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V. Joseph

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Bezeichnung der Erfindung / Title of the invention / Titre de l'invention:

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Process for manufacturing propionate products

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Process for manufacturing propionate products

The present invention pertains to a process for manufacturing propionate products via a fermentation process.

Propionic acid can be manufactured through fermentation of a
5 carbon source using a microorganism. For example,
Zhu et al (Bioresource Technology 101 (2010) 8902-8902)
describes a fermentation process for the manufacture of
calcium propionate wherein glycerol is fermented using
Propionibacterium acidipropionici. Calcium hydroxide is added
10 during the fermentation to neutralize the acid generated
during the fermentation, resulting in the formation of
calcium propionate.

Propionate salts are attractive for many purposes. For
15 example they are attractive for use in feed and food
preservation, where they provide long lasting broad spectrum
activity. Most important salts in this respect are calcium,
sodium, potassium, and ammonium salts. The calcium salt is
considered of particular interest. The propionate salt may be
20 used in solution, or in the form of a solid salt.

A problem which has been found to occur in the preparation of
propionate salts via fermentation resides in the complexity
of the fermentation process, both as regards carbon sources
25 which can be fermented, and as regards side products which
are formed during fermentation. There is need in the art for
a fermentation process for manufacturing propionate salts
which combines a high yield of propionate salt with an
efficient growth of the microorganism and the possibility of
30 obtaining a high-purity propionate salt product.

The present invention provides a process which solves this problem.

The present invention pertains to a process for manufacturing
5 propionate products through fermentation, comprising the steps of

- fermenting a carbon source selected from sugars and lactate in a fermentation medium by means of a propionic acid producing microorganism to provide a first fermentation broth

10 comprising a propionate salt,

- recovering propionic acid producing microorganism from the first fermentation broth,

- subjecting the first fermentation broth from which propionic acid producing microorganism have been recovered to

15 a water removal step to form a first propionate salt product,

- fermenting a carbon source comprising glycerol with the propionic acid producing microorganism recovered from the first fermentation broth in the presence of an inorganic alkaline salt to provide a second fermentation broth

20 comprising a propionate salt,

- subjecting the second fermentation broth to a purification step comprising at least one precipitation step, to form a second propionate salt product.

25 In the process according to the invention, there are two sequential fermentation steps. In the first fermentation step, a carbon source selected from sugars and lactate is fermented under the formation of a propionate salt product stream. This step may also be indicated herein as the sugar
30 fermentation step. Then, the microorganisms obtained in the sugar fermentation step are recovered and used to ferment a carbon source comprising glycerol, to form a further propionate salt product stream. The two propionate salt product streams are processed separately.

By applying two different fermentations, i.e. a sugar-based fermentation and a glycerol-based fermentation, and treating the resulting streams separately, it has been found that it is possible to benefit from the advantageous effects of both
5 types of fermentation. More specifically, it has been found that sugar fermentation can result in efficient cell growth, which makes for a large yield of biomass. The resulting product, however, may be relatively difficult to purify due to the presence of substantial amounts of acetate anions. In
10 contrast, the glycerol fermentation yields a product which can be purified to high purity propionate salt products. However, the glycerol fermentation results only in limited biomass formation. By combining the fermentations in the manner prescribed, an efficient process is obtained. Further
15 advantages of the process according to the invention will be discussed below.

The process according to the invention will be discussed in more detail below.

20

The first step in the process according to the invention is fermenting a carbon source selected from sugars and lactate in a fermentation medium by means of a propionic acid producing microorganism to provide a first fermentation broth
25 comprising a propionate salt.

Fermentation processes for the manufacture of propionate are known in the art and require no further elucidation here. It is within the scope of the skilled person to select, using
30 his common general knowledge, a suitable fermentation process, including fermentation conditions, and a suitable microorganism. In the selection of the microorganism, care should be taken to select an organism which is capable of fermenting sugars or lactate (in the first fermentation step

of the process according to the invention) and glycerol (in the second fermentation step of the process according to the invention).

5 The carbon source used in the first step of the process according to the invention is selected from sugars and lactate. Examples of suitable sugars and sources of sugar include glucose, sucrose, dextrose syrup, starch, hydrolysed starch, cellulose, hydrolysed cellulose, molasses, xylose,
10 lignocellulose hydrolysates, lactose and whey. Suitable lactate sources are sodium lactate, potassium lactate, ammonium lactate, magnesium lactate, calcium lactate, and lactic acid. Of the lactate sources, the use of calcium lactate may be preferred, since this will result in the
15 formation of calcium propionate, which is a preferred product of the invention. From a production point of view glucose, lactate, and sucrose may be preferred. From an availability point of view, sources like molasses and starch may be preferred.

20

At least when the carbon source is selected from sugars the fermentation is carried out in the presence of an inorganic alkaline salt. This alkaline salt is present to ensure that the pH during the fermentation does not decrease to an
25 unacceptable extent due to the manufacture of the propionic acid. Where the carbon source is a lactate salt, the addition of an inorganic base may not always be required because lactate is converted into propionate, which does not have a pH effect. Nevertheless, also in that case, an inorganic base
30 may be added for pH adjustment.

Suitable bases include hydroxides, oxides, and carbonates of calcium, magnesium, sodium, and potassium, resulting in the formation of a fermentation broth containing respectively calcium propionate, magnesium propionate, sodium propionate,

or potassium propionate. It is preferred within the process according to the invention to use an alkaline calcium salt, as this results in the formation of calcium propionate, which is a preferred product according to the invention. Suitable
5 alkaline calcium salts include hydroxides, oxides, and carbonates of calcium.

The next step is the removal of microorganism from the thus formed first fermentation broth. This step can be carried out
10 when sufficient biomass has been formed for the following glycerol fermentation step. As the microorganism is to be used in a further fermentation, care should be taken to isolate the microorganisms under such conditions that the organism is kept alive. Taking this into account, the removal
15 of microorganism may be carried out in manners known in the art, e.g., via filtration step or centrifugation step.

In the sugar fermentation, the weight of the biomass has increased, with the amount of biomass recovered being
20 generally at least 10 times the weight of the biomass originally added, in particular at least 20 times, sometimes even at least 100 times. In the present specification the weight of the biomass is calculated as dry weight, unless expressly indicated otherwise.

25 It is preferred for the removal of microorganisms from the first fermentation broth to be carried out in a relatively high yield. There are two reasons for this. In the first place, the biomass is intended to be used in the subsequent
30 glycerol fermentation. Further, biomass remaining in the fermentation broth may affect the properties of the product manufactured from this broth, e.g. in the form of a detrimental effect on product color. It is considered preferred for the biomass to be removed so that the resulting

product has a turbidity of at most 1000 NTU, in particular at most 500 NTU, more specifically at most 200 NTU. A turbidity of at most 100 NTU is preferred.

- 5 In a further step of the process according to the invention, the first fermentation broth from which propionic acid producing microorganism have been recovered is subjected to a water removal step to form a first propionate salt product, in particular a calcium propionate product. This water
10 removal step can be carried out in a number of ways.

In a first embodiment, the first fermentation broth from which propionic acid producing microorganism have been recovered is subjected to a water removal step in its
15 entirety. In this case, a first propionate salt product is formed which comprises all matter present in the first fermentation broth from which propionic acid producing microorganism have been recovered, including remaining nutrients. This drying step can be carried out in manners
20 known in the art for water removal, including spray drying, drum vacuum drying, thin film drying, e.g., agitated thin film drying, mechanically agitated drying, spray granulation, and freeze drying.

The resulting product is a solid material, in particular a
25 solid particulate material, with a propionate salt content in the range of 30-70 wt.%, in particular 40-60 wt.%, of propionate salt, in particular calcium propionate, with the remainder comprising one or more of further salts of carboxylic acids, in particular succinate and/or acetate
30 salts, and further components resulting from, e.g., the biomass and residual medium/nutrient components. In one embodiment, the resulting product comprises acetate salt in the range of 3-20 wt.%, in particular 8-15 wt.%, more in particular 10-13 wt.% and/or succinate salt in an amount of

0-10 wt.%, in particular 3-6 wt.%. It is preferred for calcium propionate, calcium succinate, and calcium acetate to make up at least 85 wt.% of the total first calcium propionate product, obtained in this manner, in particular at least 90 wt.%, more in particular between 90 and 98 wt.%. In the above, the percentages are calculated as anion (acetate, succinate, propionate) on the total anhydrous solid material.

- 10 It is noted that it is possible to include other components in the fermentation broth before spray drying, e.g., additional carboxylic acid salts such as lactates or acetates, carriers like maltodextrin, starch, milk solids, whey, flour or other functional ingredients like green tea
15 extract, nisin, and rosemary extract. This may be attractive if it is desired to manufacture a product with a particular composition.

- In another embodiment, the step of subjecting the first
20 fermentation broth from which propionic acid producing microorganism have been recovered is carried out by a process encompassing precipitation of a propionate salt, in particular calcium propionate from the first fermentation broth. Precipitation may be initiated by concentration and
25 other measures known to improve the yield of a precipitation step, e.g., reduction of temperature or addition of compounds which reduce solubility. Precipitation can be performed batchwise or continuous, in a single step or in multiple steps.

- 30 The precipitated propionate salt product, in particular calcium propionate is separated from the aqueous medium. This can be done by methods known in the art, including one or more of filtration, centrifugation, decantation, etc. It is within the scope of the skilled person to select a suitable

manner. If so desired, the propionate salt product can be washed to increase product purity. The washing liquid, if used, can, e.g., be water or a propionate salt solution. The washing liquid can be recycled to obtain a high yield. The solid calcium propionate product can be dried as desired.

In this embodiment it is possible to precipitate all types of carboxylate salts, in particular calcium carboxylate salts present in the system, in particular calcium propionate in combination with calcium succinate (if present) and/or calcium acetate (if present). In this case, a final product may, e.g., be obtained with a propionate salt content, in particular calcium propionate in the range of 30-80 wt.%, in particular 40-70 wt.%, acetate salt, in particular calcium acetate in the range of 3-20 wt.%, in particular 8-15 wt.%, more in particular 10-13 wt.% and succinate salt, in particular calcium succinate, in an amount of 0-10 wt.%, in particular 3-6 wt.%. It is preferred for calcium propionate, calcium succinate, and calcium acetate to make up at least 85 wt.% of the total first calcium propionate product, obtained in this manner, in particular at least 90 wt.%, more in particular at least 95%. In the above, the percentages are calculated as anion (acetate, succinate, propionate) on the total anhydrous solid material.

It is also possible to sequentially precipitate succinate salts and propionate salts from the first fermentation broth, this can, e.g., be done via a process for manufacturing a solid propionate salt from a mixture comprising propionate and succinate comprising the steps of

- providing an aqueous medium comprising succinate anions and propionate anions,
- providing an inorganic soluble salt in the aqueous medium in such an amount that the majority of succinate ions is

converted to solid succinate salt while the majority of propionate ions remains in solution,

- separating the aqueous medium comprising the majority of propionate ions from the solid succinate salt,

5 - increasing the concentration of salt in the aqueous medium to convert the majority of propionate ions to solid propionate salt,

- separating the solid propionate salt from the aqueous medium.

10

As indicated above, the propionic acid producing microorganism is recovered from the first fermentation broth. In a next step in the process according to the invention, a carbon source comprising glycerol is fermented with the propionic acid producing microorganism recovered from the first fermentation broth in the presence of an alkaline salt to provide a second fermentation broth comprising a propionate salt.

15

Fermentation processes for the manufacture of propionic acid starting from a carbon source comprising glycerol are known in the art and require no further elucidation here. It is within the scope of the skilled person to select, using his common general knowledge, a suitable fermentation process, including fermentation conditions.

25

The carbon source used in this fermentation step comprises glycerol. It has been found that the use of glycerol as carbon source makes it possible to obtain a fermentation process which generates relatively high amounts of propionate salt, in particular calcium propionate in combination with relatively low amounts of acetate salts. This is attractive because the separation of propionate salts and acetate salts is generally complicated. It is preferred for the carbon source to consist for at least 70 wt.% of glycerol, more in

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particular at least 80 wt.%, still more in particular at least 90 wt.%, calculated on the total amount of carbon source provided during the fermentation.

- 5 A base is added during the fermentation step to compensate for the pH decrease which would occur due to the production of the carboxylic acids.
- Suitable bases include hydroxides, oxides, and carbonates of calcium, magnesium, sodium, and potassium, resulting in the
- 10 formation of a fermentation broth containing respectively calcium propionate, magnesium propionate, sodium propionate, or potassium propionate. It is preferred within the process according to the invention to use an alkaline calcium salt, as this results in the formation of calcium propionate, which
- 15 is a preferred product according to the invention. Suitable alkaline calcium salts include hydroxides, oxides, and carbonates of calcium.
- The alkaline salts used in the two fermentation steps may be the same or different. For reasons of efficiency it may be
- 20 preferred for the alkaline salt provided in the fermenting of a carbon source selected from sugars and lactate and the alkaline salt provided in the fermenting of a carbon source comprising glycerol to be salts of the same cation. The use of calcium salts is particularly preferred.

25

Once the fermentation has run to the desired extent, the second fermentation broth is subjected to a purification step comprising at least one precipitation step, to form a second calcium propionate product.

- 30 In general, the second fermentation broth is subjected first to a biomass removal step, which can be carried out in manners known in the art. Reference is made to what is stated above for the removal of biomass from the first fermentation broth. As for the step described above, it is considered

preferred for the biomass to be removed so that the resulting product has a turbidity of at most 1000 NTU, in particular at most 500 NTU, more specifically at most 200 NTU. A turbidity of at most 100 NTU is preferred. It has been found that the
5 presence of biomass may interfere with the further precipitation step in the process according to the invention. The biomass isolated in this step may be provided to a second glycerol-based fermentation, if this is desired.

- 10 The resulting broth from which biomass has been removed is subjected to at least one precipitation step, to form a second propionate salt product.

Before the precipitation step, the medium may if so desired
15 be contacted with an adsorbent, in particular an active carbon adsorbent, to remove impurities.

The precipitation step can be carried out as described above. Precipitation may be initiated by concentration of the
20 medium, and/or by other measures known to improve the yield of a precipitation step, e.g., reduction of temperature or addition of compounds which reduce solubility. Reference is made to what has been stated above on the precipitation step. The precipitated propionate salt product, in particular
25 calcium propionate is separated from the aqueous medium. This can be done by methods known in the art, including one or more of filtration, centrifugation, decantation, etc. It is within the scope of the skilled person to select a suitable manner. If so desired, the propionate salt product can be
30 washed to increase product purity. The washing liquid, if used, can, e.g., be water or a propionate salt solution. The washing liquid can be recycled to obtain a high yield. The solid propionate salt product can be dried as desired.

In one embodiment, in addition to propionate salts, other carboxylate salts, in particular succinate and/or acetate salts are also precipitated. In particular calcium carboxylate salts present in the system may be precipitated together, in particular calcium propionate in combination with calcium succinate (if present) and/or calcium acetate (if present). In this case, a final product may, e.g., be obtained with a propionate salt content, in particular calcium propionate in the range of 50-90 wt.%, in particular 60-80 wt.%, more in particular 70-80 wt.%, acetate salt, in particular calcium acetate in the range of 0-5 wt.%, in particular 0-2 wt.%, and succinate salt, in particular calcium succinate, in an amount of 0-20 wt.%, in particular 0-10 wt.%, more in particular 0-5 wt.%. It is preferred for calcium propionate, calcium succinate, and calcium acetate to make up at least 85 wt.% of the total first calcium propionate product, obtained in this manner, in particular at least 90 wt.%, more in particular at least 95%. In the above, the percentages are calculated as anion (acetate, succinate, propionate) on the total anhydrous solid material.

It is also possible to sequentially precipitate succinate salts and propionate salts from the second fermentation broth, this can, e.g., be done via a process for manufacturing a solid propionate salt from a mixture comprising propionate and succinate comprising the steps of

- providing an aqueous medium comprising succinate anions and propionate anions,
- providing an inorganic soluble salt in the aqueous medium in such an amount that the majority of succinate ions is converted to solid succinate salt while the majority of propionate ions remains in solution,
- separating the aqueous medium comprising the majority of propionate ions from the solid succinate salt,

- increasing the concentration of salt in the aqueous medium to convert the majority of propionate ions to solid propionate salt,
- separating the solid propionate salt from the aqueous medium.

It will be clear to the skilled person that the various embodiments and preferences describes herein can be combined, unless they are presented as mutually excluding alternatives.

The present invention will be elucidated by the following examples, without being limited thereto or thereby.

Example 1: Glucose fermentation followed by glycerol fermentation

A fermenter comprising 5 kg medium was inoculated with a culture of *Propionibacterium acidipropionici*. The medium contained 80g/kg glucose and 52g/kg YEP (yeast extract paste, 50% dry solids). The fermenter was operated at 30°C and with a pH of 6.5. The pH was controlled by adding Ca(OH)₂ solution. After ±45 hours the fermentation was stopped. The fermentation broth was centrifuged at 5000RPM. The microorganism was present in the sediment of the centrifugation step.

In this step, the amount of biomass was increased from 20 gram added initially to 150 gram harvested, both calculated as dry weight.

24 grams of biomass was added to a fermenter comprising 1kg medium containing 80g/kg glycerol and 52g/kg YEP. The fermenter was operated at 30°C and with a pH of 7.0. The pH was controlled by adding Ca(OH)₂ solution.

The fermentation yielded a volumetric productivity of 1.2 gram calcium propionate per kilogram medium per hour. The total yield of the fermentation was 72 wt.%, calculated as grams propionate per gram glycerol.

5

Example 2: Work-up of glucose fermentation broth

A glucose fermentation was carried out analogous to that described in the first paragraph of Example 1. The biomass
10 was isolated therefrom by centrifugation as described therein. 1000 grams of the remaining fermentation broth was dried using a rotary evaporator, first under atmospheric pressure, then under vacuum. After 6 hours, a dry product was
15 obtained.

15

The fermentation broth comprised the following organic acids, as determined using GC

Table 1: GC organic acids in fermentation broth

component		
formic acid	[% (w/w)]	<0.02
acetic acid	[% (w/w)]	0.57
propionic acid	[% (w/w)]	2.4
ethanol	[% (w/w)]	<0.02
maleic acid	[% (w/w)]	<0.01
oxalic acid	[% (w/w)]	<0.01
sorbic acid	[% (w/w)]	<0.01
fumaric acid	[% (w/w)]	<0.01
succinic acid	[% (w/w)]	0.24
lactic acid	[% (w/w)]	0.02
pyruvic acid	[% (w/w)]	<0.03
2-hydroxy butyric acid	[% (w/w)]	<0.01

The solid product after the drying step had the following composition:

Table 2: composition of solid product

component		
propionic acid	[% (w/w)]	36
acetic acid	[% (w/w)]	9.2
succinic acid	[% (w/w)]	4.2
calcium	[% (w/w)]	18.2
water	[% (w/w)]	16.5
further solids		balance

5

This example illustrates the step of water removal to form a first calcium propionate product. In commercial operation, the water removal step will be carried out by other methods, e.g., spray drying, but analogous results may be obtained.

10

Example 3: Work-up of glycerol fermentation broth

A glycerol fermentation broth obtained analogous to the second paragraph of Example 1 is processed as follows:

15

The fermentation broth obtained from the glycerol fermentation was subjected to a centrifugation step at 5000RPM. The sediment of the centrifugation step was the microorganism. The supernatant was processed further.

20

To the supernatant, active carbon was added in an amount of 0 wt.% per gram of calcium propionate, 0.1 wt.% per gram of calcium propionate, or 10 wt.% per gram of calcium propionate. The fermentation broth to which active carbon was added was kept for one hour at 60°C. The active carbon was

25

removed by vacuum filtration using a 0.2 micron filter.

As can be seen from the following Table, an active carbon treatment did not result in the removal of calcium propionate from the system:

Starting materials	Ca propionate [%w/w]	Ca succinate [%w/w]	Ca acetate [%w/w]	Glycerol [%w/w]
102°C - no active carbon	5.35	0.22	<1	<0.15
102°C - 0.1 (%w/w) carbon	5.35	0.23	<1	<0.15
102°C - 10 (%w/w) carbon	5.48	0.23	<1	<0.15

5

Solid calcium propionate was precipitated from the purified fermentation medium by concentrating the medium through evaporation of water. Evaporation was carried out at 102-103°C (medium temperature) in an oil bath of 150°C under atmospheric pressure. After about two hours, nucleation occurred, as could be seen from the formation of a haze. At that point in time, the temperature of the oil bath was reduced to 120°C to reduce the evaporation rate during crystallization. Some cool oil was added to the oil bath to accelerate the reduction in temperature. About one hour after the start of the nucleation, the evaporation was stopped.

15

The crystals were separated from the liquid using vacuum filtration or centrifugation.

20

Active carbon treatment resulted in a substantial decrease in color of the starting material for the evaporation step. Additionally, during the evaporation step, the solution darkened. This effect was much more pronounced for the sample which had not been subjected to an evaporation step than for

25

the sample which had been subjected to the evaporation step. This can be seen from the APHA values in the following table.

Activated carbon (%w/w)	Starting material [APHA]	Material obtained [APHA]
0	280	6600
0.1	110	4200
10	<5	1900

- 5 The solid product obtained from the samples subjected to an active carbon treatment also had a much better color than the product obtained from the sample wherein no active carbon treatment was carried out.

- 10 The composition of the products was as follows:

Wet cake samples	S/L separation	Ca propionate [%w/w]	Ca succinate [%w/w]	Ca acetate [%w/w]	Glycerol [%w/w]	MC loss at 80°C ¹
102°C - blank	Vacuum filtration (Buchner funnel, 4-7 µm)	58.62	1.21	1.21	<0.1	35%
102°C - blank	Centrifuge (5000rpm, 100 µm pore size, 1 minute)	76.46	0.47	1.21	<0.15	16%
102°C - 0.1 (%w/w) carbon	Centrifuge (10000rpm, 100 µm pore size, 1 minute)	87.92	0.28	1.34	<0.1	10%
102°C - 10 (%w/w) carbon	Vacuum filtration (Buchner funnel, 4-7 µm)	61.16	1.61	1.21	<0.1	25%

1 MC loss at 80°C stands for the moisture content of the materials, determined by keeping the material for 16 hours in an oven at a temperature of 80°C.

- 5 As can be seen from the above table, the use of a centrifuge for solid liquid separation gave better results than the use of a vacuum funnel. The relevancy of this result for commercial scale operation is limited.
- 10 The wet products were dried at 80°C for 16 hours to form a dried product.

CLAIMS

1. Process for manufacturing propionate products through fermentation, comprising the steps of
 - fermenting a carbon source selected from sugars and lactate in a fermentation medium by means of a propionic acid
- 5 producing microorganism to provide a first fermentation broth comprising a propionate salt,
 - recovering propionic acid producing microorganism from the first fermentation broth,
 - subjecting the first fermentation broth from which
- 10 propionic acid producing microorganism have been recovered to a water removal step to form a first propionate salt product,
 - fermenting a carbon source comprising glycerol with the propionic acid producing microorganism recovered from the first fermentation broth in the presence of an inorganic
- 15 alkaline salt to provide a second fermentation broth comprising a propionate salt,
 - subjecting the second fermentation broth to a purification step comprising at least one precipitation step, to form a second propionate salt product.
- 20
2. Process according to claim 1, wherein the propionate salt generated in the first and the second fermentation broth are salts of the same cation and preferably calcium propionate.
- 25
3. Process according to claim 1 or 2, wherein the carbon source selected from sugars and lactate is selected from glucose, lactate, and sucrose.
- 30 4. Process according to any one of the preceding claims, wherein in the step of fermenting a carbon source selected from sugars and lactate, the weight of the biomass has

increases in such a manner that the amount of biomass recovered is generally at least 10 times the weight of the biomass originally added, in particular at least 20 times, sometimes even at least 100 times.

5

5. Process according to any one of the preceding claims, wherein the first fermentation broth from which propionic acid producing microorganism have been recovered is subjected to a water removal step in its entirety.

10

6. Process according to any one of claims 1-4, wherein the step of subjecting the first fermentation broth from which propionic acid producing microorganism have been recovered is carried out by a process encompassing precipitation of a propionate salt, in particular calcium propionate, from the first fermentation broth.

15

7. Process according to any one of the preceding claims, wherein the carbon source comprising glycerol consists for at least 70 wt.% of glycerol, more in particular at least 80 wt.%, still more in particular at least 90 wt.%, calculated on the total amount of carbon source provided during the fermentation.

20

8. Process according to any one of the preceding claims, wherein the second fermentation broth is subjected first to a biomass removal step.

25

9. Process according to any one of the preceding claims, wherein in addition to propionate salts, other carboxylate salts, in particular succinate and/or acetate salts are also precipitated.

30

10. Process according to any one of claims 1-8, wherein succinate salts and propionate salts are precipitated sequentially from the second fermentation broth.

ABSTRACT

The invention pertains to a process for manufacturing propionate products through fermentation, comprising the steps of

- fermenting a carbon source selected from sugars and lactate in a fermentation medium by means of a propionic acid producing microorganism to provide a first fermentation broth comprising a propionate salt,
- recovering propionic acid producing microorganism from the first fermentation broth,
- subjecting the first fermentation broth from which propionic acid producing microorganism have been recovered to a water removal step to form a first propionate salt product,
- fermenting a carbon source comprising glycerol with the propionic acid producing microorganism recovered from the first fermentation broth in the presence of an inorganic alkaline salt to provide a second fermentation broth comprising a propionate salt,
- subjecting the second fermentation broth to a purification step comprising at least one precipitation step, to form a second propionate salt product.

The process according to the invention allows efficient manufacture of propionate salts products.



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(54) Title: PROCESS FOR MANUFACTURING PROPIONATE PRODUCTS

(57) Abstract: The invention pertains to a process for manufacturing propionate products through fermentation, comprising the steps of - fermenting a carbon source selected from sugars and lactate in a fermentation medium by means of a propionic acid producing microorganism to provide a first fermentation broth comprising a propionate salt, - recovering propionic acid producing microorganism from the first fermentation broth, - subjecting the first fermentation broth from which propionic acid producing microorganism have been recovered to a water removal step to form a first propionate salt product, - fermenting a carbon source comprising glycerol with the propionic acid producing microorganism recovered from the first fermentation broth in the presence of an inorganic alkaline salt to provide a second fermentation broth comprising a propionate salt, - subjecting the second fermentation broth to a purification step comprising at least one precipitation step, to form a second propionate salt product. The process according to the invention allows efficient manufacture of propionate salts products.



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Process for manufacturing propionate products

The present invention pertains to a process for manufacturing propionate products via a fermentation process.

Propionic acid can be manufactured through fermentation of a carbon source using a microorganism. For example, Zhu et al (Bioresource Technology 101 (2010) 8902-8902) describes a fermentation process for the manufacture of calcium propionate wherein glycerol is fermented using *Propionibacterium acidipropionici*. Calcium hydroxide is added during the fermentation to neutralize the acid generated during the fermentation, resulting in the formation of calcium propionate.

Propionate salts are attractive for many purposes. For example they are attractive for use in feed and food preservation, where they provide long lasting broad spectrum activity. Most important salts in this respect are calcium, sodium, potassium, and ammonium salts. The calcium salt is considered of particular interest. The propionate salt may be used in solution, or in the form of a solid salt.

A problem which has been found to occur in the preparation of propionate salts via fermentation resides in the complexity of the fermentation process, both as regards carbon sources which can be fermented, and as regards side products which are formed during fermentation. There is need in the art for a fermentation process for manufacturing propionate salts which combines a high yield of propionate salt with an efficient growth of the microorganism and the possibility of obtaining a high-purity propionate salt product.

The present invention provides a process which solves this problem.

The present invention pertains to a process for manufacturing
5 propionate products through fermentation, comprising the steps of

- fermenting a carbon source selected from sugars and lactate in a fermentation medium by means of a propionic acid producing microorganism to provide a first fermentation broth
10 comprising a propionate salt,

- recovering propionic acid producing microorganism from the first fermentation broth,

- subjecting the first fermentation broth from which propionic acid producing microorganism have been recovered to
15 a water removal step to form a first propionate salt product,

- fermenting a carbon source comprising glycerol with the propionic acid producing microorganism recovered from the first fermentation broth in the presence of an inorganic alkaline salt to provide a second fermentation broth

20 comprising a propionate salt,

- subjecting the second fermentation broth to a purification step comprising at least one precipitation step, to form a second propionate salt product.

25 In the process according to the invention, there are two sequential fermentation steps. In the first fermentation step, a carbon source selected from sugars and lactate is fermented under the formation of a propionate salt product stream. This step may also be indicated herein as the sugar
30 fermentation step. Then, the microorganisms obtained in the sugar fermentation step are recovered and used to ferment a carbon source comprising glycerol, to form a further propionate salt product stream. The two propionate salt product streams are processed separately.

By applying two different fermentations, i.e. a sugar-based fermentation and a glycerol-based fermentation, and treating the resulting streams separately, it has been found that it is possible to benefit from the advantageous effects of both types of fermentation. More specifically, it has been found that sugar fermentation can result in efficient cell growth, which makes for a large yield of biomass. The resulting product, however, may be relatively difficult to purify due to the presence of substantial amounts of acetate anions. In contrast, the glycerol fermentation yields a product which can be purified to high purity propionate salt products. However, the glycerol fermentation results only in limited biomass formation. By combining the fermentations in the manner prescribed, an efficient process is obtained. Further advantages of the process according to the invention will be discussed below.

The process according to the invention will be discussed in more detail below.

20

The first step in the process according to the invention is fermenting a carbon source selected from sugars and lactate in a fermentation medium by means of a propionic acid producing microorganism to provide a first fermentation broth comprising a propionate salt.

25

Fermentation processes for the manufacture of propionate are known in the art and require no further elucidation here. It is within the scope of the skilled person to select, using his common general knowledge, a suitable fermentation process, including fermentation conditions, and a suitable microorganism. In the selection of the microorganism, care should be taken to select an organism which is capable of fermenting sugars or lactate (in the first fermentation step

30

of the process according to the invention) and glycerol (in the second fermentation step of the process according to the invention).

5 The carbon source used in the first step of the process according to the invention is selected from sugars and lactate. Examples of suitable sugars and sources of sugar include glucose, sucrose, dextrose syrup, starch, hydrolysed starch, cellulose, hydrolysed cellulose, molasses, xylose,
10 lignocellulose hydrolysates, lactose and whey. Suitable lactate sources are sodium lactate, potassium lactate, ammonium lactate, magnesium lactate, calcium lactate, and lactic acid. Of the lactate sources, the use of calcium lactate may be preferred, since this will result in the
15 formation of calcium propionate, which is a preferred product of the invention. From a production point of view glucose, lactate, and sucrose may be preferred. From an availability point of view, sources like molasses and starch may be preferred.

20

At least when the carbon source is selected from sugars the fermentation is carried out in the presence of an inorganic alkaline salt. This alkaline salt is present to ensure that the pH during the fermentation does not decrease to an
25 unacceptable extent due to the manufacture of the propionic acid. Where the carbon source is a lactate salt, the addition of an inorganic base may not always be required because lactate is converted into propionate, which does not have a pH effect. Nevertheless, also in that case, an inorganic base
30 may be added for pH adjustment.

Suitable bases include hydroxides, oxides, and carbonates of calcium, magnesium, sodium, and potassium, resulting in the formation of a fermentation broth containing respectively calcium propionate, magnesium propionate, sodium propionate,

or potassium propionate. It is preferred within the process according to the invention to use an alkaline calcium salt, as this results in the formation of calcium propionate, which is a preferred product according to the invention. Suitable
5 alkaline calcium salts include hydroxides, oxides, and carbonates of calcium.

The next step is the removal of microorganism from the thus formed first fermentation broth. This step can be carried out
10 when sufficient biomass has been formed for the following glycerol fermentation step. As the microorganism is to be used in a further fermentation, care should be taken to isolate the microorganisms under such conditions that the organism is kept alive. Taking this into account, the removal
15 of microorganism may be carried out in manners known in the art, e.g., via filtration step or centrifugation step.

In the sugar fermentation, the weight of the biomass has increased, with the amount of biomass recovered being
20 generally at least 10 times the weight of the biomass originally added, in particular at least 20 times, sometimes even at least 100 times. In the present specification the weight of the biomass is calculated as dry weight, unless expressly indicated otherwise.

25 It is preferred for the removal of microorganisms from the first fermentation broth to be carried out in a relatively high yield. There are two reasons for this. In the first place, the biomass is intended to be used in the subsequent
30 glycerol fermentation. Further, biomass remaining in the fermentation broth may affect the properties of the product manufactured from this broth, e.g. in the form of a detrimental effect on product color. It is considered preferred for the biomass to be removed so that the resulting

product has a turbidity of at most 1000 NTU, in particular at most 500 NTU, more specifically at most 200 NTU. A turbidity of at most 100 NTU is preferred.

5 In a further step of the process according to the invention, the first fermentation broth from which propionic acid producing microorganism have been recovered is subjected to a water removal step to form a first propionate salt product, in particular a calcium propionate product. This water
10 removal step can be carried out in a number of ways.

In a first embodiment, the first fermentation broth from which propionic acid producing microorganism have been recovered is subjected to a water removal step in its
15 entirety. In this case, a first propionate salt product is formed which comprises all matter present in the first fermentation broth from which propionic acid producing microorganism have been recovered, including remaining nutrients. This drying step can be carried out in manners
20 known in the art for water removal, including spray drying, drum vacuum drying, thin film drying, e.g., agitated thin film drying, mechanically agitated drying, spray granulation, and freeze drying.

The resulting product is a solid material, in particular a
25 solid particulate material, with a propionate salt content in the range of 30-70 wt.%, in particular 40-60 wt.%, of propionate salt, in particular calcium propionate, with the remainder comprising one or more of further salts of carboxylic acids, in particular succinate and/or acetate
30 salts, and further components resulting from, e.g., the biomass and residual medium/nutrient components. In one embodiment, the resulting product comprises acetate salt in the range of 3-20 wt.%, in particular 8-15 wt.%, more in particular 10-13 wt.% and/or succinate salt in an amount of

0-10 wt.%, in particular 3-6 wt.%. It is preferred for calcium propionate, calcium succinate, and calcium acetate to make up at least 85 wt.% of the total first calcium propionate product, obtained in this manner, in particular at least 90 wt.%, more in particular between 90 and 98 wt.%. In the above, the percentages are calculated as anion (acetate, succinate, propionate) on the total anhydrous solid material.

It is noted that it is possible to include other components in the fermentation broth before spray drying, e.g., additional carboxylic acid salts such as lactates or acetates, carriers like maltodextrin, starch, milk solids, whey, flour or other functional ingredients like green tea extract, nisin, and rosemary extract. This may be attractive if it is desired to manufacture a product with a particular composition.

In another embodiment, the step of subjecting the first fermentation broth from which propionic acid producing microorganism have been recovered is carried out by a process encompassing precipitation of a propionate salt, in particular calcium propionate from the first fermentation broth. Precipitation may be initiated by concentration and other measures known to improve the yield of a precipitation step, e.g., reduction of temperature or addition of compounds which reduce solubility. Precipitation can be performed batchwise or continuous, in a single step or in multiple steps.

The precipitated propionate salt product, in particular calcium propionate is separated from the aqueous medium. This can be done by methods known in the art, including one or more of filtration, centrifugation, decantation, etc. It is within the scope of the skilled person to select a suitable

manner. If so desired, the propionate salt product can be washed to increase product purity. The washing liquid, if used, can, e.g., be water or a propionate salt solution. The washing liquid can be recycled to obtain a high yield. The solid calcium propionate product can be dried as desired.

In this embodiment it is possible to precipitate all types of carboxylate salts, in particular calcium carboxylate salts present in the system, in particular calcium propionate in combination with calcium succinate (if present) and/or calcium acetate (if present). In this case, a final product may, e.g., be obtained with a propionate salt content, in particular calcium propionate in the range of 30-80 wt.%, in particular 40-70 wt.%, acetate salt, in particular calcium acetate in the range of 3-20 wt.%, in particular 8-15 wt.%, more in particular 10-13 wt.% and succinate salt, in particular calcium succinate, in an amount of 0-10 wt.%, in particular 3-6 wt.%. It is preferred for calcium propionate, calcium succinate, and calcium acetate to make up at least 85 wt.% of the total first calcium propionate product, obtained in this manner, in particular at least 90 wt.%, more in particular at least 95%. In the above, the percentages are calculated as anion (acetate, succinate, propionate) on the total anhydrous solid material.

It is also possible to sequentially precipitate succinate salts and propionate salts from the first fermentation broth, this can, e.g., be done via a process for manufacturing a solid propionate salt from a mixture comprising propionate and succinate comprising the steps of

- providing an aqueous medium comprising succinate anions and propionate anions,
- providing an inorganic soluble salt in the aqueous medium in such an amount that the majority of succinate ions is

converted to solid succinate salt while the majority of propionate ions remains in solution,

- separating the aqueous medium comprising the majority of propionate ions from the solid succinate salt,

5 - increasing the concentration of salt in the aqueous medium to convert the majority of propionate ions to solid propionate salt,

- separating the solid propionate salt from the aqueous medium.

10

As indicated above, the propionic acid producing microorganism is recovered from the first fermentation broth. In a next step in the process according to the invention, a carbon source comprising glycerol is fermented with the propionic acid producing microorganism recovered from the first fermentation broth in the presence of an alkaline salt to provide a second fermentation broth comprising a propionate salt.

15

Fermentation processes for the manufacture of propionic acid starting from a carbon source comprising glycerol are known in the art and require no further elucidation here. It is within the scope of the skilled person to select, using his common general knowledge, a suitable fermentation process, including fermentation conditions.

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The carbon source used in this fermentation step comprises glycerol. It has been found that the use of glycerol as carbon source makes it possible to obtain a fermentation process which generates relatively high amounts of propionate salt, in particular calcium propionate in combination with relatively low amounts of acetate salts. This is attractive because the separation of propionate salts and acetate salts is generally complicated. It is preferred for the carbon source to consist for at least 70 wt.% of glycerol, more in

30

particular at least 80 wt.%, still more in particular at least 90 wt.%, calculated on the total amount of carbon source provided during the fermentation.

5 A base is added during the fermentation step to compensate for the pH decrease which would occur due to the production of the carboxylic acids.

Suitable bases include hydroxides, oxides, and carbonates of calcium, magnesium, sodium, and potassium, resulting in the
10 formation of a fermentation broth containing respectively calcium propionate, magnesium propionate, sodium propionate, or potassium propionate. It is preferred within the process according to the invention to use an alkaline calcium salt, as this results in the formation of calcium propionate, which
15 is a preferred product according to the invention. Suitable alkaline calcium salts include hydroxides, oxides, and carbonates of calcium.

The alkaline salts used in the two fermentation steps may be the same or different. For reasons of efficiency it may be
20 preferred for the alkaline salt provided in the fermenting of a carbon source selected from sugars and lactate and the alkaline salt provided in the fermenting of a carbon source comprising glycerol to be salts of the same cation. The use of calcium salts is particularly preferred.

25 Once the fermentation has run to the desired extent, the second fermentation broth is subjected to a purification step comprising at least one precipitation step, to form a second calcium propionate product.

30 In general, the second fermentation broth is subjected first to a biomass removal step, which can be carried out in manners known in the art. Reference is made to what is stated above for the removal of biomass from the first fermentation broth. As for the step described above, it is considered

preferred for the biomass to be removed so that the resulting product has a turbidity of at most 1000 NTU, in particular at most 500 NTU, more specifically at most 200 NTU. A turbidity of at most 100 NTU is preferred. It has been found that the presence of biomass may interfere with the further precipitation step in the process according to the invention. The biomass isolated in this step may be provided to a second glycerol-based fermentation, if this is desired.

- 10 The resulting broth from which biomass has been removed is subjected to at least one precipitation step, to form a second propionate salt product.

Before the precipitation step, the medium may if so desired be contacted with an adsorbent, in particular an active carbon adsorbent, to remove impurities.

The precipitation step can be carried out as described above. Precipitation may be initiated by concentration of the medium, and/or by other measures known to improve the yield of a precipitation step, e.g., reduction of temperature or addition of compounds which reduce solubility. Reference is made to what has been stated above on the precipitation step. The precipitated propionate salt product, in particular calcium propionate is separated from the aqueous medium. This can be done by methods known in the art, including one or more of filtration, centrifugation, decantation, etc. It is within the scope of the skilled person to select a suitable manner. If so desired, the propionate salt product can be washed to increase product purity. The washing liquid, if used, can, e.g., be water or a propionate salt solution. The washing liquid can be recycled to obtain a high yield. The solid propionate salt product can be dried as desired.

In one embodiment, in addition to propionate salts, other carboxylate salts, in particular succinate and/or acetate salts are also precipitated. In particular calcium carboxylate salts present in the system may be precipitated together, in particular calcium propionate in combination with calcium succinate (if present) and/or calcium acetate (if present). In this case, a final product may, e.g., be obtained with a propionate salt content, in particular calcium propionate in the range of 50-90 wt.%, in particular 60-80 wt.%, more in particular 70-80 wt.%, acetate salt, in particular calcium acetate in the range of 0-5 wt.%, in particular 0-2 wt.%, and succinate salt, in particular calcium succinate, in an amount of 0-20 wt.%, in particular 0-10 wt.%, more in particular 0-5 wt.%. It is preferred for calcium propionate, calcium succinate, and calcium acetate to make up at least 85 wt.% of the total first calcium propionate product, obtained in this manner, in particular at least 90 wt.%, more in particular at least 95%. In the above, the percentages are calculated as anion (acetate, succinate, propionate) on the total anhydrous solid material.

It is also possible to sequentially precipitate succinate salts and propionate salts from the second fermentation broth, this can, e.g., be done via a process for manufacturing a solid propionate salt from a mixture comprising propionate and succinate comprising the steps of

- providing an aqueous medium comprising succinate anions and propionate anions,
- providing an inorganic soluble salt in the aqueous medium in such an amount that the majority of succinate ions is converted to solid succinate salt while the majority of propionate ions remains in solution,
- separating the aqueous medium comprising the majority of propionate ions from the solid succinate salt,

- increasing the concentration of salt in the aqueous medium to convert the majority of propionate ions to solid propionate salt,
- separating the solid propionate salt from the aqueous medium.

It will be clear to the skilled person that the various embodiments and preferences describes herein can be combined, unless they are presented as mutually excluding alternatives.

The present invention will be elucidated by the following examples, without being limited thereto or thereby.

Example 1: Glucose fermentation followed by glycerol fermentation

A fermenter comprising 5 kg medium was inoculated with a culture of *Propionibacterium acidipropionici*. The medium contained 80g/kg glucose and 52g/kg YEP (yeast extract paste, 50% dry solids). The fermenter was operated at 30°C and with a pH of 6.5. The pH was controlled by adding Ca(OH)₂ solution. After ±45 hours the fermentation was stopped. The fermentation broth was centrifuged at 5000RPM. The microorganism was present in the sediment of the centrifugation step.

In this step, the amount of biomass was increased from 20 gram added initially to 150 gram harvested, both calculated as dry weight.

24 grams of biomass was added to a fermenter comprising 1kg medium containing 80g/kg glycerol and 52g/kg YEP. The fermenter was operated at 30°C and with a pH of 7.0. The pH was controlled by adding Ca(OH)₂ solution.

The fermentation yielded a volumetric productivity of 1.2 gram calcium propionate per kilogram medium per hour. The total yield of the fermentation was 72 wt.%, calculated as grams propionate per gram glycerol.

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Example 2: Work-up of glucose fermentation broth

A glucose fermentation was carried out analogous to that described in the first paragraph of Example 1. The biomass
10 was isolated therefrom by centrifugation as described therein. 1000 grams of the remaining fermentation broth was dried using a rotary evaporator, first under atmospheric pressure, then under vacuum. After 6 hours, a dry product was obtained.

15

The fermentation broth comprised the following organic acids, as determined using GC

Table 1: GC organic acids in fermentation broth

component		
formic acid	[% (w/w)]	<0.02
acetic acid	[% (w/w)]	0.57
propionic acid	[% (w/w)]	2.4
ethanol	[% (w/w)]	<0.02
maleic acid	[% (w/w)]	<0.01
oxalic acid	[% (w/w)]	<0.01
sorbic acid	[% (w/w)]	<0.01
fumaric acid	[% (w/w)]	<0.01
succinic acid	[% (w/w)]	0.24
lactic acid	[% (w/w)]	0.02
pyruvic acid	[% (w/w)]	<0.03
2-hydroxy butyric acid	[% (w/w)]	<0.01

20

The solid product after the drying step had the following composition:

Table 2: composition of solid product

component		
propionic acid	[% (w/w)]	36
acetic acid	[% (w/w)]	9.2
succinic acid	[% (w/w)]	4.2
calcium	[% (w/w)]	18.2
water	[% (w/w)]	16.5
further solids		balance

5

This example illustrates the step of water removal to form a first calcium propionate product. In commercial operation, the water removal step will be carried out by other methods, e.g., spray drying, but analogous results may be obtained.

10

Example 3: Work-up of glycerol fermentation broth

A glycerol fermentation broth obtained analogous to the second paragraph of Example 1 is processed as follows:

15

The fermentation broth obtained from the glycerol fermentation was subjected to a centrifugation step at 5000RPM. The sediment of the centrifugation step was the microorganism. The supernatant was processed further.

20

To the supernatant, active carbon was added in an amount of 0 wt.% per gram of calcium propionate, 0.1 wt.% per gram of calcium propionate, or 10 wt.% per gram of calcium propionate. The fermentation broth to which active carbon was added was kept for one hour at 60°C. The active carbon was

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removed by vacuum filtration using a 0.2 micron filter.

As can be seen from the following Table, an active carbon treatment did not result in the removal of calcium propionate from the system:

Starting materials	Ca propionate [%w/w]	Ca succinate [%w/w]	Ca acetate [%w/w]	Glycerol [%w/w]
102°C – no active carbon	5.35	0.22	<1	<0.15
102°C – 0.1 (%w/w) carbon	5.35	0.23	<1	<0.15
102°C – 10 (%w/w) carbon	5.48	0.23	<1	<0.15

5

Solid calcium propionate was precipitated from the purified fermentation medium by concentrating the medium through evaporation of water. Evaporation was carried out at 102-103°C (medium temperature) in an oil bath of 150°C under atmospheric pressure. After about two hours, nucleation occurred, as could be seen from the formation of a haze. At that point in time, the temperature of the oil bath was reduced to 120°C to reduce the evaporation rate during crystallization. Some cool oil was added to the oil bath to accelerate the reduction in temperature. About one hour after the start of the nucleation, the evaporation was stopped.

The crystals were separated from the liquid using vacuum filtration or centrifugation.

Active carbon treatment resulted in a substantial decrease in color of the starting material for the evaporation step. Additionally, during the evaporation step, the solution darkened. This effect was much more pronounced for the sample which had not been subjected to an evaporation step than for

25

the sample which had been subjected to the evaporation step. This can be seen from the APHA values in the following table.

Activated carbon (%w/w)	Starting material [APHA]	Material obtained [APHA]
0	280	6600
0.1	110	4200
10	<5	1900

- 5 The solid product obtained from the samples subjected to an active carbon treatment also had a much better color than the product obtained from the sample wherein no active carbon treatment was carried out.

- 10 The composition of the products was as follows:

Wet cake samples	S/L separation	Ca propionate [%w/w]	Ca succinate [%w/w]	Ca acetate [%w/w]	Glycerol [%w/w]	MC loss at 80°C ¹
102°C - blank	Vacuum filtration (Buchner funnel, 4-7 µm)	58.62	1.21	1.21	<0.1	35%
102°C - blank	Centrifuge (5000rpm, 100 µm pore size, 1 minute)	76.46	0.47	1.21	<0.15	16%
102°C - 0.1 (%w/w) carbon	Centrifuge (10000rpm, 100 µm pore size, 1 minute)	87.92	0.28	1.34	<0.1	10%
102°C - 10 (%w/w) carbon	Vacuum filtration (Buchner funnel, 4-7 µm)	61.16	1.61	1.21	<0.1	25%

1 MC loss at 80°C stands for the moisture content of the materials, determined by keeping the material for 16 hours in an oven at a temperature of 80°C.

- 5 As can be seen from the above table, the use of a centrifuge for solid liquid separation gave better results than the use of a vacuum funnel. The relevancy of this result for commercial scale operation is limited.
- 10 The wet products were dried at 80°C for 16 hours to form a dried product.

CLAIMS

1. Process for manufacturing propionate products through fermentation, comprising the steps of

- fermenting a carbon source selected from sugars and lactate in a fermentation medium by means of a propionic acid

5 producing microorganism to provide a first fermentation broth comprising a propionate salt,

- recovering propionic acid producing microorganism from the first fermentation broth,

10 - subjecting the first fermentation broth from which propionic acid producing microorganism have been recovered to a water removal step to form a first propionate salt product,

- fermenting a carbon source comprising glycerol with the propionic acid producing microorganism recovered from the first fermentation broth in the presence of an inorganic

15 alkaline salt to provide a second fermentation broth comprising a propionate salt,

- subjecting the second fermentation broth to a purification step comprising at least one precipitation step, to form a second propionate salt product.

20

2. Process according to claim 1, wherein the propionate salt generated in the first and the second fermentation broth are salts of the same cation and preferably calcium propionate.

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3. Process according to claim 1 or 2, wherein the carbon source selected from sugars and lactate is selected from glucose, lactate, and sucrose.

30 4. Process according to any one of the preceding claims, wherein in the step of fermenting a carbon source selected from sugars and lactate, the weight of the biomass has

increases in such a manner that the amount of biomass recovered is generally at least 10 times the weight of the biomass originally added, in particular at least 20 times, sometimes even at least 100 times.

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5. Process according to any one of the preceding claims, wherein the first fermentation broth from which propionic acid producing microorganism have been recovered is subjected to a water removal step in its entirety.

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6. Process according to any one of claims 1-4, wherein the step of subjecting the first fermentation broth from which propionic acid producing microorganism have been recovered is carried out by a process encompassing precipitation of a propionate salt, in particular calcium propionate, from the first fermentation broth.

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7. Process according to any one of the preceding claims, wherein the carbon source comprising glycerol consists for at least 70 wt.% of glycerol, more in particular at least 80 wt.%, still more in particular at least 90 wt.%, calculated on the total amount of carbon source provided during the fermentation.

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8. Process according to any one of the preceding claims, wherein the second fermentation broth is subjected first to a biomass removal step.

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9. Process according to any one of the preceding claims, wherein in addition to propionate salts, other carboxylate salts, in particular succinate and/or acetate salts are also precipitated.

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10. Process according to any one of claims 1-8, wherein succinate salts and propionate salts are precipitated sequentially from the second fermentation broth.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/055753A. CLASSIFICATION OF SUBJECT MATTER
INV. C12P7/52
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

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 practicable, search terms used)

EPO-Internal, FSTA, BIOSIS, EMBASE, WPI Data, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DUARTE ET AL: "Microbial production of propionic and succinic acid from sorbitol using Propionibacterium acidipropionici", AMB EXPRESS, vol. 5, 20 February 2015 (2015-02-20), pages 1-8, XP002738774, * See pages 1-2 (Introduction) and page 6 (Table 5); online publication * ----- -/--	1-10



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 application or patent but published on or after the international filing date

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"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

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Date of the actual completion of the international search

12 April 2016

Date of mailing of the international search report

26/04/2016

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2016/055753

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DISHISHA ET AL: "Improved propionic acid production from glycerol: combining cyclic batch- and sequential batch fermentations with optimal nutrient composition", BIORESOURCE TECHNOLOGY, vol. 176, 11 November 2014 (2014-11-11), pages 80-87, XP029103745, * See pages 80-81 (Introduction); early online publication *	1-10
A	STOWERS ET AL: "Development of an industrializable fermentation process for propionic acid production", JOURNAL OF INDUSTRIAL MICROBIOLOGY AND BIOTECHNOLOGY, vol. 41, 2014, pages 837-852, XP035330832, * See page 841 (Table 1) *	1-10
A	DISHISHA ET AL: "An economical biorefinery process for propionic acid production from glycerol and potato juice using high cell density fermentation", BIORESOURCE TECHNOLOGY, vol. 135, 2013, pages 504-512, XP028579581, * See pages 504-505 (Introduction) *	1-10
A	LIU ET AL: "Glycerol/glucose co-fermentation: One more proficient process to produce propionic acid by Propionibacterium acidipropionici", CURRENT MICROBIOLOGY, vol. 62, 2011, pages 152-158, XP019869364, * See page 154 (Table 1) and page 157 (Figure 4) *	1-10
A	KOSMIDER ET AL: "Propionic acid production by Propionibacterium freudenreichii ssp. shermanii using crude glycerol and whey lactose industrial wastes", POLISH JOURNAL OF ENVIRONMENTAL STUDIES, vol. 19, 2010, pages 1249-1253, XP002738776, * See pages 1252-1253 (Conclusions) *	1-10
A	JIANG ET AL: "Enhanced propionic acid production from whey lactose with immobilized Propionibacterium acidipropionici and the role of trehalose synthesis in acid tolerance", GREEN CHEMISTRY, vol. 17, 13 August 2014 (2014-08-13), pages 250-259, XP002756359, * See page 250 (Abstract); early online publication *	1-10

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To:

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Overschiestraat 180
1062 XK Amsterdam
PAYS-BAS

Date of mailing (day/month/year) 11 April 2016 (11.04.2016)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference WO23743OG/pb	
International application No. PCT/EP2016/055753	International filing date (day/month/year) 17 March 2016 (17.03.2016)

1. The following indications appeared on record concerning:		
☐ the applicant	☒ the inventor	☐ the agent
☐ the common representative		
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2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:		
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