

Señor Superintendente
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
E. S. D.



No. 09-062890- -00006-0000

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Evento : 379
Actuación : 412

Referencia : Expediente No. **09.062.890**
Asunto : Solicitud de patente para: **TISÚ PARA HIGIENE QUE COMPRENDE UNA COMPOSICIÓN INHIBIDORA DE MICROBIOS**
Solicitante : SCA HYGIENE PRODUCTS AB
Fecha solicitud : 17 de junio de 2009

I. RECURSO VÍA GUBERNATIVA

Yo, Luz Clemencia DE PÁEZ, en mi condición de apoderada de la sociedad **SCA HYGIENE PRODUCTS AB** solicitante de la patente de la referencia, por el presente escrito atentamente manifiesto a usted que debidamente notificada de la Resolución No. **14618** del 1 de abril de 2013, interpongo en su contra el recurso de **REPOSICIÓN**, para que sea revocada y en consecuencia se conceda el privilegio de patente de invención para **“TISÚ PARA HIGIENE QUE COMPRENDE UNA COMPOSICIÓN INHIBIDORA DE MICROBIOS”** a favor de **SCA HYGIENE PRODUCTS AB**.

II. OPORTUNIDAD DE LA IMPUGNACIÓN

La Resolución No. **14618** del 1 de abril de 2013, con fecha de publicación listado único 9 de abril de 2013, fecha de vencimiento 9 de mayo de 2013, y fecha del término legal 17 de mayo de 2013.

III. LA DECISIÓN RECURRIDA

3.1 Mediante la Resolución objeto de la presente impugnación, el Superintendente de Industria y Comercio decidió:

“EXCELENCIA LEGAL EN UN MUNDO SIN FRONTERAS”

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3.1.1 Denegar la patente de invención a la creación titulada: **“TISÚ PARA HIGIENE QUE COMPRENDE UNA COMPOSICIÓN INHIBIDORA DE MICROBIOS”**

3.2 En la parte motiva de la providencia, se mencionaron los siguientes fundamentos:

3.2.1 “Las características esenciales del producto de la reivindicación 1 habían sido divulgadas previamente en el documento D4 (Resumen; Reivindicación 6; Reivindicación 14), el cual da a conocer un tisú higiénico que comprende en:

Una composición inhibidora de microbios.

3.2.2 “Las características esenciales del producto de la reivindicación 1 habían sido divulgadas previamente en el documento D4, por lo tanto, la materia reivindicada no es nueva.

3.2.3 “Por su parte, las reivindicaciones dependientes 2 a 10 no mencionan alguna característica que haga nuevo al producto de la reivindicación 1, por lo tanto, no son nuevas.

3.2.4 “Es oportuno aclarar que el capítulo reivindicatorio presentado bajo el N° 09-062890-00003-0000 del 7 de noviembre de 2012, continua con problemas de claridad en la definición de la materia que se desea proteger. Tal es el caso de la reivindicación 1 que continua definiendo un *‘producto extracelular’* que no se especifica y solo lo caracteriza por un resultado en su proceso de obtención como es por ser un *‘sobrenadante’*. De hecho, el *‘producto extracelular’* es una frase de carácter general que sugiere de forma imprecisa que la protección se extiende a otras posibles variaciones o modificaciones. Por su parte, el término *‘sobrenadante’* se trata de un resultado a alcanzar; dado que después de un proceso de centrifugación o filtración siempre se encontrarán dos fracciones, en donde una de ellas resulta un sobrenadante y la otra un precipitado; lo anterior también se puede sustentar en lo mencionado por el solicitante en el capítulo descriptivo en el folio 52, en donde menciona: *‘el producto extracelular es obtenido de cultivos de células bacterianas por, por ejemplo filtradas, centrifugación, o ambos de estos, y el sobrenadante resultante que comprende el producto extracelular posee actividad antimicrobiana útil en una composición inhibidora de microbios’*.

- 3.2.5 “Por lo tanto, como respuesta a lo afirmado por la solicitante con respecto al documento D4 que menciona, ‘D4 no describe o sugiere el uso de productos extracelulares de bacterias probióticas’, la Oficina aclara que contrario a lo afirmado por la solicitante el documento D4 menciona una composición que comprende una preparación de ácido láctico producido por una bacteria (Párrafo 0016). Siendo claro que el producto extracelular es el ácido láctico. En este sentido, es importante mencionar que la bacteria mencionada en la solicitud como productora de ácido láctico es el *Lactobacillus plantarium* LB 931 que también es mencionada en D4 (Párrafo 003), por lo tanto la obtención de un producto extracelular reportado como la diferencia con la solicitud con D4, no es correcta ya que la misma bacteria produciría el mismo producto extracelular como sobrenadante, solo que este como ya se menciona es resultado a alcanzar en los procesos de centrifugación y filtración de los medios de cultivo de la bacteria reportada como productora de ácido láctico.

IV. OBJETO DEL RECURSO

De acuerdo con los argumentos que expongo a continuación, solicito atentamente a su Despacho lo siguiente:

1. Revocar la Resolución No. **14618** del 1 de abril de 2013.
2. Conceder el privilegio de patente de invención para **“TISÚ PARA HIGIENE QUE COMPRENDE UNA COMPOSICIÓN INHIBIDORA DE MICROBIOS”** a favor de **SCA HYGIENE PRODUCTS AB**.

V. FUNDAMENTOS DEL RECURSO

Respetuosamente me permito disentir de lo expuesto por ese Despacho en la Resolución objeto del presente recurso, toda vez que la presente solicitud sí cumple con los requerimientos de novedad y claridad del pliego reivindicatorio exigidos en la Decisión 486, como se demostrará a continuación:

5.1 La presente solicitud sí tiene novedad

El artículo 16 de la Decisión 486 señala lo siguiente:

Artículo 16.- Una invención es nueva cuando no está comprendida en el estado de la técnica. El estado de la técnica comprenderá todo lo que haya sido accesible al público, por una descripción escrita u oral, por una utilización o cualquier otro medio antes de la fecha de presentación de la solicitud de patente o, en su caso, de la prioridad reconocida.

Siguiendo entonces los lineamientos de la Jurisprudencia aplicable al análisis de novedad de una solicitud de patente, se considera que cuando las características técnicas esenciales son idénticas a las características de la materia divulgada en cada documento del estado de la técnica, puede afectar dicho requerimiento de patentabilidad, en cuyo caso se considera que no tiene novedad. Adicionalmente, se considerará nueva la invención si se encuentra alguna diferencia o un sólo aspecto que no hubiese sido revelado en el arte previo.

Con base en lo anterior, respetuosamente manifestamos nuestro desacuerdo con el análisis llevado a cabo por el Examinador y con las conclusiones del mismo y solicitamos atentamente la reconsideración de las razones por las cuales se objeta la novedad, con base en la siguiente información:

En primer lugar, nos referimos a la objeción elevada con respecto a la expresión '*sobrenadante*' considerada como un resultado a alcanzar por el señor Examinador. Amablemente señalamos que la expresión '*sobrenadante*' que corresponde a un '*producto extracelular*' no es un resultado a alcanzar, ya que el objeto de la presente solicitud no es proteger o reivindicar dicho producto, sino por el contrario el objeto de la presente invención es un tisú o pañuelo higiénico que comprende un componente y una serie de elementos que lo hacen novedoso e inventivo. De esta forma, dicho *sobrenadante* es aplicado al tisú higiénico para proporcionarle una característica que lo hace diferente y ventajoso frente a otras invenciones. Con respecto a lo anterior y debido a que el '*producto celular*' corresponde al '*sobrenadante*', es indudable afirmar que dicho producto no es de carácter general y tampoco puede ser considerado como un resultado a alcanzar.

En segundo lugar, nos referimos a la objeción elevada con relación a la falta de novedad de la presente invención con respecto al documento D4. A este respecto, amablemente manifestamos nuestro desacuerdo, ya que como bien fue mencionado por el Examinador, el documento D4 se refiere a *'una composición que comprende una preparación de ácido láctico producido por una bacteria'*. Dicho producto extracelular específico correspondiente al *'ácido láctico'* no es igual a un producto extracelular que corresponde a un sobrenadante obtenido a partir de la bacteria *Lactobacillus plantarium* LB 931. Es importante tener en cuenta que para poder obtener ácido láctico a partir de un microorganismo como lo es una bacteria, se debe llevar a cabo un procedimiento de separación y purificación de dicho producto extracelular, el cual es diferente al de un sobrenadante. Dejamos en claro el procedimiento en el cual se obtiene un sobrenadante es diferente y además su contenido es distinto, ya que dicho sobrenadante puede contener tanto ácido láctico pero adicionalmente varias sustancias resultado del proceso de separación, que como muy bien decía el señor Examinador, puede ser por medio de filtración o centrifugación. Por lo tanto, respetuosamente señalamos que es muy bien sabido por una persona del oficio normalmente versada en la materia, que el proceso de separación, recuperación y purificación del ácido láctico es muy diferente y mucho más complejo en comparación al proceso de obtención de un sobrenadante a partir de una bacteria.

De esta forma nos referimos al artículo adjunto (Narayanan *et al.*, 2004) el cual muestra en la página 175 el proceso de separación y purificación del ácido láctico para su uso industrial, el cual dice lo siguiente:

En la mayoría de los procesos, el ácido láctico es recuperado bajo la forma de lactato de calcio o sal de lactato, y los tratamientos posteriores van a depender de la pureza deseada e incluyen: tratamiento con carbón activo, purificación con resina de intercambio iónico, extracción con solventes, separación por absorción, separación por destilación al vacío, y separación de o esterificación con metanol seguido por destilación e hidrólisis.

En vista de lo anterior, el producto extracelular correspondiente a un sobrenadante, el cual es aplicado al tisú higiénico, de la presente invención, es diferente del producto extracelular correspondiente a ácido láctico del documento D4. En consecuencia, la invención de la presente solicitud es nueva e inventiva frente al documento D4.

Conclusión

El documento D4 no resulta anterioridad válida capaz de afectar la novedad del tisú higiénico de la presente solicitud.

Por todo lo anterior, consideramos que la presente solicitud cumple a cabalidad con los requerimientos de patentabilidad exigidos por la legislación actual, por lo que atentamente solicitamos en consideración lo siguiente:

VI. PETICIÓN

Por todo lo anterior, solicito a ese Despacho lo siguiente:

6.1 Que al considerar las razones precedentes se revoque la decisión contenida en la Resolución No. **14618** del 1 de abril de 2013, en cuanto resuelve negar el privilegio de patente de invención.

6.2 Que se otorgue el privilegio de patente de invención para: que sea revocada y en consecuencia se conceda el privilegio de patente de invención para **“TISÚ PARA HIGIENE QUE COMPRENDE UNA COMPOSICIÓN INHIBIDORA DE MICROBIOS”** a favor de **SCA HYGIENE PRODUCTS AB**.

VII. NOMBRE Y DIRECCIÓN

El nombre y la dirección del solicitante recurrente son los siguientes:

SCA HYGIENE PRODUCTS AB

405 03 Göteborg,
Suecia

VIII. ANEXOS

Artículo : Narayanan, N., Roychoudhury, P. & Srivastava, A. (2004). L (+) lactic acid fermentation and its product polymerization. Electronic Journal of Biotechnology. Vol. 7(2) : 167-179.

IX. NOTIFICACIONES

Atentamente manifiesto a usted que recibiré notificaciones en la Secretaria de su Despacho o en mi oficina de abogada situada en la Carrera 4ª. No. 72-35, piso 4º, de esta ciudad de Bogotá, D.C.

Señor Superintendente,


LUZ CLEMENCIA DE PÁEZ
C.C. 35.456.344 de Usaquén
T.P.A. No. 23.555

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L (+) lactic acid fermentation and its product polymerization

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Keywords: bioprocesses, fermentation, L (+) lactic acid, *Lactobacillus* sp., polymerization.

Lactic acid has been first introduced to us as early as 1780 as a sour component of milk. Ever since we have found its applications in food, pharmaceutical, cosmetic industries etc. Now there are emerging uses as a potential feedstock for the biodegradable polymer industry. The microorganisms being used for lactic acid fermentation, the raw materials reported, the various novel fermentation processes and its processing methods have been reviewed. The properties and applications of lactic acid, its derivatives and polymer have been discussed. The various routes to polymerization and the companies presently involved in lactic acid production have been covered.

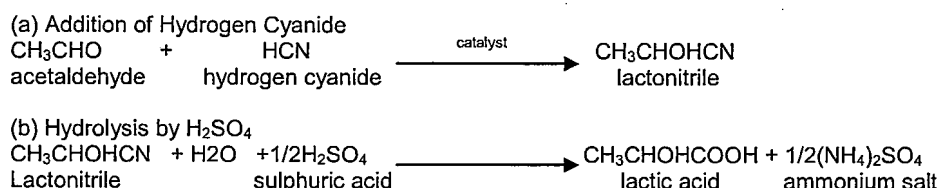
Lactic acid (2-hydroxy prop ionic acid) is the most widely occurring carboxylic acid in nature. The Swedish chemist Scheele first discovered it in 1780, but it was first produced commercially by Charles E. Avery at Littleton,

Massachusetts, USA in 1881. Lactic acid can be manufactured by (a) Chemical synthesis or (b) Carbohydrate fermentation.

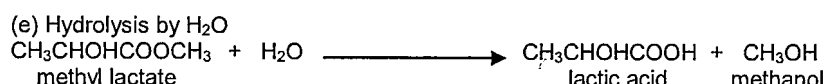
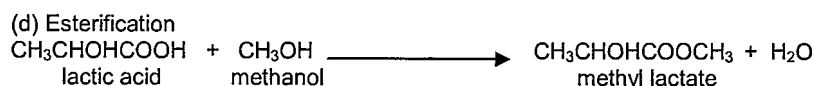
CHEMICAL SYNTHESIS

The commercial process for chemical synthesis is based on lactonitrile. Hydrogen cyanide is added to acetaldehyde in the presence of a base to produce lactonitrile. This reaction occurs in liquid phase at high atmospheric pressures. The crude lactonitrile is recovered and purified by distillation. It is then hydrolyzed to lactic acid, either by concentrated HCl or by H₂SO₄ to produce the corresponding ammonium salt and lactic acid. Lactic acid is then esterified with methanol to produce methyl lactate, which is removed and purified by distillation and hydrolyzed by water under acid catalyst to produce lactic acid and the methanol, which is recycled.

This process is represented by the following reactions.



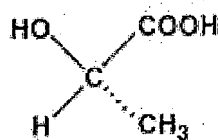
* Corresponding author



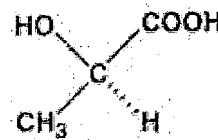
The chemical synthesis method produces a racemic mixture of lactic acid. Two companies Musashino, Japan and Sterling Chemicals Inc., USA are using this technology.

Other possible routes are base catalyzed degradation of sugars, oxidation of propylene glycol, reaction of acetaldehyde, carbon monoxide and water at elevated temperature and pressures, hydrolysis of chloropropionic acid, carbohydrate fermentation, nitric acid oxidation of propylene.

forms.



L (+) Lactic acid



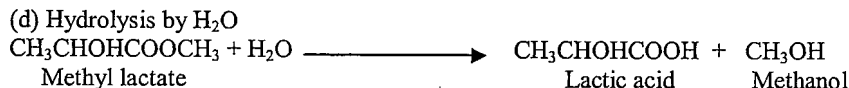
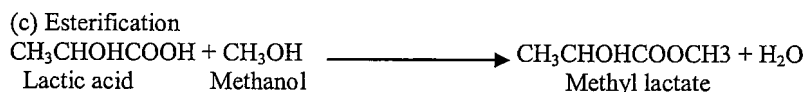
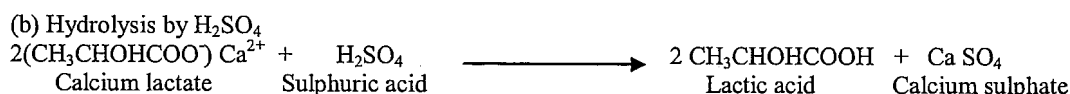
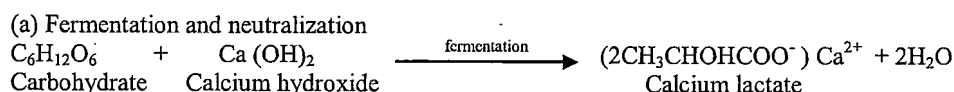
D (-) Lactic acid

FERMENTATION

Though chemical synthesis produces a racemic mixture, stereo specific acid can be made by carbohydrate fermentation depending on the strain being used. It can be described by

Lactic acid is soluble in water and water miscible organic solvents but insoluble in other organic solvents. It exhibits low volatility. Other properties of lactic acid are summarized in Table 1.

The various reactions characteristic of an alcohol which



The broth containing calcium lactate is filtered to remove cells, carbon treated, evaporated and acidified with sulphuric acid to get lactic acid and calcium sulphate. The insoluble calcium sulphate is removed by filtration; lactic acid is obtained by hydrolysis, esterification, distillation and hydrolysis.

lactic acid (or it esters or amides) may undergo are
 Table 1. Physical properties of lactic acid.

Molecular weight	90.08
Melting point	16.8°C
Boiling point	82°C at 0.5 mm Hg 122°C at 14 mm Hg
Dissociation constant, K_a at 25°C	1.37×10^{-4}
Heat of combustion, ΔH_c	1361 KJ/mole
Specific Heat, C_p at 20°C	190 J/mole/°C

PROPERTIES, USES AND APPLICATIONS

Lactic acid is a three carbon organic acid: one terminal carbon atom is part of an acid or carboxyl group; the other terminal carbon atom is part of a methyl or hydrocarbon group; and a central carbon atom having an alcohol carbon group. Lactic acid exists in two optically active isomeric

xanthation with carbon bisulphide, esterification with organic acids and dehydrogenation or oxygenation to form pyruvic acid or its derivatives. The acid reactions of lactic acid are those that form salts. It also undergoes

esterification with various alcohols.

Lactic acid can be analyzed by NAD⁺ linked lactate dehydrogenase enzyme assay. A colorimetric method has been described in many papers. Gas chromatography can be used after esterification of lactic acid but is unsatisfactory for quantitative. Liquid chromatography and its various techniques can be used for quantitative analysis and separation of its optical isomers.

Lactic acid is used as acidulant/ flavouring/ pH buffering agent or inhibitor of bacterial spoilage in a wide variety of processed foods. In contrast to other food acids it has a mild acidic taste. It is non-volatile odorless and is classified as GRAS (generally regarded as safe) by FDA in the US. It is a very good preservative and pickling agent. Addition of lactic acid aqueous solution to the packaging of poultry and fish increases their shelf life (Anon, 1992). The esters of

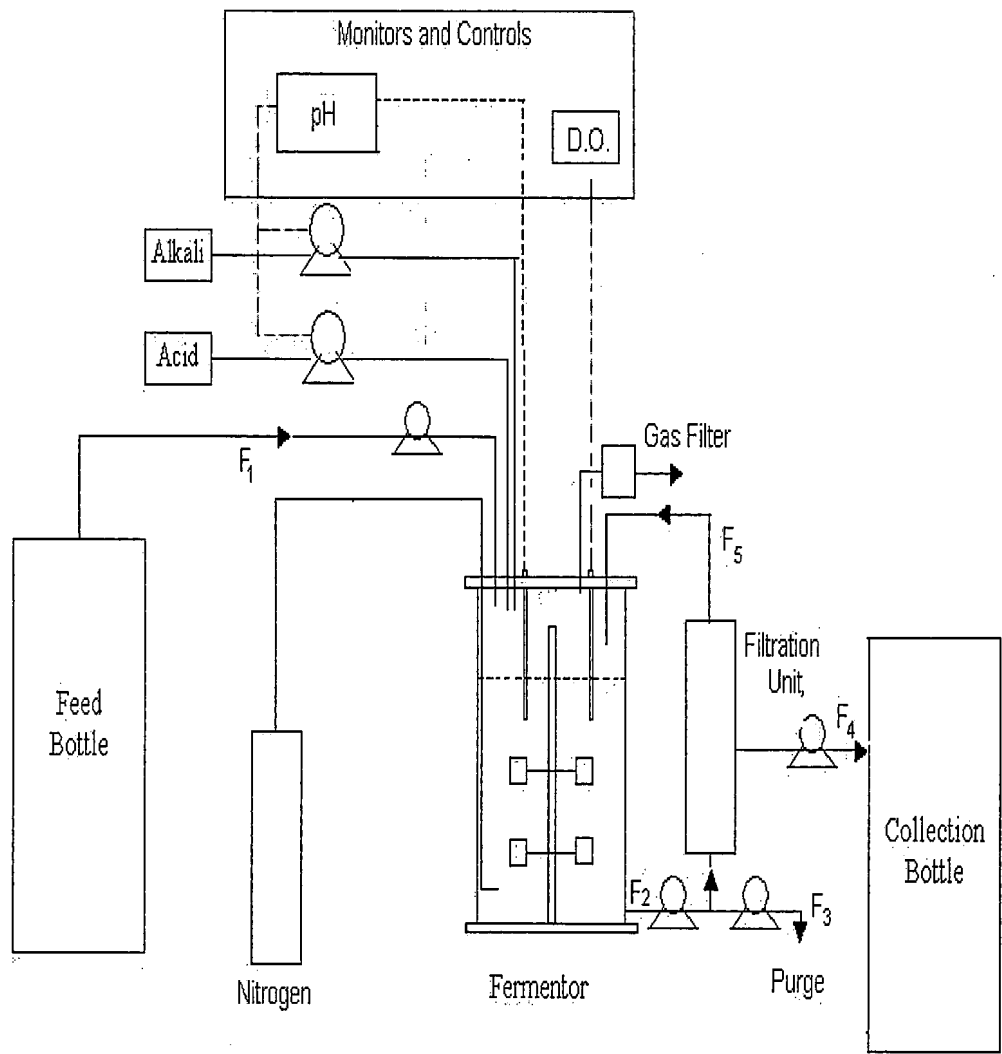


Figure 1. Sketch of the fermentor is shown with all the connection for monitoring and control of the continuous lactic acid fermentation process with continuous cell recycling.

F₁: feed rate (the dilution rate was maintained by maintaining the feed rate);

F₂: rate at which broth was taken out of the fermentor;

F₃: rate of purging (the sample from the purge were regularly analyzed for biomass and metabolites concentrations inside the fermentor);

F₄: rate at which permeate was taken out from the filtration unit;

F₅: rate at which biomass was recycled back to the fermentor.

lactic acid are used as emulsifying agents in baking foods (stearoyl-2-lactylate, glyceryl lactostearate, glyceryl lactopalmitate). The manufacture of these emulsifiers requires heat stable lactic acid, hence only the synthetic or the heat stable fermentation grades can be used for this application (Datta, 1995; Sodegard, 1998).

Technical grade lactic acid is used as an acidulant in vegetable and leather tanning industries. Various textile finishing operant and acid dying of food require low cost technical grade lactic acid to compete with cheaper inorganic acid. Lactic acid is being used in many small scale applications like pH adjustment hardening baths for cellophanes used in food packaging, terminating agent for phenol formaldehyde resins, alkyd resin modifier, solder flux, lithographic and textile printing developers, adhesive formulations, electroplating and electropolishing baths, detergent builders.

Lactic acid has many pharmaceutical and cosmetic applications and formulations in topical ointments, lotions, anti acne solutions, humectants, parenteral solutions and dialysis applications, for anti carries agent. Calcium lactate can be used for calcium deficiency therapy and as anti carries agent. Its biodegradable polymer has medical applications as sutures, orthopaedic implants, controlled drug release etc. Polymers of lactic acids are biodegradable thermoplastics. These polymers are transparent and their degradation can be controlled by adjusting the composition, and the molecular weight. Their properties approach those of petroleum derived plastics. Lactic acid esters like ethyl/butyl lactate can be used as green solvents. They are high boiling, non-toxic and degradable components. Poly L-lactic acid with low degree of polymerization can help in controlled release or degradable mulch films for large-scale

agricultural applications (Datta, 1995).

LACTIC ACID BACTERIA

Lactic acid bacteria are among the best studied microorganisms. Important new developments have been made in the research of lactic acid bacteria in the areas of multidrug resistance, bacteriocins, quorum sensing, osmoregulation, autolysins and bacteriophages. Progress has also been made in the construction of food grade genetically modified Lactic acid bacteria. These have opened new potential applications for these microorganisms in various industries (Konings et al. 2000).

The desirable characteristics of industrial microorganisms are their ability to rapidly and completely ferment cheap raw materials, requiring minimal amount of nitrogenous substances, providing high yields of preferred stereo specific lactic acid under conditions of low pH and high temperature, production of low amounts of cell mass and negligible amounts of other byproducts.

The choice of an organism primarily depends on the carbohydrate to be fermented. *Lactobacillus delbreuckii* subspecies *delbreuckii* are able to ferment sucrose. *Lactobacillus delbreuckii* subspecies *bulgaricus* is able to use lactose. *Lactobacillus helveticus* is able to use both lactose and galactose. *Lactobacillus amylophilus* and *Lactobacillus amylovirus* are able to ferment starch. *Lactobacillus lactis* can ferment glucose, sucrose and galactose. *Lactobacillus pentosus* have been used to ferment sulfite waste liquor.

Lactobacillus has complex nutritional requirements, as they are those groups of microorganisms that have lost their

Table 2. Overview of lactic acid based polymers prepared by direct polycondensation or by polycondensation followed by chain extension.

Molecular size prepared in kDa	Monomer/monomers	Linking agent	Macro molecular form	Reference
Low or medium molar mass < 70 kDa	L-lactic acid	nil	Linear homopolymer	Espartero et al. 1996; Ajioka et al. 1998
High molecular size >70 kDa	L-lactic acid	HMDI	Linear homopolymer	Hiltunen et al. 1997; Woo et al. 1995
High molecular size >70 kDa	L-lactic acid	Dipentaerythritol	Star shaped homopolymer	Kim and Kim, 1999
Low molar mass < 20 kDa	L-lactic acid with 6-hydroxycapric acid; L-lactic acid with caprolactone	Nil with 6hydroxycapric acid, and HMDI for caprolactone monomer polymerization	Linear copolymer	Kylma et al. 1997; Kawasaki et al. 1998
High molecular size > 70 kDa	L-lactic acid with Butandiol; L-lactic acid with mandelic acid	HMDI; IPDI	Copolymers	Kylma and Seppala, 1997
High molecular size > 70 kDa	L-lactic acid with butandiol; 4 hydroxybenzoic acid	HMDI; IPDI	Copolymers	Kylma and Seppala, 1997
High molecular size > 70 kDa	L-lactic acid with butandiol; 4 acetoxybenzoic acid	HMDI; IPDI	Copolymers	Kylma and Seppala, 1997
High molecular size > 70 kDa	L-lactic acid with butandiol; 4	HMDI; IPDI	Copolymers	Kylma and Seppala, 1997
High molecular size > 70 kDa	L-lactic acid	HMDI; IPDI	Copolymers	Kylma and Seppala, 1997

ability to synthesize their own growth factors. They cannot grow solely on carbon source and inorganic nitrogen salts. Organisms such as *Rhizopus oryzae* have less limiting nutritional requirements and can utilize starch feed stocks. They are able to produce pure L (+) lactic acid (Skory et al. 1998). Studies have also been carried out with *Saccharomyces cerevisiae* and *Kluyveromyces lactis* for production of pure L (+) lactic acid because of their ability to tolerate high concentration of hydrogen ions (Porro et al.

1997), which is desirable.

ENZYMES FOR LACTIC ACID FERMENTATION

Lactic acid is produced in the form of L (+) or D (-) lactic acid or as its racemic mixture. Organisms that form the L (+) form or D (-) form have two lactate dehydrogenases (LDH), which differ in their stereospecificity. Some *Lactobacilli* produce L (+) form, which on accumulation

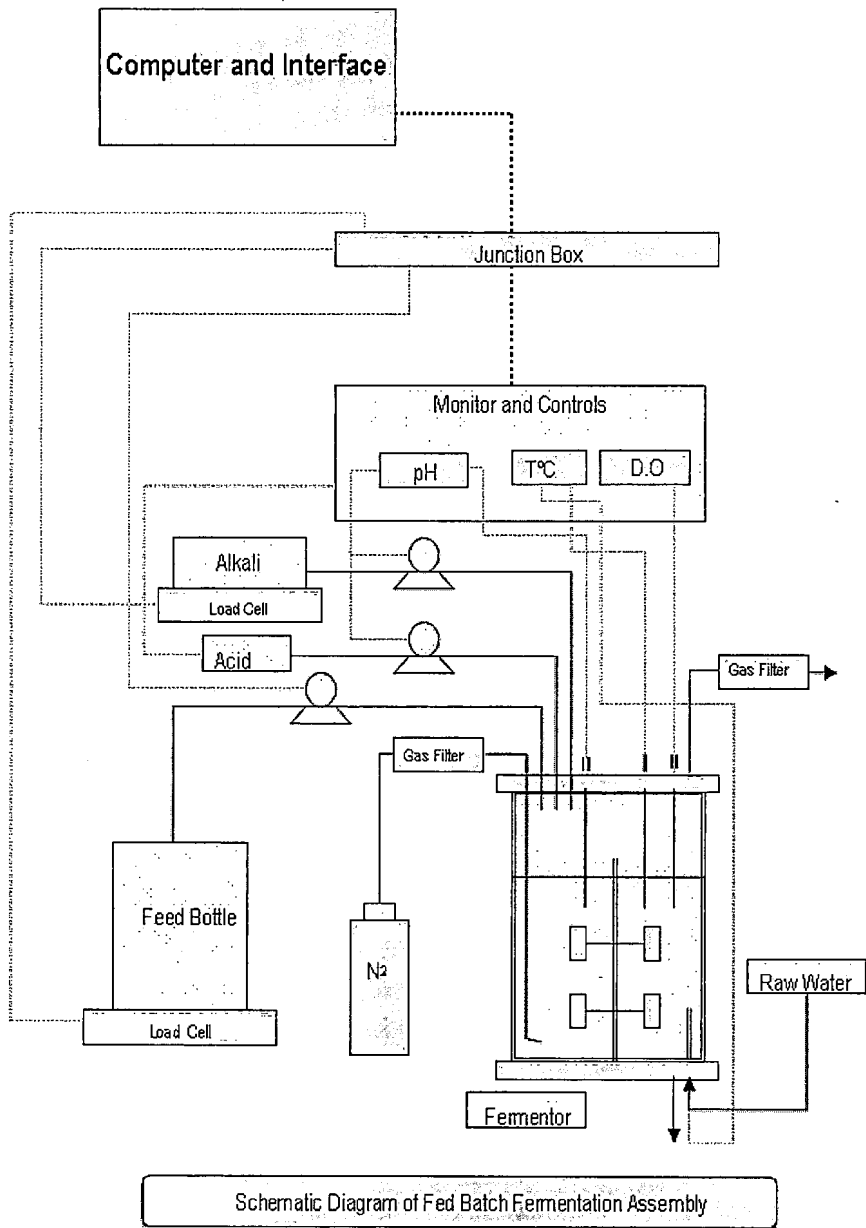


Figure 2. Schematic of lactic acid fermentation in fed-batch fermenter.

induces a racemase, which converts it into D (-) lactic acid until equilibrium is obtained.

The L- lactate dehydrogenase in *L. casei* have been found to be an allosteric enzyme with fructose 1,6- bisphosphate (FDP). In some cases Mn^{2+} acts as the cofactor. The LDH in *L. casei* and eukaryotes and in *L. casei* and vertebrates show 37% and 76% similarity respectively, but the active sites show 70% and 86% similarity respectively which shows that the essential parts of this enzyme has been conserved. In comparison to the vertebrate enzymes *L. casei* is found to lack 12-amino acid residues at the N-terminus, which is found to be a common characteristic of bacterial enzymes irrespective of the allosteric behaviour. *L. casei* also carries 7 additional amino acid residues at the C end but it is not known whether this is also characteristic of bacterial enzymes as there are no complete sequence of other bacterial enzymes available.

Despite the differences in primary structure, crystallographic analysis shows that the overall structure of the allosteric enzymes in *L. casei* and the non-allosteric enzymes in vertebrates are similar. Therefore, probably the minor alterations in the primary structure are responsible for its allosteric behaviour. The absence of the first 12 amino acids at the N- terminus indicates a possible effector binding site, also accounting for the dissociation inhibiting effect of Mn^{2+} or (Mn^{2+} + FDP) on the enzyme. The tetrameric enzyme dissociates into dimers showing the free solvent accessibility to tyrosine residues, which may not be located in the subunit contact region. Tryptophan residues are in UV absorption and protein fluorescence by effector binding but protein fluorescence was found to be destroyed in dimethyl sulfonium bromide, and also there is no influence on FDP binding. Therefore, it may be due to some remote tyrosine residue. However, the metabolic pathways of *L. casei* were found to be controlled by the kind of carbohydrates available, which determine the amount of FDP and triose phosphate intermediates. These control the activity of LDH and other enzymes to produce metabolites other than lactic acid. Also FDP independent control of Lactate dehydrogenase has been reported in *L. bulgaricus*. When this organism was grown in continuous culture, a shift in pH from acidic to alkaline causes it to catabolize sugar in a heterofermentation mode by the phosphoketolase split pathway. This implied that lactate dehydrogenases in lactic acid bacteria were under the control of not only allosteric affects but also gene expressions

GENETICALLY MODIFIED LACTIC ACID BACTERIA FOR IMPROVED L (+) LACTIC ACID BACTERIA

A few attempts have been made to improve L (+) lactic acid production by metabolic engineering in lactobacilli

producing both L (+) and D (-) lactic acids.

In *Lactobacillus helveticus* inactivation of *ldhD* (D-lactate dehydrogenase gene) led to a two fold increase in the amount of L (+) lactic acid, thereby restoring the total amount of lactic acid to the level in the wild type strain. Two stable *ldhD* negative strains of *Lactobacillus helveticus* were constructed by the gene replacement method. One strain was constructed by an internal deletion of the promoter region thereby preventing the transcription of the *ldhD* gene. The second construct was prepared by replacing the *ldhD* gene with *ldhL*, thus duplicating the gene dosage. The L-lactate dehydrogenase activity was increased by 53% and 93% respectively in the two modified strains than in the wild type strain. The two D-lactate dehydrogenase negative strains produced only L (+) lactate in an amount equal to the total lactate produced by the wild type strain (Nikkila et al. 2000).

The gene encoding L (+) lactate dehydrogenase was isolated from *Lactobacillus plantarum* and cloned into *Escherichia coli*. This gene was sequenced and used to construct *Lactobacillus plantarum* strains by either over expressing or not expressing *ldhL*. A multicopy plasmid bearing *ldhL* gene was introduced into *Lactobacillus plantarum* without modification of its expression signals. This increased the L-lactate dehydrogenase activity 13-fold but it hardly had any effect on the production of L (+) lactate or D (-) lactate. A stable chromosomal deletion in the *ldhL* gene resulted in the absence of L-lactate dehydrogenase activity and in exclusive production of the D-isomer of lactate (Ferain et al. 1994).

In *Lactococcus lactis*, when the copy number of the *lac* operon in which the *ldhL* gene was increased, it resulted in a slight increase in lactic acid production (Davidson et al. 1995).

The D-lactate dehydrogenase gene (*ldhD*) of *Lactobacillus johnsonii* was isolated, and an *in vitro* truncated copy of that gene was used to inactivate the genomic copy of the wild strain. For this an 8-bp deletion was generated within the cloned *ldhD* gene to inactivate its function. The plasmid containing the altered *ldhD* was transferred to *Lactobacillus johnsonii* via conjugative comobilisation with *Lactococcus lactis*. Crossover integrations of the plasmid at the genomic *ldhD* site were selected, and appropriated resolution of the structures resulted in mutants completely lacking D-lactate dehydrogenase activity. The lower remaining L-lactate dehydrogenase activity rerouted pyruvate to L-lactate with a marginal increase in the secondary end products acetaldehyde, acetoin and diacetyl (Lapierre et al. 1999).

E. coli is a facultative anaerobe, which carries out mixed fermentation of glucose dehydrogenase activity, was also not able to grow on glucose. However, an alcohol

dehydrogenase (*adh*), phosphotransacetylase (*pta*) double mutant was able to grow anaerobically on glucose by lactate fermentation producing D- lactate and a small amount of succinate. An additional mutation in the phosphoenol pyruvate carboxylase gene made the mutant produce D-lactate like a homofermentative in which the principal products are formate, acetate, d-lactate, succinate and ethanol. A *pta*⁻ mutant, that is not able to synthesize phosphotransacetylase responsible for acetate formation, was not able to grow on glucose. An *adh*⁻ mutant are not having alcohol in lactic acid bacteria (Narayanan et al. 2004). An L- Lactate dehydrogenase gene was introduced into this mutant lacking D-lactate dehydrogenase gene, this resulted in producing L-lactate dehydrogenase as the major fermentation product (Chang et al. 1999).

Rhizopus oryzae has ethanol fermentative enzymes that allow the fungus to grow for short periods in the absence of oxygen. A mutant was isolated that expressed only 5% of the wild type alcohol dehydrogenase activity under O₂ limiting conditions. Thus pyruvate was shuttled to lactic acid formation (Skory et al. 1998).

RAW MATERIALS

Over the years authors have studied a large number of carbohydrates and nitrogenous materials for production of lactic acid. They have been investigated on the basis of high lactic acid yields, optimum biomass production, negligible by product formation, fast fermentation rate, less pre-treatment, easy down stream processing, low cost, ease of availability etc. The choice of the raw material to be used depends on the microorganisms studied and also on the product desired.

Sucrose (from syrups, juices and molasses), lactose (from whey), maltose (produced by specific enzymatic starch conversion processes), glucose (from starch conversion processes, mannitol etc have been commercially used. Molasses are cheap but give low yields of lactic acid and laborious purification procedures. Whey is also cheap and easily available but like molasses have expensive purification processes. These have stimulated the development of modern technologies like ultra filtration and electrodialysis (Kulozik and Wilde, 1999). Hydrolyzed potato starch, corn, straw, whey, cottonseed hulls, grapefruit, sulphite waste liquor etc. have also been investigated. Studies have also been made for the production of L (+) lactic acid by *R. oryzae* using cornstarch and corncobs in an air-lift bioreactor and fibrous bed bioreactor.

Studies are also being carried out to develop microbial processes for the production of high purity L (+) lactic acid at low cost from sago starch which is in abundance in Sarawak, Malaysia, Riau and Indonesia. Lactic acid has

also been produced by simultaneous saccharification and fermentation of pre-treated alpha fibre.

A number of nitrogenous materials like whey permeate, yeast extract, malt sprouts, malt combining nuts, grass extract, peptones, beef extract, casein hydrolysate, corn steep liquor, N-Z-amine, soybean hydrolysate with supplementation of vitamins to supplement carbohydrate sources to give fast and heavy growth have been studied. However, yeast extract seems to be the most effective supplement. Eleven different nitrogen sources were tested. Various amounts of B vitamins were studied to replace yeast extract (Hujanen and Linko, 1996). These are kept at minimal levels to simplify the recovery process. Additional minerals are occasionally required when the carbohydrate and nitrogenous sources lack sufficient quantities

FERMENTATION PROCESSES.

Lactic acid fermentation is known to be end product inhibited fermentation by an undissociated form of lactic acid. Several studies have been carried out to overcome this problem. It has found that using extractive lactic acid fermentation technique could give a lactic acid yield of 0.99g/l and lactic acid productivity of 1.67 g/l/h over a conventional batch reactor which gave a yield of 0.83 g/l and lactic acid productivity of 0.31 g/l/h (Srivastava et al. 1992). Ion exchange resin amberlite IRA-400 was used for lactate separation. As lower temperature favours adsorption and higher temperature favours lactic acid production, a temperature of 39°C was found optimal for lactic acid production by extractive lactic acid fermentation. Anion exchange method has been used for lactic acid recovery from lactic acid-glucose solution in an ion exchange membrane based extractive fermentation system (Ziha and Kefung, 1995). Roychoudhury et al. 1995 have described the different extractive lactic acid fermentation processes.

It has been demonstrated that hydrogen ion had a negative effect on the metabolism of *Lactococcus lactis* cells during electro dialysis bioprocess, in which culture filtrate was circulated through the cathode compartment (Nomura et al. 1998). They investigated the stimulation of the rate of L-lactate fermentation by periodic electrodialysis. Electro dialysis bioprocess has been studied wherein lactate and acetate are removed simultaneously which maintains a low level of lactate in the broth, which reduces end product inhibition. Hydrogen ions have an inhibitory effect on the metabolism of the cells; therefore use of a standard electrodialyser enables circulating the culture filtrate through the dialysis compartment so that the culture does not contact the cathode. This enabled a complete consumption of xylose in lesser time.

Mainly the two reactor systems results in high yields and productivities of lactic acid: - a continuous cell recycle

fermentation process (Figure 1) and a fed batch fermentation (Figure 2). A high volumetric productivity of 117 g/l/h using membrane cell recycle bioreactor is reported but it does not result in high product concentration, and are run under continuous manner with continuous bleeding of cells to prevent the change in fluidity that occurs when cell concentration goes too high. To overcome this problem, CSTR have been used in series (Kulozik et al. 1992). This increased the productivity and concentration of lactic acid. The increased lactic acid yield also found at the expense of biomass formation at a latter stage. A high purity of the lactic acid isomer L (+) lactic acid also increased via increased population of fresh cells. The performance of a seven staged cascade reactor with cell recycle has been investigated. Membrane Cell Recycle Bioreactors (MCRB) in series has been studied where high cell density with high lactic acid productivity of 5.7 g/l/h, and 92 g/l lactic acid concentration were obtained (Kwon et al. 2001). Continuous production of ammonium lactate in a 3-staged reactor has been investigated (Borgardt et al. 1998). Various retention times examined showed higher lactate productivity and higher lactose utilization. Continuous fermentations using whey permeates have been reported with high productivities. Experiments with cell recycling have been studied. A volumetric productivity of 76 kg/m³/h was determined with an effluent lactic acid concentration. Lactic acid production has been studied with immobilized cell systems. *Lactobacillus delbreuckii* were immobilized in calcium alginate beads and used them in continuous flow column reactors and have got yield of 0.97 g/g lactic acid. *Lactobacillus delbreuckii* were immobilized in a hollow fibre reactor. 100 kg/m³/h lactate productivity was observed. Excessive growth of the organisms reduced the long-term operation of the reactor system. The kinetics of growth and lactic acid production of *Lactobacillus casei* and *Lactobacillus lactis* have been studied for lignocellulosic hydrolysate of crushed corncobs in the cell retention continuous culture with an ultra filtration module retaining all biomass and allowing the continuous removal of metabolites (Melzoch et al. 1996). Biofilms are a natural form of cell immobilization. It has been demonstrated that lactic acid production was enhanced when biofilm fermentation was carried out with chips of plastic composite support PCS containing 75% (w/w) polypropylene (PP) and 25% (w/w) agricultural material (Demirci and Pometto, 1995). 24 PCS disc blends have been containing 50% (w/w) PP and 50% agricultural materials for L (+) lactic acid biofilm fermentation in minimal media with no pH control. Each PCS blend was evaluated for biofilm development, slow release of nutrients, surface contact angle, hydrophobic compatibility with *Lactobacillus casei*, porosity and lactic acid absorption. The PCS disc that consistently demonstrated the highest performance contained 50% (w/w) PP, 35% (w/w) soybean hulls, 5% (w/w) yeast extract, 5% (w/w) dried bovine albumin and mineral salts. The biofilm population is

affected by the contact angle and relative hydrophobicity of the supports. Use of plastic composite supports gave high biofilm population, cell density and lactic acid concentrations.

Solvent extraction has been used for the purification of carboxylic acid such as lactic acid and succinic acid. But these solvents *in-situ* are toxic as they rupture the cell membrane causing the metabolite to leak out. Long chain alcohols such as 1-octanol and 1-decanol were found to be less toxic than other diluents. It has also been shown that Colloidal Liquid Aprons (CCA) cause little difference in the equilibrium distribution with the solvent alone. They reduce the toxicity of solvents on the cells.

A high productivity can be obtained using membrane recycle reactor, but it has a potential drawback of fouling. At high cell densities the cells are put under stress and start producing the D-isomer of the product. High cell densities can be obtained by using immobilized cells but controlled pH is a prerequisite. A stirred tank reactor provides efficient control on pH but often leads to attrition of the support. An adhesive strain of *L. casei* was inoculated onto two packed bed reactors that were operating in a continuous manner. In packed bed reactors large pH gradients are generated and a substantial fraction of cells do not experience optimal pH. Adsorption to a support provides a simpler and better entrapment of cells. The multiplying cells are liberated to the medium leading to the presence of cells suspended in the medium (Bruno et al. 1999).

L (+) lactic acid is commercially produced in fermentation processes using lactic acid bacteria or fungi such as *Rhizopus oryzae* in submerged culture. *Rhizopus sp.* can produce L (+) lactic acid from starch but the yield is very less in comparison with lactic acid bacteria. Using an air-lift bioreactor under optimum conditions could produce L (+) lactic acid with a yield of 85%. The mycelial morphology not being conducive to fermentation as they increase the viscosity of the medium wrap around impellers and cause blockage during sampling and in overflows lines. Regulating the inoculated spore concentration in pre culture produced small mycelial pellets of *R. oryzae*. However, pellets have problem of inadequate mass transfer. Mineral supports can be used to get cotton like floc morphology (Sun et al. 1999).

Perfusion cultivation of microorganisms is an efficient technique for achieving high productivity of extra cellular products. The stirred ceramic membrane reactor (SCMR) equipped with an asymmetric membrane tube was found to be effective in maintaining a high permeability for long periods of time. However, the production rate gradually decreased during the repeated batch fermentation. Nevertheless, the long lasting, high-filtration performance of the SCMR enabled the replenishment of the culture

supernatant in a short period of time (Ohashi et al. 1999).

VARIOUS OPTIONS FOR LACTIC ACID /LACTATE SALT SEPARATION; ADVANTAGES AND DISADVANTAGES

The fermented medium contains either pure lactic acid or its salt or the mixture of the two. A class of advantageous processing approaches involves removal of lactic acid from the fermentation broth or other mixture, while leaving the soluble lactate behind in the fermentation broth. The separation can, in some instance occur within the fermenter or it can be conducted on solution material removed from the fermenter.

A number of approaches can be used for separation of lactate salt from fermented medium, which are extraction by solvents, ion-exchange separation, separation by adsorption, separation by vacuum distillation, and the membrane separation (Eyal et al. 2001). Each of these exhibits some advantages and disadvantages that are also described with fermentation processes earlier in this review. The choice of the separation process should be based on the efficient and economical usage of these extractants (Roychoudhury et al. 1995).

According to the Eyal et al. 2001; a preferred process for the lactic acid products from the mixture containing free lactic acid and the dissolved lactate salt comprises of following steps: - (a) lowering down of the pH of fermented broth (3.0 to 4.2); (b) Use of hydrophilic membrane and the volatile amine weak base (VAWB) to separate lactic acid from the fermented broth through the hydrophilic membrane to VAWB; (c) Regeneration of lactic acid from salts of weak amine base by selectively vaporizing the volatile amine base. This process can be repeated to ensure the efficient separation of free lactic acid and its salt.

LACTIC ACID POLYMERS BY POLYCONDENSATION

Lactic acid polymers consist of mainly lactyl units, of only one stereoisomer or combinations of D and L lactyl units in various ratio. A disadvantage of polycondensation is that a low molar mass polymer is obtained. There have been studies to obtain high molar mass polymer by manipulating the equilibrium between lactic acid, water and polylactic acid in an organic solvent (Ajioka et al. 1995) or a multifunctional branching agent was used to give star-shaped polymers (Kim and Kim, 1999). In the presence of bifunctional agents (dipoles and diacids) they form telechelic polymers, which can be further linked to give high molar mass polymers using linking agents like diisocyanate (Hiltunen et al. 1997). An overview of the different lactic acid based polymers prepared by

polycondensation and polycondensation followed by chain extension are given in Table 2.

LACTIC ACID POLYMERS BY RING-OPENING POLYMERIZATION

The ring opening polymerization route includes polycondensation of lactic acid followed by a depolymerisation into the dehydrated cyclic dimer, lactide which can be ring opening polymerized to high molar mass polymers. The depolymerisation is conventionally done by increasing the polycondensation temperature and lowering the pressure, and distilling off the produced lactide. Solution polymerization, bulk polymerization, melt polymerization and suspension polymerization are the various methods of ring opening polymerization (Niewenhuis, 1992). The polymerization mechanism can be cationic, anionic, coordination or free radical polymerization. It is catalyzed by compounds of transition metals: - tin, aluminium, lead, zinc, bismuth, iron and yttrium (Nijenhuis et al. 1992). Other ring formed monomers can also be incorporated into the lactic acid based polymer by ring opening copolymerisation. The most utilized comonomers are glycolide, caprolactone, valerolactone, dioxypenone and trimethyl carbonate. The advantage of ring opening polymerization is that the chemistry of the reaction can be accurately controlled thus varying the properties of the resultant polymer in a more controlled manner.

Various authors have studied the synthesis of different molecular weight polymers. It has been reported that high molecular weight of poly lactic acid can be synthesized by one step polycondensation if appropriate azeotropic solvents are employed. The catalyst concentration, polymerization time and temperature cause profound effects on the polymer yield, molecular weight and optical rotation.

The synthesis of polylactic acid through polycondensation of the lactic acid monomer gave weight average molecular weights lower than 1.6×10^4 , whereas ring opening polymerisation of lactides gave average molecular weights ranging from 2×10^4 to 6.8×10^5 (Hyon et al. 1997). The monomer conversion and average molecular weights showed a maximum at a catalyst concentration of stannous octoate of 0.05%. It increases linearly with polymerisation time up to a monomer conversion of 80% to a maximum but thermal depolymerisation of resultant polylactides is observed with prolonged times at higher polymerization temperatures.

The synthesis of star shaped copolymers depends on the ratio of monomer to initiator and monomer to catalyst and monomer conversion (Dong et al. 2001). For the polymerisation of polylactide with methylglycolide using

trimethylolpropane initiator depends on the molar ratio of monomer to initiator and the monomer conversion producing three or four armed star shaped polymers.

There has been an interesting study for the selection > 99:1 of stereoisomers of lactic acid. Diels-Alder reactions of the acrylate of ethyl lactate with cyclopentadiene proceed with diastereoface-selectivity of up to 85:15 (non-catalyzed) and 93:7 (TiCl₄ promoted). Depending on the lewis acid, products of inverse configuration are obtained. This can be used as a method for large-scale practical applications of the asymmetric Diels Alder reaction. The influences of the relative proportion of lactide and glycolide in the mixture and the catalyst concentrations have been found to be statistically significant. The influence of time, temperature and lauryl alcohol on the molecular weight, composition and chain structure have also been studied by authors (Dorta et al. 1993)

EFFORTS IN MANUFACTURING LACTIC ACID AND LACTIC ACID BASED POLYMERS

Technological advancements in the major process components – fermentation, primary and secondary purification, polymerization, chemical conversion of lactic acid and its derivatives would enable low cost large volume and environment friendly production of lactic acid. Recent advancements in membrane based separation and purification would enable lactic acid production without producing salt or gypsum by products. In recently issued patents, an osmotolerant strain of lactic acid bacteria and a configuration of desalting electrodialysis, water splitting electrodialysis and ion exchange purification, a concentrated lactic acid product containing less than 0.1% proteinaceous components can be produced by a carbohydrate fermentation. This process gives no byproduct salt gypsum but only a small amount of salt during ion exchange regeneration. It also claims to have small power requirement.

Ecochem, a Dupont ConAgra partnership has developed a recovery and purification process that produces a byproduct ammonium salt, which can be sold as fertilizer (Anon, 1992). This plant has a capacity of 1000 tons/year. A continuous process has been developed for manufacture of lactide polymers with controlled optical purity (Gruber, 1992). The process uses a configuration of multistage evaporation followed by polymerization to a low molecular weight prepolymer, which is then catalytically converted to dilactide. The purified dilactide is recovered in a distillation system with partial condensation and recycle. The dilactide can be used to make high molecular weight polymers and copolymers. A novel process to make cyclic esters, dilactide and glycolide has been developed. This process uses an inert gas to sweep away the cyclic esters from the reaction mass and then it recovers and purifies the

volatilized ester by scrubbing with an appropriate organic acid and finally separates the cyclic ester from the liquid by precipitation or crystallization and filtration of the solids producing high purity lactide with minimal losses due to racemization. Recycle and reuse of the lactic acid moiety in the various process streams have been claimed to be feasible.

Hydrogenolysis reaction technology to produce alcohol from organic acids or esters has also advanced recently, new catalysts and processes yield high selectivity and rates and operate at moderate pressures. This technology has been commercialized to produce 1,4 butanediol, tetrahydrofuran and other four carbon chemical intermediates from maleic anhydride. In the future, such technologies could be integrated with low cost processes for the production of lactic acid to make propylene glycol and other intermediate chemicals.

The L-lactic acid based polymers may produce polymer which is a linear homopolymer of the molecular size >70 kDa. The main application field of lactic acid polymer has been medical applications and a number of companies have made their efforts in manufacturing lactic acid based polymers and their products. These medical applications include its usage if carrying different properties in terms of tensile strength, viscosity, purity etc. L-lactic acid polymer exist in three different forms solids that can be used for filling the gaps in bones, solid with tensile strength to produce sutures (stitching material), and the glue form that is mainly applied in joining membranes or thin skins in humans (Shikinami et al. 2002). Another important property of poly lactic acid is its high strength against UV radiation. The biosorbable glue or sticky form of lactic acid comprises of copolymer of two or more biosorbable monomers: - L-lactic acid with dioxanone, with tri methylene carbonate and with ϵ -caprolactone.

Dow Chemicals and Cargill have the largest polylactide (PLA) producing company with an annual capacity of 140,000 tonnes located in Blair, USA (Anon, 1992). The PLA is produced by ROP and their main application is in fibres, packaging materials and as solvents. It has a joint venture with PURAC, Netherlands for lactic acid production in corn milling plant. It has made PLA business development collaboration with Mitsubishi Polymers. Apack, Germany is a food packaging company uses the polylactide technology of former Nestle Chemicals in collaboration with Fortum Oyj, Finland (Kivimaki, 2000). Galactic, Belgium produces 1500 tons of lactic acid annually from beet sugar. Brussels Biotech, a subsidiary of Galactic works on the research and development aspects of lactic acid products (Bronnbann and Yoshida, 2000). Hycail, Netherlands a joint venture between Dairy Farmers, USA and the Dutch State University Groningen, plans to construct a pilot plant for lactic acid production of capacity

400 tons per year from whey and converting the lactic acid to PLA. Mitsui Chemicals, Japan is producing PLA by a direct polycondensation route. Shimadzu Corporation, Japan is producing PLA by ROP. Birmingham Polymers, USA and Phusi, France are some of the other active producers of PLA (Ohrlander et al. 1999).

The research on lactic acid related materials have attracted several universities and institutes in Europe, Asia and USA. There are a number of small-scale production facilities of polylactic acid.

When we say pure L (+) lactic acid polymer industries, we have only few names as Yipu, Dahuachem International, Sinochem Hebei Qinhuangdao Imp and Exp Corp., Zechem, and Qingdao FTZ united international Inc. in China; and PURAC, Macropore Biosurgery, ECOCHEM etc. in USA. Most of them have adopted the semi-natural process for L (+) lactic acid polymer production. The process comprises of isolation of L (+) lactic acid from its recemic mixture produced via fermentation by adopting enzymatic process for L (+) lactic acid production from its recemic mixtures followed by separation using expensive High performance liquid Chromatography (HPLC) techniques (Oxoid, USA; and Cargill Co., USA).

CONCLUDING REMARKS

The proven degradability in biological systems, biocompatibility and the possibility of tailoring the properties to a wide range have made lactic acid derivatives well suited for a range of applications. The environmental issues that have gained importance during the last decade have resulted in efforts to applying the lactic acid polymers for medical applications and as packaging materials. Only fermentation produces natural L (+) lactic acid. In the international market, natural form of polymers is preferred to be used for medical purposes compared to that produced by chemical or enzymatic process. Lactic acid can be derived from a wide range of renewable materials and can easily fit into municipal waste management systems. Several major agriculture processing and chemical industries have identified and built lactic acid plants and has plans for major large-scale plants in future. Several novel processes are being deployed for facile production and separation of lactic acid and their manufacturing costs and economics have attractive potential in large scale operations.

REFERENCES

- AJIOKA, I.; ENOMOTO, K.; SUZUKI, K. and YAMAGUCHI, A. The basic properties of poly lactic acid produced by the direct condensation polymerisation of lactic acid. *Journal of Environmental polymer degradation*, 1995, vol. 3, no. 8, p. 225-234.
- AJIOKA, I.; SUZUKI, H.; HIGUCHI, C. and KASHIMA T. Aliphatic polyesters and their copolymers synthesized through the direct condensation polymerization. *Polymer Degradation and Stability*, 1998, vol. 59, no. 1-3, p. 137-143.
- ANON. Ecochem (Dupont and Conagra Joint Venture, Wilmington, DE) completes lactic acid plant. *Chemical Engineering News*, August 1992, p. 20-25.
- BOHLMANN, G.M. and YOSHIDA, Y. CEH Marketing Research Report. *Biodegradable Polymers*, February 2000, p. 1-5.
- BORGARDTS, P.; KRISCHKE, W.; TROSCH, W. and BRUNNER, H. Integrated bioprocess for the simultaneous production of lactic acid and dairy sewage treatment. *Bioprocess Engineering*, 1998, vol. 19, no. 5, p. 321-329.
- BRUNO, J.M.; RAGOUT, A.L.; CORDOBA, P.R. and SENERIZ, F. Continuous production of L (+) lactic acid by *Lactobacillus casei* in two-stage systems. *Applied Microbiology Biotechnology*, March 1999, vol. 51, p. 316-24.
- CHANG, D.E.; JUNG, H.C.; RHEE, J.S. and PAN, J.G. Homofermentative production of D- or L-lactate in metabolically engineered *E. coli* RR. *Applied and Environmental Microbiology*, 1999, vol. 65, p. 1384-1389.
- DATTA, R. Technological and economical potential of polylactic acid and lactic acid derivatives. *FEMS Microbiology Reviews*, 1995, vol. 16, no. 1-3, p. 221-231.
- DAVIDSON, B.E.; LIANOS, R.M.; CANCELLA, M.R.; REDMAN, N.C. and HILLIER, A.J. Current research on the genetics of lactic acid production by lactic acid bacteria. *International Dairy Journal*, 1995, vol. 5, no. 8, p. 763-784.
- DEMIRCI, A. and POMETTO, A.L. Repeated fed-batch fermentation in biofilm reactors with plastic composite supports for lactic acid production. *Applied Microbiology and Biotechnology*, 1995, vol. 43, no. 4, p. 585-589.
- DONG, C.M.; QIU, K.Y.; GU, Z.W. and FENG, X.D. Synthesis of star shaped poly (D, L-lactic acid-alt-glycolic acid) with multifunctional initiator and SnOct₂ catalyst. *Polymer*, 2001, vol. 42, no. 16, p. 6891-6896.
- DORTA, M.J.; MUNGULIA, O. and LLABRES, M. Effects of polymerisation variables on PGLA properties: molecular weight, composition and chain structure. *International Journal of Pharmaceutics*, 1993, vol. 100, no. 1-3, p. 9-14.

- ESPARTERO, J.L.; RASHKOV, I.; LI, S.M.; MANOLOVA, N. and VERT, M. NMR analysis of polylactic acid macromolecules. *Macromolecules*, 1996, vol. 59, p. 3535-3539.
- EYAL, A.M.; STARR, J.N.; FISHER, R.; HAZEN, B.; CANARI, R.; WITZKE, D.R.; GRUBER, P.R. and KOLSTAD, J.J. Lactic Acid processing; methods; arrangements; and, product. *U.S. Patent no. 6320077*, U.S.A., 2001.
- FERAIN, T.; GRMYN, D.; BERNARD, N.; HOES, P. and DELCOUR, J. *Lactobacillus plantarum* *ldhL* gene: Overexpression and deletion. *Journal of Bacteriology*, 1994, vol. 176, p. 596-601.
- GRUBER, P.R. *U.S. Patent no. 5142023*. Cargil, Inc., U.S.A., 1992.
- HILTUNEN, K.; SEPPALA, J.V. and HARKONEN, M. Effect of catalyst and polymerization conditions on the preparation of low molecular weight lactic acid polymer. *Macromolecules*, 1997, vol. 30, no. 3, p. 1091-1100.
- HUJANEN, M. and LINKO, Y.Y. Effect of temperature and various nitrogen sources on L (+) Lactic acid production by *Lactobacillus casei*. *Applied Microbiology and Biotechnology*, 1996, vol. 45, no. 3, p. 307-313.
- HYON, S.H.; JAMSHIDI, K. and IKADA, Y. Synthesis of polylactides with different molecular weights. *Biomaterials*, 1997, vol. 18, no. 22, p. 1503-1508.
- KAWASAKI, N.; NAKAYAMA, A.; MAEDA, Y.; HAYASHI, K., YAMAMOTO, N. and AIBA, S. Synthesis of a new biodegradable co polyester amide poly lactic acid co caprolactam. *Macromolecular Chemistry and Physics*, 1998, vol. 199, p. 2407-2415.
- KIM, S.H. and KIM, Y.H. Direct condensation of polymerisation of lactic acid. *Macromolecular Symposium*, 1999, vol. 144, p. 227-287.
- KIVIMAKI-MAJANEN M, editor. Fortumin biomuovien valmistuslisenksi Saksaan. *Kemia-Kemi*, 2000; vol. 27, p. 395.
- KONINGS, W.N.; KOK, J.; KUITERS, O.P. and POOLMAN, V. Lactic acid bacteria: The bugs of new millennium. *Current Opinions in Microbiology*, 2000, vol. 3, p. 276-282.
- KULOZIK, U.; HAMMELEHLE, B. and KESSLER H. High reaction rate continuous bioconversion process in a tubular reactor with narrow residence time distributions for the production of lactic acid. *Journal of Biotechnology*, 1992, vol. 22, no. 1-2, p. 107-116.
- KULOZIK, U; and WILDE, J. Rapid lactic acid production at high cell concentration in whey ultrafiltrate by *Lactobacillus helveticus*. *Enzyme and Microbial Technology*, 1999, vol. 24, no. 5-6, p. 297-302.
- KWON, S.; YOO, I.K.; LEE, W.G.; CHANG, H.N. and CHANG Y.K. High-rate continuous production of lactic acid by *Lactobacillus rhamnosus* in a two-stage membrane cell recycle bioreactor. *Biotechnology and Bioengineering*, 2001, vol. 73, no. 1, p. 25-34.
- KYLMA, J. and SEPPALA, J.V. Synthesis and characterization of a biodegradable polyesters. *Macromolecules*, 1997, vol. 30, no. 10, p. 2876-2882.
- KYLMA, J.; HARKONEN, M. and SEPPALA, J.V. The modification of lactic acid based polymer by copolymerization. *Journal of applied polymer science*, 1997, vol. 63, no. 13, p. 1865-1872.
- LAPIERRE, L.; GERMOND, J.E.; OTT, A.; DELLEY M. and MOLLET, B. D-Lactate dehydrogenase gene (*ldhD*) inactivation and resulting metabolic effects in the *Lactobacillus johnsonii* strains La 1 and N 312. *Applied and Environmental Microbiology*, 1999, vol. 65, no. 9, p. 4002-4007.
- MELZUCH, K.; VOTRUBA, J.; HABOVA, V. and RYCHTERA, M. Lactic acid production in a cell retention continuous culture using lignocellulosic hydrolysate as a substrate. *Folia Microbiologica*, 1996, vol. 41, no. 2, p. 211-215.
- NARAYANAN, N.; ROYCHOUDHURY, P.K. and SRIVASTAVA, A. Isolation of *adh* mutant of *Lactobacillus rhamnosus* for production of L(+) Lactic acid. *Electronic Journal of Biotechnology* [online]. 15 April 2004, vol. 7, no. 1]. Available from Internet: <http://www.ejbiotechnology.info/content/vol7/issue1/full/7/index.html>. ISSN 0717 3458.
- NIEUWENHUIS, J. Synthesis of polylactides, polyglycolides and their copolymers. *Clinical Materials*, 1992, vol. 10, p. 59-67.
- NIJENHUIS, A.J.; GRIJPM, D.W. and PENNING, A.J. Lewis acid catalyzed polymerisation of L-lactide. Kinetics and mechanism of esters of bulk polymerisation. *Macromolecules*, 1992, vol. 25, no. 24, p. 6419-6424.

- NIKKILA, K.K.; HUJANEN, M.; LEISOLA, M. and PALVA, A. Metabolic engineering of *Lactobacillus helveticus* CNRZ32 for production of pure L-(+) lactic acid. *Applied and Environmental Microbiology*, 2000, vol. 66, p. 3835-3841.
- NOMURA, Y.; IWAHARA, M.; HALLSWORTH, J.E.; TANAKA, T. and ISHIZAKI, A. High-speed conversion of xylose to L-lactate by electrodialysis bioprocess. *Journal of Biotechnology*, 1998, vol. 60, no. 1-2, p. 131-135.
- OHASHI, R.; YAMAMOTO, T. and SUZUKI, T. Continuous production of lactic acid from molasses by perfusion culture of *Lactococcus lactis* using a stirred ceramic membrane reactor. *Journal of Bioscience and Bioengineering*, 1999, vol. 87, p. 647-654.
- OHRLANDER, M.; PALMGREN, R.; WIRSEN, A. and ALBERTSSON, A. The grafting of acrylamide onto poly (E-caprolactone) and poly (1,5-dioxypn-2-one) using electron-beam pre-irradiation. III. The grafting and *in vitro* degradation of chemically cross linked poly (1,5-dioxypn-2-one). *Journal of Polymer Science Part A: Polymer Chemistry*, 1999, vol. 37, no. 11, p. 1659-1663.
- PORRO, D.; BIANCHI, M.M.; BRAMBILLA, L.; MENGHINI, R.; BOLZANI, D.; CARRERA, V.; LIEVENSE, J.; LIU, C.; RANZI, B.M.; FRONTALI, L. and ALBERGHINA, L. Replacement of a metabolic pathway for large scale production of lactic acid from engineered yeasts. *Applied and Environmental Microbiology*, 1997, vol. 65, p. 4211-4215.
- ROYCHOUDHURY, P.K.; SRIVASTAVA A. and SAHAI V. Extractive bioconversion of lactic acid. In: FIECHTER, A. ed. *Advances in Biochemical Engineering/Biotechnology*. Springer-Verlag, 1995, vol. 53, p. 61-87. ISBN 354059308X.
- SHIKINAMI, Y.; KWARADA, H.; HIROYUKI, N. and CHIKA, N. Biosorbable polymeric clayey and sticky substance. *U.S. Patent 6387391*, U.S.A., 2002.
- SKORY, C.D.; FREER, S.N. and BOTHAST, V. Production of L-Lactic acid by *Rhizopus oryzae* under oxygen limiting conditions. *Biotechnology Letters*, 1998, vol. 20, no. 2, p. 191-194.
- SODEGARD, A. Preparation of poly (L-lactide-graft-acrylic acid) by pre-irradiation grafting. *Polymer Preparation*, 1998, vol. 39, p. 215-216.
- SRIVASTAVA, A.; ROYCHOUDHURY, P.K. and SAHAI, V. Extractive Lactic acid fermentation using ion exchange resins. *Biotechnology and Bioengineering*, 1992, vol. 39, p. 607-613.
- SUN, Y.; LI, Y. and BAI, S. Modelling of continuous L (+)-lactic acid production with immobilized *R. oryzae* in an airlift bioreactor. *Biochemical Engineering Journal*, February 1999, vol. 3, p. 87-90.
- WOO, S.I.; KIM, B.O.; JUN, H.S. and CHANG, H.S.N. Polymerization of lactic acid to prepare high molecular weight poly lactic acid by chain extending and hexamethylene diisocyanate. *Polymer bulletin*, 1995, vol. 35, no. 4, p. 415-421.
- ZIHA, O.W. and KEFUNG, Z. Kinetics and mass transfer for lactic acid recovered with anion exchange method in fermentation solutions. *Biotechnology and Bioengineering*, 1995, vol. 47, no. 1, p. 1-7.