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(71) Applicant *(for all designated States except US)*: **KOREA ADVANCED INSTITUTE OF SCIENCE AND TECHNOLOGY** [KR/KR]; 373-1, Guseong-dong, Yuseong-gu, Daejeon 305-701 (KR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **HONG, Won Hi** [KR/KR]; 107-1703, Hanbit Apt., Eoeun-dong, Yuseong-gu, Daejeon 305-755 (KR). **LEE, Sang Yup** [KR/KR]; 212-702, Expo Apt., Jeonmin-dong, Yuseong-gu, Daejeon 305-761 (KR). **HONG, Yeon Ki** [KR/KR]; 105-403, Hyundai Apt., Chilgeum-dong, Chungju-si, Chungbuk 380-776 (KR). **WON, Hyo Jin** [KR/KR]; 806-1402, Munchon Maeul 8-danji, Juyeop 2-dong, Ilsan-gu, Goyang-si, Gyeonggi-do 411-748 (KR). **HUH, Yun Suk** [KR/KR]; 33/2, 651-40, Hakha-dong, Yuseong-gu, Daejeon 305-360 (KR). **SONG, Hyohak** [KR/KR]; 112-1105, Hanaro Apt., Wolpyeong 3-dong, Seo-gu, Daejeon 302-283 (KR). **LEE, Eun Zoo** [KR/KR]; 137-203, Hanbit Apt., Eoeun-dong, Yuseong-gu, Daejeon 305-755 (KR).

(74) Agent: **LEE, Cheo Young**; 11th, Won Bldg., 648-26, Yeoksam-dong, Gangnam-gu, Seoul 135-080 (KR).

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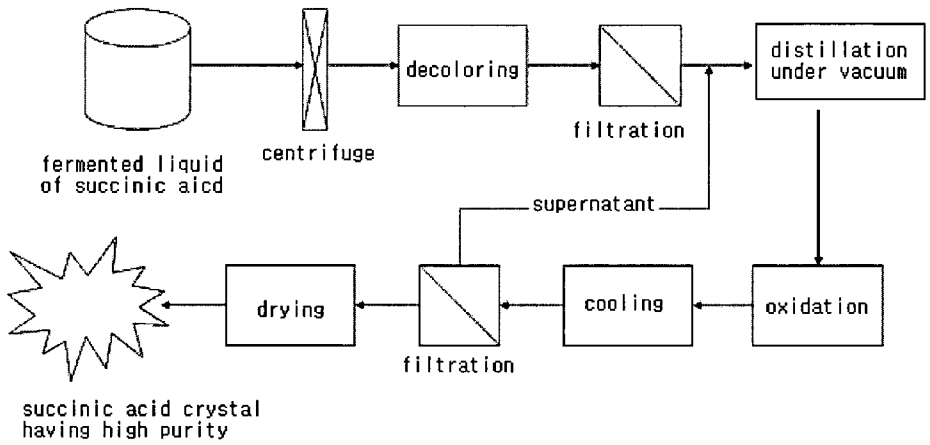
[Continued on next page]

(54) Title: METHOD FOR PURIFYING SUCCINIC ACID BY CRYSTALLIZATION OF CULTURE BROTH

FIG. 1



(57) Abstract: The present invention relates to a method for separating and purifying succinic acid with high purity and high yield by crystallization of culture broth and, more particularly, a method for recovering succinic acid with high purity and high yield, which comprises concentrating culture broth from which succinic acid-producing microorganism is removed and then adding an acid solution at low temperature, thus directly crystallizing without other pretreatment processes. According to the present invention, succinic acid is separated and purified using culture broth, from which succinic acid producing microorganism is removed, without other pretreatment processes, and thus a cost-saving effect due to process simplification, and an effect of environmental pollution prevention due to the prevention of sludge generation during succinic acid recovery process, can be achieved. In addition, succinic acid was recovered with high purity and high yield, thus making it possible to achieve a technical effect unprecedented in the prior art in terms of cost efficiency.

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Method for Purifying Succinic Acid by Crystallization of Culture Broth

TECHNICAL FIELD

The present invention relates to a method for separating and purifying succinic acid with high purity and high yield by crystallization of culture broth and, more particularly, a method for recovering succinic acid with high purity and high yield, which comprises concentrating culture broth from which succinic acid-producing microorganism is removed, and then adding an acid solution at low temperature, thus directly crystallizing without other pretreatment processes.

BACKGROUND ART

Succinic acid, which is a divalent organic acid consisting of 4 carbons, has been widely used in producing foods, medicines, cosmetics, solvents and the like, and has a high utility as a precursor of various, industrially important chemical products, and thus, the demand for succinic acid is expected to be dramatically increased (Zeikus *et al., Appl. Microbiol. Biotechnol.*, 51:545, 1999; Song *et al., Enzyme Microbial Technol.*, 39: 352, 2006; Shekhawat *et al., Biores. Technol.*, 97: 342, 2006; McKinlay *et al., Appl. Microbiol. Biotechnol.*, 76:727, 2007). Particularly, succinic acid has been drawing a great attention as a main source of biodegradable polymers which can overcome a disadvantage of synthetic polymers, non-biodegradability (Gottschalk *et al.*, *Bacterial. Metabolism.*, 2nd ed., *Springer-Verlag,* NY, USA, 1986; Wood, A., *Chem. Week*, 166:15, 2004).

Most of succinic acid for industrial use is currently produced by chemical synthesis using petroleum-based feedstocks and only a small quantity of succinic acid used for foods and medicines is produced by microbial fermentation. However, under

recent circumstances of a sharp increase in petroleum prices and enhanced regulation of environmental pollution, there is an urgent need to develop a process for efficiently producing, separating and purifying succinic acid using microorganisms, which can replace the chemical synthesis process. With microbial culture technology, prediction by using in silico models and various metabolic engineering technologies required for strain improvement, the technology for succinic acid production using microorganisms has been developing rapidly throughout the world (Kim *et al.*, *Biotech. Bioeng.*, 97:657, 2007; Song *et al.*, *Enzyme Microbial Technol.*, 39:352, 2006).

The present inventors isolated an excellent succinic acid-producing rumen bacterium, *Mannheimia succiniciproducens* MBEL55E (KCTC 0769BP), from the rumen of Korean cow, and completed its full genome sequence and characterized metabolic properties thereof (Hong *et al., Nature Biotechnol.*, 22:1275, 2004). Also, the present inventors have constructed its mutants, *M. succiniciproducens* LPK (KCTC 10558BP) by disrupting a gene encoding lactate dehydrogenase(*ldhA*) and a gene encoding pyruvate formate-lyase(*pfl*) from *M. succiniciproducens* MBEL55E and *M. succiniciproducens* LPK7 (KCTC 10626BP) by disrupting a phosphotransacetylase gene(*pta*) and an acetate kinase gene(*ackA*) in the mutant strain, *M. succiniciproducens* LPK (WO 2005/052135 A1; Lee *et al., Appl. Environ. Microbiol.*, 72:1939, 2006). In addition to that, the present inventors have constructed a mutant strain, *M. succiniciproducens* PALK (KCTC 10973BP) (PCT/KR2007/003574) by disrupting a lactate dehydrogenase gene(*ldhA),* a phosphotransacetylase gene(*pta)* and an acetate kinase gene(*ackA)* in the *M. succiniciproducens* MBEL55E strain, *M. succiniciproducens* ALKt (PCT/KR2008/000012) by overexpressing a phosphotransacetylase gene (*pta)* in the PALK strain, and a mutant strain, *M. succiniciproducens* ALK (PCT/KR2008/000012) by disrupting a lactate dehydrogenase gene(*ldhA)* and an acetate kinase gene (*ackA)* in the *M. succiniciproducens* MBEL55E strain.

When producing a bio-based material through microbial fermentation using renewable raw materials, since various metabolites such as organic acids, proteins and the like together with the target material are excessively produced as byproducts and thus present in the final fermentation broth, there is a desperate need for the development of a process for efficiently separating and purifying the final target material with high purity. Particularly, it is well known to whom those skilled in the art that, when producing organic acids using microbial fermentation, cost of separation and purification process accounts for 50-70% of total production cost. Thus, in order to produce bio-based succinic acid at prices competitive in the market, there is an urgent need to develop an efficient and economical separation and purification process (King *et al., Chemtech.,* 22:285, 1992).

Although, various separation and purification processes for succinic acid, such as liquid-liquid extraction, membrane separation, reactive extraction and the like have been developed till now, most of the aforementioned processes have lots of limitations to be directly utilized as a final separation and purification process for bio-based succinic acid production due to very low levels of yield or purity of succinic acid (Choi *et al., Int. J. Chemical Kinetics*, 28:37, 1996; Choi *et al., J. Chemical Eng. Jpn.*, 32:184, 1999; Han *et al., Sep. Sci. Techn.*, 31:1123, 1996; Zeikus *et al., Chem. Proc.*, 58:71, 1995; Tamada *et al., Ph.D. Thesis,* Univ. of California at Berkeley, 1989; Hong *et al., Korean J. Chem. Eng.*, 21:488, 2004; Huh *et al., Proc. Biochem.*, 41:1461, 2006). Moreover, when the conventional precipitation or crystallization method is used, large amounts of sludge as a waste material are produced during the process and succinic acid is recovered in the salt form, so that a re-acidification process using high concentration of acid is additionally required (Vick Roy *et al., In. comprehensive Biotechnol.*, Murray Moo-Young eds., Vol. 3, Pergamon Press, 761, 1985; Bessling *et al., Chem. Eng. Technol.*, 21:393, 1998; Huh, *et al., Proc. Biochem.*, 41:1461, 2006). Therefore, there is a great demand to develop a simple and inexpensive separation and purification process enabling high purity succinic acid recovery with high

efficiency without other pretreatment processes of fermentation broth, in order for bio-based succinic acid production process to be competitive against current petroleum-based succinic acid production process. Particularly, in order to reduce environmental burden, it is very important to develop an environmentally friendly separation and purification process which minimizes sludge generation or generates no sludge during the process.

Meanwhile, the present inventors had developed a method for recovering succinic acid with 99.76% purity and 73.09% yield by crystallizing through vacuum distillation and crystallization processes after a pretreatment process for extracting microbial fermentation broth using an amine extractant, but the method could not solve the problems of complicated pretreatment processes of fermentation broth and relatively large amounts of remaining organic acids even after purification process of succinic acid (Korean Patent Registration No.10-672813).

Accordingly, the present inventors have made extensive efforts to solve the above-mentioned problems occurring in the prior art, and, as a result, confirmed that, when crystallization of succinic acid, which is produced through fermentation by microorganism, *Mannheimia* sp., is carried out at low temperature by adding an acidic solution without any particular pretreatment processes, succinic acid with more than 99.99% purity and more than 74.56% yield could be recovered produced, thereby completing the present invention.

SUMMARY OF INVENTION

It is an object of the present invention to provide a method for separating and purifying succinic acid with high purity and high yield using crystallization of culture broth.

To achieve the above objects, the present invention provides a method for

separating and purifying succinic acid from a culture broth of a succinic acid-producing microorganism, which comprises the steps of: (a) concentrating the culture broth from which a succinic acid-producing microorganism is removed; (b) acidifying the concentrated culture broth; and (c) recovering the crystal of succinic acid by cooling down the acidified culture broth.

In addition, the present invention provides a method for separating and purifying succinic acid from a culture broth of *M. succiniciproducens* PALK, which comprises the steps of: (a) concentrating the culture broth, from which *M. succiniciproducens* PALK strain is removed, to a succinic acid concentration of 100~300 g/L; (b) acidifying the concentrated culture broth to pH 1.0~3.0; and (c) recovering the crystal of succinic acid by cooling down the acidified culture broth to 2~20℃.

Other features and aspects of the present invention will be apparent from the following detailed descriptions and the appended claims.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a schematic diagram showing the whole process of recovering the crystal of succinic acid from culture broth.

FIG. 2 is a graph showing the fermentation profiles of *M. succiniciproducens* PALK strain during succinic acid production in a fed-batch mode.

FIG. 3 is a photograph showing the removal of impure pigments present in culture broth according to the activated carbon concentration used.

FIG. 4 is a graph showing changes in the yield of succinic acid crystal according to succinic acid concentration in the concentrated culture broth in a crystallization process using HCl.

FIG. 5 is a graph showing changes in the yield of succinic acid crystal according to succinic acid concentration in the concentrated culture broth in a

crystallization process using H_2SO_4.

FIG. 6 is a graph showing changes in the yield of succinic acid crystal according to cooling temperature of the concentrated culture broth in a crystallization process using HCl.

FIG. 7 is a graph showing changes in the yield of succinic acid crystal according to cooling temperature of the concentrated culture broth in a crystallization process using H_2SO_4.

FIG. 8 is a graph showing changes in the yield of succinic acid crystal according to pH of the concentrated culture broth in a crystallization process using HCl.

FIG. 9 is a graph showing changes in the yield of succinic acid crystal according to pH of the concentrated culture broth in a crystallization process using H_2SO_4.

FIG. 10 is a diagram of an HPLC analysis for the initial culture broth and succinic acid crystal.

DETAILED DESCRIPTION OF THE INVENTION, AND PREFERRED EMBODIMENTS

In one aspect, the present invention relates to a method for recovering succinic acid from a culture broth of a succinic acid-producing microorganism, which comprises the steps of (a) concentrating the culture broth from which a succinic acid-producing microorganism is removed; (b) acidifying the concentrated culture broth; and (c) recovering the crystal of succinic acid by cooling down the acidified culture broth.

Particularly, the present invention relates to a method capable of separating and purifying succinic acid with high purity and high yield by a simple process by acidifying a culture broth, obtained by culturing a succinic acid producing microorganism, at low temperature without other pretreatment processes and then

directly crystallizing succinic acid, compared to the prior art, thus reducing separation and purification costs of succinic acid.

In addition, the culture broth used in the present invention is preferably a culture broth from which a succinic acid producing microorganism is removed and thus a pretreatment of the culture broth is not necessary, thereby resulting in an effect of simplifying the process.

In the present invention, the method additionally comprises a step of: decolorizing the culture broth of the succinic acid producing microorganism before the concentration the step, and the decolorization is preferably performed using 1.8~2.5%(w/v) activated carbon.

In the decolorization step using activated carbon, if activated carbon concentration is less than 1.8%(w/v), the impure pigments are not sufficiently removed from the culture broth, and if activated carbon concentration is more than 2.5%(w/v), a loss rate of succinic acid sharply increases, and thus, it is preferable to use 1.8~2.5%(w/v) activated carbon.

In the present invention, the concentration of succinic acid is preferably performed by vacuum distillation, and succinic acid concentration of the culture broth concentrated in the step (a) is preferably 100~300g/L.

In the present invention, the acidification step (b) is preferably performed in the range of pH 1.0~3.0 using hydrochloric acid and sulphuric acid. At this time, if the pH is less than 1.0 or greater than 3.0, the final purity and yield of succinic acid dramatically decreases. Therefore, it is preferable to perform the acidification in a pH rage of 1.0~3.0.

In the present invention, the cooling in the step (c) is preferably performed in the

temperature range of 2 to 20℃. At this time, if the cooling temperature is lower than 2℃, the final succinic acid yield is sharply decreased, and if the cooling temperature is higher than 20℃, the crystallization did not occur. Therefore, the cooling temperature is preferably 2~20℃.

In the present invention, the succinic acid producing microorganism is preferably *Mannheimia* sp., but any microorganism can be used without limitations as long as it is a microorganism capable of producing succinic acid.

In the present invention, said *Mannheimia* sp. is preferably selected from the group consisting of *M. succiniciproducens* LPK, *M. succiniciproducens* LPK7, *M. succiniciproducens* PALK, *M. succiniciproducens* ALKt, and *M. succiniciproducens* ALK strains.

In addition, the culture broth of the succinic acid producing microorganism can be prepared by various types of culture including batch culture, fed-batch culture, continuous culture etc., which are generally known in the conventional culture processes of microorganisms.

In another aspect, the present invention relates to a method for separating and purifying succinic acid from a culture broth of *M. succiniciproducens* PALK, which comprises the steps of (a) concentrating the culture broth, from which *M. succiniciproducens* PALK strain is removed, to a succinic acid concentration of 100~300g/L; (b) acidifying the concentrated culture broth to pH 1.0~3.0; and (c) recovering the crystal of succinic acid by cooling down the acidified culture broth to 2~20℃.

In the present invention, said *M. succiniciproducens* PALK is preferably cultured in a chemically defined synthetic culture medium.

In the present invention, the method preferably additionally comprises a step of decolorizing the culture broth of *M. succiniciproducens* PALK strain prior to the concentration step.

According to a preferable embodiment of the present invention, as shown in FIG.1, microorganisms are removed from the culture broth obtained by culturing an excellent succinic acid-producing mutant strain, *M. succiniciproducens* PALK in a fed-batch mode, by centrifugation, and then impure pigments present in the culture broth are removed using activated carbon. Then, succinic acid concentration in the culture broth is increased by vacuum distillation, and the pH thereof is reduced using HCl and H_2SO_4, followed by cooling down to low temperature, thereby recovering succinic acid crystal with high purity and high yield as a final product.

As described above, in the inventive method for separating and purifying succinic acid, since it is possible to obtain succinic acid with high purity and high yield through a simple process by directly crystallizing succinic acid using an acidic solution at low temperature without other ~~a~~ pretreatment processes due to the use of the culture broth from which a succinic acid-producing microorganisms are removed, costs of separating and purifying succinic acid from the culture broth of microorganisms can be significantly reduced.

The separation and purification method according to the present invention highly contributes to the commercialization of succinic acid through such a cost reduction, as well as fundamentally prevents excess sludge generation and thus provides the effect of preventing environmental pollution.

The separation and purification method according to the present invention enables an increase in the yield and purify of succinic acid up to 74.65% and 99.99% , respectively, thus making it possible to achieve technical effect unprecedented in the prior art, as well as, has advantages in cost reduction and environmental aspects.

Therefore, the separation and purification method of the present invention is expected to highly contribute to replace current chemical processes for succinic acid production with bio-based succinic acid production processes.

Examples

Hereinafter, the present invention will be described in further detail with reference to examples. It is to be understood, however, that these examples are for illustrative purposes only and are not to be construed to limit the scope of the present invention.

Particularly, the following examples illustrate only a succinic acid-producing microorganism *Mannheimia* sp., and fed-batch culture method, but, it is obvious to a person whom skilled in the art that other kinds of succinic acid-producing microorganisms and culture methods can also be used.

In addition, the following examples illustrate only activated carbon, but it is obvious to a person whom skilled in the art that any material can be used without any limitations as long as it can remove impure pigments present in culture broth. Moreover, the following examples illustrate only a concentration method by vacuum distillation, but it is obvious to a person whom skilled in the art that any concentration method can be used without any limitations as long as it enables them to carry out the concentration of succinic acid in the culture broth.

Example 1: Fed-batch culture of *M. succiniciproducens* PALK strain

1mL of *M. succiniciproducens* PALK (KCTC 10973BP) stored in a 15% glycerol solution at -70°C was inoculated into 19mL of complex medium containing 50mM of glucose, and cultured in anaerobic conditions at 39°C for 8hr, and then 2.5mL of the culture broth was transferred to 250mL of complex medium containing 50mM

of glucose and cultured again at 39°C for 8hr. Fermentation was performed by inoculating 250mL of the culture broth into a bioreactor containing 2.25L of chemically defined synthetic medium under culture conditions of an initial glucose concentration of 100mM, an initial glycerol concentration of 50mM at 39°C and 200rpm. In order to maintain anaerobic conditions during the whole period of fermentation, carbon dioxide was continuously supplied at a flow rate of 0.2vvm (500mL/min). pH during the fermentation was adjusted to 6.5 by adding 28%(w/v) ammonia solution. During the fermentation, when glucose concentration in the culture broth was decreased less than 5g/L, 700g/L of concentrated glucose solution was supplied to maintain glucose concentration in the culture broth at a range of 5~10g/L. The chemically defined synthetic medium consisted of 1.0g/L of NaCl, 1.0g/L of $(NH_4)_2SO_4$, 8.708g/L of K_2HPO_4, 9.996g/L of $NaHCO_3$, 0.02g/L of $CaCl_2 \cdot 2H_2O$, 0.2g/L of $MgCl_2 \cdot 6H_2O$, 5mL/L of trace metal solution (Lee *et al., J. Environ. Polymer Degrad.*, 4:131, 1996), 0.5g/L of cysteine, 0.5g/L of methionine, 0.5g/L of alanine, 0.5g/L of asparagine, 0.5g/L of aspartic acid, 0.5g/L of proline, 0.5g/L of serine, 0.005g/L of nicotinic acid, 0.005g/L of Ca-pantothenate, 0.005g/L of pyridoxine·HCl, 0.005g/L of thiamine, 0.005g/L of ascorbic acid, and 0.005g/L of biotin.

The concentration of cells in the culture broth was measured with a spectrophotometer, and then calculated using the previously measured light absorption by the spectrophotometer (OD_{600}) and the verification test for dried-cell weight. During the fermentation, samples were collected at a regular time interval from the bioreactor. The collected samples were centrifuged at 13,000 rpm and 4°C for 10 minutes, and then the supernatants were used to analyze the concentrations of ethanol, organic acids including succinic acid, produced as metabolites during the culture, and glucose and glycerol used as carbon sources by using a High-Performance Liquid Chromatography.

As a result, as shown in FIG.2 and Table 2, it could be confirmed that *M.*

succiniciproducens PALK strain produced 60.36g/L of succinic acid as a final product and 3.385g/L of pyruvic acid, 1.539g/L of acetic acid and trace amounts of other organic acids as byproducts.

Example 2: Influence of activated carbon content on loss of succinic acid and removal of impure pigments from culture broth

The culture broth obtained in Example 1 was centrifuged to remove microorganisms and decolorization was performed prior to crystallization in order to remove impure pigments from the culture broth to produce white crystallized succinic acid as a final product. Herein, for the decolorization, activated carbon was used. Decolorization was performed by adding 1.0~3.0% (w/v) activated carbon to the culture broth, and then mixing the resultant mixture at a stirring speed of less than 50 rpm using a stirrer at room temperature for 1 hour.

As a result, as shown in FIG. 3, it could be confirmed that the more the amount of the activated carbon is, the more transparent the color of the culture broth becomes, suggesting that this is because impure pigments are removed by activated carbon.

Table 1 shows the absolute amount of succinic acid decreased and relative loss rate of succinic acid in the culture broth according to the activated carbon concentration used, when culture broth containing succinic acid having an initial concentration of 60.36g/L was decolorized with activated carbon, and it was found that as the concentration of activated carbon added increases, the concentration of succinic acid decreases and the loss rate of succinic acid increases in the culture broth.

As shown in FIG.3 and Table 1, it could be seen that, in order to effectively remove impure pigments from the culture broth while minimizing loss of succinic acid, it is preferable to use 2.0%(w/v) of activated carbon.

Table 1: Comparison of the loss rate of succinic acid during the removal of impure pigments with activated carbon from the culture broth

	Initial culture broth	Amount of activated carbon added %(w/v)				
		1.0	1.5	2.0	2.5	3.0
Concentration of succinic acid (g/L)	60.36	60.07	59.24	58.45	58.43	58.24
Loss rate of succinic acid (%)	-	0.49	1.87	3.18	3.20	3.53

Example 3: Influence of succinic acid concentration of culture broth through concentration process on crystallization process

The culture broth decolorized in Example 2 was concentrated through vacuum distillation and then succinic acid was crystallized therefrom. First, succinic acid crystallization was performed by concentrating the culture broth containing succinic acid to a succinic acid concentration of 100~300g/L using vacuum distillation, and separately adding HCl and H_2SO_4 to the concentrated culture broth, to adjust pH to 1.5, and then cooling down the culture broth to 2°C. Succinic acid crystal was finally obtained through filtration, and moisture was completely removed therefrom in an oven at 80°C, followed by measuring the weight thereof to obtain a final succinic acid yield.

As a result, as shown in FIG. 4 and Fig. 5, when crystallization process was performed using HCl and H_2SO_4, separately, as succinic acid concentration in the concentrated culture broth increased, the yield of final purified succinic acid crystal increased, and particularly, it was confirmed that, when succinic acid concentration in the culture broth was 200g/L, the yield of succinic acid crystal sharply increased.

Therefore, in order to effectively crystallize succinic acid from the culture broth, when considering the economic costs of vacuum distillation process and the yield of succinic acid crystal, it was found that the crystallization process is preferably

performed at a succinic acid concentration of about 200g/L in the concentrated culture broth.

Example 4: Influence of temperature and pH on succinic acid crystallization

Optimum cooling temperature and pH was determined by examining the influence of cooling temperature on succinic acid crystallization using the concentrated culture broth containing 200g/L of succinic acid, obtained in Example 3. First, in order to examine the influence of cooling temperature on succinic acid crystallization, HCl and H_2SO_4 were separately added to the concentrated culture broth containing the 200g/L of succinic acid to adjust pH to 1.5, and then each sample was cooled down to 2°C, 4°C, 7°C, 10°C, 15°C, and 20°C. Then, precipitated succinic acid crystal was obtained through filtration, and moisture was completely removed therefrom in an oven at 80°C, and then each sample was measured for the weight of succinic acid crystal to obtain a final succinic acid yield.

As a result, as shown in FIG. 6 and FIG. 7, the highest yield of succinic acid crystal was shown at 2°C.

Generally, the pH value of initial culture broth is higher than dissociation constants of organic acids in the culture broth and thus the organic acids exist in dissociated form. Therefore, in a range of pH higher than their dissociation constants, solubility of the organic acids increases, and on the contrary, in a range of pH lower than their dissociation constants, organic acids receive an H^+ ion to form carboxylic acids as original organic acids, thus decreasing the solubility thereof. In the present invention, HCl or H_2SO_4 were added to the culture broth to adjust the pH value at the value less than their dissociation constants, so that the solubility of succinic acid is reduced, thus making it possible to produce non-dissociated form of succinic acid crystal.

In addition, in order to examine the influence of pH on succinic acid crystallization, HCl and H_2SO_4 were separately added to the concentrated culture broth containing 200g/L of succinic acid to adjust pH to 1.0, 1.5, 2.0, 2.5 and 3.0, and the culture broth was cooled down to a temperature of 2°C at which the highest yield of succinic acid is observed, and then precipitated succinic acid crystal was obtained through filtration, followed by completely removing moisture therefrom in an oven at 80°C, to obtain the yield of succinic acid by measuring the weight of succinic acid crystal at each pH.

As a result, as shown in FIG. 8 and FIG. 9, it was confirmed that, in the range of pH 1.5~2.0, the yield of succinic acid crystal was sharply increased and, in the range of pH value less than the aforementioned pH value, the increase rate of the yield was significantly decreased.

Thus, in order to perform an efficient crystallization process, it could be seen that, when considering economic costs of adding acid and succinic acid yield, the crystallization process is preferably performed in the range of pH 1.5~2.0.

Example 5: Succinic acid crystallization from culture broth using optimum separation and purification process

Using the culture broth prepared in Example 1, succinic acid crystal was recovered by removing impure pigments using 2.0%(w/w) of activated carbon, concentrating culture broth to a succinic acid concentration of 200g/L through vacuum distillation, adjusting pH to 1.5 using HCl or H_2SO_4, crystallizing succinic acid through cooling down to 2°C, and filtering the culture broth.

Finally, in order to examine the yield and purity of succinic acid crystal recovered from the culture broth, moisture was completely removed therefrom in an oven at 80°C to measure the weight thereof to obtain the yield thereof, and also its purity

was obtained using HPLC.

Table 2 shows the composition of organic acids including succinic acid present in initial culture broth, and a comparison of dry weight of organic acids including succinic acid between before and after the inventive separation and purification process, based on 1.0L of the culture broth obtained in Example 1. FIG. 10 shows the results of HPLC analysis for the culture broth obtained in Example 1 and succinic acid crystal finally obtained after crystallization process.

As shown in Table 2 and FIG. 10, almost all organic acids except for succinic acid were completely removed through the above separation and purification process, and succinic acid crystal with more than 99.99% purity and a high yield of 74.65% could be recovered.

Table 2: The composition of organic acids present in initial culture broth, and a comparison of dry weight of organic acids including succinic acid between before and after the inventive separation and purification process, based on 1.0L of the culture broth obtained in Example 1.

Composition of organic acids in culture broth	Culture broth	Crystallization process	
	Dry weight (g)	Dry weight (g)	Purity (%)
Succinic acid	60.364	45.0617	99.997
Maleic acid	0.004	-	-
Pyruvic acid	3.385	-	-
Acetic acid	1.539	0.0014	0.003
Fumaric acid	0.004	-	-
Total	65.296	45.0631	100

INDUSTRIAL APPLICABILITY

As described in detail above, according to the present invention, succinic acid is

separated and purified using culture broth from which succinic acid producing microorganism is removed without other separate pretreatment processes, and thus a cost-saving effect due to process simplification, and an effect of environmental pollution prevention due to the prevention of sludge generation during succinic acid recovery process, can be achieved. In addition, succinic acid was separated and purified with high purity and high yield, thus making it possible to achieve a technical effect unprecedented in the prior art in terms of cost efficiency.

Although the present invention has been described in detail with reference to the specific features, it will be apparent to whom those skilled in the art that this description is only for a preferred embodiment and does not limit the scope of the present invention. Thus, the substantial scope of the present invention will be defined by the appended claims and equivalents thereof.

THE CLAIMS

What is Claimed is:

1. A method for recovering succinic acid from a culture broth of a succinic acid-producing microorganism, the method comprising the steps of:

(a) concentrating the culture broth from which a succinic acid-producing microorganism is removed;

(b) acidifying the concentrated culture broth; and

(c) recovering the crystal of succinic acid by cooling down the acidified culture broth.

2. The method for recovering succinic acid according to claim 1, which additionally comprises a step of: decolorizing the culture broth of a succinic acid producing microorganism prior to the concentrating step.

3. The method for recovering succinic acid according to claim 2, wherein the decolorization is performed using activated carbon.

4. The method for recovering succinic acid according to claim 1, wherein the concentration of the step (a) is performed by vacuum distillation.

5. The method for recovering succinic acid according to claim 1, wherein succinic acid concentration in the culture broth concentrated in the step (a) is 100~300g/L.

6. The method for recovering succinic acid according to claim 1, wherein the acidification of the step (b) is performed in a range of pH 1.0~3.0 using hydrochloric acid or sulphuric acid.

7. The method for recovering succinic acid according to claim 1, wherein the cooling in the step (c) is performed in a temperature range of 2 to 20℃.

8. The method for recovering succinic acid according to claim 1, wherein the succinic acid producing microorganism is *Mannheimia* sp.

9. The method for recovering succinic acid according to claim 8, wherein said *Mannheimia* sp. is selected from the group consisting of *M. succiniciproducens* LPK, *M. succiniciproducens* LPK7, *M. succiniciproducens* PALK, *M. succiniciproducens* ALKt, and *M. succiniciproducens* ALK strains.

10. A method for recovering succinic acid from a culture broth of *M. succiniciproducens* PALK, the method comprising the steps of:

(a) concentrating the culture broth, from which *M. succiniciproducens* PALK strain is removed, to a succinic acid concentration of 100~300 g/L;

(b) acidifying the concentrated culture broth to pH 1.0~3.0; and

(c) recovering the crystal of succinic acid by cooling down the acidified culture broth to 2~20℃.

11. The method for recovering succinic acid according to claim 10, which additionally comprises a step of: decolorizing the culture broth of *M. succiniciproducens* PALK strain prior to the concentrating step.

12. The method for recovering succinic acid according to claim 10, wherein said *M. succiniciproducens* PALK is cultured in a chemically defined synthetic culture medium.

DRAWINGS

1/5

FIG. 1

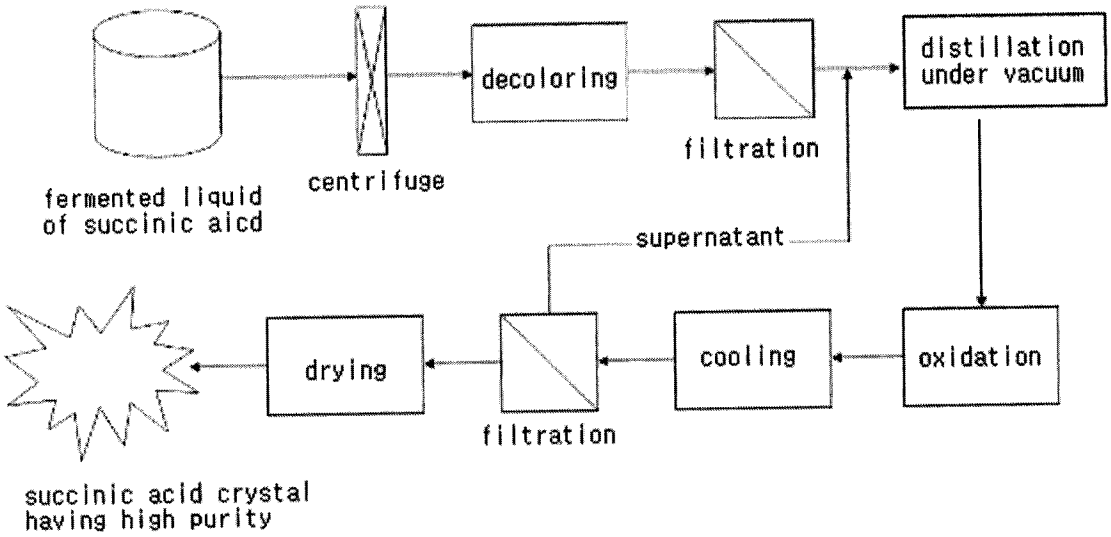


FIG. 2

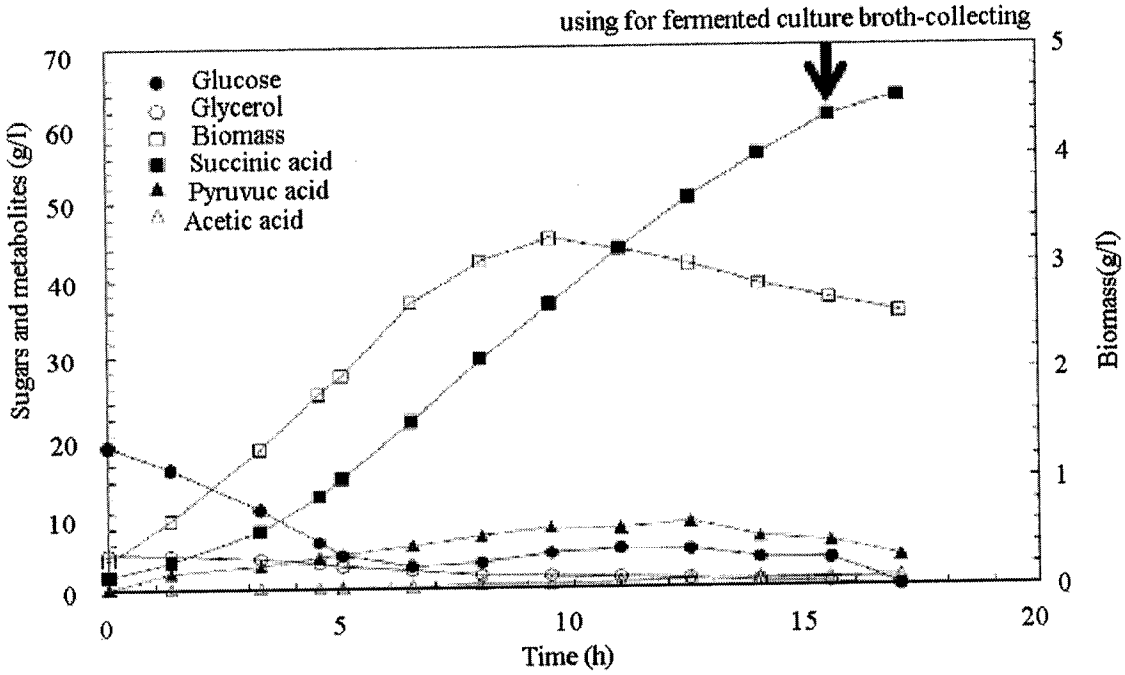


FIG. 3

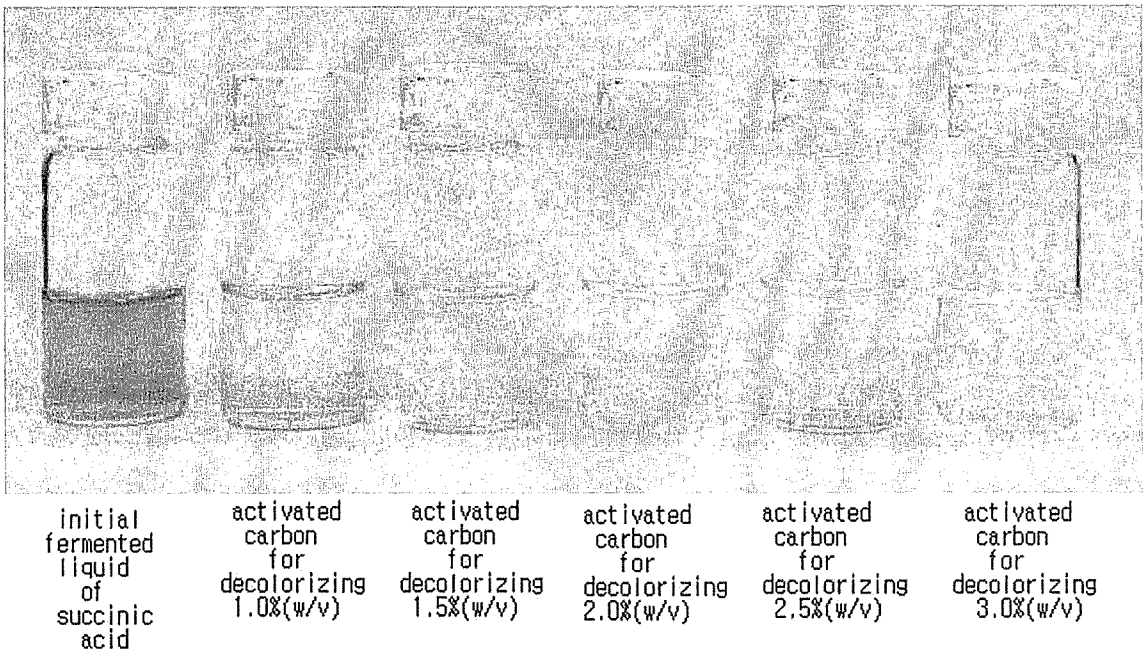

initial fermented liquid of succinic acid
activated carbon for decolorizing 1.0%(w/v)
activated carbon for decolorizing 1.5%(w/v)
activated carbon for decolorizing 2.0%(w/v)
activated carbon for decolorizing 2.5%(w/v)
activated carbon for decolorizing 3.0%(w/v)

FIG. 4

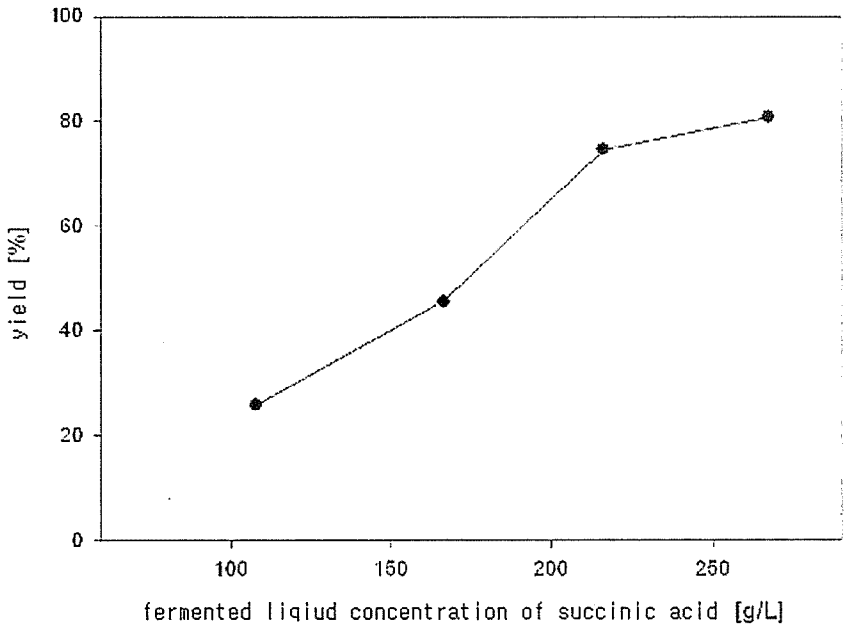

100
80
60
40
20
0
yield [%]
100
150
200
250
fermented liqiud concentration of succinic acid [g/L]

FIG. 5

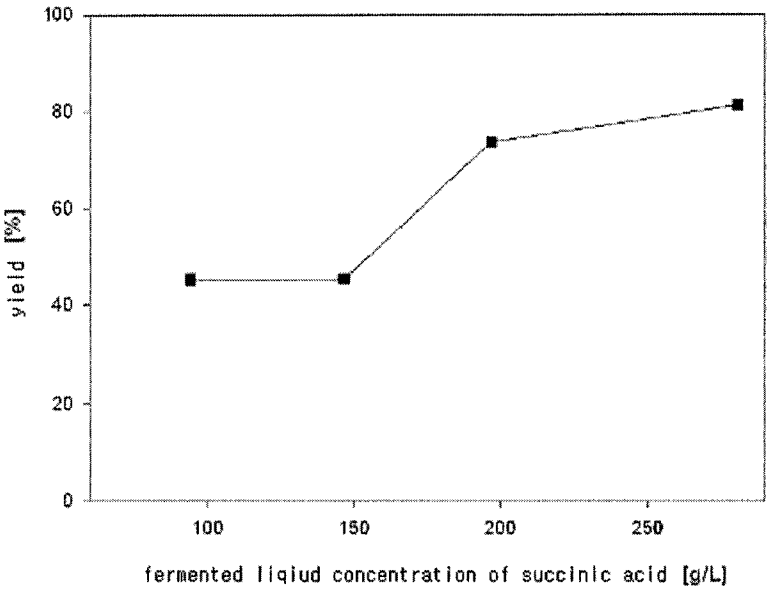

100
80
60
40
20
0
yield [%]
100
150
200
250
fermented liqiud concentration of succinic acid [g/L]

FIG. 6

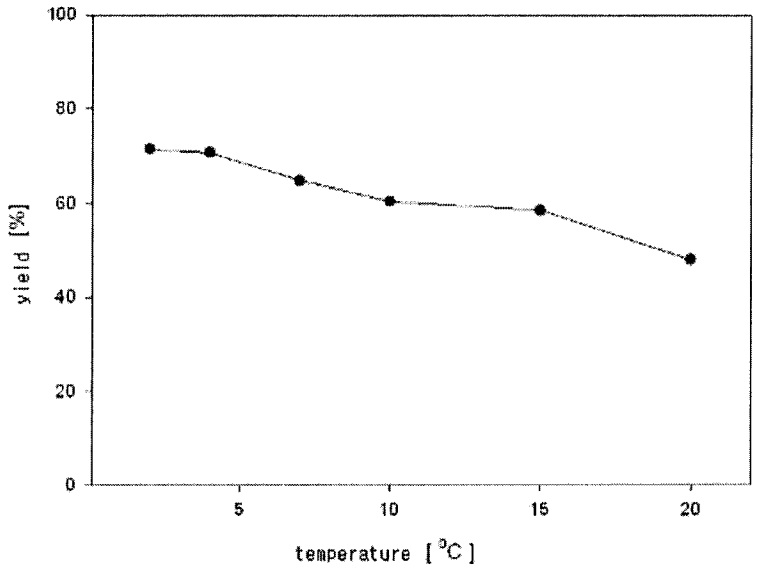

100
80
60
40
20
0
yield [%]
5
10
15
20
temperature [°C]

FIG. 7

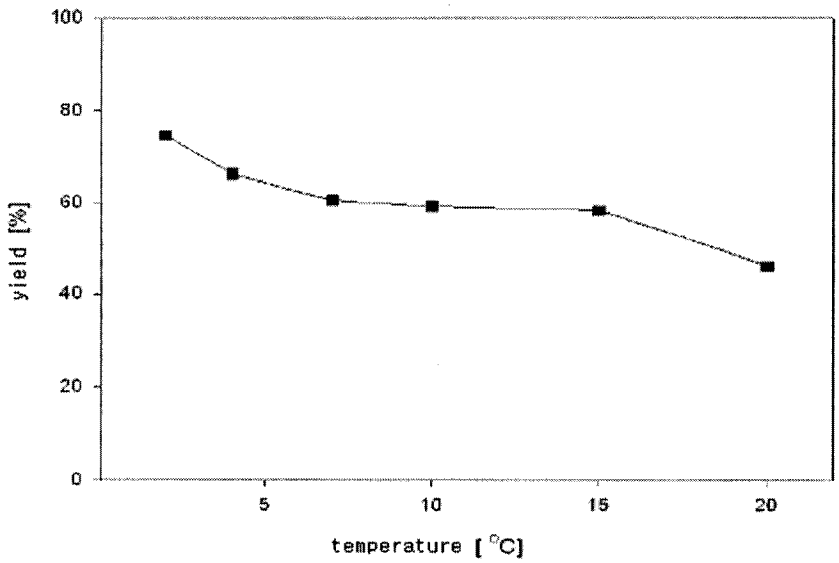

100
80
60
40
20
0
yield [%]
5
10
15
20
temperature [°C]

FIG. 8

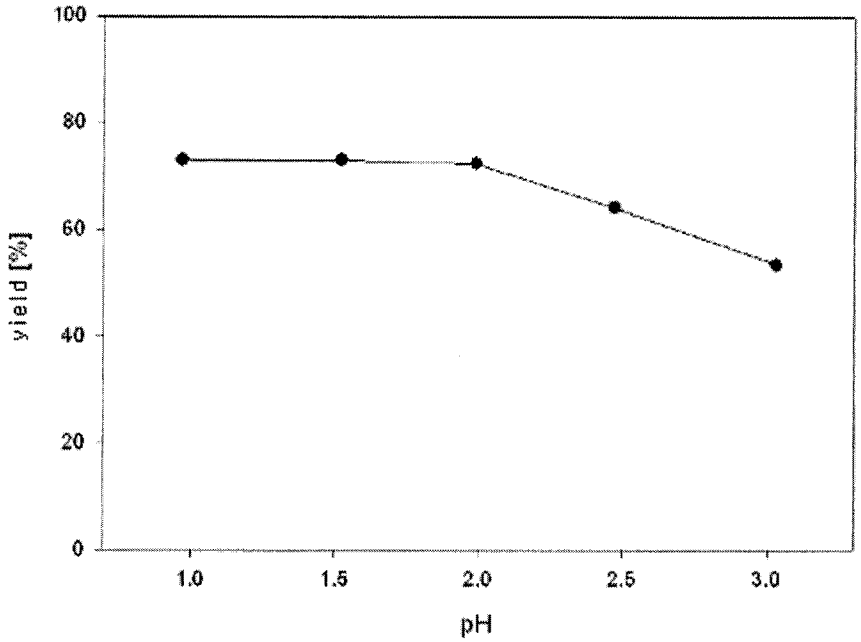

100
80
60
40
20
0
yield [%]
1.0
1.5
2.0
2.5
3.0
pH

FIG. 9

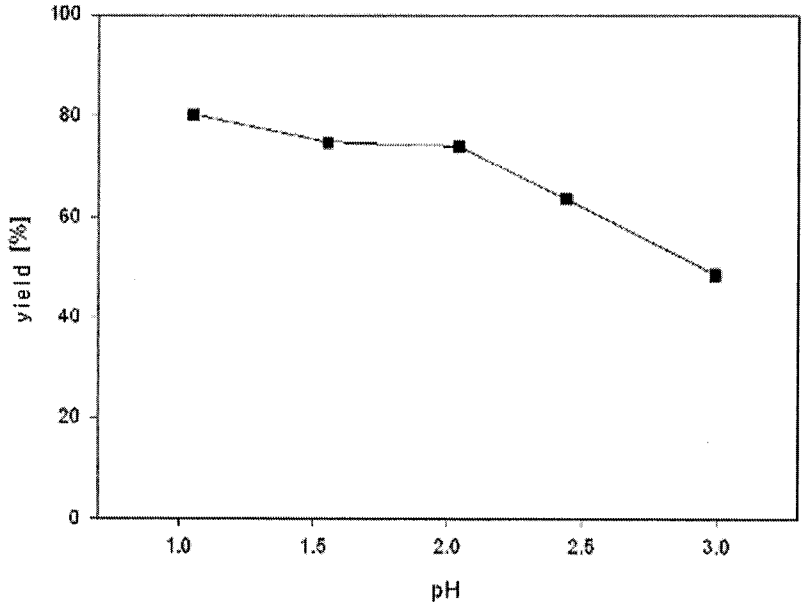
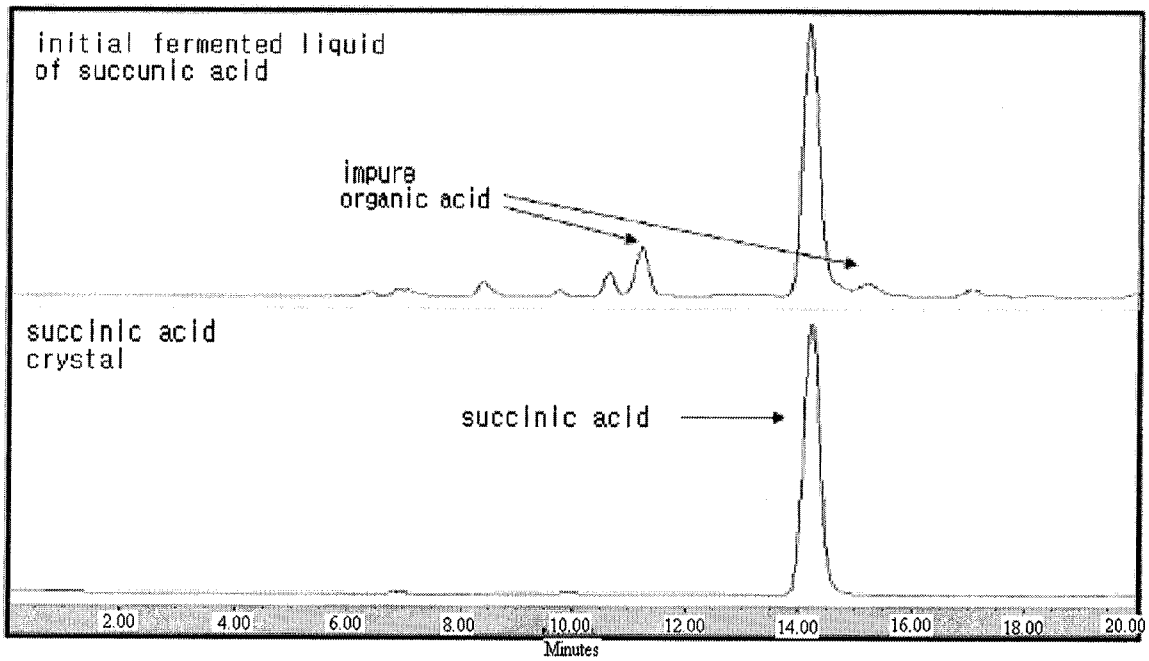

100
80
60
40
20
0
yield [%]
1.0
1.5
2.0
2.5
3.0
pH

FIG. 10


initial fermented liquid
of succunic acid
impure
organic acid
succinic acid
crystal
succinic acid
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
Minutes

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2008/000238

A. CLASSIFICATION OF SUBJECT MATTER

C12P 7/40(2006.01)i

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)

IPC 8 as above

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)

eKIPASS(KIPO internal), Delphion, Pubmed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KR 2006-083729 A (KOREA ADVANCED INSTITUTE OF SCIENCE AND TECHNOLOGY) 21 JULY 2006 See the whole document.	1, 4, 6-10
A	US 6,265,190 B1 (Yedur, S. et al.) 24 JULY 2001 See the whole document.	1-12
A	JP 2005-333886 A (Showa Denko KK) 08 DECEMBER 2005 See the whole document.	1-12
A	WO 2005/030973 A1 (AJINOMOTO CO., INC. et al.) 07 APRIL 2005 See the whole document.	1-12

☐ 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
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 AUGUST 2008 (27.08.2008)	**28 AUGUST 2008 (28.08.2008)**
Name and mailing address of the ISA/KR Korean Intellectual Property Office Government Complex-Daejeon, 139 Seonsa-ro, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer KIM, EUN HEE Telephone No. 82-42-481-5609

Form PCT/ISA/210 (second sheet) (July 2008)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2008/000238

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
KR 2006-083729 A	21.07.2006	none	
US 6265190 B1	24.07.2001	none	
JP 2005-333886 A	08.12.2005	none	
WO 2005/030973 A1	07.04.2005	BR 200414764 A	28.11.2006
		CN 1860237 A	08.11.2006
		EP 1669459 A1	14.06.2006
		US 2006276674 A	07.12.2006