



- (51) **International Patent Classification:**
C12P 17/04 (2006.01) *C07D 307/68* (2006.01)
- (21) **International Application Number:**
PCT/NL2014/050411
- (22) **International Filing Date:**
20 June 2014 (20.06.2014)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
2011027 24 June 2013 (24.06.2013) NL
- (71) **Applicant:** TECHNISCHE UNIVERSITEIT DELFT [NL/NL]; Stevinweg 1, NL-2628 CN Delft (NL).
- (72) **Inventors:** VAN SPRONSEN, Jacob; p/a TU Delft, Valorisation Centre, Postbus 5, NL-2600 AA Delft (NL). WITKAMP, Geert-Jan; p/a TU Delft, Valorisation Centre, Postbus 5, NL-2600 AA Delft (NL). DE GRAAFF, Bart; p/a TU Delft, Valorisation Centre, Postbus 5, NL-2600 AA Delft (NL).
- (74) **Agent:** JANSEN, C.M.; V.O., Johan de Wittlaan 7, NL-2517 JR Den Haag (NL).
- (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, BN, 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, IR, IS, JP, KE, KG, 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, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, 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, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, 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, CL, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) **Title:** PROCESS FOR THE PREPARATION OF 2,5-FURANDICARBOXYLIC ACID

(57) **Abstract:** The invention is directed to a process for the preparation of 2,5-furandicarboxylic acid (FDCA) by fermentation comprising fermenting a suitable starting compound to produce an aqueous solution of a salt of FDCA having a pH of at least 7, optionally removing solids from said solution, e.g. by filtration, subsequently freeze crystallizing the said salt of FDCA from said solution at said pH, isolating the said FDCA salt in the form of solid crystals, preparing an aqueous solution of said salt and crystallizing FDCA as the free acid from said solution at an acidic pH.

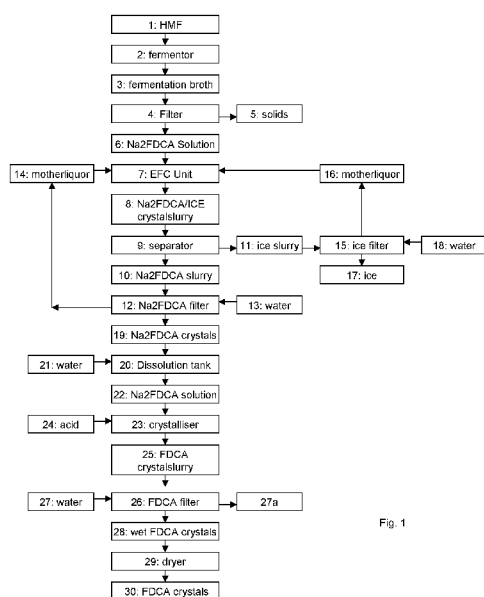


Fig. 1

Title: Process for the preparation of 2,5-furandicarboxylic acid

The invention is directed to a process for producing 2,5 furan dicarboxylic acid (FDCA) by fermentation followed by purification of the resulting fermentation broth to produce substantially pure FDCA as the free acid.

FDCA is a very promising compound which can be produced via
5 biological pathways and which is a useful starting compound for various chemical compounds.

Various methods for the production of FDCA are known, some of them by various chemical synthesis steps, others by fermentation of suitable starting compounds, such as hydroxymethyl furfural (HMF) (WO 2012/064195).

10 One of the disadvantages of the enzymatic preparation of FDCA is the difficulty of removing contaminants from the product to produce a substantially pure FDCA. More in particular it has been found to be very difficult to remove the monocarboxylic acid from the final product, for example when using the conventional acid precipitation..

15 It is one of the objects of the invention to provide a process for producing substantially pure FDCA by fermentation having a very low mono carboxylic acid content..

The invention is accordingly directed to a process for the preparation of 2,5-furandicarboxylic acid (FDCA) by fermentation comprising fermenting a
20 suitable starting compound to produce an aqueous solution of a salt of FDCA having a pH of at least 7, optionally removing solids from said solution, e.g. by filtration, subsequently freeze crystallizing the said salt of FDCA from said solution at said pH, isolating the said FDCA salt in the form of solid crystals, preparing an aqueous solution of said salt and crystallizing FDCA as the free acid from said
25 solution at an acidic pH.

The process of the invention has the very important and unexpected advantage of providing FDCA in very high purity, almost completely free of the mono carboxylic acid.

The present invention comprises various steps, namely first the fermentation to produce a fermentation broth containing a salt of FDCA. Pretreatment of the fermentation broth like sterilization, centrifugation and decolourization is subsequently done to remove cell lysis and solid particles (if
5 necessary) before carrying out the crystallization experiments.

The resulting aqueous solution is subjected to freeze crystallization, preferably eutectic freeze crystallization. The products of this are ice crystals, FDCA salt crystals and a mother liquor. The FDCA salt is then dissolved to produce an aqueous solution. From this solution FDCA is crystallized as the free
10 acid under acidic conditions.

There are various ways to crystallize FDCA as the free acid from the said solution. An important aspect thereby is that the pH is at an acidic value, preferably below pH of 3. One method is the addition of a salt to the said solution. By the lowering of the pH value the free salt of FDCA crystallizes. Another method
15 is the simultaneous addition of the salt and an acid (or acidic solution). A preferred way of crystallizing is to provide an amount of water in the crystallizer, to which simultaneously the salt solution and the acid are added. It has been found that this method, contrary to direct addition of acid to the salt solution, produces crystals of the free acid of FDCA without inclusions of contaminants in the crystals.

The FDCA crystals are then separated from the mother liquor, which
20 also contains dissolved salt, as will be explained later. The crystals may be washed to remove mother liquor. The mother liquor may be treated in a conventional way to remove or recover the said salt and to produce water, for example by eutectic freeze crystallization or another method. In case the salt is
25 useful (as will be discussed below) it may be reused in the process.

The production of FDCA starts via the bio-catalytic conversion of suitable starting materials, such as 5-hydroxymethylfurfural [HMF].

In that case this can be done by aerobic growth of Gram-negative bacterium *Cupriavidus basilensis*. This can be carried out in a batch seed
30 fermenter or in a continuous main fermenter at constant temperature (for example between 30 and 37°C).

The fermentation occurs at a pH of 7 or higher by the addition of at least one alkaline compound, such as sodium hydroxide, sodium bicarbonate or ammonia.

5 The resulting fermentation broth is then treated to make it suitable for the recovery of the FDCA from it. This treatment generally includes removal of solids, possibly sterilization, and decolourization.

From the aqueous solution, a salt of FDCA is then preferably recovered by eutectic freeze crystallization, which is a process based on separation of components at a eutectic freezing point. Eutectic freeze crystallisation has been
10 described in EP-A 1,230,194 and in Chem.Eng.Proc. **37**, (1998), pp 207-213.

In freeze crystallisation at a eutectic freezing point (Eutectic freeze crystallisation: EFC) on the one hand the crystalline salt of FDCA is obtained, and on the other hand ice crystals. Depending on the nature of the cation of the alkaline compound used in the fermentation, the corresponding salt is obtained.

15 The crystals of the FDCA salt (generally sodium or ammonium salt), optionally after washing, for example with water or a saturated FDCA salt solution, are redissolved in water and subsequently the free acid is crystallized from the aqueous solution of the FDCA salt. As indicated above, various methods may be used for this, but the preferred way is the simultaneous injection of acid and the
20 FDCA salt solution into a crystallizer.

The free acid of FDCA is crystallized and a salt solution is obtained. Depending on the nature of the cations of the FDCA salt and the acid used, a specific salt is produced. In case of sodium and the use of sulphuric acid, a sodiumsulfate solution is obtained. This solution may be treated, for example by
25 EFC, to produce solid sodium sulphate and water. In the case of ammonia, ammonium sulphate is obtained. When carbon dioxide is used to acidify, sodium bicarbonate is obtained. It is for example possible to recycle this to the fermentation step to keep the pH at the correct level.

The FDCA crystals can be separated from the mother liquor (salt
30 solution) and washed to remove remaining, adhering salt.

The invention is now further explained on the basis of the attached figure, showing a flow sheet of one variant of the process.

In the figure, HMF is fed via line 1 to a fermenter 2. The fermentation broth is introduced via line 3 into filtration unit 4 to remove solids from the broth. The solids are removed via line 5. The clear solution is introduced via line 6 into eutectic freeze crystallization unit 7. The Na₂FDCA/Ice slurry obtained therein is introduced via line 8 into separator 9. In the separator ice slurry is removed with part of the mother liquor and introduced via line 11 into filter 15. The ice crystals are washed with water via line 18 and are removed from the system via line 17.

The remaining Na₂FDCA slurry, comprising Na₂FDCA crystals in mother liquor, is fed from separator 9 into filter 12, via line 10 and washed, introduced into filter 12, via line 13. The mother liquor is recycled via line 14 to EFC unit 7. If necessary a small bleed stream may be used to remove part of the mother liquor from line 14 or 16, to prevent build-up of contaminants in the system.

The Na₂FDCA crystals are preferably washed with water or a saturated Na₂FDCA solution (to minimize losses a saturated solution is preferred) and fed via line 19 into dissolution tank 20, wherein the Na₂FDCA salt crystals are dissolved in water, introduced via line 21. The FDCA solution thus obtained, is introduced in crystallizer 23, via line 22. Acid or an acidic solution is introduced simultaneously via line 24 into crystallizer 23.

In the crystallizer, crystals of the free acid of FDCA dispersed in an aqueous salt solution are obtained. This dispersion is introduced via line 25 into separation unit 26, where it is filtered or centrifuged to produce the free acid crystals and a mother liquor (salt solution). The crystals are washed with water introduced via line 27. The mother liquor and wash liquor are removed via line 27^a. The product crystals are obtained via line 28. If required they may be dried in dryer 29. The crystals are removed from the dryer via line 30.

The invention is now elucidated on the basis of the following, non-limiting examples.

EXAMPLE 1:

Isolation of the disodium salt of FDCA from fermentation broth.

5 P. Putida S12 equipped with the hmfH oxidoreductase gene from
bacterium Cupriavidus basilensis was fermented in a fedbatch experiment for the
production of FDCA from HMF. Glycerol and HMF were fed into the fermentor.
The pH was maintained at 7 with 10 N NaOH. Dissolved oxygen tension was
maintained at 30% air saturation. Temperature was kept at 25°C. After the
10 fermentation the solids were removed by centrifugation. 1 l. Of the resulting
solution was cooled down. At -6°C ice started to crystallise from the solution. At
the eutectic point at -7°C a second solid, the disodium salt of FDCA, started to
crystallize from solution. After cooling for another hour at -7°C the crystals of the
disodium salt of FDCA were removed from the crystallisation mixture by filtration.
15 The crystals of the disodium salt of FDCA were washed with 50 ml of a saturated
disodium FDCA solution resulting in 30 gram of wet crystals.

Recrystallization of the disodium salt of FDCA into FDCA.

20 10 Gram of the disodium salt of FDCA was dissolved in 100 ml. of
water. The resulting solution was added to 50 ml. of water over 1 hour at 25°C.
The pH of water phase was kept at 1 with 6N sulphuric acid. After stirring for
another hour at 25°C 5 gram of substantially pure FDCA crystals were isolated by
filtration, washing with 30 ml of water and drying on the air at 50°C. The purity of
25 the FDCA crystals was >99% with the content of 5-(hydroxymethyl)furoic acid
<0,1%.

EXAMPLE 2 (comparative):

Direct isolation of FDCA from fermentation broth.

- 5 P. Putida S12 equipped with the hmfH oxidoreductase gene from
bacterium Cupriavidus basilensis was fermented in a fedbatch experiment for the
production of FDCA from HMF. Glycerol and HMF were fed into the fermentor.
The pH was maintained at 7 with 10 N NaOH. Dissolved oxygen tension was
maintained at 30% air saturation. Temperature was kept at 25°C. After the
10 fermentation the solids were removed by centrifugation. 1 l. of the resulting
solution was added to 50 ml. of water over 1 hour at 25°C. The pH of water phase
was kept at 1 with 6N sulphuric acid. After stirring for another hour at 25°C 15
gram of FDCA crystals were isolated by filtration, washing with 30 ml of water and
drying on the air at 50°C. The purity of the FDCA crystals was 87% with the
15 content of 5-(hydroxymethyl)furoic acid 1%.

Comparing this result with the result of the process according to the
invention, as exemplified in Example 1, it is clear that there is a marked and
important improvement in the purity of the final product, FDCA.

Claims

1. Process for the preparation of 2,5-furandicarboxylic acid (FDCA) by fermentation comprising fermenting a suitable starting compound to produce an aqueous solution of a salt of FDCA having a pH of at least 7, optionally removing solids from said solution, e.g. by filtration, subsequently freeze crystallizing the
5 said salt of FDCA from said solution at said pH, isolating the said FDCA salt in the form of solid crystals, preparing an aqueous solution of said salt and crystallizing FDCA as the free acid from said solution at an acidic pH.
2. Process according to claim 1, wherein the crystallization of the free acid of FDCA is done at a pH below 3.
- 10 3. Process according to claim 1 or 2, wherein at least one acid is added to said solution of the salt of FDCA to provide for an acidic pH.
4. Process according to claim 1 or 2, wherein the crystallization is done by adding the aqueous solution and an acid simultaneously to a crystallizer.
5. Process according to claim 4, wherein the crystallizer already contains an
15 amount of aqueous liquid to which the solution and the acid are added.
6. Process according to claim 1-5, wherein the starting compound is 5-hydroxy methyl furfural.
7. Process according to claim 1-6, wherein the crystallization of the free acid of FDCA produces a salt solution as mother liquor, which salt is sodium
20 bicarbonate and which is at least partly recycled to the fermentation.
8. Process according to claim 1-7, wherein the freeze crystallization of the FDCA salt is done at the eutectic freezing point of the solution.

1/1

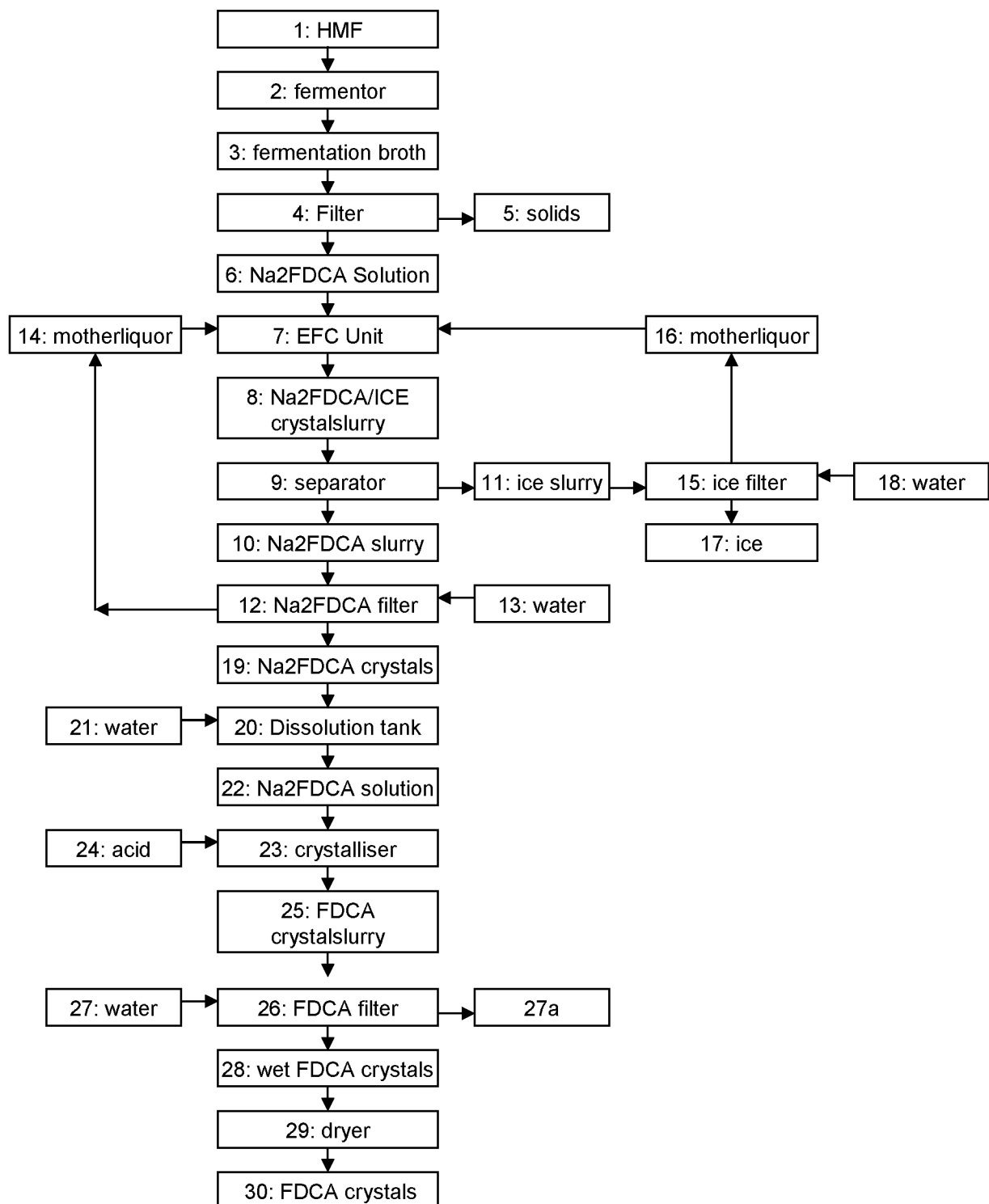


Fig.

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2014/050411

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12P17/04 C07D307/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12P C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2012/064195 A2 (BIRD ENGINEERING B V [NL]; PURAC BIOCHEM BV [NL]; WIERCKX NICK JOHANN) 18 May 2012 (2012-05-18) abstract; claims 17-22 page 24, lines 1-13, 20-25 -----	1-3,6,8
Y	WO 2008/113395 A1 (LAROX AG [FI]; EVERS J A [NL]) 25 September 2008 (2008-09-25) abstract; claims 1-3, 10 page 4, lines 6-31 -----	1-3,6,8
A	WO 2011/026913 A1 (DSM IP ASSETS BV [NL]; RUIJSSENAARS HARALD JOHAN [NL]; WIERCKX NICK JO) 10 March 2011 (2011-03-10) abstract page 14, line 16 - page 15, line 8 page 21, lines 5-11 ----- -/--	1-8



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

"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

15 October 2014

Date of mailing of the international search report

23/10/2014

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

Schröder, Gunnar

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2014/050411

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2013/025106 A1 (PURAC BIOCHEM BV [NL]; DE HAAN ANDRE BANIER [NL]; VAN BREUGEL JAN [NL]) 21 February 2013 (2013-02-21) abstract; claims 1, 2, 6, 12 -----	1-3,7
A	WO 2009/023174 A2 (ARCHER DANIELS MIDLAND CO [US]; HANKE PAUL D [US]) 19 February 2009 (2009-02-19) abstract; claim 19 -----	1-3,6
A	FRANK KOOPMAN ET AL: "Efficient whole-cell biotransformation of 5-(hydroxymethyl)furfural into FDCA, 2,5-furandicarboxylic acid", BIORESOURCE TECHNOLOGY, vol. 101, no. 16, 1 August 2010 (2010-08-01), pages 6291-6296, XP055008790, ISSN: 0960-8524, DOI: 10.1016/j.biortech.2010.03.050 abstract -----	1-3,6
A	EP 1 094 047 A1 (UNIV DELFT TECH [NL]; KEMIRA AGRO OY [FI]) 25 April 2001 (2001-04-25) cited in the application abstract; claims 1, 6, 8 paragraphs [0001] - [0004], [0025] -----	1,8

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/NL2014/050411

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012064195 A2	18-05-2012	AU 2012202889 A1 CA 2829078 A1 CN 103502441 A EA 201300999 A1 EP 2683815 A2 JP 2014508531 A KR 20140015439 A NL 2006359 C US 2014073019 A1 WO 2012064195 A2	26-09-2013 18-05-2012 08-01-2014 28-02-2014 15-01-2014 10-04-2014 06-02-2014 24-04-2012 13-03-2014 18-05-2012
WO 2008113395 A1	25-09-2008	WO 2008113387 A1 WO 2008113395 A1	25-09-2008 25-09-2008
WO 2011026913 A1	10-03-2011	AU 2010291208 A1 CA 2777503 A1 CN 102834386 A EA 201200398 A1 EP 2473494 A1 JP 2013503614 A KR 20120083367 A US 2012309918 A1 WO 2011026913 A1	29-03-2012 10-03-2011 19-12-2012 28-09-2012 11-07-2012 04-02-2013 25-07-2012 06-12-2012 10-03-2011
WO 2013025106 A1	21-02-2013	NONE	
WO 2009023174 A2	19-02-2009	US 2009053780 A1 WO 2009023174 A2	26-02-2009 19-02-2009
EP 1094047 A1	25-04-2001	AT 329891 T AU 1737901 A CA 2388812 A1 DE 60028806 T2 DK 1230194 T3 EP 1094047 A1 EP 1230194 A1 ES 2266006 T3 PT 1230194 E US 2004060322 A1 WO 0128958 A1	15-07-2006 30-04-2001 26-04-2001 18-01-2007 18-09-2006 25-04-2001 14-08-2002 01-03-2007 31-10-2006 01-04-2004 26-04-2001