor a stream (A) comprising at least an alcohol optionally in aqueous solution and an inert component,
- b) contacting said stream with a catalyst in said reactor at conditions effective to dehydrate at least a portion of the alcohol to make an olefin,
- c) recovering from said reactor a stream (B) comprising: the inert component and at least an olefin, water and optionally unconverted alcohol,
- d) optionally fractionating the stream (B) to recover the unconverted alcohol and recycling said unconverted alcohol to the reactor of step a),
- e) optionally fractionating the stream (B) to recover the inert component and the olefin and recycling said inert component to the reactor of step a), wherein, the inert component is selected among ethane, the hydrocarbons having from 3 to 10 carbon atoms, naphthenes and CO_2 , the proportion of the inert component is

such as the reactor operates essentially adiabatically. It also relates to a similar process as above but the catalyst is :

- a crystalline silicate having a ratio Si/Al of at least 100, or
- a dealuminated crystalline silicate, or
- 5 • a phosphorus modified zeolite, the WHSV of the alcohol is at least 2 h^{-1} wherein the catalyst is a crystalline silicate having a ratio Si/Al of at least 100 or a dealuminated crystalline silicate. Advantageously the pressure of the dehydration reactor is high enough to help the recovery of the inert component and recycling thereof in the reactor of step a) without a gas compressor but only
- 10 a pump.

As regards the propanol stream, the propanol may be subjected to dehydration alone or in mixture with an inert medium. The inert component is any component provided there is no adverse effect on the catalyst. Because the

15 dehydration is endothermic the inert component can be used to bring energy. The inert component may be selected among the saturated hydrocarbons having up to 10 carbon atoms, naphtenes, nitrogen and CO_2 . Advantageously it is a saturated hydrocarbon or a mixture of saturated hydrocarbons having from 3 to 7 carbon atoms, more advantageously having from 4 to 6 carbon atoms

20 and is preferably pentane. An example of inert component can be any individual saturated compound, a synthetic mixture of the individual saturated compounds as well as some equilibrated refinery streams like straight naphtha, butanes etc. Advantageously the inert component is a saturated hydrocarbon having from 3 to 6 carbon atoms and is preferably pentane. The weight proportions of

25 respectively propanol, water and inert component are, for example, 5-100/0-95/0-95 (the total being 100). The alcohol stream, containing substantially propanol may also contain ethanol and butanols. The propanol concentration within the alcohols should be at least 50 wt%; the ethanol concentration between 0 and 50 wt% and the butanol between 0 and 50 wt%.

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As regards the dehydration reactor, it can be a fixed bed reactor, a moving bed reactor or a fluidized bed reactor. A typical fluid bed reactor is one of the FCC type used for fluidized-bed catalytic cracking in the oil refinery. A typical moving bed reactor is of the continuous catalytic reforming type. The dehydration may be performed continuously in a fixed bed reactor configuration using a pair of parallel "swing" reactors. The various preferred catalysts of the present invention have been found to exhibit high stability. This enables the dehydration process to be performed continuously in two parallel "swing" reactors wherein when one reactor is operating, the other reactor is undergoing catalyst regeneration. The catalyst of the present invention also can be regenerated several times.

As regards the dehydration pressure, it can be any pressure but it is more easy and economical to operate at moderate pressure. By way of example the pressure of the reactor ranges from 0.5 to 30 bars absolute (50 kPa to 3 MPa), advantageously from 0.5 to 5 bars absolute (50 kPa to 0.5 MPa), more advantageously from 1.2 to 5 bars absolute (0.12 MPa to 0.5 MPa) and preferably from 1.2 to 4 bars absolute (0.12 MPa to 0.4 MPa). Advantageously the partial pressure of the alcohols (propanol and eventually ethanol and butanol) is from 1.2 to 4 bars absolute (0.12 MPa to 0.4 MPa), more advantageously from 1.2 to 3.5 bars absolute (0.35 MPa).

As regards the dehydration temperature, and the first embodiment it ranges from 200°C to 600°C, advantageously from 300°C to 580°C, more advantageously from 350°C to 580°C. As regards the temperature and the second embodiment it ranges from 320°C to 600°C, advantageously from 320°C to 580°C, more advantageously from 350°C to 580°C.

These reaction temperatures refer substantially to average catalyst bed temperature. The propanol dehydration is an endothermic reaction and requires the input of reaction heat in order to maintain catalyst activity sufficiently high and shift the thermodynamic equilibrium to sufficiently high conversion levels.

In case of fluidised bed reactors: (i) for stationary fluidised beds without catalyst circulation, the reaction temperature is substantially homogeneous throughout the catalyst bed; (ii) in case of circulating fluidised beds where catalyst circulates between a converting reaction section and a catalyst regeneration section, depending on the degree of catalyst backmixing the temperature in the catalyst bed approaches homogeneous conditions (a lot of backmixing) or approaches plug flow conditions (nearly no backmixing) and hence a decreasing temperature profile will install as the conversion proceeds.

10 In case of fixed bed or moving bed reactors, a decreasing temperature profile will install as the conversion of the alcohols proceeds. In order to compensate for temperature drop and consequently decreasing catalyst activity or approach to thermodynamic equilibrium, reaction heat can be introduced by using several catalyst beds in series with interheating of the reactor effluent from the first bed to higher temperatures and introducing the heated effluent in a second catalyst bed, etc. When fixed bed reactors are used, a multi-tubular reactor can be used where the catalyst is loaded in small-diameter tubes that are installed in a reactor shell. At the shell side, a heating medium is introduced that provides the required reaction heat by heat-transfer through the wall of the reactor tubes to the catalyst.

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As regards the dehydration WHSV of the alcohols, it ranges advantageously from 1 to 20 h⁻¹, preferably from 3 to 15 h⁻¹, more preferably from 4 to 10 h⁻¹.

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As regards the dehydration effluent stream, it comprises essentially water, olefin, the inert component (if any) and unconverted alcohols. Said unconverted alcohols is supposed to be as less as possible. The olefin is recovered by usual fractionation means. Advantageously the inert component, if any, is recycled in the stream (A) as well as the unconverted alcohols, if any.

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Unconverted alcohols, if any, is recycled to the reactor in the stream (A).

- As regards the dehydration catalyst**, it is by way of example,
- a crystalline silicate of the group FER (ferrierite, FU-9, ZSM-35), MWW (MCM-22, PSH-3, ITQ-1, MCM-49), EUO (ZSM-50, EU-1), MFS (ZSM-57), ZSM-48, MTT (ZSM-23), MFI (ZSM-5 or silicalite), MEL (ZSM-11) or TON (ZSM-22, Theta-1, NU-10),
- 5 or a dealuminated crystalline silicate of the group FER (ferrierite, FU-9, ZSM-35), MWW (MCM-22, PSH-3, ITQ-1, MCM-49), EUO (ZSM-50, EU-1), MFS (ZSM-57), ZSM-48, MTT (ZSM-23), MFI (ZSM-5 or silicalite), MEL (ZSM-11) or TON (ZSM-22, Theta-1, NU-10),
- 10 or a phosphorus modified crystalline silicate of the group FER (ferrierite, FU-9, ZSM-35), MWW (MCM-22, PSH-3, ITQ-1, MCM-49), EUO (ZSM-50, EU-1), MFS (ZSM-57), ZSM-48, MTT (ZSM-23), MFI (ZSM-5 or silicalite), MEL (ZSM-11) or TON (ZSM-22, Theta-1, NU-10),
- or a silicoaluminophosphate molecular sieve of the group AEI, CHA or AEL,
- 15 or gamma-, beta-, eta- or delta-alumina and amorphous alumina
or silica-alumina
or a alkalized, silicated, zirconated or titanated or fluorinated alumina.

- The crystalline silicate can be subjected to various treatments before use in the
- 20 dehydration including, ion exchange, modification with metals (in a not restrictive manner alkali, alkali-earth, transition, or rare earth elements), external surface passivation, modification with phosphorus-compounds, steaming, acid treatment or other dealumination methods, or combination thereof.

- 25 Another suitable catalyst for the present process is the silicoaluminophosphate molecular sieves of the AEI, CHA or AEL group with typical example the SAPO-18, SAPO-34 or SAPO-11 molecular sieve. The SAPO molecular sieve is based on the ALPO, having essentially an Al/P ratio of 1 atom/atom. During the synthesis silicon precursor is added and insertion of
- 30 silicon in the ALPO framework results in an acid site at the surface of the

micropores of the 10-membered ring sieve. The silicon content ranges from 0.1 to 10 atom% (Al + P + Si is 100).

In another specific embodiment the crystalline silicate or silicoaluminophosphate molecular sieve is mixed with a binder, preferably an inorganic binder, and shaped to a desired shape, e.g. pellets. The binder is selected so as to be resistant to the temperature and other conditions employed in the dehydration process of the invention. The binder is an inorganic material selected from clays, silica, metal silicates, metal oxides such as ZrO_2 and/or metals, or gels including mixtures of silica and metal oxides. If the binder which is used in conjunction with the crystalline silicate is itself catalytically active, this may alter the conversion and/or the selectivity of the catalyst. Inactive materials for the binder may suitably serve as diluents to control the amount of conversion so that products can be obtained economically and orderly without employing other means for controlling the reaction rate. It is desirable to provide a catalyst having a good crush strength. This is because in commercial use, it is desirable to prevent the catalyst from breaking down into powder-like materials. Such clay or oxide binders have been employed normally only for the purpose of improving the crush strength of the catalyst. A particularly preferred binder for the catalyst of the present invention comprises silica. The relative proportions of the finely divided crystalline silicate material and the inorganic oxide matrix of the binder can vary widely. Typically, the binder content ranges from 5 to 95% by weight, more typically from 20 to 75% by weight, based on the weight of the composite catalyst. Such a mixture of the crystalline silicate and an inorganic oxide binder is referred to as a formulated crystalline silicate. In mixing the catalyst with a binder, the catalyst may be formulated into pellets, extruded into other shapes, or formed into spheres or a spray-dried powder. Typically, the binder and the crystalline silicate are mixed together by a mixing process. In such a process, the binder, for example silica, in the form of a gel is mixed with the crystalline silicate material and the resultant mixture is extruded into the desired shape, for example cylindrical or multi-lobe bars. Spherical shapes can

be made in rotating granulators or by oil-drop technique. Small spheres can further be made by spray-drying a catalyst-binder suspension. Thereafter, the formulated crystalline silicate is calcined in air or an inert gas, typically at a temperature of from 200 to 900°C for a period of from 1 to 48 hours.

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Another family of suitable catalysts for the dehydration of propanol into propylene are alumina's (amorphous, gamma-, beta-, eta- or delta-alumina's) as such or alumina's that have been modified by surface treatment with alkali's, silicon, zirconium or titanium and silica-alumina's. Alumina's are generally characterised by a rather broad acid strength distribution and having both Lewis-type and Bronsted-type acid sites. The presence of a broad acid strength distribution makes the catalysis of several reactions, requiring each a different acid strength, possible. This often results in low selectivity for the desired product. Deposition of alkali's, silicon, zirconium or titanium on the surface of alumina allows rendering the catalyst significantly more selective. For the preparation of the alumina based catalyst, suitable commercial alumina's can be used, preferably eta or gamma alumina, having a surface area of 10 to 500 m²/gram. The catalyst according to the present invention is prepared by adding 0.05 to 10% of alkali (earth) metal, silicon, zirconium or titanium. The addition of these metals can be done during the preparation of the alumina or can be added to the existing alumina, eventually already activated. Addition of the metal during the preparation of the alumina can be done by dissolving the metal precursor together with the aluminium precursor before precipitation of the final alumina or by addition of the metal precursor to the aluminium hydroxide gel. A preferred method is adding metal precursors to the shaped alumina. Metal precursors are dissolved in a suitable solvent, either aqueous or organic, and contacted with the alumina by incipient wetness impregnation or by wet impregnation or by contacting with an excess of solute during a given time, followed by removing the excess solute. The alumina can also be contacted with vapour of the metal precursor. Suitable metal precursors are halides of alkali (earth) metals, silicon, zirconium or titanium, oxyhalides of zirconium or

titanium; alcoxides of alkali (earth) metals, silicon, zirconium or titanium; acetates, oxalates or citrates of alkali (earth) metals, zirconium or titanium; nitrates, sulfates or phosphates of alkali (earth) metals, or mixtures of the above. The solvent is selected according to the solubility of the metal precursor.

- 5 The contacting can be done at temperature of 0°C to 500°C, most preferred from 10°C to 200°C. After the contacting, the alumina is eventually washed, dried and finally calcined in order to enhance the surface reaction between the alkali (earth) metals, silicon, zirconium or titanium and the alumina and the removal of the metal precursor ligands. The use of alkalized, silicated, zirconated or titanated or fluorinated alumina's for the dehydration of propanol into propylene is preferably done in the presence of water. The weight ratio of water to propanol ranges from 1/25 to 3/1. Fluorinated alumina is known in itself and can be made according to the prior art.

- 15 One skilled in the art will also appreciate that the propylene made by the dehydration process of the present invention can be, by way of example, polymerized into polypropylene, copolymers of ethylene, hexane, octane or decene and propylene, ethylene-propylene rubbers (EPR), ethylene-propylene-diene polymers (EPDM), can be used for alkylation of benzene to make cumene, a precursor for phenol production, can be oxidized to acrylic acid, can be ammoxidised into acrylonitrile, can be oxidized to propylene oxide for making polyether polyols and propanediol and can be hydroformylated to n-butylaldehyde.

- 20 The present invention relates also to said polypropylene, ethylene-propylene rubbers (EPR), ethylene-propylene-diene polymers (EPDM), cumene, acrylic acid, acrylonitrile, propyleneoxide and n-butylaldehyde.

CLAIMS

1. A process for making propylene by dehydration of propanol, involving the following steps:
1. Gasifying carbonaceous solid or liquid feedstock or reforming gaseous carbonaceous feedstock into synthesis gas,
 2. Removing contaminants from the synthesis gas
 3. Fermenting the synthesis gas by means of a microorganism into substantially propanol in which the microorganism
 - i. Is a wild strain having the natural capability to ferment synthesis gas into substantially propanol or
 - ii. Is a microorganism, possessing the required nucleic acid sequence information to express the enzymes for the biosynthesis of C₃-oxygenates, modified by conferring it with the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway), or
 - iii. Is a microorganism, possessing the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes for the biosynthesis of C₃-oxygenates.
 4. Fractionating and purifying the stream containing predominantly propanol
 5. Dehydrating said above stream in a reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene,
 6. recovering from said reactor the propylene containing stream.
2. A process for making propylene by dehydration of propanol, involving the following steps:

1. Gasifying carbonaceous solid or liquid feedstock or reforming gaseous carbonaceous feedstock into synthesis gas,
 2. Removing contaminants from the synthesis gas
 3. Co-Fermenting the synthesis gas with at least one liquid oxygenate, like carbohydrates, glycerol, propanediol, lactaldehyde, lactic acid, acetol, methanol, ethanol, acetic acid, propionic acid, propanal and acetone, by means of a microorganism into substantially propanol in which the microorganism
 - i. Is a wild strain having the natural capability to ferment synthesis gas into substantially propanol or
 - ii. Is a microorganism, possessing the required nucleic acid sequence information to express the enzymes for the biosynthesis of C₃-oxygenates, modified by conferring it with the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway) or
 - iii. Is a microorganism, possessing the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes for the biosynthesis of C₃-oxygenates.
 4. Fractionating and purifying the stream containing predominantly propanol
 5. Dehydrating said above stream in a reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene,
 6. recovering from said reactor the propylene containing stream
3. A process according to claim 1 or 2, wherein the fermentation or the co-fermentation step (3), produces a mixture of alcohols, containing at least 50

wt% of propanol, between 0 and 50 wt% of ethanol and between 0 and 50 wt% of butanol and where in the fractionation and purification step (4) the propanol, ethanol and butanol are fractionated and purified and where in the dehydration step (5) propanol in presence of ethanol and butanol, if any,
5 are dehydrated simultaneously and where in the recovery step (6) the propylene and ethylene and butenes, if any, are recovered from said reactor.

4. A process according to claim 1 or 2, wherein the C₃-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway) and the aceton/isopropanol pathway, involving at least
10 an acetyl-CoA synthase, a pyruvate synthase and acetoacetate decarboxylase, and producing mainly isopropanol.

5. A process according to claim 1 or 2, wherein the C₃-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway) and the dicarboxylic pathway, involving at least an
15 acetyl-CoA synthase, a pyruvate synthase and an methylmalonyl-CoA carboxytransferase or an propionyl-CoA succinate transferase, and producing mainly n-propanol.

6. A process according to claim 1 or 2, wherein the C₃-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway) and the acrylate pathway, involving at least an acetyl-
20 CoA synthase, a pyruvate synthase and an acryloyl-CoA reductase and producing mainly n-propanol.

7. A process according to claim 1 or 2, wherein the C₃-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway) and the threonine degradation pathway, involving at
25 least an acetyl-CoA synthase, a pyruvate synthase and a 2-ketobutyrate formate lyase and producing mainly n-propanol.

8. A process according to claim 1 or 2, wherein the C₃-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway) and the citramalate pathway, involving at least an acetyl-CoA synthase, a pyruvate synthase and a citramalate synthase and producing mainly n-propanol.
9. A process according to claim 1 or 2, wherein the C₃-oxygenates are produced via a combination of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway) and the propanediol reduction pathway, involving at least an acetyl-CoA synthase, a pyruvate synthase, a propanediol oxidoreductase and a propanediol dehydratase and producing mainly n-propanol.
10. Process according to any one of the preceding claims wherein the dehydration is made over an acidic catalyst at a temperature of at least 200°C and a WHSV of at least 1 h⁻¹.
11. Process according to claim 10 wherein dehydration is be made by introducing in a reactor a stream comprising at least propanol, optionally water, optionally an inert component, contacting said stream with a catalyst in said reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene.
12. Use of the propylene made according any one of the preceding claims to make polypropylene.
13. Use of the propylene made according any one of the claims 1 to 11 to make propylene copolymers, containing ethylene, hexene, octene or decene as comonomer.

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14. Use of the propylene made according any one of claims 1 to 11 to make ethylene-propylene rubbers (EPR).
- 5 15. Use of the propylene made according any one of claims 1 to 11 to make ethylene-propylene-diene copolymers (EPDM).
16. Use of the propylene made according any one of claims 1 to 11 to make acrylonitrile.
- 10 17. Use of the propylene made according any one of claims 1 to 11 to make acrylic acid.
18. Use of the propylene made according any one of claims 1 to 11 to make cumene.
- 15 19. Use of the propylene made according any one of claims 1 to 11 to make propylene-oxide.
- 20 20. Use of the propylene made according any one of claims 1 to 11 to make n-butylaldehyde.

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ABSTRACT

Process for making propylene by dehydration of propanol, involving the following steps:

- 5 ○ Gasifying carbonaceous solid or liquid feedstock or reforming gaseous carbonaceous feedstock into synthesis gas,
- Removing contaminants from the synthesis gas
- Fermenting the synthesis gas by means of a microorganism into substantially propanol or
- 10 ○ Co-Fermenting the synthesis gas with at least one liquid oxygenate, like carbohydrates, glycerol, propanediol, lactaldehyde, lactic acid, acetol, methanol, ethanol, acetic acid, propionic acid, propanal and acetone, by means of a microorganism into substantially propanol in which the
- 15 microorganism
- - Is a wild strain having the natural capability to ferment synthesis gas into substantially propanol or
 - Is a microorganism, possessing the required nucleic acid sequence information to express the enzymes for the biosynthesis of C₃-oxygenates, modified by conferring it with the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway), or
 - 20
 - 25 ▪ Is a microorganism, possessing the required nucleic acid sequence information to express the enzymes of the Wood-Ljungdahl pathway (reduced Acetyl-CoA pathway), modified by conferring it with the required nucleic acid sequence information to express the enzymes for the
 - 30 biosynthesis of C₃-oxygenates.

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- Fractionating and purifying the stream containing predominantly propanol
- Dehydrating said above stream in a reactor at conditions effective to dehydrate at least a portion of the propanol to make propylene,
- 5 ○ recovering from said reactor the propylene containing stream.

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Figure 1

Reducing equivalents generation

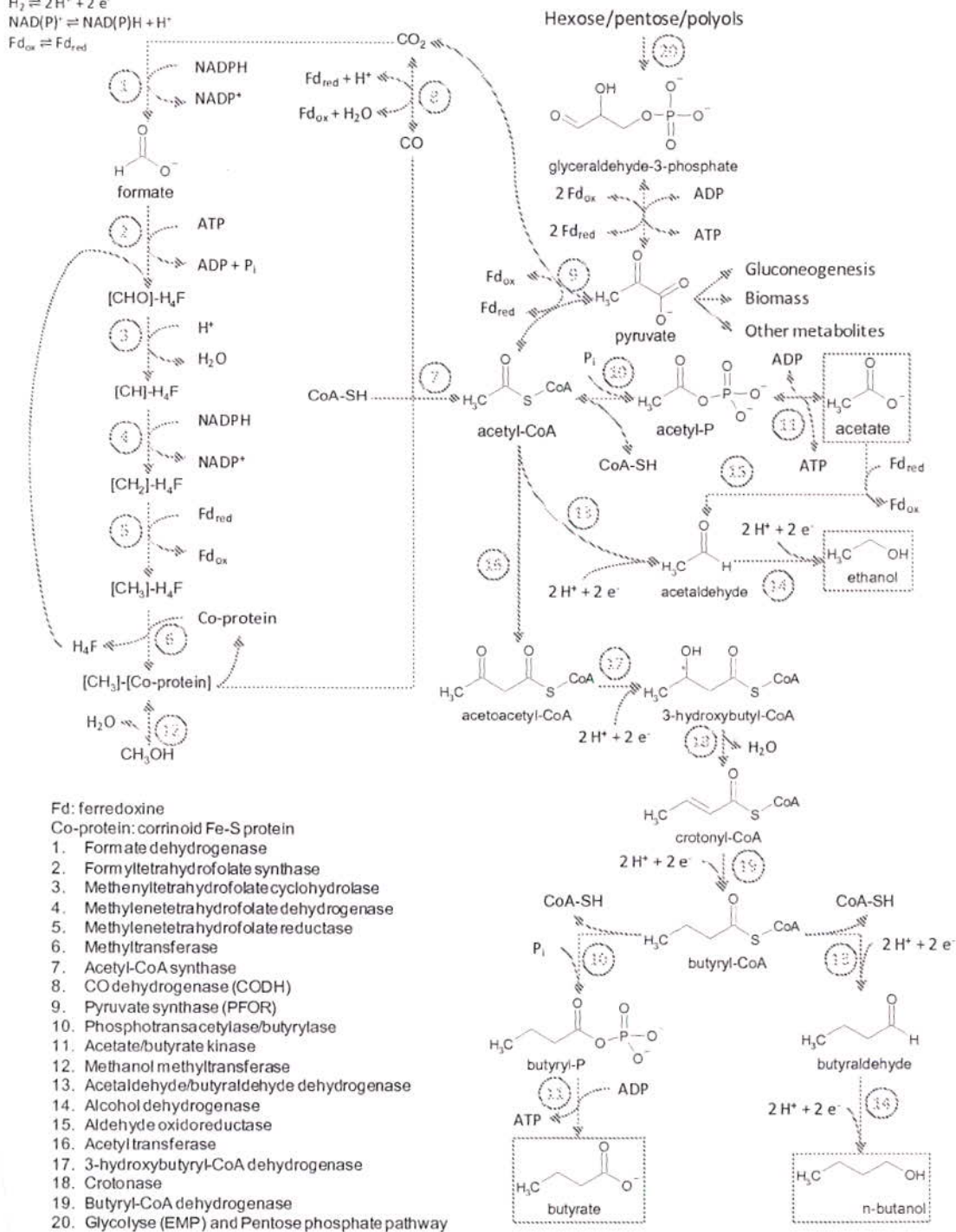
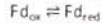
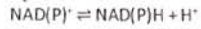
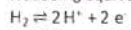


Figure 2

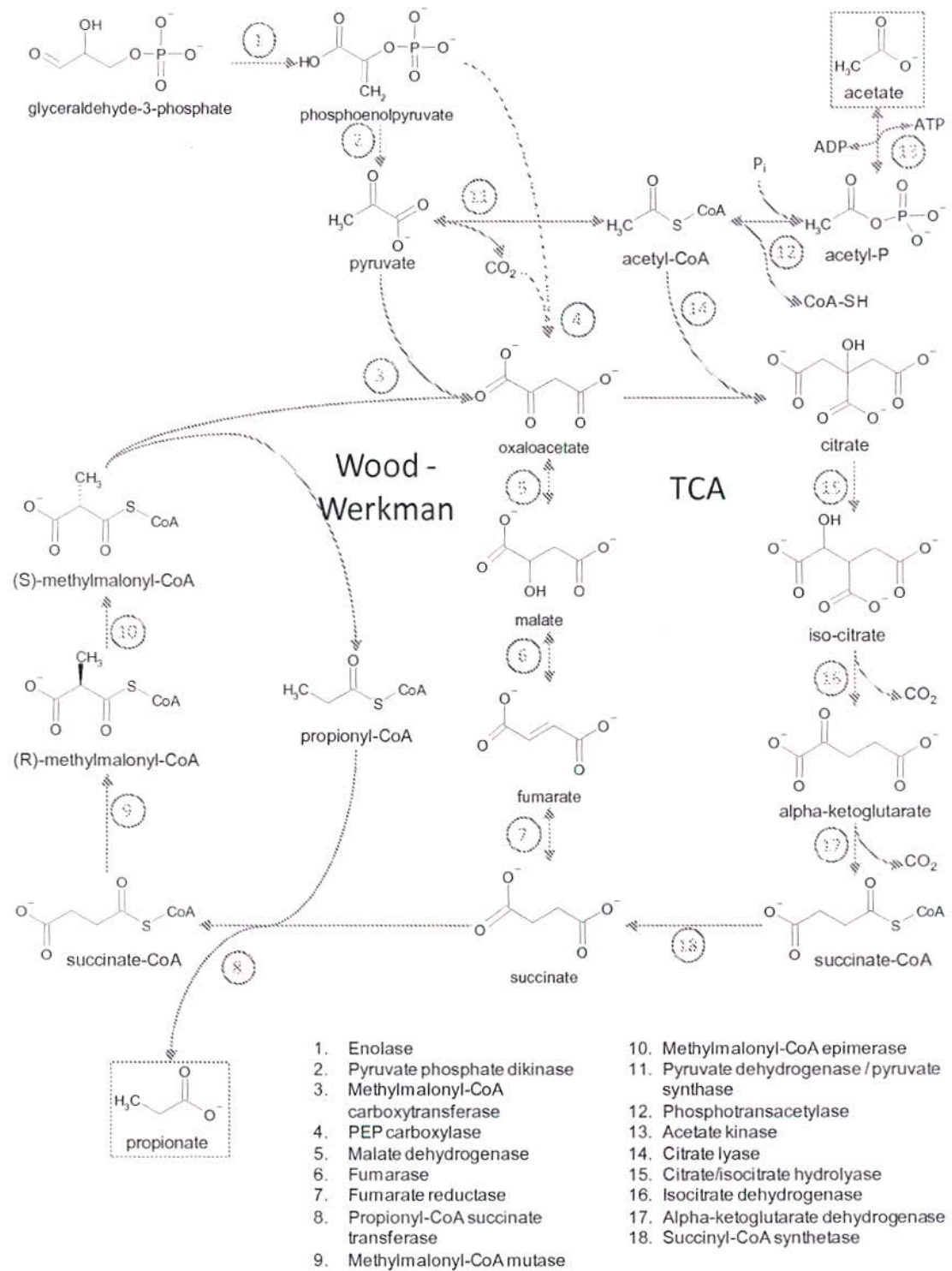
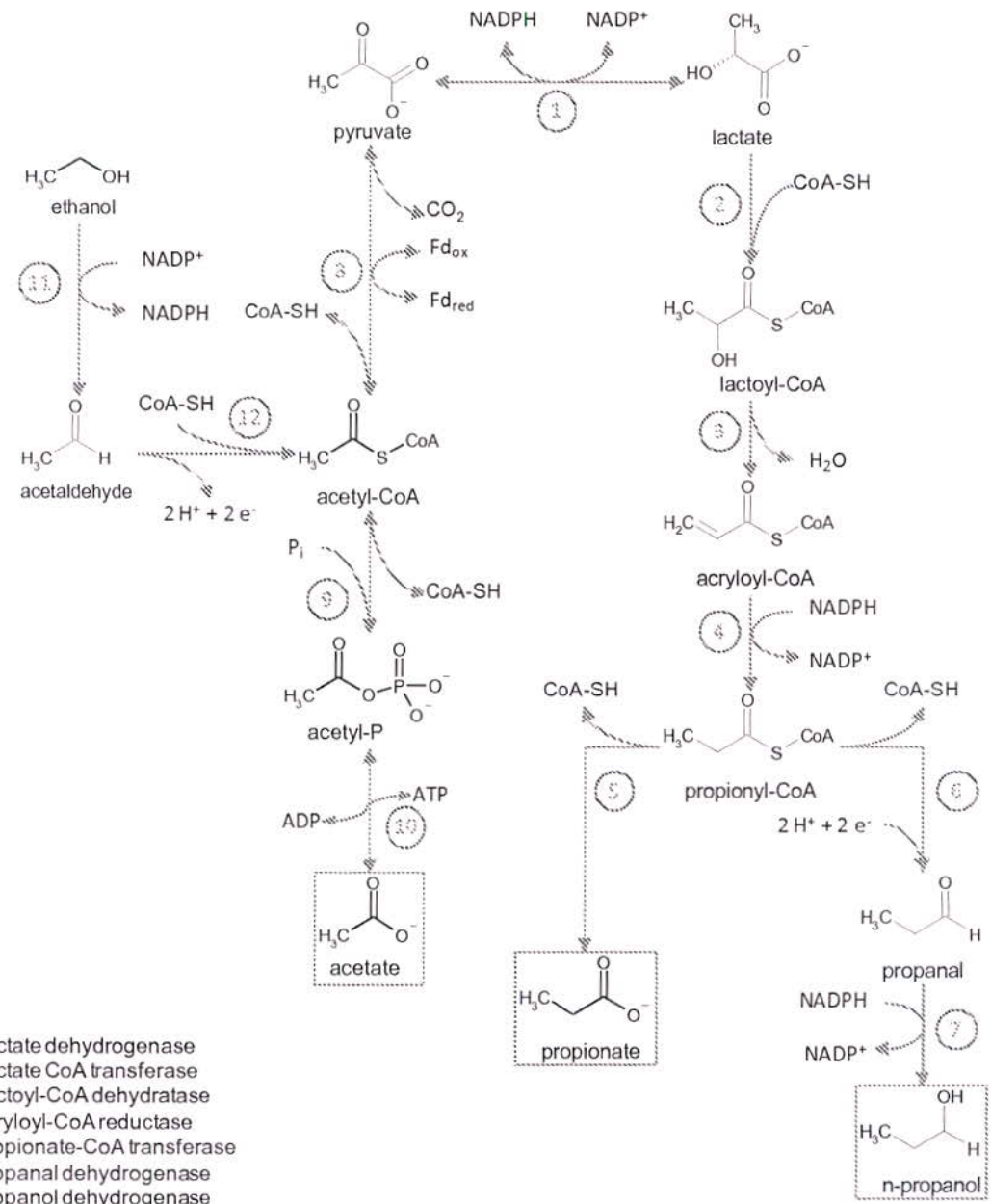


Figure 3



1. Lactate dehydrogenase
2. Lactate CoA transferase
3. Lactoyl-CoA dehydratase
4. Acryloyl-CoA reductase
5. Propionate-CoA transferase
6. Propanal dehydrogenase
7. Propanol dehydrogenase
8. Pyruvate dehydrogenase / pyruvate synthase
9. Phosphotransacetylase
10. Acetate kinase
11. Ethanol dehydrogenase
12. Acetaldehyde dehydrogenase

Figure 4

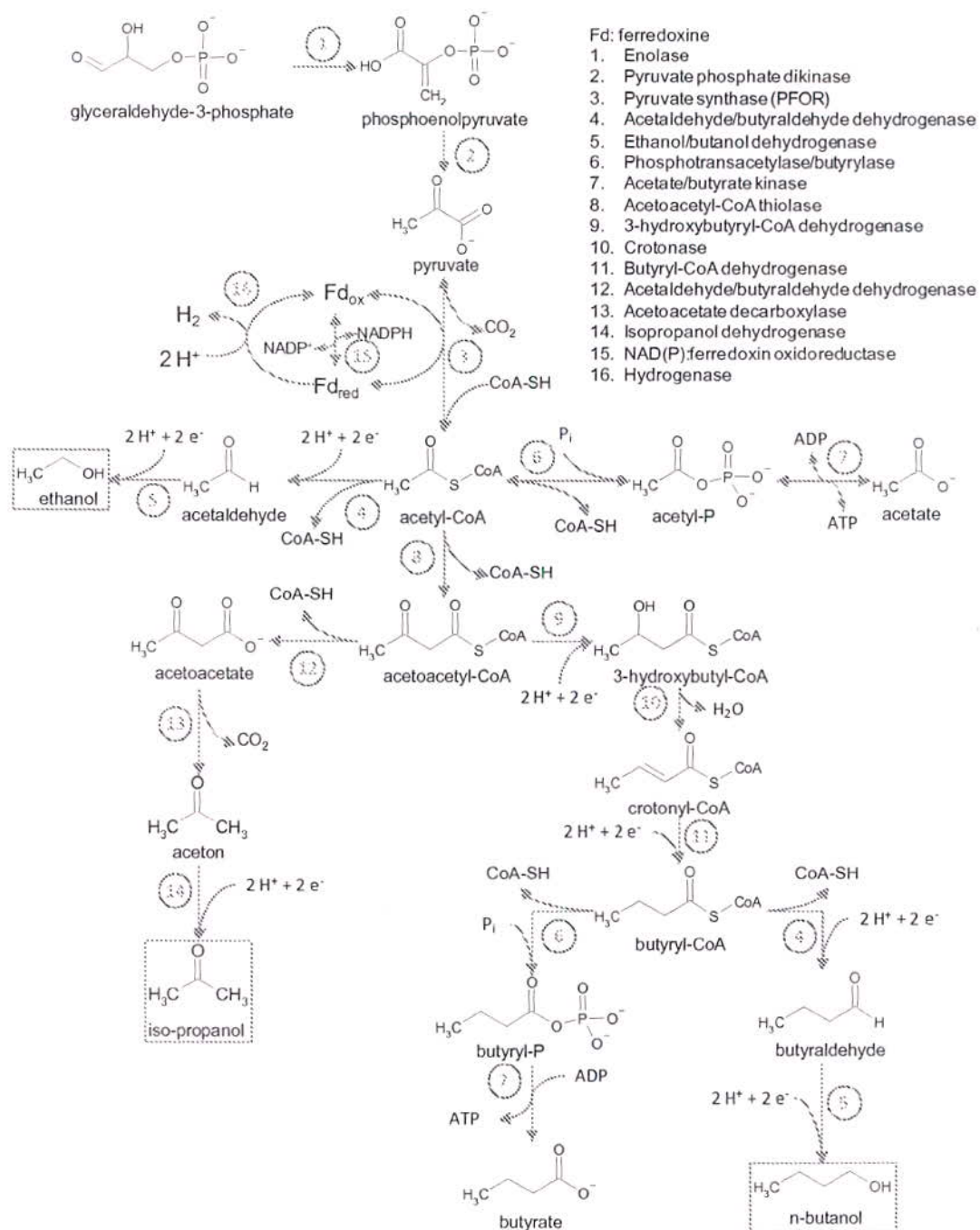
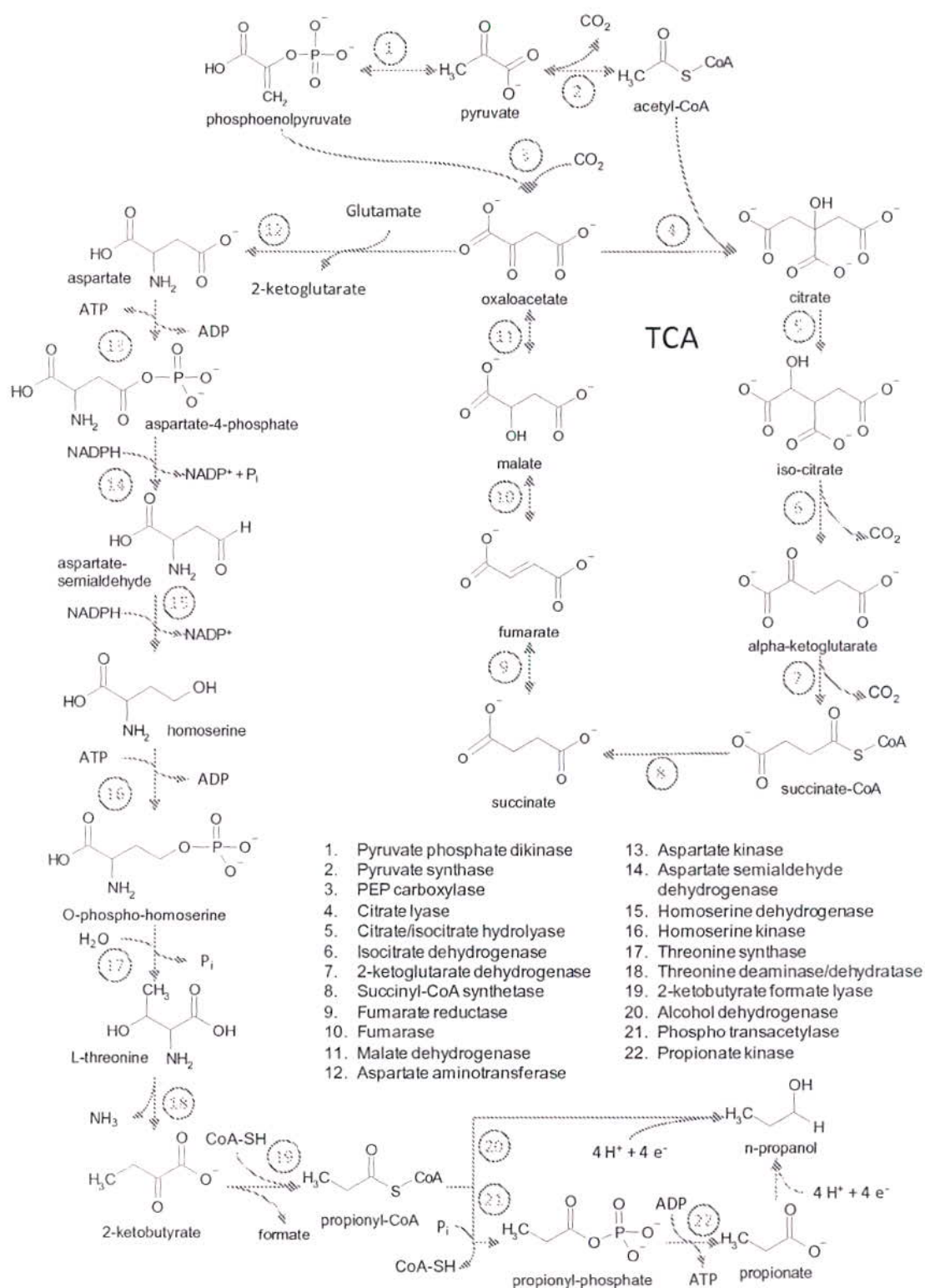


Figure 5



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Figure 6

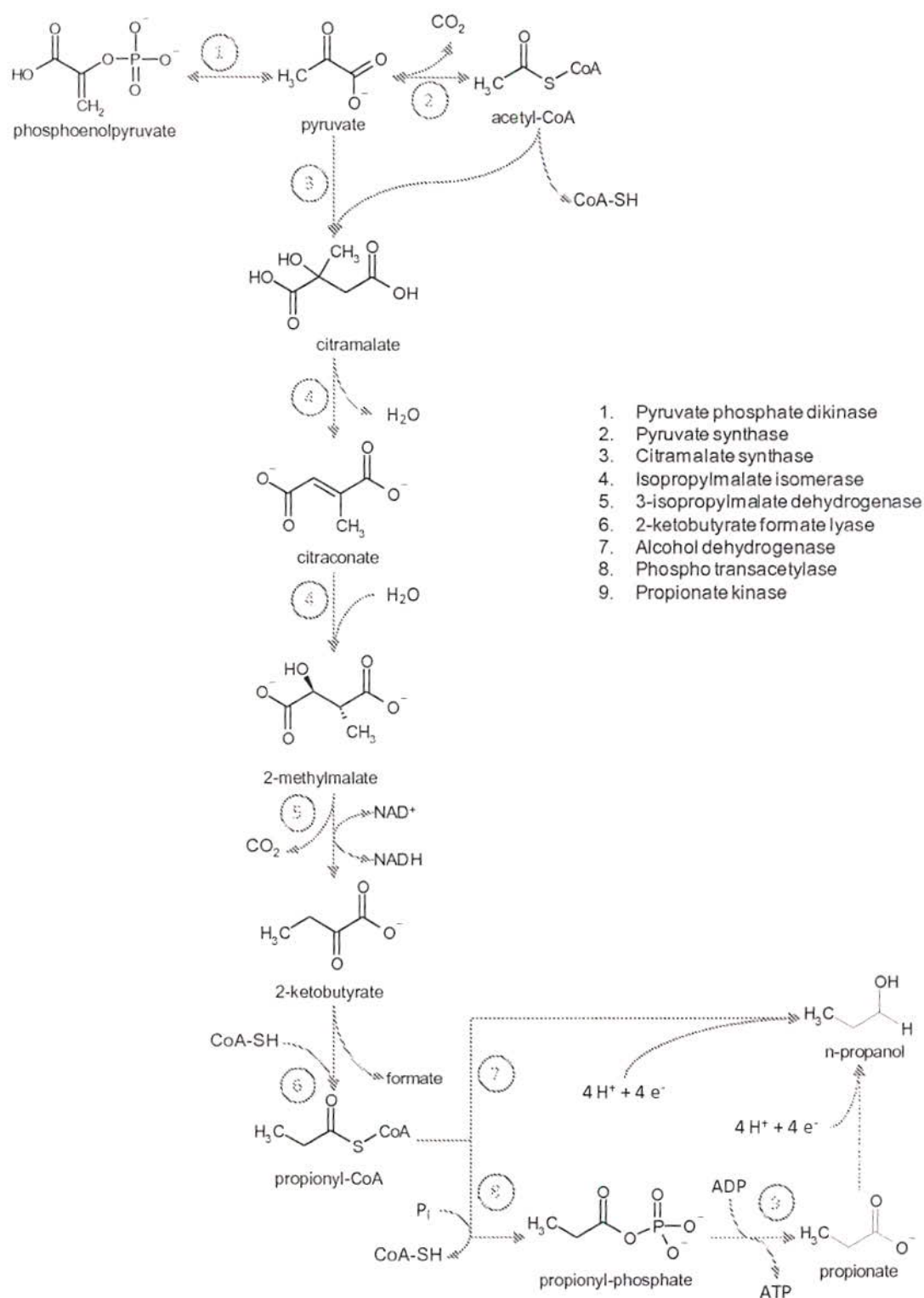


Figure 7

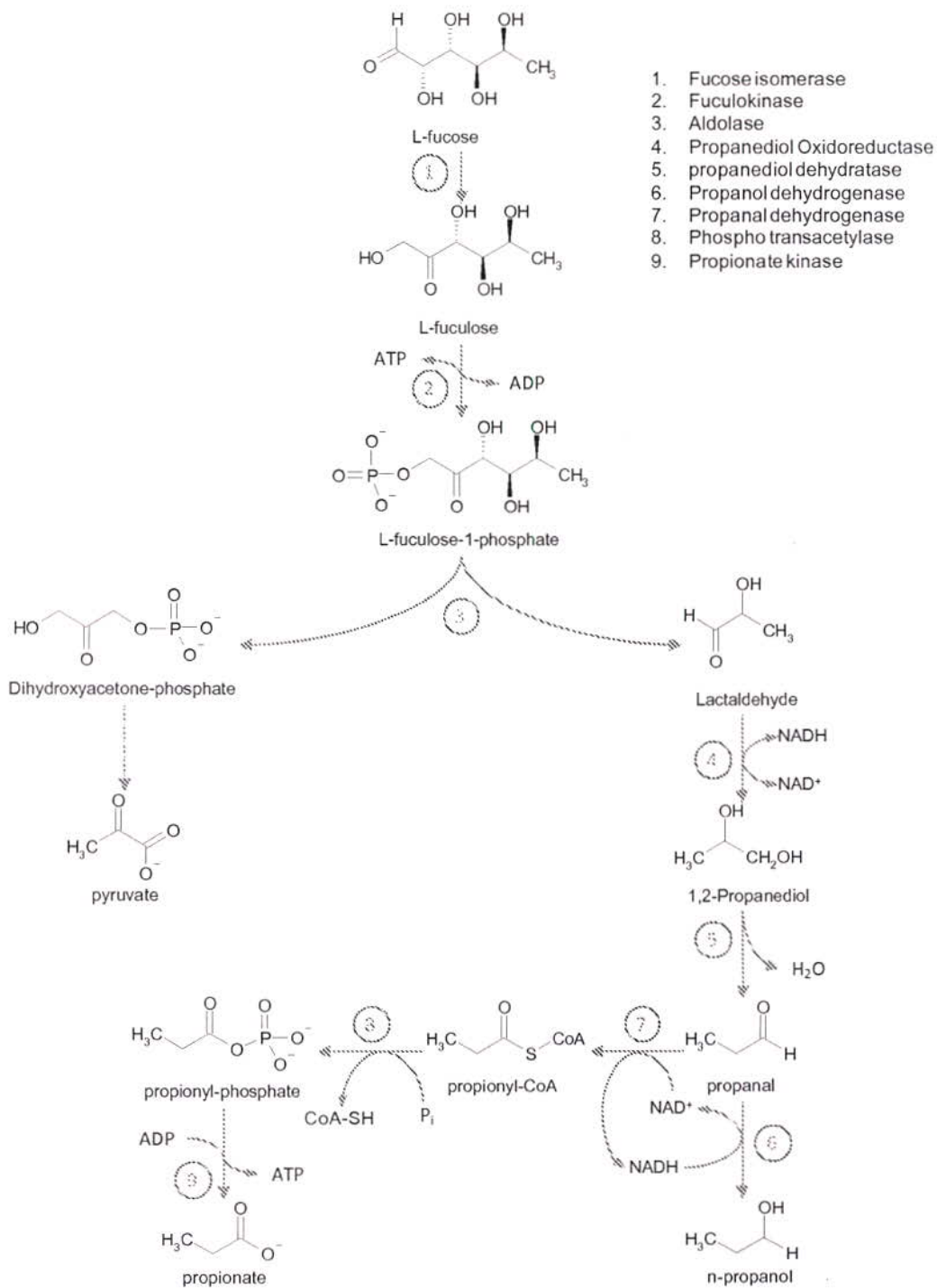
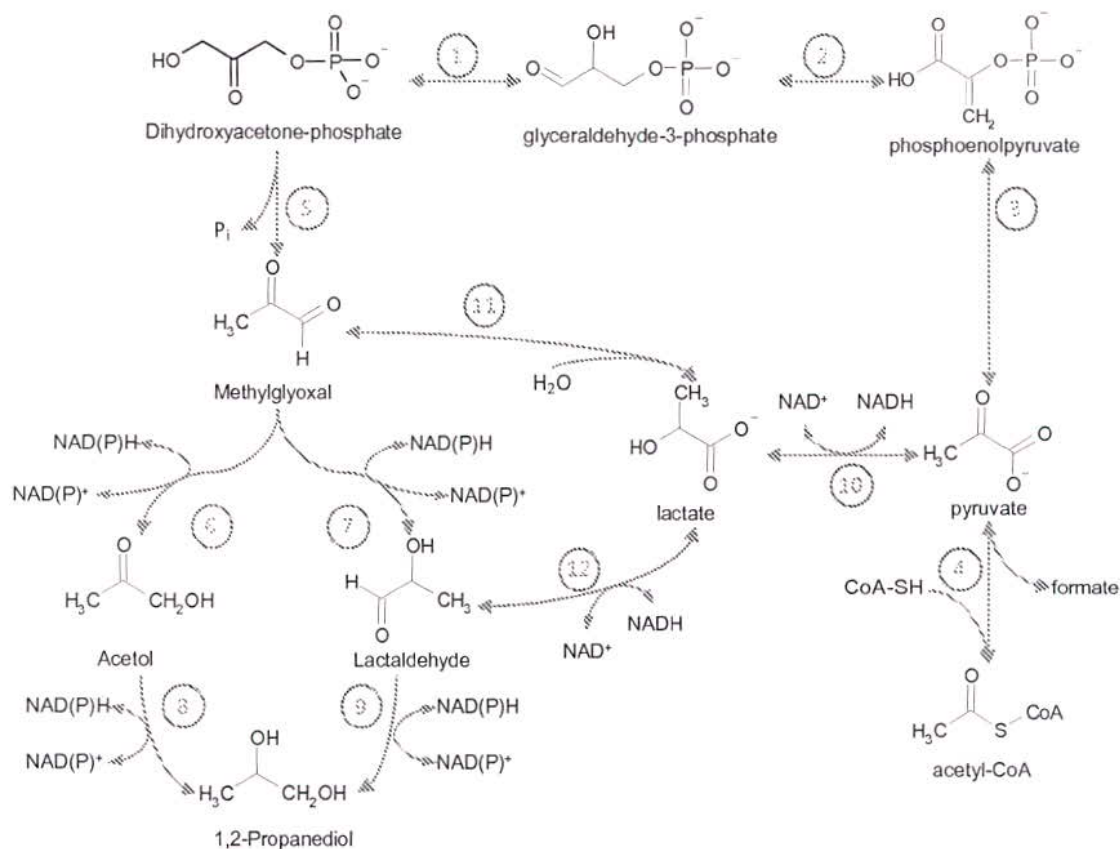


Figure 8



1. Triose phosphate isomerase
2. Enolase
3. Pyruvate phosphate dikinase
4. Pyruvate synthase
5. Methylglyoxal synthase
6. Methylglyoxal reductase
7. Glycerol dehydrogenase
8. Glycerol dehydrogenase
9. Propanediol oxidoreductase
10. Lactate dehydrogenase
11. Glyoxalases
12. Lactaldehyde dehydrogenase

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
- / -



RECIBO DE CAJA

No. 13 - 0059324

Bogotá D.C., Junio 17 de 2013 - 15:36:44

RECIBIDO DE : POSSE HERRERA & RUIZ S.A. NI 830.022.196

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR. PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	562578111	17/06/2013	4.125.000.00

*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr. UNIDITARIO	Vr. CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE INVENCIÓN	500.000.00	500.000.00
				\$500.000.00

SON: **QUINIENTOS MIL PESOS MONEDA CORRIENTE**

Responsable: _____

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 13-144231- -00000-0000

Fecha: 2013-06-17 15:59:47 Dep. 2020 DIR. NUEVASCR
Tra. 11 PATENTE IYII Eve: 378 FASENACIONAL I
Act. 411 PRESENTACION Folios: 253

Sede Centro: Carrera 13 No. 27 - 00 Pisos 3,4,5 y 10 Bogotá, D.C.- Colombia

Web: www.sic.gov.co e-mai: info@sic.gov.co Conmutador: (571) 5870000 Fax: (571) 5870284 Línea: 018000-910165 Call Center: (571) 6513240

, Junio 17 de 2013 - 15:36:46

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
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TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR. PAGO
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*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
10	50005-01-01 SOLICITUDES	1607 REIVINDICACION PATENTE UNITARIA ADICIONAL A LAS 10 INIC	30.000.00	300.000.00
				\$300.000.00

SON: **TRESCIENTOS MIL PESOS MONEDA CORRIENTE***

Responsable: _____

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 13-144231- -00000-0000
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Junio 17 de 2013 - 15:36:46

PATENTE DE INVENCION ☐

MODELO DE UTILIDAD ☐

Art 33 Decisión 486/00

☐	Indicación que se solicita una patente.
☐	Datos de identificación del solicitante o de la persona que presenta la solicitud
☐	Descripción de la invención
☐	Dibujos de ser estos pertinentes
☐	Comprobante de pago de las tasas establecidas (De ser el caso formato de descuento)
Completa ☐	Incompleta ☐

~~PATENTE DE INVENCION PCT ☐~~

~~MODELO DE UTILIDAD PCT ☐~~

Art.33 Decisión 486/00, Circular Única

~~☐~~	~~Indicación que se solicita una PCT~~
~~☐~~	~~Copia de la solicitud en español, tal como fue presentada inicialmente (capítulo descriptivo, reivindicatorio, resumen)~~
~~☐~~	~~Dibujos de ser estos pertinentes~~
~~☐~~	~~Comprobante de pago de las tasas establecidas (de ser el caso formato de descuento)~~
~~Completa ☐~~	~~Incompleta ☐~~

DISEÑO INDUSTRIAL ☐

(Art. 119 Decisión 486/00)

☐	Indicación que se solicita Diseño industrial
☐	Datos de identificación del solicitante o de la persona que presenta la solicitud
☐	Representación gráfica y fotográfica del Diseño industrial o muestra del material que incorpora el diseño
☐	Comprobante de pago de las tasas establecidas
Completa ☐	Incompleta ☐

ESQUEMA DE TRAZADO ☐

(Art. 92 Decisión 486/00)

☐	Indicación que se solicita un esquema de trazado
☐	Datos de identificación del solicitante o de la persona que presenta la solicitud
☐	Representación gráfica de un esquema de trazado
☐	Comprobante de pago de las tasas establecidas
Completa ☐	Incompleta ☐

PI02-F08 (2009-11-18)

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Fecha: 2013-06-17 15:59:47 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 253 255