HU000034273T2

(19) **HU**

(11) Lajstromszám: **E 034 273**

(13) **T2**

MAGYARORSZÁG
Szellemi Tulajdon Nemzeti Hivatala

EURÓPAI SZABADALOM
SZÖVEGÉNEK FORDÍTÁSA

(21) Magyar ügyszám: **E 10 766273**
(22) A bejelentés napja: **2010. 10. 14.**

(96) Az európai bejelentés bejelentési száma:
EP 20100766273
(97) Az európai bejelentés közzétételi adatai:
EP 2488651 A1 **2011. 04. 21.**
(97) Az európai szabadalom megadásának meghirdetési adatai:
EP 2488651 B1 **2017. 05. 24.**

(51) Int. Cl.: ***C12P 1/00*** *(2006.01)*
C12P 7/56 *(2006.01)*
C12P 7/46 *(2006.01)*

(86) A nemzetközi (PCT) bejelentési szám:
PCT/EP 10/065395
(87) A nemzetközi közzétételi szám:
WO 11045365

(30) Elsőbbségi adatok:
09172984 **2009. 10. 14.** **EP**

(72) Feltaláló(k):
VAN BREUGEL, Jan, NL-4285 WN Woudrichem (NL)
GROOT, Willem Jacob, NL-3315 DH Dordrecht (NL)
JANSEN, Peter Paul, NL-5342 AM Oss (NL)

(73) Jogosult(ak):
PURAC Biochem BV, 4206 AC Gorinchem (NL)

(74) Képviselő:
SBGK Szabadalmi Ügyvivői Iroda, Budapest

(54) **Vízelvétellel járó, csökkentett nyomású fermentációs eljárás**

Az európai szabadalom ellen, megadásának az Európai Szabadalmi Közlönyben való meghirdetésétől számított kilenc hónapon belül, felszólalást lehet benyújtani az Európai Szabadalmi Hivatalnál. (Európai Szabadalmi Egyezmény 99. cikk(1))

A fordítást a szabadalmas az 1995. évi XXXIII. törvény 84/H. §-a szerint nyújtotta be. A fordítás tartalmi helyességét a Szellemi Tulajdon Nemzeti Hivatala nem vizsgálta.

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 2 488 651 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
24.05.2017 Bulletin 2017/21

(21) Application number: **10766273.6**

(22) Date of filing: **14.10.2010**

(51) Int Cl.:
C12P 1/00 *(2006.01)* ***C12P 7/46*** *(2006.01)*
C12P 7/56 *(2006.01)*

(86) International application number:
PCT/EP2010/065395

(87) International publication number:
WO 2011/045365 (21.04.2011 Gazette 2011/16)

(54) **FERMENTATION PROCESS AT REDUCED PRESSURE WITH WATER REMOVAL**

FERMENTATIONSVERFAHREN BEI VERMINDERTEM DRUCK MIT WASSERENTFERNUNG

PROCÉDÉ DE FERMENTATION SOUS PRESSION RÉDUITE AVEC ÉLIMINATION D'EAU

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

(30) Priority: **14.10.2009 EP 09172984**

(43) Date of publication of application:
22.08.2012 Bulletin 2012/34

(73) Proprietor: **PURAC Biochem BV**
4206 AC Gorinchem (NL)

(72) Inventors:
- **VAN BREUGEL, Jan**
  NL-4285 WN Woudrichem (NL)
- **GROOT, Willem Jacob**
  NL-3315 DH Dordrecht (NL)
- **JANSEN, Peter Paul**
  NL-5342 AM Oss (NL)

(74) Representative: **De Vries & Metman**
Overschiestraat 180
1062 XK Amsterdam (NL)

(56) References cited:
- **BALLONGUE ET AL: "Enhancement of solvents production by Clostridium acetobutylicum cultivated on a reducing compounds depletive medium", BIOMASS, vol. 10, 1986, pages 121-129, XP002588300,**
- **CHENG ET AL: "Proteomic insights into adaptive responses of Saccharomyces cerevisiae to the repeated vacuum fermentation", APPLIED AND MICROBIAL BIOTECHNOLOGY, vol. 83, 2 June 2009 (2009-06-02), pages 909-923, XP019705627,**
- **YANCHEVSKII: "Composition of semi-manufactured products during single-phase vacuum processing of molasses mash (TRANSLATED)", FERMENTNAYA I SPIRTOVAYA PROMYSHLENNOST (UKRAINE), vol. 3, 1984, pages 6-9, XP008125515,**
- **VIRKAR ET AL: "Glycerol production by anaerobic vacuum fermentation of molasses on pilot scale", BIOTECHNOLOGY AND BIOENGINEERING, vol. 29, 1987, pages 773-774, XP002597142,**
- **DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1990, Sakaguchi et al: "The influence of reduced pressure on the growth of yeast cells and the production of volatile compounds / Hakkokogaku Kaishi; 68(4), pages 261-266, 1990", XP002597143, Database accession no. PREV199090085949**
- **VAN DEN BOSCH ET AL / WAGENINGEN UNIVERSITY: "Growth of Caldicellulosiruptor saccharolyticus under reduced pressure", , page 1, XP002597144, Retrieved from the Internet: URL:www.biohydrogen.nl/downloadattachment/20602/posterfonsstam2.pdf [retrieved on 2010-08-18]**

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

EP 2 488 651 B1

Description

[0001] Fermentation processes are well known in the art. They are used to manufacture a number of different components such as acids, including lactic acid, acetic acid, propionic acid, and succinic acid, alcohols, such as ethanol, propanol, butanol, and erythritol, and numerous other components of biological origin such as vitamins.

[0002] In the art, there is a continuous need for improved fermentation processes. The present invention now provides an improved fermentation process, which solves a number of problems associated with various fermentation processes known in the art.

[0003] The present invention pertains to a fermentation process wherein a carbohydrate source is contacted under fermentation conditions with a microorganism in an aqueous fermentation broth to form a fermentation product which is a salt or a product with a boiling point above the boiling point of water, wherein the fermentation process is carried out at a pressure which is below atmospheric pressure and at least at the value where the reaction medium is at its boiling point at the fermentation temperature, with water being evaporated and removed from the reactor during the fermentation in an amount which is at least 20% of the volume of liquid present in the reactor at the start of the fermentation.

[0004] The crux of the present invention is that the fermentation process is carried out at reduced pressure, that is, at a pressure below atmospheric pressure, resulting in the evaporation and removal of a substantial amount of water. The lower limit of the pressure is provided by the consideration that the reaction medium should be in the liquid phase under fermentation conditions. This means that at the fermentation temperature the pressure should be such that the fermentation medium is at its boiling temperature, or higher. This will be discussed in more detail below.

[0005] The fermentation process according to the invention has a number of advantages.

[0006] It is one feature of the invention that it solves the problem of a surplus of water associated with some fermentation processes known in the art. In a fermentation process the feed source, generally a carbohydrate source, is added to the reactor in combination with water, for example in dissolved form. Further, additional streams such as pH regulating agents may sometimes be added, again in the form of aqueous solutions. This may lead to the formation of dilute product streams, which may necessitate the use of water removal steps, e.g., during the process. However, this has a number of disadvantages. In the first place, where reaction medium is removed, this may be associated with the removal of liquid product, which leads to a yield loss in the process. Further, when the volume of water removed during or after the fermentation is large, this requires the use of large apparatus which, by its nature, is expensive. Accordingly, there is need for a fermentation process which reduces the surplus water in the fermentor in a cost-effective way.

[0007] It has now been found that this problem can be solved in an elegant manner by carrying out the fermentation under reduced pressure. The reduction of pressure leads to an increased evaporation of water which is then removed, and therewith to more concentrated product streams.

[0008] A further advantage of the process according to the invention resides in the removal of heat generated during the fermentation.

[0009] Conventionally, fermentation processes are carried out at constant temperature. The heat generated during the fermentation needs to be removed from the system. In the process according to the invention the application of a vacuum results in evaporation of liquid, and this leads to the removal of heat from the system. Accordingly, it is an advantage of the process according to the invention that it reduces the need for removal of heat generated during the fermentation process.

[0010] To keep the fermentation temperature at the required value, it may be necessary to provide additional heat to the system. When low quality waste heat is used to provide this additional heat, the process according to the invention can be carried out in a process-efficient manner. Suitable low quality waste heat may derive from the fermentation process itself, from concentration steps further on in the procedure, or from other available sources. Therefore, in one embodiment, the process according to the invention can be provided with low quality waste heat. Within the context of the present specification, the wording low quality waste heat refers to heat derived from hot utility streams with a temperature of below 100°C, more in particular below 70°C. The use of streams with a supply temperature between 5 and 25°C above the fermentation temperature, more in particular between 5 and 15°C is particularly attractive.

[0011] It is noted that fermentation processes have been described in the art wherein a reduced pressure is applied to remove a reaction product with a higher volatility than water, and wherein co-evaporated water is partly condensed and fed back to the reaction vessel, e.g., using a condensor. It will be evident to the skilled person that where the present specification mentions that water is removed, it is meant that it is indeed removed, and not evaporated, condensed, and fed back directly to the reactor.

[0012] It is also noted that J. Ballonge et al. (Enhancement of Solvents Production by Clostridium acebutylicum Cultivated on a Reducing Compounds Depletive Medium, Biomass, 1986, pp. 121-129) describes the formation of acetone through a fermentation process. It is stated that reduced pressure may be applied to remove hydrogen from the medium to suppress the formation of the side-product n-butanol which is toxic for the microorganism. This reference does not disclose water being evaporated and removed from the reactor during the fermentation in an amount which is at least 20% of the volume of liquid present in the reactor at the start of the fermen-

tation.

[0013] It is a further feature of the present invention that an increased yield of fermentation product may be obtained as compared to fermentation systems where water is removed through other means, e.g., as a side stream. This may go for either or both of the yield of desired product per gram substrate and the yield of desired product per liter fermentation medium. This is because removal of the surplus water through evaporation as a result of the application of reduced pressure is not accompanied by removal of fermentation product, in contrast with removal through other means, e.g., as a side stream. Further, because in the process according to the invention the water is removed through evaporation, the water stream removed from the reactor may not require additional purification.

[0014] Additionally, it has been found that the process of the present invention may lead to an improved fermentation quality. Not wishing to be bound by any theory it is suspected that the application of reduced pressure may result in the evaporation of low-boiling contaminants which inhibit the microorganism, or which cause off-odours, off-flavours, or other undesirable properties in the final product.

[0015] The process of the present invention is applicable to the formation of fermentation products which are salts, or which have a boiling point above the boiling point of water. If the fermentation product has a boiling point below the boiling point of water, it will be evaporated and removed with the water at reduced pressure. This does not solve the problem underlying the present invention, which is the provision of a fermentation broth with reduced water content and increased product yield.

[0016] The process according to the invention finds application in numerous types of fermentation processes. It has been found to be particularly suitable for the fermentation processes which are carried out under anaerobic conditions.

[0017] Suitable products that may be manufactured via the process according to the invention include organic acids (or the salts thereof), such as lactic acid, acetic acid, propionic acid, and succinic acid, alcohols, such as butanol, and erythritol, and numerous other components of biological origin such as vitamins. The manufacture of organic acids as main product is preferred, with the manufacture of lactic acid and succinic acid (or the salts thereof) being particularly preferred.

[0018] In the process of the invention it is intended that fermentation product or products towards which the fermentation is aimed, also indicated as the main fermentation products, are salt(s) and/or have a boiling point above the boiling point of water. That is, they are to remain in the fermentation medium in liquid or solid form. This does not exclude the formation of by-products which are not salts and have a boiling point below the boiling point of water, and which will thus be removed in whole or in part during the fermentation process due to the application of the vacuum. The total yield of main fermentation product (or products) will generally be at least 20 wt.%, calculated as gram of product per gram of converted substrate, in particular, at least 30 wt.%, more in particular at least 50 wt.%. Depending on the nature of the fermentation, the total yield of main fermentation product (or products) may be at least 60 wt.%, calculated as gram of product per gram of converted substrate, sometimes at least 80 wt.%, more in particular at least 80 wt.%, or even at least 90wt.%.

[0019] It is noted that the present invention is not directed to fermentation processes aimed at the manufacture of ethanol, wherein the total of ethanol and carbon dioxide makes up 70 wt.% or more of the product, calculated on the amount of converted substrate. It is noted that the present invention is not directed to fermentation processes wherein hydrogen is the main product.

[0020] As discussed above, the fermentation is carried out at a pressure which is below atmospheric pressure. The minimum pressure at which the process can be carried out is determined by the fermentation temperature. The pressure should be at least so high that the fermentation medium is at or below its boiling point. If temperature of the fermentation medium is above its boiling point, the fermentation medium will vapourise and the temperature will drop until the medium is at its boiling point. In other words, the minimum pressure for the process according to the invention is the vapour pressure of the liquid at fermentation temperature.

[0021] The boiling point of a reaction medium is the temperature at which the vapour pressure of the liquid equals the environmental pressure surrounding the liquid. Boiling is the process in which molecules anywhere in the liquid escape into the vapour phase, resulting in the formation of bubbles in the liquid. The boiling point may also be indicated as the saturation temperature, namely the temperature where the liquid, at a given pressure, is saturated with thermal energy, and wherein the addition of any energy will result in a phase transition.

[0022] The fermentation will be effected at a fermentation temperature which is selected on a case by case basis. Considerations include maximum yield of the desired product and maximum conversion. It is within the scope of the skilled person to select appropriate conditions. The temperature may vary over the fermentation process, for example to optimise growth at the beginning of the process and yield nearer the end of the process. This may result in the variation of fermentation pressure.

[0023] Incidentally, it should be noted that surprisingly it has been found possible to carry out the process of the present invention at a pressure at which the fermentation medium boils. Apparently, the microorganism is not detrimentally affected by being surrounded by a boiling medium. Accordingly, it is a particular embodiment of the present invention for the pressure to be selected such that the aqueous fermentation medium boils at the temperature at which fermentation is carried out.

[0024] The pressure applied in the process according to the invention is below atmospheric pressure, to in-

crease evaporation of water as compared to a process carried out at atmospheric pressure. For example, the pressure may be selected to be between 20 mbar and 950 mbar, more in particular between 40 and 500 mbar.

[0025] In the process according to the invention water is evaporated and removed from the reactor during the fermentation in an amount which is at least 20% of the volume of liquid present in the reactor at the start of the fermentation. If the amount of water which is removed is less, the advantages associated with the present invention may not be obtained to such an extent that they balance the costs associated therewith. The amount of water that is desired to be removed will depend on a number of features. One feature is the concentration of the substrate steam. If the substrate is provided in a relatively dilute form, e.g., for cellulose hydrolysates, it will be desirable to remove more water than when the substrate is provided in a more concentrated form. If a dilute stream is added during the fermentation, e.g., a dilute stream of a pH regulating agent such as a base, it will be desirable to remove more water than when no such stream is provided, or when a more concentrated stream is provided. The upper limit for the amount of water removed will depend on the amount of water remaining in the reaction vessel. This should be sufficient to allow proper mixing of the reactor. It is within the scope of the skilled person to determine the suitable amount of water to be removed, taking the above into account.

[0026] Depending on these various parameters, the amount of water removed during the fermentation may be at least 40% of the volume of liquid present in the reactor at the start of the fermentation, more in particular at least 50%. In the case of dilute substrate streams or other liquids provided to the reactor, the amount of water may be much higher, e.g., at least 75%, or in some cases at least 100%.

[0027] In general, the amount of water removed in the process according to the invention is regulated by selecting the time during which the pressure is reduced and the amount of heat that is supplied to the fermentation medium. Taking the above into account it is within the scope of the skilled person to select the fermentation conditions such that the desired amount of water is removed.

[0028] In one embodiment of the present invention, water is added during the fermentation, as will be discussed in more detail below. In this embodiment it may be preferred for the pressure to be selected such that the volume of water removed from the reactor by evaporation is at least 1%, in particular at least 5%, more in particular at least 10% of the total of water added during the fermentation. In this embodiment the volume of water removed is often at most 50% of the total of water added during the fermentation.

[0029] In some fermentation processes, aqueous solutions are added during the fermentation, for example to add pH control agents or in fed batch processes. In these processes, the removal of water may be of particular interest, because in some cases the amount of water added during fermentation may be quite substantial. As an example the situation may be mentioned where a high substrate concentration inhibits the fermentation, but where the product does not, for example because it is solid.

[0030] In one embodiment the present invention pertains to a fermentation process to manufacture a product comprising a salt of an acid. In these fermentation processes, the microorganism produces an acid, and a solution of a base is added to the fermentation medium to keep the pH within the range required for the microorganism at issue, converting the acid in whole or in part to its corresponding salt. This base addition may be accompanied by the addition of undesirably large amounts of water.

[0031] Acids that may be manufactured via the process according to the invention include carboxylic acids, in particular carboxylic acids selected from the group consisting of mono-, di-, and tricarboxylic acids having 2-8 carbon atoms. Examples include lactic acid, propionic acid, citric acid, malic acid, maleic acid, fumaric acid, adipic acid, succinic acid, tartaric acid, alpha-ketoglutaric acid, oxaloacetic acid, acetic acid, and acrylic acid, or salts thereof. At this point in time fermentation processes aimed at manufacturing lactic acid or succinic acid or salts thereof are considered preferred.

[0032] In one embodiment according to the invention, a fermentation procedure is used wherein the product aimed for is in the form of its salt, or has a boiling point above that of water, while a main byproduct has a boiling point below that of water. In this case performing the fermentation under reduced pressure ensures the removal of substantial amounts of the undesirable byproduct. An example of this type of fermentation would be a mixed acid fermentation where the formation of acid, in particular acetic acid, is accompanied by the production of ethanol.

[0033] As discussed above, during the fermentation, the formation of acid results in a decrease in the pH. To counter this and keep the pH within the range where the microorganism can perform, a basic solution is typically added during the fermentation. Suitable basic solutions contain solutions comprising one or more of calcium (hydr)oxide, calcium carbonate, calcium bicarbonate, magnesium (hydr)oxide, sodium hydroxide, ammonium hydroxide, potassium hydroxide, magnesium carbonate, sodium bicarbonate, potassium bicarbonate. Depending on the solubility of the base, the basic solution mentioned above may be a true solution in the sense that the base is completely dissolved and the solution does not contain solid components. However, the basic solution may also be a slurry, which contains solid particles in addition to dissolved base. Within the present specification the word solution is intended to encompass both embodiments.

[0034] Generally, the basic solution is added in an amount effective to control the pH of the broth between about 3 and 9, more specifically between 5.5 and about

7.0.

[0035] It has been found that solutions of magnesium salts, in particular solutions of magnesium hydroxide, have to be relatively dilute to allow adequate pumpability. Accordingly, in this case, the concentration of the basic solution will be relatively low, as a result of which a relatively large amount of water is added.

[0036] Accordingly, in one embodiment, the invention pertains to a process for fermentative manufacture of an acid where a basic solution of a magnesium salt is used to keep the pH in a predetermined range.

[0037] The manufacture of lactic acid is a preferred embodiment of the present invention. Accordingly, in one embodiment, the present invention pertains to a process as defined in claim 1 for manufacturing lactic acid, while a basic solution is added to the fermentation broth during fermentation to keep the pH in a predetermined range.

[0038] Fermentative lactic acid manufacture is well known in the art. The following description is given as general elucidation only.

[0039] The carbohydrate source in lactic acid manufacture generally comprises one or more of sugars, (liquefied) starch, sugar syrup, or cheese whey, glucose, fructose, or galactose, or disaccharides such as sucrose or lactose, hexoses and pentoses in hydrolysates of plant origin, such as biowaste, wood, straw, etc.

[0040] Suitable microorganisms include Lactobacillus sp. such as L. delbrueckii, Bacillus coagulants. Bacillus thermoamylovarans, Bacillus smithii, Geobacillus stearothermophilus, and Escherichia Coli.

[0041] Each microorganism has its own optimum pH and temperature range. The pressure that may be used depends on the optimum temperature for the microorganism.

[0042] If so desired, enzymes may be added during the fermentation process to help convert the carbohydate source into compounds which can be processed by the microorganism.

[0043] It is within the scope of the skilled person to select an appropriate combination of substrate, microorganism, and reaction conditions to obtain a fermentation process suitable for use in the present invention.

[0044] In one embodiment of the present invention, the fermentation is a simultaneous saccharification and fermentation (SSF) process wherein a feed is contacted with a microorganism and an enzyme.

[0045] In a simultaneous saccharification and fermentation process, a low-value feed source is subjected to enzymatic processing to produce sugar, which is simultaneously fermented to the final fermentation product, e.g., lactic acid, using a microorganism. It has been found that an SSF process is an attractive candidate for fermentation under reduced pressure, because in this process undesirable byproducts resulting from the enzymatic reaction are at least partly removed from the system by volatilisation.

[0046] The low-value feed source may be selected from starch and partial hydrolysates of starch, the latter also known as liquefied starch, malto-dextrines or maltooligosaccharides. Suitable enzymes that may be added to the fermentation medium include one or more of alpha-amylases or glucoamylases or pullulanase. Alternatively, the low-value feed source may be selected from cellulose-containing materials, such as biowaste. In this case, suitable enzymes include cellulases.

[0047] Accordingly, in one embodiment, the present invention pertains to a method for the production of lactic acid or a salt thereof wherein a Low-value feed source is subjected to a process of simultaneous saccharification and fermentation, the method comprising saccharifying the low-value feed source in a medium comprising at least an enzyme capable of converting the low-value feed source to a sugar component, and simultaneously fermenting the sugar component using a microorganism, and optionally isolating lactic acid from the medium, wherein the fermentation process is carried out at a pressure which is below atmospheric pressure and at least at a value where the reaction medium is at its boiling point at the fermentation temperature.

[0048] For further information on a suitable SSF process, in particular a process where the low-value feed source is selected from starch and partial hydrolysates of starch, the latter also known as liquefied starch, malto-dextrines or maltooligosaccharides and suitable enzymes that may be added to the fermentation medium. include one or more of alpha-amylases or glucoamylases or pullulanase, reference is made to WO03095659.

[0049] In general, the use of reduced pressure does not affect the properties and conditions of the fermentation. Accordingly, the present invention may be applied to any fermentation process wherein it is desired to reduce water content of the fermentation product and increase product yield, wherein the product is a salt or a product with a boiling point above the boiling point of water.

[0050] It is within the scope of the skilled person to determine whether a particular fermentation procedure can suitably be adapted by applying the present invention.

[0051] The present invention will be elucidated by the following example, without being limited thereto or thereby.

Example 1

[0052] A 7 liter stirred fermenter was equipped with an external condenser coupled to the head space. The condenser was kept at 4°C with a cryostate. The fermenter and condenser could be evacuated with a vacuum pump. In the fermenter, a fermentation medium was prepared containing 1000 grams of sucrose and 2700 grams of demineralised water. Diammonium phosphate and diammonium sulphate were added as nitrogen source. Vitamins and trace elements were added as is conventional in the art.

[0053] A 10% inoculum of a lactic acid producing mi-

croorganism was added to the fermentation medium and the fermentation medium was brought to reaction temperature of 55°C. The microorganism produced lactic acid. The pH of the medium was continuously monitored and maintained at a value of 6.4 by addition of a slurry of magnesium hydroxide.

[0054] After 24 hours, the pressure above the fermentation medium was reduced, to a value of 120 mbar. The temperature of the fermentation medium was maintained at 55°C. It was observed that the fermentation medium boiled during the fermentation. However, the lactic acid production profile appeared to be comparable to a fermentation at atmospheric conditions. In 6 hours, 1800 ml of water was evaporated and condensed in the external condenser. The fermentation was left to proceed. When all sugar was consumed, the fermentation broth had a volume of 3200 ml. As compared to a conventional atmospheric fermentation, performing the fermentation at reduced pressure resulted in a reduction of the volume of the fermentation liquid by almost 50%. Additionally, the yield of the fermentation, in grams of solid magnesium lactate per liter final fermentation broth, was found to have increased.

[0055] In this example the amount of water being evaporated and removed from the reactor during the fermentation is 67%, calculated on the volume of liquid present in the reactor at the start of the fermentation.

Example 2

[0056] A 70 liter stainless steel stirred fermenter was equipped with an external plate and frame condenser and condensate collection vessel coupled to the head space. The fermenter and condenser could be evacuated with a variable drive membrane vacuum pump. The fermenter was double walled and was kept at the desired temperature with a water-heating bath. In the fermenter, a fermentation medium was prepared containing 18 kg of sucrose and 50 L of demineralised water. Diammonium phosphate and diammonium sulphate were added as nitrogen source. Vitamins and trace elements were added as is conventional in the art.

[0057] 5.8 L inoculum of a lactic acid producing microorganism was added to the fermentation medium and the fermentation medium was brought to the reaction temperature of 55°C. The microorganism produced lactic acid. The pH of the medium was continuously monitored and maintained at a value of 6.4 by addition of a 20.7 wt% magnesium hydroxide slurry.

[0058] After 18 hours, the pressure above the fermentation medium was reduced, to a value of 110-125 mbar. This pressure was maintained by a digital vacuum meter coupled to the vacuum pump. The temperature of the fermentation medium was maintained at 55°C by a temperature probe coupled to the water-heating bath. The condenser was kept at 20°C with cooling water. It was observed that the fermentation medium boiled during the fermentation. 100 ml of antifoaming agent was added to reduce foaming. After 14 hours, 32 L of water had been evaporated and condensed in the external condenser. The lactic acid production profile remained comparable to fermentation at atmospheric conditions. When all sugar was consumed, the fermentation broth had a volume of 66 L. As compared to a conventional atmospheric fermentation, performing the fermentation at a reduced (vacuum) pressure resulted in a reduction of the liquid volume of the fermentation broth by about 43%. The yield of the fermentation, in grams solid magnesium lactate per liter final fermentation broth, was found to have increased. The total amount of lactate produced by the fermentation process performed under reduced pressure was comparable to the total amount produced by a comparable process carried out under atmospheric conditions.

[0059] In this example the amount of water being evaporated and removed from the reactor during the fermentation is 64%, calculated on the volume of liquid present in the reactor at the start of the fermentation.

Claims

1. Fermentation process wherein a carbohydrate source is contacted under fermentation conditions with a microorganism in an aqueous fermentation broth to form a fermentation product which is a salt or a product with a boiling point above the boiling point of water, wherein the fermentation process is carried out at a pressure which is below atmospheric pressure and at least at the value where the reaction medium is at its boiling point at the fermentation temperature, with water being evaporated and removed from the reactor during the fermentation in an amount which is at least 20% of the volume of liquid present in the reactor at the start of the fermentation.

2. Fermentation process according to claim 1, wherein the pressure is selected such that the aqueous fermentation broth boils at the temperature at which fermentation is carried out.

3. Fermentation process according to any one of the preceding claims wherein water is added during the fermentation and the pressure is selected such that the volume of water evaporated and removed from the reactor is at least 1% of the total of water added during the fermentation.

4. Fermentation process according to claim 3, wherein the pressure is selected such that the volume of water evaporated and removed from the reactor is at least 5% of the total of water added during the fermentation.

5. Fermentation process according to claim 4, wherein the pressure is selected such that the volume of wa-

ter evaporated and removed from the reactor is at least 10% of the total of water added during the fermentation.

6. Fermentation process according to anyone of the preceding claims, wherein a carbohydrate source is contacted under fermentation conditions with a microorganism in an aqueous fermentation broth to form an acid fermentation product, while a basic solution is added to the fermentation broth during fermentation to keep the pH in a predetermined range.

7. Fermentation process according to claim 6, wherein the acid fermentation product comprises one or more carboxylic acids selected from the group consisting of mono-, di-, and tricarboxylic acids having 2-8 carbon atoms, or salts thereof.

8. Fermentation process according to claim 7, wherein the acid fermentation product comprises one or more carboxylic acids selected from lactic acid, propionic acid, citric acid, malic acid, maleic acid, fumaric acid, adipic acid, succinic acid, tartaric acid, alpha-ketoglutaric acid, oxaloacetic acid, and acetic acid, or salts thereof.

9. Fermentation process according to claim 8, wherein the acid fermentation product comprises lactic acid or succinic acid or salts thereof.

10. Fermentation process according to claim 9, wherein the acid fermentation product comprises lactic acid or a salt thereof.

11. Fermentation process according to any one of claims 6-10, wherein the basic solution comprises one or more of calcium (hydr)oxide, calcium carbonate, calcium bicarbonate, magnesium (hydr)oxide, sodium hydroxide, and potassium hydroxide.

12. Fermentation process according to claim 11, wherein the basic solution comprises magnesium hydroxide.

13. Fermentation process according to any one of the preceding claims, wherein the fermentation process is a simultaneous saccharification and fermentation process wherein a feed is contacted with a microorganism and an enzyme.

Patentansprüche

1. Fermentationsverfahren, bei dem eine Kohlenhydratquelle unter Fermentationsbedingungen zum Bilden eines Fermentationsprodukts, das ein Salz oder ein Produkt mit einem Siedepunkt oberhalb des Siedepunkts von Wasser ist, in einer wässrigen Fermentationslösung mit einem Mikroorganismus in Kontakt gebracht wird, wobei der Fermentationsverfahren bei einem Druck durchgeführt wird, der unterhalb des Atmosphärendrucks und mindestens bei dem Wert liegt, bei dem das Reaktionsmedium bei der Fermentationstemperatur an seinem Siedepunkt ist, wobei Wasser verdampft und während der Fermentation in einer Menge, die mindestens 20 % des Volumens der in dem Reaktor am Beginn der Fermentation vorhandenen Flüssigkeit umfasst, aus dem Reaktor entfernt wird.

2. Fermentationsverfahren gemäß Anspruch 1, wobei der Druck derart ausgewählt ist, dass die wässrige Fermentationslösung bei der Temperatur, bei der die Fermentation durchgeführt wird, siedet.

3. Fermentationsverfahren gemäß irgendeinem der vorangegangenen Ansprüche, wobei Wasser während der Fermentation hinzugefügt wird und der Druck derart ausgewählt ist, dass das verdampfte und aus dem Reaktor entfernte Wasser mindestens 1 % der Gesamtmenge des während der Fermentation hinzugefügten Wassers ist.

4. Fermentationsverfahren gemäß Anspruch 3, wobei der Druck derart ausgewählt ist, dass das Volumen des verdampften und aus dem Reaktor entfernten Wassers mindestens 5 % der Gesamtmenge des während der Fermentation hinzugefügten Wassers ist.

5. Fermentationsverfahren gemäß Anspruch 4, wobei der Druck derart ausgewählt ist, dass das Volumen des verdampften und aus dem Reaktor entfernten Wassers mindestens 10 % der Gesamtmenge des während der Fermentation hinzugefügten Wassers ist.

6. Fermentationsverfahren gemäß irgendeinem der vorangegangenen Ansprüche, wobei eine Kohlenhydratquelle unter Fermentationsbedingungen in einer wässrigen Fermentationslösung zum Bilden eines sauren Fermentationsprodukts mit einem Mikroorganismus in Kontakt gebracht wird, während eine basische Lösung während der Fermentation zu der Fermentationslösung zum Aufrechterhalten des pH-Werts in einem vorher festgelegten Bereich hinzugefügt wird.

7. Fermentationsverfahren gemäß Anspruch 6, wobei das saure Fermentationsprodukt eine oder mehrere Carbonsäuren, ausgewählt aus der Gruppe bestehend aus Mono-, Di- und Tricarbonsäuren, die 2-8 Kohlenstoffatome aufweisen, oder deren Salze, aufweist.

8. Fermentationsverfahren gemäß Anspruch 7, wobei

das saure Fermentationsprodukt eine oder mehrere Carbonsäuren, ausgewählt aus Milchsäure, Propionsäure, Zitronensäure, Apfelsäure, Maleinsäure, Fumarsäure, Adipinsäure, Bernsteinsäure, Weinsäure, Alpha-Ketoglutarsäure, Oxalessigsäure und Essigsäure oder deren Salze, aufweist.

9. Fermentationsverfahren gemäß Anspruch 8, wobei das saure Fermentationsprodukt Milchsäure oder Bernsteinsäure oder deren Salze aufweist.

10. Fermentationsverfahren gemäß Anspruch 9, wobei das saure Fermentationsprodukt Milchsäure oder eines ihrer Salze aufweist.

11. Fermentationsverfahren gemäß irgendeinem der Ansprüche 6-10, wobei die basische Lösung eines oder mehrere von Calcium(hydr)oxid, Calciumcarbonat, Calciumbicarbonat, Magnesium(hydr)oxid, Natriumhydroxid und Kaliumhydroxid aufweist.

12. Fermentationsverfahren gemäß Anspruch 11, wobei die basische Lösung Magnesiumhydroxid aufweist.

13. Fermentationsverfahren gemäß irgendeinem der vorangegangenen Ansprüche, wobei das Fermentationsverfahren eine gleichzeitige Verzuckerung und ein Fermentationsverfahren ist, bei dem eine Nährsubstanz mit einem Mikroorganismus und einem Enzym in Kontakt gebracht wird.

Revendications

1. Procédé de fermentation dans lequel une source de glucides est mise en contact dans des conditions de fermentation avec un microorganisme dans un bouillon de fermentation aqueux afin de former un produit de fermentation qui est un sel ou un produit ayant un point d'ébullition situé au-dessus du point d'ébullition de l'eau, dans lequel le procédé de fermentation est réalisé à une pression qui se trouve sous la pression atmosphérique et au moins à la valeur où le milieu de réaction est à son point d'ébullition à la température de fermentation, l'eau étant évaporée et éliminée du réacteur durant la fermentation en une quantité qui fait au moins 20 % du volume de liquide présent dans le réacteur au début de la fermentation.

2. Procédé de fermentation selon la revendication 1, dans lequel la pression est sélectionnée de telle sorte que le bouillon de fermentation aqueux bout à la température à laquelle la fermentation est réalisée.

3. Procédé de fermentation selon l'une quelconque des revendications précédentes dans lequel de l'eau est ajoutée durant la fermentation et la pression est sélectionnée de telle sorte que le volume d'eau évaporée et éliminée du réacteur fait au moins 1 % du total d'eau ajoutée durant la fermentation.

4. Procédé de fermentation selon la revendication 3, dans lequel la pression est sélectionnée de telle sorte que le volume d'eau évaporée et éliminée du réacteur fait au moins 5 % du total d'eau ajoutée durant la fermentation.

5. Procédé de fermentation selon la revendication 4, dans lequel la pression est sélectionnée de telle sorte que le volume d'eau évaporée et éliminée du réacteur fait au moins 10 % du total d'eau ajoutée durant la fermentation.

6. Procédé de fermentation selon l'une quelconque des revendications précédentes, dans lequel une source de glucides est mise en contact dans des conditions de fermentation avec un microorganisme dans un bouillon de fermentation aqueux afin de former un produit de fermentation acide, alors qu'une solution basique est ajoutée au bouillon de fermentation durant la fermentation afin de maintenir le pH dans une plage prédéterminée.

7. Procédé de fermentation selon la revendication 6, dans lequel le produit de fermentation acide comprend un ou plusieurs acides carboxyliques sélectionnés dans le groupe constitué d'acides mono-, di- et tricarboxyliques ayant 2-8 atomes de carbone, ou des sels de ceux-ci.

8. Procédé de fermentation selon la revendication 7, dans lequel le produit de fermentation acide comprend un ou plusieurs acides carboxyliques sélectionnés parmi l'acide lactique, l'acide propionique, l'acide citrique, l'acide malique, l'acide maléique, l'acide fumarique, l'acide adipique, l'acide succinique, l'acide tartrique, l'acide alpha-cétoglutarique, l'acide oxaloacétique et l'acide acétique, ou des sels de ceux-ci.

9. Procédé de fermentation selon la revendication 8, dans lequel le produit de fermentation acide comprend de l'acide lactique ou de l'acide succinique ou des sels de ceux-ci.

10. Procédé de fermentation selon la revendication 9, dans lequel le produit de fermentation acide comprend de l'acide lactique ou un sel de celui-ci.

11. Procédé de fermentation selon l'une quelconque des revendications 6-10, dans lequel la solution basique comprend un ou plusieurs parmi l'(hydr)oxyde de calcium, le carbonate de calcium, le bicarbonate de calcium, l'(hydr)oxyde de magnésium, l'hydroxyde de sodium et l'hydroxyde de potassium.

12. Procédé de fermentation selon la revendication 11, dans lequel la solution basique comprend de l'hydroxyde de magnésium.

13. Procédé de fermentation selon l'une quelconque des revendications précédentes, dans lequel le procédé de fermentation est un procédé de fermentation et de saccharification simultanées dans lequel une charge est mise en contact avec un microorganisme et une enzyme.

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- WO 03095659 A **[0048]**

Non-patent literature cited in the description

- **J. BALLONGE et al.** *Enhancement of Solvents Production by Clostridium acebutylicum Cultivated on a Reducing Compounds Depletive Medium, Biomass,* 1986, 121-129 **[0012]**

Vízelvétellel járó, csökkentett nyomású fermentációs eljárás

Szabadalmi igénypontok

1. Fermentációs eljárás, amelynek során egy szénhidrát forrást fermentációs körülmények között egy mikroorganizmussal érintkeztetünk egy vizes fermentációs tápközegben egy olyan fermentációs termék előállítására, amely egy olyan só vagy termék, amelynek forráspontja a víz forráspontjánál magasabb, ahol a fermentációs eljárást csökkentett nyomáson hajtjuk végre, amely nyomás a légköri nyomásnál alacsonyabb, és legalább egy olyan érték, ahol a reakcióközeg a fermentációs hőmérsékleten a forráspontján van, miközben a reaktorból a fermentáció során vizet párologtatunk el és vonunk ki olyan mennyiségben, amely legalább a reaktorban a fermentáció kezdetén lévő folyadék térfogatának a 20 %-a.

2. Az 1. igénypont szerinti fermentációs eljárás, ahol a nyomást úgy választjuk meg, hogy a vizes fermentációs tápközeg azon a hőmérsékleten forrjon, amelyen a fermentációt végezzük.

3. Az előző igénypontok valamelyike szerinti fermentációs eljárás, amelynek során a fermentáció közben vizet adunk, és a nyomást úgy választjuk meg, hogy a reaktorból elpárologtatott és eltávolított víz térfogata legalább a fermentáció során adott összes víz 1 %-a.

4. A 3. igénypont szerinti fermentációs eljárás, amelynek során a nyomást úgy választjuk meg, hogy a reaktorból elpárologtatott és eltávolított víz térfogata legalább a fermentáció során adott összes víz 5 %-a.

5. A 4. igénypont szerinti fermentációs eljárás, amelynek során a nyomást úgy választjuk meg, hogy a reaktorból elpárologtatott és eltávolított víz térfogata legalább a fermentáció során adott összes víz 10 %-a.

6. Az előző igénypontok bármelyike szerinti fermentációs eljárás, amelynek során egy szénhidrát forrást fermentációs körülmények között egy mikroorganizmussal érintkeztetünk egy vizes fermentációs tápközegben egy sav fermentációs termék előállítására, miközben a fermentációs tápközeghez egy lúgos oldatot adunk a fermentáció során a pH érték egy előre meghatározott tartományban történő tartására.

SZTNH-100064013

7. A 6. igénypont szerinti fermentációs eljárás, ahol a sav fermentációs termék egy vagy több az alábbi csoportból választott karbonsavat tartalmaz: 2-8 szénatomos mono-, di- és trikarbonsavak vagy ezek sói.

8. A 7. igénypont szerinti fermentációs eljárás, ahol a sav fermentációs termék egy vagy több az alábbiak közül választott karbonsavat tartalmaz: tejsav, propionsav, citromsav, almasav, maleinsav, fumársav, adipinsav, szukcinsav, borostyánkősav, alfa-ketoglutársav, oxalocetsav és ecetsav, vagy ezek sói.

9. A 8. igénypont szerinti fermentációs eljárás, ahol a sav fermentációs termék tejsavat vagy szukcinsavat vagy ezek sóit tartalmazza.

10. A 9. igénypont szerinti fermentációs eljárás, ahol a sav fermentációs termék tejsavat vagy ennek sóit tartalmazza.

11. A 6-11 igénypontok bármelyike szerinti fermentációs eljárás, ahol a lúgos oldat egy vagy több alábbi vegyületet tartalmaz: kalcium-(hidr)oxid, kalcium karbonát, kalcium bikarbonát, magnézium-(hidr)oxid, nátrium-hidroxid és kálium-hidroxid.

12. A 11. igénypont szerinti fermentációs eljárás, ahol a lúgos oldat magnézium-hidroxidot tartalmaz.

13. Az előző igénypontok bármelyike szerinti fermentációs eljárás, ahol a fermentációs eljárás egy egyidejű szacharifikálás és egy fermentációs eljárás, a betáplálást egy mikroorganizmussal és egy enzimmel érintkeztetjük.