

(51) International Patent Classification:
C07C 51/487 (2006.01) C12P 7/46 (2006.01)(21) International Application Number:
PCT/US2011/028997(22) International Filing Date:
18 March 2011 (18.03.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/320,063 1 April 2010 (01.04.2010) US
61/327,789 26 April 2010 (26.04.2010) US(71) Applicant (for all designated States except US):
BIOAMBER S.A.S. [FR/CA]; 1250 Rene-levesque
Blvd., West, Suite 4110, Montreal, QC H3B 4W8 (CA).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **FRUCHEY, Olan, S.** [US/US]; 13 Greenbrier Avenue, Hurricane, WV 25526 (US). **KEEN, Brian, T.** [US/US]; 108 Hughes Drive, Pinch, WV 25156 (US). **ALBIN, Brooke, A.** [US/US]; 1026 Gordon Drive, Charleston, WV 25303 (US). **CLINTON, Nye, A.** [US/US]; 16 Christopher Drive, Hurricane, WV 25526 (US). **DUNUWILA, Dilum** [US/US]; 394 Franklin Avenue, Princeton, NJ 08540 (US). **DOMBEK, Bernard, D.** [US/US]; 1620 Woodvale Drive, Charleston, WV 25314 (US).(74) Agents: **CHRISTENBURY, T., Daniel** et al.; Dla Piper LLP (US), One Liberty Place, 1650 Market Street, Suite 4900, Philadelphia, PA 19103 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: PROCESSES FOR PRODUCING SUCCINIC ACID FROM FERMENTATION BROTHS CONTAINING DIAMMONIUM SUCCINATE

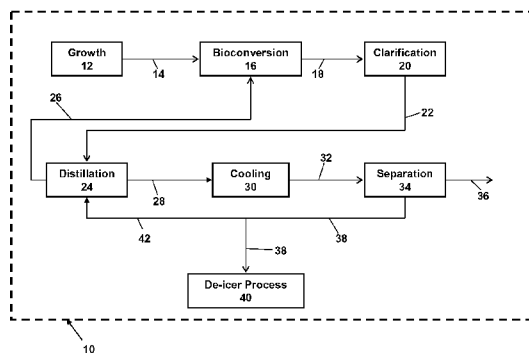


Fig. 1

(57) Abstract: Processes for making SA from either a clarified DAS-containing fermentation broth or a clarified MAS-containing fermentation broth that include distilling the broth under super atmospheric pressure at a temperature of >100°C to about 3000C to form an overhead that comprises water and ammonia, and a liquid bottoms that includes SA, and at least about 20 wt% water; cooling the bottoms to a temperature sufficient to cause the bottoms to separate into a liquid portion and a solid portion that is substantially pure SA; and separating the solid portion from the liquid portion. A method also reduces the broth distillation temperature and pressure by adding an ammonia separating and/or water azeotrope solvent to the broth.

PROCESSES FOR PRODUCING SUCCINIC ACID FROM FERMENTATION BROTHS CONTAINING DIAMMONIUM SUCCINATE

RELATED APPLICATIONS

[0001] This application claims the benefit of US Provisional Application Nos.
5 61/320,063, filed April 1, 2010 and 61/327,789, filed April 26, 2010, the subject matter of
which is hereby incorporated by reference.

TECHNICAL FIELD

[0002] This disclosure relates to processes for the direct production of succinic
acid (SA) from fermentation broths containing diammonium succinate (DAS),
10 monoammonium succinate (MAS), and/or SA.

BACKGROUND

[0003] Certain carbonaceous products of sugar fermentation are seen as
replacements for petroleum-derived materials for use as feedstocks for the manufacture of
carbon-containing chemicals. One such product is SA.

15 [0004] Succinic acid can be produced by microorganisms using fermentable
carbon sources such as sugars as starting materials. However, most commercially viable,
succinate producing microorganisms described in the literature neutralize the fermentation
broth to maintain an appropriate pH for maximum growth, conversion and productivity.
Typically, the pH of the fermentation broth is maintained at or near a pH of 7 by introduction
20 of ammonium hydroxide into the broth, thereby converting the succinic acid to DAS.

[0005] Kushiki (Japanese Published Patent Application, Publication No. 2005-
139156) discloses a method of obtaining MAS from an aqueous solution of DAS that could
be obtained from a fermentation broth to which an ammonium salt is added as a counter ion.
Specifically, MAS is crystallized from an aqueous solution of DAS by adding acetic acid to
25 the solution to adjust the pH of the solution to a value between 4.6 and 6.3, causing impure
MAS to crystallize from the solution.

[0006] Masuda (Japanese Unexamined Application Publication P2007-254354,
Oct. 4, 2007) describes partial deammoniation of dilute aqueous solutions of “ammonium
succinate” of the formula $\text{H}_4\text{NOOCCH}_2\text{CH}_2\text{COONH}_4$. From the molecular formula
30 disclosed, it can be seen that “ammonium succinate” is diammonium succinate. Masuda
removes water and ammonia by heating solutions of the ammonium succinate to yield a solid
succinic acid-based composition containing, in addition to ammonium succinate, at least one
of monoammonium succinate, succinic acid, monoamide succinate, succinimide, succinamide

or ester succinate. Thus, it can be inferred that like Kushiki, Masuda discloses a process that results in production of impure MAS. The processes of both Kushiki and Masuda lead to materials that need to be subjected to various purification regimes to produce high purity MAS.

5 [0007] It would be desirable to have a process for the direct production of substantially pure SA from a DAS-containing fermentation broth.

SUMMARY

10 [0008] We provide a process for making SA from a clarified DAS-containing fermentation broth including distilling the broth under super atmospheric pressure at a temperature of $>100^{\circ}\text{C}$ to about 300°C to form an overhead that comprises water and ammonia, and a liquid bottoms that comprises SA and at least about 20 wt% water, cooling and/or evaporating the bottoms to attain a temperature and composition sufficient to cause the bottoms to separate into a liquid portion and a solid portion that is substantially pure SA, and separating the solid portion from the liquid portion.

15 [0009] We also provide a process for making SA from a clarified DAS-containing fermentation broth including adding an ammonia separating solvent and/or a water azeotroping solvent to the broth, distilling the broth at a temperature and pressure sufficient to form an overhead that comprises water and ammonia, and a liquid bottoms that comprises SA and at least about 20 wt% water, cooling and/or evaporating the bottoms to attain a temperature and composition sufficient to cause the bottoms to separate into a liquid portion and a solid portion that is substantially pure SA, and separating the solid portion from the liquid portion.

20 [0010] We further provide a process for making SA from a clarified MAS-containing fermentation broth including distilling the broth under super atmospheric pressure at a temperature of $>100^{\circ}\text{C}$ to about 300°C to form an overhead that comprises water and ammonia, and a liquid bottoms that comprises SA and at least about 20 wt% water, cooling and/or evaporating the bottoms to attain a temperature and composition sufficient to cause the bottoms to separate into a liquid portion and a solid portion that is substantially pure SA, and separating the solid portion from the liquid portion.

30 [0011] We further provide a process for making SA from a clarified MAS-containing fermentation broth including adding an ammonia separating solvent and/or a water azeotroping solvent to the broth, distilling the broth at a temperature and pressure sufficient

to form an overhead that includes water and ammonia, and a liquid bottoms that comprises SA, and at least about 20 wt% water, cooling and/or evaporating the bottoms to attain a temperature and composition sufficient to cause the bottoms to separate into a liquid portion and a solid portion that is substantially pure SA, and separating the solid portion from the liquid portion.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Fig. 1 is a block diagram of the process for making SA from a DAS containing broth.

[0013] Fig. 2 is a graph showing the solubility of SA as a function of temperature in both water and a 20 wt% aqueous MAS solution.

DETAILED DESCRIPTION

[0014] It will be appreciated that at least a portion of the following description is intended to refer to representative examples of processes selected for illustration in the drawings and is not intended to define or limit the disclosure, other than in the appended claims.

[0015] Our processes may be appreciated by reference to Fig. 1, which shows in block diagram form one representative example, 10, of our methods.

[0016] A growth vessel 12, typically an in-place steam sterilizable fermentor, may be used to grow a microbial culture (not shown) that is subsequently utilized for the production of the DAS, MAS, and/or SA -containing fermentation broth. Such growth vessels are known in the art and are not further discussed.

[0017] The microbial culture may comprise microorganisms capable of producing SA from fermentable carbon sources such as carbohydrate sugars. Representative examples of microorganisms include *Escherichia coli* (*E. coli*), *Aspergillus niger*, *Corynebacterium glutamicum* (also called *Brevibacterium flavum*), *Enterococcus faecalis*, *Veillonella parvula*, *Actinobacillus succinogenes*, *Mannheimia succiniciproducens*, *Anaerobiospirillum succiniciproducens*, *Paecilomyces Varioti*, *Saccharomyces cerevisiae*, *Bacteroides fragilis*, *Bacteroides ruminicola*, *Bacteroides amylophilus*, *Alcaligenes eutrophus*, *Brevibacterium ammoniagenes*, *Brevibacterium lactofermentum*, *Candida brumptii*, *Candida catenulate*, *Candida mycoderma*, *Candida zeylanoides*, *Candida paludigena*, *Candida sonorensis*, *Candida utilis*, *Candida zeylanoides*, *Debaryomyces hansenii*, *Fusarium oxysporum*, *Humicola lanuginosa*, *Kloeckera apiculata*, *Kluyveromyces lactis*, *Kluyveromyces*

wickerhamii, *Penicillium simplicissimum*, *Pichia anomala*, *Pichia besseyi*, *Pichia media*, *Pichia guilliermondii*, *Pichia inositovora*, *Pichia stipidis*, *Saccharomyces bayanus*, *Schizosaccharomyces pombe*, *Torulopsis candida*, *Yarrowia lipolytica*, mixtures thereof and the like.

5 **[0018]** A preferred microorganism is an *E. coli* strain deposited at the ATCC under accession number PTA-5132. More preferred is this strain with its three antibiotic resistance genes (*cat*, *amphl*, *tetA*) removed. Removal of the antibiotic resistance genes *cat* (coding for the resistance to chloramphenicol), and *amphl* (coding for the resistance to kanamycin) can be performed by the so-called “Lambda-red (λ-red)” procedure as described
10 in Datsenko KA and Wanner BL., Proc. Natl. Acad. Sci. U S A 2000 Jun 6; 97(12) 6640-5, the subject matter of which is incorporated herein by reference. The tetracycline resistant gene *tetA* can be removed using the procedure originally described by Bochner et al., J Bacteriol. 1980 August; 143(2): 926-933, the subject matter of which is incorporated herein by reference. Glucose is a preferred fermentable carbon source for this microorganism.

15 **[0019]** A fermentable carbon source (e.g., carbohydrates and sugars), optionally a source of nitrogen and complex nutrients (e.g., corn steep liquor), additional media components such as vitamins, salts and other materials that can improve cellular growth and/or product formation, and water may be fed to the growth vessel 12 for growth and sustenance of the microbial culture. Typically, the microbial culture is grown under aerobic
20 conditions provided by sparging an oxygen-rich gas (e.g., air or the like). Typically, an acid (e.g., sulphuric acid or the like) and ammonium hydroxide are provided for pH control during the growth of the microbial culture.

[0020] In one example (not shown), the aerobic conditions in growth vessel (provided by sparging an oxygen-rich gas) are switched to anaerobic conditions by changing
25 the oxygen-rich gas to an oxygen-deficient gas (e.g., CO₂ or the like). The anaerobic environment triggers bioconversion of the fermentable carbon source to succinic acid *in situ* in growth vessel 12. Ammonium hydroxide is provided for pH control during bioconversion of the fermentable carbon source to SA. The SA that is produced is at least partially if not totally neutralized to DAS due to the presence of the ammonium hydroxide, leading to the
30 production of a broth comprising DAS. The CO₂ provides an additional source of carbon for the production of SA.

[0021] In another example, the contents of growth vessel 12 may be transferred via stream 14 to a separate bioconversion vessel 16 for bioconversion of a carbohydrate source to SA. An oxygen-deficient gas (e.g., CO₂ or the like) is sparged in bioconversion vessel 16 to provide anaerobic conditions that trigger production of SA. Ammonium hydroxide is provided for pH control during bioconversion of the carbohydrate source to SA. Due to the presence of the ammonium hydroxide, the SA produced is at least partially neutralized to DAS, leading to production of a broth that comprises DAS. The CO₂ provides an additional source of carbon for production of SA.

[0022] In yet another example, the bioconversion may be conducted at relatively low pH (e.g., 3 – 6). A base (ammonium hydroxide or ammonia) may be provided for pH control during bioconversion of the carbohydrate source to SA. Depending on the desired pH, due to the presence or lack of the ammonium hydroxide, either SA is produced or the SA produced is at least partially neutralized to MAS, DAS or a mixture comprising SA, MAS and/or DAS. Thus, the SA produced during bioconversion can be subsequently neutralized, optionally in an additional step, by providing either ammonia or ammonium hydroxide leading to a broth comprising DAS. As a consequence, a “DAS-containing fermentation broth” generally means that the fermentation broth comprises DAS and possibly any number of other components such as MAS and/or SA, whether added and/or produced by bioconversion or otherwise. Similarly, a “MAS-containing fermentation broth” generally means that the fermentation broth comprises MAS and possibly any number of other components such as DAS and/or SA, whether added and/or produced by bioconversion or otherwise.

[0023] The broth resulting from the bioconversion of the fermentable carbon source (in either vessel 12 or vessel 16, depending on where the bioconversion takes place), typically contains insoluble solids such as cellular biomass and other suspended material, which are transferred via stream 18 to clarification apparatus 20 before distillation. Removal of insoluble solids clarifies the broth. This reduces or prevents fouling of subsequent distillation equipment. The insoluble solids can be removed by any one of several solid-liquid separation techniques, alone or in combination, including but not limited to centrifugation and filtration (including, but not limited to ultra-filtration, micro-filtration or depth filtration). The choice of filtration can be made using techniques known in the art.

Soluble inorganic compounds can be removed by any number of known methods such as, but not limited to, ion-exchange, physical adsorption and the like.

[0024] An example of centrifugation is a continuous disc stack centrifuge. It can be useful to add a polishing filtration step following centrifugation such as dead-end or cross-flow filtration that may include the use of a filter aide such as diatomaceous earth or the like or, more preferably, ultra-filtration or micro-filtration. The ultra-filtration or micro-filtration membrane can be ceramic or polymeric, for example. One example of a polymeric membrane is SelRO MPS-U20P (pH stable ultra-filtration membrane) manufactured by Koch Membrane Systems (850 Main Street, Wilmington, MA, USA). This is a commercially available polyethersulfone membrane with a 25,000 Dalton molecular weight cut-off which typically operates at pressures of 0.35 to 1.38 MPa (maximum pressure of 1.55 MPa) and at temperatures up to 50°C. As an alternative to using centrifugation and a polishing filtration in combination, cross-flow filtration may be employed alone using ultra- or micro-filtration membranes.

[0025] The resulting clarified DAS-containing broth or MAS-containing broth, substantially free of the microbial culture and other solids, is transferred via stream 22 to distillation apparatus 24.

[0026] The clarified broth should contain DAS and/or MAS in an amount that is at least a majority of, preferably at least about 70 wt%, more preferably 80 wt% and most preferably at least about 90 wt% of all the ammonium dicarboxylate salts in the broth. The concentration of DAS and/or MAS as a weight percent (wt%) of the total dicarboxylic acid salts in the fermentation broth can be easily determined by high pressure liquid chromatography (HPLC) or other known means.

[0027] Water and ammonia are removed from distillation apparatus 24 as an overhead, and at least a portion is optionally recycled via stream 26 to bioconversion vessel 16 (or growth vessel 12 operated in the anaerobic mode).

[0028] Distillation temperature and pressure are not critical as long as the distillation is carried out in a way that ensures that the distillation overhead contains water and ammonia, and the distillation bottoms preferably comprises at least some MAS and at least about 20 wt% water. A more preferred amount of water is at least about 30 wt% and an even more preferred amount is at least about 40 wt%. The rate of ammonia removal from the distillation step increases with increasing temperature and also can be increased by injecting

steam (not shown) during distillation. The rate of ammonia removal during distillation may also be increased by conducting distillation under a vacuum, under pressure or by sparging the distillation apparatus with a non-reactive gas such as air, nitrogen or the like.

[0029] Removal of water during the distillation step can be enhanced by the use of an organic azeotroping agent such as toluene, xylene, methylcyclohexane, methyl isobutyl ketone, cyclohexane, heptane or the like, provided that the bottoms contains at least about 20 wt% water. If the distillation is carried out in the presence of an organic agent capable of forming an azeotrope consisting of the water and the agent, distillation produces a biphasic bottoms that comprises an aqueous phase and an organic phase, in which case the aqueous phase can be separated from the organic phase, and the aqueous phase used as the distillation bottoms. Byproducts such as succinamide and succinimide are substantially avoided provided the water level in the bottoms is maintained at a level of at least about 30 wt%.

[0030] A preferred temperature for the distillation step is in the range of about 50 to about 300° C, depending on the pressure. A more preferred temperature range is about 150 to about 240° C, depending on the pressure. A distillation temperature of about 170 to about 230° C is preferred. "Distillation temperature" refers to the temperature of the bottoms (for batch distillations this may be the temperature at the time when the last desired amount of overhead is taken).

[0031] Adding a water miscible organic solvent or an ammonia separating solvent facilitates deammoniation over a variety of distillation temperatures and pressures as discussed above. Such solvents include aprotic, bipolar, oxygen-containing solvents that may be able to form passive hydrogen bonds. Examples include, but are not limited to, diglyme, triglyme, tetraglyme, sulfoxides such as dimethylsulfoxide (DMSO), amides such as dimethylformamide (DMF) and dimethylacetamide, sulfones such as dimethylsulfone, gamma-butyrolactone (GBL), sulfolane, polyethyleneglycol (PEG), butoxytriglycol, N-methylpyrrolidone (NMP), ethers such as dioxane, methyl ethyl ketone (MEK) and the like. Such solvents aid in the removal of ammonia from the DAS or MAS in the clarified broth. Regardless of the distillation technique, it is preferable that the distillation be carried out in a way that ensures that at least some MAS and at least about 20 wt% water remain in the bottoms and even more advantageously at least about 30 wt%. The distillation can be performed at atmospheric, sub-atmospheric or super-atmospheric pressures.

[0032] Under other conditions such as when the distillation is conducted in the absence of an azeotropic agent or ammonia separating solvent, the distillation is conducted at super atmospheric pressure at a temperature of $>100^{\circ}\text{C}$ to about 300°C to form an overhead that comprises water and ammonia and a liquid bottoms that comprises SA and at least about 20 wt% water. Super atmospheric pressure typically falls within a range of $>\text{ambient}$ atmosphere up to and including about 25 atmospheres. Advantageously the amount of water is at least about 30 wt%.

[0033] The distillation can be a one-stage flash, a multistage distillation (i.e., a multistage column distillation), multiple columns or the like. The one-stage flash can be conducted in a flasher (e.g., a wiped film evaporator, thin film evaporator, thermosiphon flasher, forced circulation flasher and the like). The multistages of the distillation column can be achieved by using trays, packing or the like. The packing can be random packing (e.g., Raschig rings, Pall rings, Berl saddles and the like) or structured packing (e.g., Koch-Sulzer packing, Intalox pack-ing, Mellapak and the like). The trays can be of any design (e.g., sieve trays, valve trays, bubble-cap trays and the like). The distillation can be performed with any number of theoretical stages.

[0034] If the distillation apparatus is a column, the configuration is not particularly critical, and the column can be designed using well known criteria. The column can be operated in either stripping mode, rectifying mode or fractionation mode. Distillation can be conducted in either batch, semi-continuous or continuous mode. In the continuous mode, the broth is fed continuously into the distillation apparatus, and the overhead and bottoms are continuously removed from the apparatus as they are formed. The distillate from distillation is an ammonia/water solution, and the distillation bottoms is a liquid, aqueous solution of MAS and SA, which may also contain other fermentation by-product salts (i.e., ammonium acetate, ammonium formate, ammonium lactate and the like) and color bodies.

[0035] The distillation bottoms can be transferred via stream 28 to cooling apparatus 30 and cooled by conventional techniques. Cooling technique is not critical. A heat exchanger (with heat recovery) can be used. A flash vaporization cooler can be used to cool the bottoms down to about 15°C . Cooling to 0°C typically employs a refrigerated coolant such as, for example, glycol solution or, less preferably, brine. A concentration step can be included prior to cooling to help increase product yield. Further, both concentration

and cooling can be combined using known methods such as vacuum evaporation and heat removal using integrated cooling jackets and/or external heat exchangers.

[0036] We found that the presence of some MAS in the liquid bottoms facilitates cooling-induced separation of the bottoms into a liquid portion in contact with a solid portion that at least “consists essentially” of SA (meaning that the solid portion is at least substantially pure crystalline SA) by reducing the solubility of SA in the liquid, aqueous, MAS-containing bottoms. Fig. 2 illustrates the reduced solubility of SA in an aqueous 20 wt% MAS solution at various temperatures ranging from 5 to 45°C. We discovered, therefore, that SA can be more completely crystallized out of an aqueous solution if some MAS is also present in that solution. A preferred concentration of MAS in such a solution is about 20 wt%. This phenomenon allows crystallization of SA (i.e., formation of the solid portion of the distillation bottoms) at temperatures higher than those that would be required in the absence of MAS.

[0037] The distillation bottoms, after cooling, is fed via stream 32 to separator 34 for separation of the solid portion from the liquid portion. Separation can be accomplished via pressure filtration (e.g., using Nutsche or Rosenmond type pressure filters), centrifugation and the like. The resulting solid product can be recovered as product 36 and dried, if desired, by standard methods.

[0038] After separation, it may be desirable to treat the solid portion to ensure that no liquid portion remains on the surface(s) of the solid portion. One way to minimize the amount of liquid portion that remains on the surface of the solid portion is to wash the separated solid portion with water and dry the resulting washed solid portion (not shown). A convenient way to wash the solid portion is to use a so-called “basket centrifuge” (not shown). Suitable basket centrifuges are available from The Western States Machine Company (Hamilton, OH, USA).

[0039] The liquid portion of the separator 34 (i.e., the mother liquor) may contain remaining dissolved SA, any unconverted MAS, any fermentation byproducts such as ammonium acetate, lactate, or formate, and other minor impurities. This liquid portion can be fed via stream 38 to a downstream apparatus 40. In one instance, apparatus 40 may be a means for making a de-icer by treating in the mixture with an appropriate amount of potassium hydroxide, for example, to convert the ammonium salts to potassium salts. Ammonia generated in this reaction can be recovered for reuse in the bioconversion vessel 16

(or growth vessel 12 operating in the anaerobic mode). The resulting mixture of potassium salts is valuable as a de-icer and anti-icer.

[0040] The mother liquor from the solids separation step, 34, can be recycled (or partially recycled) to distillation apparatus 24 via stream 42 to further enhance recovery of SA, as well as further convert MAS to SA.

[0041] The solid portion of the cooling-induced crystallization is substantially pure SA and is, therefore, useful for the known utilities of SA.

[0042] HPLC can be used to detect the presence of nitrogen-containing impurities such as succinamide and succinimide. The purity of SA can be determined by elemental carbon and nitrogen analysis. An ammonia electrode can be used to determine a crude approximation of SA purity.

[0043] Depending on the circumstances and various operating inputs, there are instances when the fermentation broth may be a clarified MAS-containing fermentation broth or a clarified SA-containing fermentation broth. In those circumstances, it can be advantageous to add MAS, DAS and/or SA and, optionally, ammonia, and/or ammonium hydroxide to those fermentation broths to facilitate the production of substantially pure SA. For example, the operating pH of the fermentation broth may be oriented such that the broth is a MAS-containing broth or a SA-containing broth. MAS, DAS, SA, ammonia, and/or ammonium hydroxide may be optionally added to those broths to attain a broth pH preferably less than about 6 to facilitate production of the above-mentioned substantially pure SA. In one particular form, it is especially advantageous to recycle SA, MAS and water from the liquid bottoms resulting from the distillation step 24, and/or the liquid portion from the separator 34, into the fermentation broth and/or clarified fermentation broth. In referring to the MAS-containing broth, such broth generally means that the fermentation broth comprises MAS and possibly any number of other components such as DAS and/or SA, whether added and/or produced by bioconversion or otherwise.

EXAMPLES

[0044] The processes are illustrated by the following non-limiting representative examples. In the first two examples, a synthetic, aqueous DAS solution was used in place of an actual clarified DAS-containing fermentation broth. The other examples employed an actual clarified DAS-containing fermentation broth.

[0045] For the first two examples, the use of a synthetic DAS solution is believed to be a good model for the behavior of an actual broth in our processes because of the solubility of the typical fermentation by-products found in actual broth. The major by-products produced during fermentation are ammonium acetate, ammonium lactate and ammonium formate. Ammonium acetate, ammonium lactate and ammonium formate are significantly more soluble in water than SA, and each is typically present in the broth at less than 10% of the DAS concentration. In addition, even when the acids (acetic, formic and lactic acids) were formed during the distillation step, they are miscible with water and will not crystallize from water. This means that the SA reaches saturation and crystallizes from solution (i.e., forming the solid portion), leaving the acid impurities dissolved in the mother liquor (i.e., the liquid portion).

EXAMPLE 1

[0046] This experiment shows the conversion of DAS to SA in an aqueous media.

[0047] An experiment was conducted in a 300 ml Hastelloy C stirred Parr reactor using a 15% (1.0 M) synthetic DAS solution. The reactor was charged with 200 g of solution and pressurized to 200 psig. The contents were then heated to begin distillation, bringing the temperature to approximately 200°C. Ammonia and water vapor were condensed overhead with cooling water and collected in a reservoir. Fresh water was pumped back to the system at a rate equal to the make rate (approximately 2 g/min) to maintain a constant succinate concentration and volume of material. The run continued for 7 hours. At the end of the run, analysis of the mother liquor showed 59% conversion to SA, 2.4% to succinamic acid, and 2.9% to succinimide. Cooling the mother liquor would result in a liquid portion and a solid portion that would be substantially pure SA.

EXAMPLE 2

[0048] This example demonstrates the effect of solvents on ammonia evolution from aqueous DAS. Run 10 is the control experiment where no solvent is present.

[0049] The outer necks of a three neck 1-L round bottom flask were fitted with a thermometer and a stopper. The center neck was fitted with a five tray 1" Oldershaw section. The Oldershaw section was topped with a distillation head. An ice cooled 500 mL round bottom flask was used as the receiver for the distillation head. The 1-L round bottom flask was charged with distilled water, the solvent being tested, SA and concentrated ammonium hydroxide solution. The contents were stirred with a magnetic stirrer to dissolve all the

solids. After the solids dissolved, the contents were heated with the heating mantle to distill 350g of distillate. The distillate was collected in the ice cooled 500 mL round bottom flask. The pot temperature was recorded as the last drop of distillate was collected. The pot contents were allowed to cool to room temperature and the weight of the residue and weight of the distillate were recorded. The ammonia content of the distillate was then determined via titration. The results were recorded in Tables 1 and 2.

Table 1

Run #	1	2	3	4	5
Name of Acid charged	Succinic	Succinic	Succinic	Succinic	Succinic
Wt Acid Charged (g)	11.8	11.81	11.83	11.8	11.78
Moles Acid Charged	0.1	0.1	0.1	0.1	0.1
Wt 28% NH3 Solution Charged (g)	12.76	12.78	12.01	12.98	13.1
Moles NH3 Charged	0.21	0.21	0.2	0.215	0.217
Name of Solvent	DMSO	DMF	NMP	sulfolane	triglyme
Wt Solvent Charged (g)	400.9	400	400	400	400
Wt Water Charged (g)	400	400	400	400	401
Wt Distillate (g)	350.1	365.9	351.3	352.1	351.2
Wt Residue (g)	467.8	455	460.5	457.1	473
%Mass Accountability	99.1	99.6	98.5	98.1	99.8
Wt% NH3 in distillate (titration)	0.91	0.81	0.78	0.71	0.91
Moles NH3 in distillate	0.187	0.174	0.161	0.147	0.188
%NH3 removed in Distillate	89	83	81	66	86
%First NH3 removed in Distillate	100	100	100	100	100
%Second NH3 removed in Distillate	78	66	62	32	72
Final Pot Temp (°C)	138	114	126	113	103
Final DAS/MAS/SA ratio	0/22/78	0/34/66	0/38/62	0/68/32	0/28/72

Table 2

Run #	6	7	8	9	10
Name of Acid charged	Succinic	Succinic	Succinic	Succinic	Succinic
Wt Acid Charged (g)	11.84	11.81	11.8	11.81	11.8
Moles Acid Charged	0.1	0.1	0.1	0.1	0.1
Wt 28% NH3 Solution Charged (g)	12.11	12.11	12.1	12.15	12.1
Moles NH3 Charged	0.2	0.2	0.2	0.2	0.2
Name of Solvent	Dowanol TPM	Tetraglyme	Tetra PentaEG	HeavyMe GlyEther	none
Wt Solvent Charged (g)	400.1	400	400	400.1	0
Wt Water Charged (g)	400	400	400	400.1	800
Wt Distillate (g)	350	345	350	349	351
Wt Residue (g)	468.4	473.8	465	470.4	466
%Mass Accountability	99.3	99.4	98.9	99.4	99.2

Run #	6	7	8	9	10
Wt% NH3 in distillate (titration)	0.58	0.62	0.55	0.6	0.13
Moles NH3 in distillate	0.119	0.126	0.113	0.123	0.027
%NH3 removed in Distillate	60	63	57	62	13.4
%First NH3 removed in Distillate	100	100	100	100	27
%Second NH3 removed in Distillate	20	26	14	24	0
Final Pot Temp (°C)	104	110	115	113	100
Final DAS/MAS/SA ratio	0/80/20	0/74/26	0/86/14	0/76/24	83/27/0

EXAMPLE 3

[0050] This example used a DAS-containing, clarified fermentation broth derived from a fermentation broth containing *E. coli* strain ATCC PTA-5132.

5 **[0051]** The initial fermentation broth was clarified, thereby resulting in a clarified fermentation broth containing ~4.5% diammonium succinate (DAS). That clarified broth was used to produce crystalline SA as follows. The broth was first concentrated to approximately 9% using an RO membrane and then subjected to distillation at atmospheric pressure to further concentrate the broth to around 40%.

10 **[0052]** The concentrated broth was used as the starting material for conversion of DAS to SA, carried out batchwise in a 300 ml Parr reactor. A 200 g portion of the solution was reacted at 200°C/200 psig for 11 hours. As the reaction proceeded, water vapor and ammonia liberated from the DAS were condensed and collected overhead. Condensate was collected at about 2 g/min, and makeup water was fed back to the system at approximately
15 the same rate.

[0053] Multiple samples were taken throughout the experiment. Samples taken early in the reaction indicated the presence of succinamide, succinamic acid, and succinimide. However, these nitrogen-containing byproducts decreased throughout the experiment. Conversion to SA was observed to be 55% in the final bottoms sample. The
20 final solution was concentrated by evaporation and cooled to 4°C. The resulting crystalline solids were isolated via vacuum filtration, washed with ice water and dried under vacuum. The product (7 g) was essentially pure SA as determined by HPLC.

EXAMPLE 4

[0054] A 500 mL round bottom flask was charged with 80g of an aqueous 36% DAS solution and 80g of triglyme. The flask was fitted with a 5 tray 1" glass Oldershaw column section which was topped with a distillation head. An addition funnel containing 3300g of water was also connected to the flask. The flask was stirred with a magnetic stirrer and heated with a heating mantel. The distillate was collected in an ice cooled receiver. When the distillate started coming over the water in the addition funnel was added to the flask at the same rate as the distillate was being taken. A total of 3313g of distillate was taken. The distillate contained 4.4g of ammonia, as determined by titration. This means ~37% of the DAS was converted to SA with the rest being converted to MAS. The residue in the flask was then placed in an Erlenmeyer flask and cooled to -4°C while stirring. After stirring for 30 minutes the slurry was filtered while cold yielding 7.1g of solids. The solids were dissolved in 7.1g of hot water and then cooled in an ice bath while stirring. The cold slurry was filtered and the solids dried in a vacuum oven at 100°C for 2 hrs yielding 3.9g of SA. HPLC analysis indicated that the solids were SA with 0.099% succinamic acid present.

EXAMPLE 5

[0055] A pressure distillation column was made using an 8 ft long 1.5" 316 SS Schedule 40 pipe that was packed with 316 SS Propak packing. The base of the column was equipped with an immersion heater to serve as a reboiler. Nitrogen was injected into the reboiler via a needle valve to pressure. The overhead of the column had a total take-off line which went to a 316 SS shell and tube condenser with a receiver. The receiver was equipped with a pressure gauge and a back pressure regulator. Material was removed from the overhead receiver via blowcasing through a needle valve. Preheated feed was injected into the column at the top of the packing via a pump along with a dilute 0.4% sodium hydroxide solution. Preheated water was also injected into the reboiler via a pump. This column was first operated at 50 psig pressure which gave a column temperature of 150°C. The top of the column was fed a 4.7% DAS containing broth at a rate of 8 mL/min along with 0.15 mL/min of 0.4% sodium hydroxide solution. Water was fed to the reboiler at a rate of 4 mL/min. The overhead distillate rate was taken at 8 mL/min and the residue rate was taken at 4 mL/min. A total of 2565g of broth was fed to the column along with 53g of 0.4% sodium hydroxide solution. A total of 2750g of distillate was taken and 1269g of residue taken during the run. Titration of the distillate indicated that ~71% of the total ammonia contained in the DAS was

removed (i.e. the residue was a 42/58 mixture of SA/MAS). The composited residue was then fed back to the same column the next day under the following conditions; pressure 100 psig and temperature 173 °C. The composited residue was fed to the top of the column at 4 mL/min along with 0.15 mL/min of 0.4% sodium hydroxide solution. The reboiler was fed
5 water at 9.2 mL/min. A total of 1240g of residue from the previous day was fed to the column along with 58g of sodium hydroxide solution and 2890g of water. A total of 3183g of distillate was taken along with 1132g of residue during the run. Titration of the distillate revealed an additional ~14% of the ammonia was removed yielding a 70/30 mixture of SA/MAS in the residue.

10 **[0056]** Although our processes have been described in connection with specific steps and forms thereof, it will be appreciated that a wide variety of equivalents may be substituted for the specified elements and steps described herein without departing from the spirit and scope of this disclosure as described in the appended claims.

CLAIMS

1. A process for making SA from a clarified DAS-containing fermentation broth comprising:
 - (a) distilling the broth under super atmospheric pressure at a temperature of $>100^{\circ}\text{C}$ to about 300°C to form an overhead that comprises water and ammonia, and a liquid bottoms that comprises SA, and at least about 20 wt% water;
 - (b) cooling and/or evaporating the bottoms to attain a temperature and composition sufficient to cause the bottoms to separate into a liquid portion and a solid portion that is substantially pure SA; and
 - (c) separating the solid portion from the liquid portion.
2. The process of claim 1, wherein the liquid bottoms contains at least some MAS and/or the solid portion is substantially free of succinamic acid, succinamide and succinimide.
3. The process of claim 1, further comprising recycling the SA and water and, optionally, MAS from the liquid bottoms to the clarified broth.
4. The process of claim 1, further comprising removing water from the liquid bottoms to increase concentration of SA in the liquid bottoms.
5. The process of claim 1, wherein distilling the broth is carried out in the presence of an ammonia separating solvent which is at least one selected from the group consisting of diglyme, triglyme, tetraglyme, sulfoxides, amides, sulfones, polyethyleneglycol (PEG), butoxytriglycol, N-methylpyrrolidone (NMP), ethers, and methyl ethyl ketone (MEK) or in the presence of a water azeotroping solvent which is at least one selected from the group consisting of toluene, xylene, methylcyclohexane, methyl isobutyl ketone, hexane, cyclohexane and heptane.
6. A process for making SA from a clarified DAS-containing fermentation broth comprising:
 - (a) adding an ammonia separating and/or water azeotroping solvent to the broth;
 - (b) distilling the broth at a temperature and pressure sufficient to form an overhead that comprises water and ammonia, and a liquid bottoms that comprises SA, and at least about 20 wt% water;

- (c) cooling and/or evaporating the bottoms to attain a temperature and composition sufficient to cause the bottoms to separate into a liquid portion and a solid portion that is substantially pure SA; and
- (d) separating the solid portion from the liquid portion.

7. The process of claim 6, wherein the liquid bottoms contains at least some MAS and/or the solid portion is substantially free of succinamic acid, succinamide and succinimide.

8. The process of claim 6, further comprising recycling the SA, water, and the solvent and, optionally, MAS from the liquid bottoms to the clarified broth.

9. The process of claim 6, wherein distilling the broth is carried out in the presence of an ammonia separating solvent which is at least one selected from the group consisting of diglyme, triglyme, tetraglyme, sulfoxides, amides, sulfones, polyethyleneglycol (PEG), butoxytriglycol, N-methylpyrrolidone (NMP), ethers, and methyl ethyl ketone (MEK) or in the presence of a water azeotroping solvent which is at least one selected from the group consisting of toluene, xylene, methylcyclohexane, methyl isobutyl ketone, hexane, cyclohexane and heptane.

10. The process of claim 6, further comprising removing water from the liquid bottoms to increase concentration of SA in the liquid bottoms.

11. A process for making SA from a clarified MAS-containing fermentation broth comprising:

- (a) distilling the broth under super atmospheric pressure at a temperature of $>100^{\circ}\text{C}$ to about 300°C to form an overhead that comprises water and ammonia, and a liquid bottoms that comprises SA, and at least about 20 wt% water;
- (b) cooling and/or evaporating the bottoms to attain a temperature and composition sufficient to cause the bottoms to separate into a liquid portion and a solid portion that is substantially pure SA; and
- (c) separating the solid portion from the liquid portion.

12. The process of claim 11, wherein the liquid bottoms contains at least some MAS and/or the solid portion is substantially free of succinamic acid, succinamide and succinimide.

13. The process of claim 11, further comprising recycling the SA and water and optionally MAS from the liquid bottoms to the clarified broth.

14. The process of claim 11, further comprising removing water from the liquid bottoms to increase concentration of SA in the liquid bottoms.

15. The process of claim 11, wherein distilling the broth is carried out in the presence of an ammonia separating solvent which is at least one selected from the group consisting of diglyme, triglyme, tetraglyme, sulfoxides, amides, sulfones, polyethyleneglycol (PEG), butoxytriglycol, N-methylpyrrolidone (NMP), ethers, and methyl ethyl ketone (MEK) or in the presence of a water azeotroping solvent which is at least one selected from the group consisting of toluene, xylene, methylcyclohexane, methyl isobutyl ketone, hexane, cyclohexane and heptane.

16. A process for making SA from a clarified MAS-containing fermentation broth comprising:

- (a) adding an ammonia separating and/or water azeotroping solvent to the broth;
- (b) distilling the broth at a temperature and pressure sufficient to form an overhead that comprises water and ammonia, and a liquid bottoms that comprises SA, and at least about 20 wt% water;
- (c) cooling and/or evaporating the bottoms to attain a temperature and composition sufficient to cause the bottoms to separate into a liquid portion and a solid portion that is substantially pure SA; and
- (d) separating the solid portion from the liquid portion.

17. The process of claim 16, wherein the liquid bottoms contains at least some MAS and/or the solid portion is substantially free of succinamic acid, succinamide and succinimide.

18. The process of claim 16, further comprising recycling the SA, water, and the solvent and, optionally, MAS from the liquid bottoms to the clarified broth.

19. The process of claim 16, wherein distilling the broth is carried out in the presence of an ammonia separating solvent which is at least one selected from the group consisting of diglyme, triglyme, tetraglyme, sulfoxides, amides, sulfones, polyethyleneglycol (PEG), butoxytriglycol, N-methylpyrrolidone (NMP), ethers, and methyl ethyl ketone (MEK) or in the presence of a water azeotroping solvent which is at least one selected from the group consisting of toluene, xylene, methylcyclohexane, methyl isobutyl ketone, hexane, cyclohexane and heptane.

20. The process of claim 16, further comprising removing water from the liquid bottoms to increase concentration of MAS in the liquid bottoms.

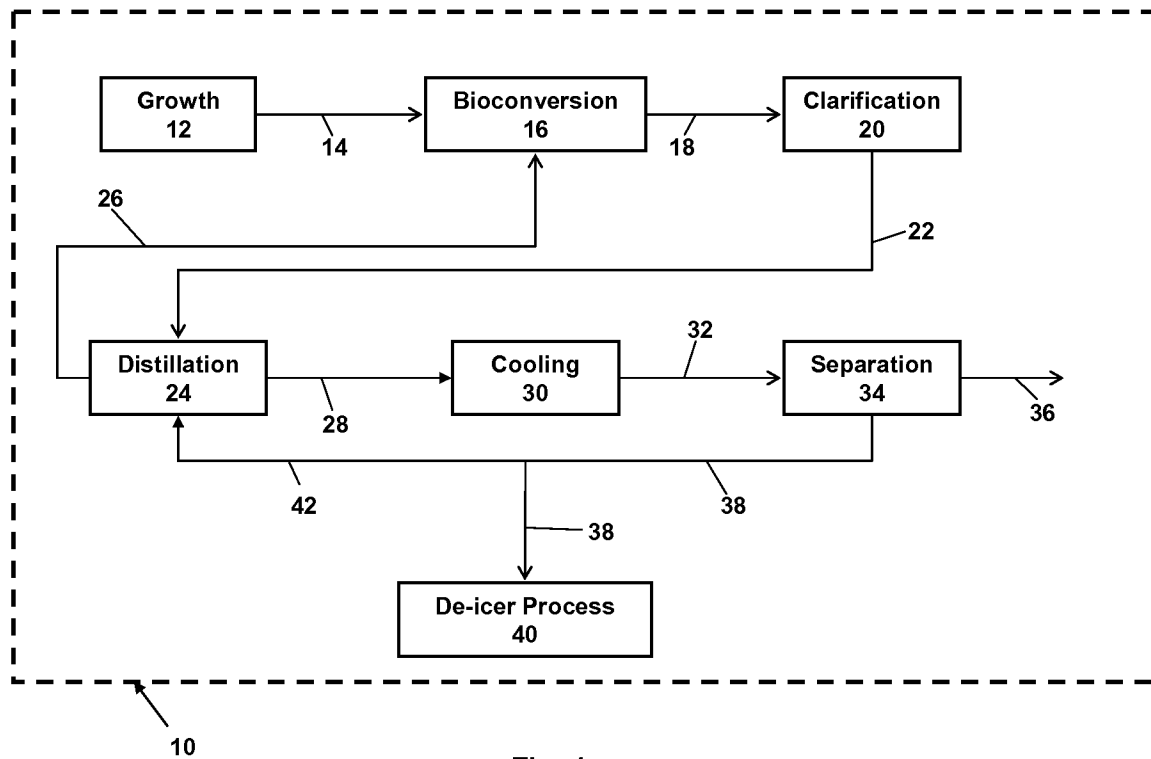
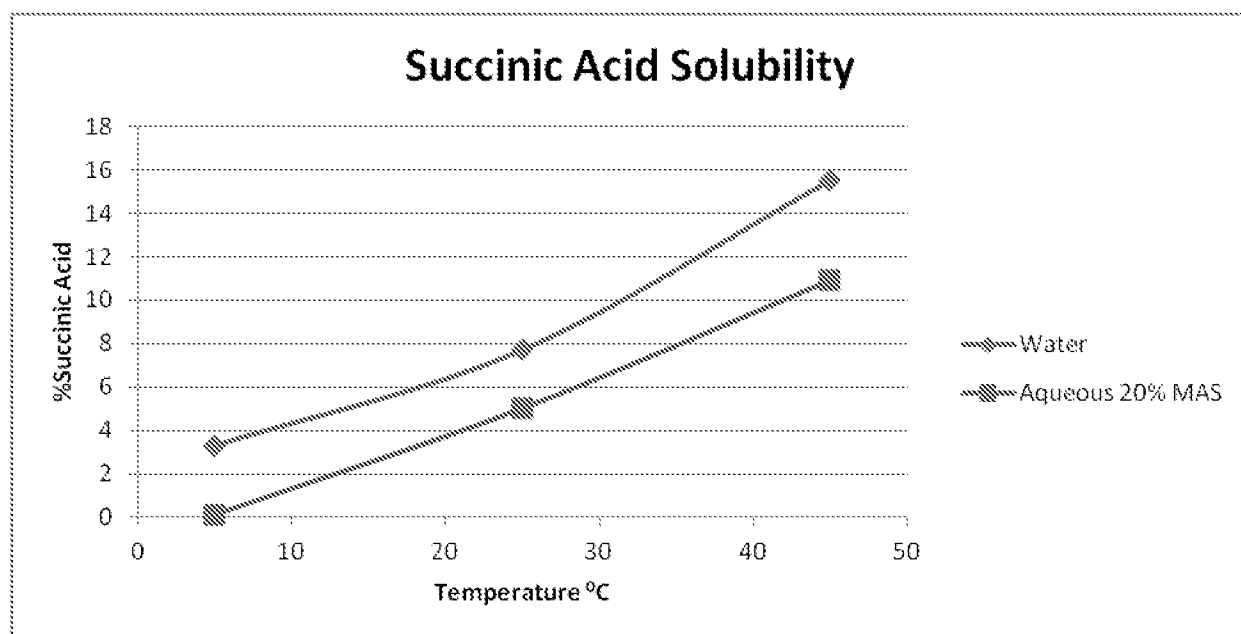


Fig. 1

**Fig. 2**

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/028997

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07C51/487 C12P7/46
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)
C07C 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 practical, search terms used)

EPO-Internal, BEILSTEIN Data, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2007 254354 A (NAT INST OF ADV IND & TECHNOL; SHOWA HIGHPOLYMER) 4 October 2007 (2007-10-04) cited in the application examples -----	1-20

☐

Further documents are listed in the continuation of Box C.

☒

See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

21 June 2011

Date of mailing of the international search report

30/06/2011

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Pérez Carlón, Raquel

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2011/028997

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 2007254354 A	04-10-2007	NONE	
