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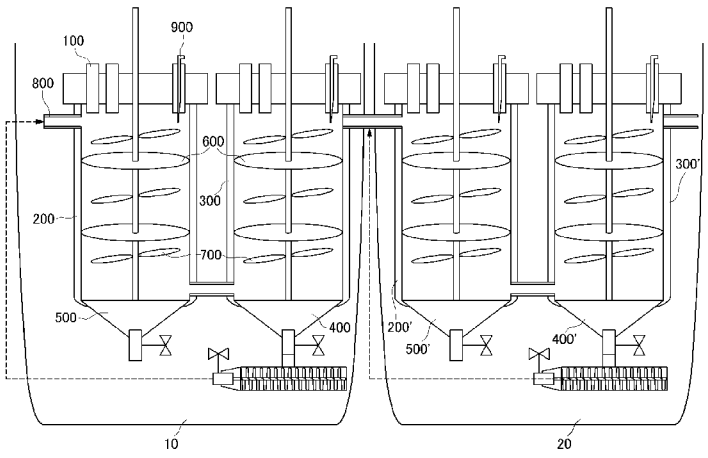
(54) Title: CONTINUOUS FERMENTATION APPARATUS AND MULTI-STEP CONTINUOUS FERMENTATION PROCESS USING THE SAME

[Fig. 1]



(57) Abstract: The present disclosure relates to a continuous fermentation apparatus and a multi-step continuous fermentation process using the same.

WO 2013/062372 A1

Description

Title of Invention: CONTINUOUS FERMENTATION APPARATUS AND MULTI-STEP CONTINUOUS FERMENTATION PROCESS USING THE SAME

Technical Field

[1] The present disclosure relates to a continuous fermentation apparatus and a multi-step continuous fermentation process using the same.

Background Art

[2] Biomass is a general term to refer to all organic materials currently being used as energy sources including dedicated energy crops and trees, agricultural products and forage crops, agricultural wastes and residues, forest product wastes and wood chips, water plants, excretion of animals, industrial wastes including municipal wastes, and other renewable organic materials extracted from the above-described wastes. The biomass is a huge energy storage that keeps solar energy. If it is possible to effectively use the biomass, a current energy crisis caused by overuse of fossil fuels can be solved easily. Since the biomass is cultured to produce energy to be used as much as needed, it is possible to prevent excessive accumulation of carbon dioxide in the air. Therefore, it may be possible to solve global warming being currently brought up as a very serious problem.

[3] Bioethanol as one of alternative energy sources using the biomass is extracted from plants such as sugar cane, corn, and the like and can be used as an automotive fuel in a combined manner with gasoline or solely. Therefore, the bioethanol has drawn attention as a representative renewable energy source together with biodiesel. Biomass ethanol is described in documents as follows: DiPardo, Journal of Outlook for Biomass Ethanol Production and Demand (EIA Forecasts), 2002; Sheehan, Biotechnology Progress, 15 8179, 1999; Martin, Enzyme Microbes Technology, 31 274, 2002; Greer, BioCycle, 61-65, April 2005; Lynd, Microbiology and Molecular Biology Reviews, 66 3, 506-577, 2002; and Lynd et al in "Consolidated Bioprocessing of Cellilosic Biomass: An Update, "Current Opinion in Biotechnology, 16 577-583, 2005.

[4] However, as the bioethanol has drawn attention as alternative energy, a demand for corn, sugar crane, and wheat as sources of the bioethanol has been sharply increased, which becomes a factor of a sharp rise in crop prices. Accordingly, a method of obtaining bioethanol from crops causes the above-mentioned problem of a sharp rise in crop prices and cannot avoid criticism that such crops should be supplied to many starving people as food. Therefore, a global bioethanol market has turned its attention from crops to wood. However, there is no way of making a breakthrough since the

wood needs to go through a complicated process of removing lignin or the like.

[5] Accordingly, recently, marine algae have drawn attention as a source of the bioethanol. Since the marine algae have a high growth rate, it is possible to mass-produce them without any fertilizer or agricultural water. Further, since the marine algae are rich in various sugar components and alginic acid, they are suitable to be converted into energy. Furthermore, the marine algae are about 1.5 to 2 times richer in carbohydrates than wood.

[6] Moreover, since the marine algae have less dense structure than lignin, as compared with conventional biomass, it is relatively easy to perform saccharification on the marine algae, resulting in a great amount of output. Besides, since relatively abundant marine resources can be utilized, the marine algae have great potential.

Disclosure of Invention

Technical Problem

[7] The present disclosure provides a continuous fermentation apparatus capable of collecting microbial cells and reusing them during a continuous fermentation process to produce biofuel and a multi-step continuous fermentation process using the same apparatus.

[8] However, the problems to be solved by the present disclosure are not limited to the above description and other problems can be clearly understood by those skilled in the art from the following description.

Solution to Problem

[9] In accordance with a first aspect of the present disclosure, there is provided a continuous fermentation apparatus including a source input unit; a fermentation unit including a downward fermentation tube and an upward fermentation tube; and a microbial cell collection unit formed on a lower part of the upward fermentation tube.

[10] In accordance with an illustrative embodiment of the present disclosure, a plurality of fermentation units is included. If the source is sugar mixture, the number of fermentation unit may adjust according to the number of sugar contained in the sugar mixture

[11] In accordance with an illustrative embodiment of the present disclosure, the source includes, but is not limited to, one selected from the group consisting of marine algae, cellulosic biomass, and the combination thereof.

[12] In accordance with an illustrative embodiment of the present disclosure, the source includes, but is not limited to, a sugar mixture selected from the group consisting of glucose, galactose, sucrose, xylose, and the combinations thereof.

[13] In accordance with an illustrative embodiment of the present disclosure, continuous fermentation apparatus further includes, but is not limited to, a collection unit for

collecting microbial cells and precipitates on a lower part of the downward fermentation tube.

[14] In accordance with an illustrative embodiment of the present disclosure, the downward fermentation tube and the upward fermentation tube further includes, but is not limited to, an impeller and a screen.

[15] In accordance with an illustrative embodiment of the present disclosure, the microbial cells collected by the microbial cell collection unit of the upward fermentation tube are supplied into the downward fermentation tube to be recycled, but is not limited thereto.

[16] In accordance with a second aspect of the present disclosure, there is a provided a multi-step continuous fermentation process of sugar mixture using the continuous fermentation apparatus in the first aspect.

[17] In accordance with an illustrative embodiment of the present disclosure, the process comprising the steps of supplying the sugar mixture into a source input unit; and fermenting the sugar mixture in a fermentation unit including a downward fermentation tube and an upward fermentation tube, but is not limited thereto.

[18] In accordance with an illustrative embodiment of the present disclosure, microbial cells are collected from the upward fermentation tube and supplied into the downward fermentation tube to be recycled, but is not limited thereto.

[19] In accordance with an illustrative embodiment of the present disclosure, the step of fermenting is carried out at least one time according to the number of sugar contained in the sugar mixture, but is not limited thereto.

Advantageous Effects of Invention

[20] In accordance with the present disclosure, it is possible to continuously produce ethanol through continuous fermentation and the present disclosure can be applied to a fermentation process using sugar mixtures. Conventionally, sugars extracted from marine algae are not monosaccharides but sugar mixtures. Thus, even if a simple batch fermentation process and a conventional continuous fermentation process are carried out, a sugar shock occurs for a certain time, which results in an increase in a total fermentation time and also affects a fermentation yield. However, the present disclosure can solve such problems. Further, there is provided a separate harvesting unit at a lower part of a U-shaped tube, and, thus, it is possible to control respective strains, which maximizes the fermentation yield and makes it possible to perform the continuous fermentation process. Furthermore, microbial cells are collected and reused during a series of continuous fermentation processes, so that the total fermentation time can be reduced. Moreover, a specific sugar (glucose) from a saccharification liquid containing various sugars including the glucose is first fermented and the

remaining sugars are successively fermented, so that it is possible to prevent a shock caused by a difference in metabolic pathways within a strain due to a difference in kinds of sugars.

Brief Description of Drawings

[21] Fig. 1 is a schematic diagram of a continuous fermentation apparatus in accordance with an illustrative embodiment of the present disclosure; and

[22] Fig. 2 is a schematic view of a fermentation unit of a continuous fermentation apparatus in accordance with an illustrative embodiment of the present disclosure.

[23] Fig. 3 is a schematic view of a screen of a continuous fermentation apparatus in accordance with an illustrative embodiment of the present disclosure.

[24] Fig. 4 shows graphs of (a) sugar mixture consumption and (b) ethanol production under various ratio of galactose and glucose in accordance with example of the present disclosure.

[25] Fig. 5 shows graphs of (a) mono-sugar consumption and (b) ethanol production in a continuous fermentation process in accordance with example of the present disclosure.

[26] Fig. 6 is a graph showing time course of cell growth during the continuous fermentation process in accordance with example of the present disclosure.

[27] Fig. 7 is a graph showing galactose consumption during the continuous fermentation process in accordance with example of the present disclosure.

Best Mode for Carrying out the Invention

[28] Hereinafter, embodiments of the present disclosure will be described in detail with reference to the accompanying drawings so that the present disclosure may be readily implemented by those skilled in the art. However, it is to be noted that the present disclosure is not limited to the embodiments but can be embodied in various other ways. In drawings, parts irrelevant to the description are omitted for the simplicity of explanation, and like reference numerals denote like parts through the whole document.

[29] Hereinafter, embodiments of the present disclosure will be described in detail with reference to the accompanying drawings so that the present disclosure may be readily implemented by those skilled in the art. However, it is to be noted that the present disclosure is not limited to the embodiments but can be embodied in various other ways. In drawings, parts irrelevant to the description are omitted for the simplicity of explanation, and like reference numerals denote like parts through the whole document.

[30] Through the whole document, the term "on" that is used to designate a position of one element with respect to another element includes both a case that the one element is adjacent to the another element and a case that any other element exists between

these two elements.

[31] Further, the term "comprises or includes" and/or "comprising or including" used in the document means that one or more other components, steps, operation and/or existence or addition of elements are not excluded in addition to the described components, steps, operation and/or elements unless context dictates otherwise. The term "about or approximately" or "substantially" are intended to have meanings close to numerical values or ranges specified with an allowable error and intended to prevent accurate or absolute numerical values disclosed for understanding of the present disclosure from being illegally or unfairly used by any unconscionable third party. Through the whole document, the term "step of" does not mean "step for".

[32] Through the whole document, the term "combination of" included in Markush type description means mixture or combination of one or more components, steps, operations and/or elements selected from a group consisting of components, steps, operation and/or elements described in Markush type and thereby means that the disclosure includes one or more components, steps, operations and/or elements selected from the Markush group.

[33] The present disclosure provides a column-type continuous fermentation apparatus including at least one fermentation unit including U-shaped downward and upward fermentation tubes each formed by connecting cylindrical column straight tubes are successively connected to one another. Each U-shaped downward and upward fermentation tubes may include a screw that pushes downwards and a screw that pushes upwards on its right and left sides, respectively. At a lower part of respective U-shaped upward fermentation tubes, a separated microbial cell collection unit capable of separating cells may be provided, and the cells may be recycled. In accordance with an illustrative embodiment, if a sugar mixture containing both galactose and glucose is fermented, yeast first consumes the glucose and then the galactose. In this case, a galactose shock may occur and the fermentation may be delayed.

[34] In accordance with an illustrative embodiment of the present disclosure, during a first-step continuous fermentation process in a first fermentation unit, glucose is first fermented and then microbial cells are separately collected. A culture medium in which a glucose is removed and only the galactose remains is transferred to second fermentation unit including U-shaped downward and upward fermentation tubes. Microbial cells collected in the U-shaped upward fermentation tube of the first fermentation unit are supplied to a U-shaped downward fermentation tube of the first fermentation unit to ferment glutose. In the same manner, fermented broth in which most of glutose is consumed is transferred to the U-shaped downward and upward fermentation tubes of the second fermentation unit, and, thus, the yeast can consume only the galactose without a shock. After the fermentation, microbial cells collected by a

microbial cell collection unit provided at a lower part of the upward fermentation tube of the second fermentation unit are supplied to the inlet of the downward fermentation tube of the second fermentation unit, so that it is possible to suppress a delay in fermentation of the sugar mixture and also possible to reduce a total time for a continuous fermentation process. This can be applied to other sugar mixtures.

[35] The above-described fermentation process is performed to convert monosaccharides remaining after saccharification into biofuel by fermenting the monosaccharides with microorganisms such as yeast.

[36] The microorganisms for fermentation may include any one selected from the group consisting of, but not limited to, Brettanomyces bruxellensis, Saccharomyces pastorianus, Saccharomyces cerevisiae, Sarcina ventriculi, Kluyveromyces fragilis, Zymomonas mobilis, Kluyveromyces marxianus IMB3, Brettanomyces custersii, Clostridium acetobutylicum, Clostridium beijerinckii, Clostridium aurantibutylicum, Clostridium tetanomorphum, variants thereof, and combinations thereof.

[37] As fermentation conditions, a temperature of a fermentation unit is maintained in a range of from about 20℃ to about 70℃ and may be maintained to be lower than about 45℃, for example, lower than about 42℃ (from about 34℃ to about 38℃, or about 37℃). In some cases, the fermentation unit may be maintained at a temperature higher than about 5℃, for example, a temperature higher than about 15℃.

[38] Hereinafter, a continuous fermentation apparatus in accordance with an illustrative embodiment will be explained in detail with reference to Figs. 1 and 2. However, the present disclosure is not limited thereto.

[39] Referring to Figs. 1 and 2, a continuous fermentation apparatus in accordance with an illustrative embodiment includes a source input unit 100, a first fermentation unit 10 including a downward fermentation tube 200 and an upward fermentation tube 300, a second fermentation unit including a downward fermentation tube 200' and an upward fermentation tube 300' and a microbial cell collection unit 400, 400'.

[40] The source input unit 100 as a apparatus for controlling a speed of a pre-fermentation process is configured to supply a source. For example, the source input unit 100 may supply the source with certain flow rate per hour. The source may include, but is not limited to, marine algae including macro-algae and micro-algae and terrestrial cellulosic biomass. For example, the source may include, but is not limited to, sugar mixture selected from the group consisting of glucose, galactose, sucrose, xylose, and the combinations thereof.

[41] The downward fermentation tube 200, 200' may include a screw-type impeller 700 for preventing microbial cells from sedimentation, a driving unit at an upper part thereof, a carbon dioxide outlet 900 for discharging carbon dioxide generated during fermentation, and a screen 600 for preventing upper and lower solutions within the fer-

mentation tube from being mixed with each other. The screen 600 may be in form of porous plate, and blades in shape of Right-angled triangle may be formed on surface of the screen 600. The screen 600 may prevent the microbial cells from sedimentation during rotation in the downward fermentation tube to prevent loss of the microbial cells. The porous plate has pores having size from about 0.1 mm to about 10 mm, and the pores are formed in shape which upper part is narrow and lower part is broad.

[42] The downward fermentation tube 200, 200' may include a collection unit 500, 500' for collecting microbial cells and precipitates on a lower part of the downward fermentation tube.

[43] The upward fermentation tube 300, 300' may include a screw-type impeller 700, a driving unit at an upper part thereof, a carbon dioxide outlet 900 for discharging carbon dioxide generated during fermentation, and a screen 600 for preventing upper and lower solutions within the fermentation tube from being mixed with each other. The screen 600 may be in form of porous plate, in particular may be use the porous plate identical with that of the downward fermentation tube. Blades in shape of Right-angled triangle may be formed on surface of the screen 600, and pores of the porous plate are range of from about 0.1 mm to about 10 mm. The screen 600 may be used as overturned form with respect to that of the downward fermentation tube. The screen 600 may prevent a rise of the microbial cells.

[44] At a lower part of the upward fermentation tube 300, 300' the microbial cell collection unit 400, 400' may be provided. A guard and a screen 600 for suppressing a rise of microbial cells may be provided within the fermentation tube. Collected microbial cells can be supplied into a microbial cell input 800, and recycled in the downward fermentation tube 200, 200'. Therefore, the present disclosure can provide a recycling system for circulating collected microbial cells in a fermentation process.

[45] There may be provided a apparatus for suppressing a rise of microbial cells in an upward fermentation unit equipped with the microbial cell collection unit 400, 400'.

[46] The present disclosure can be applied to fermentation of a sugar mixture by connecting the same fermentation unit systems depending on the number of sugar included in the sugar mixture.

Mode for the Invention

[47] [EXAMPLE]

[48] EXAMPLE 1

[49] A sugar mixture including glucose and galactose was fermented using B. custersii in a continuous fermentation apparatus of Fig. 1.

[50] Fig. 4 shows graphs of (a) sugar mixture consumption and (b) ethanol production under various ratio of galactose and glucose with 1% N-source. It can be confirmed

that fermentation rate of the sugar mixture are decreased.

[51] Fig. 5 shows graphs of (a) mono-sugar consumption and (b) ethanol production using 20% sugar with 1% N-source. Temporary fermentation delay was occurred due to conversion of monosugar of sugar mixture as carbon source during a continuous fermentation process using recycled microbial cells, but fermentation rate is shortly recovered.

[52] Fig. 6 is a graph showing time course of cell growth during the continuous fermentation process under 200 g/L galactose with 1% N-source, and Fig. 7 is a graph showing galactose consumption by Brettanomyces custersii during the continuous fermentation process depending on the recycle number of microbial cells. As the recycle number of microbial cells is increased, a cell mass is increased and sugar consumption is rapidly descreased, and thus a conversion rate of fermentation is improved.

[53] The above description of the present disclosure is provided for the purpose of illustration, and it would be understood by those skilled in the art that various changes and modifications may be made without changing technical conception and essential features of the present disclosure. Thus, it is clear that the above-described embodiments are illustrative in all aspects and do not limit the present disclosure. For example, each component described to be of a single type can be implemented in a distributed manner. Likewise, components described to be distributed can be implemented in a combined manner.

[54] The scope of the present disclosure is defined by the following claims rather than by the detailed description of the embodiment. It shall be understood that all modifications and embodiments conceived from the meaning and scope of the claims and their equivalents are included in the scope of the present disclosure.

[55] [Explanation of Codes]

[56] 10: first fermentation unit

[57] 20: second fermentation unit

[58] 100: Input unit

[59] 200, 200': Downward fermentation tube

[60] 300, 300': Upward fermentation tube

[61] 400, 400': Microbial cell collection unit

[62] 500, 500': collection unit

[63] 600: screen

[64] 700: impeller

[65] 800: microbial cell input

[66] 900: carbon dioxide outlet

Claims

[Claim 1] A continuous fermentation apparatus comprising:
a source input unit;
a fermentation unit including a downward fermentation tube and an upward fermentation tube; and
a microbial cell collection unit formed on a lower part of the upward fermentation tube.

[Claim 2] The continuous fermentation apparatus of claim 1,
wherein a plurality of fermentation units is included.

[Claim 3] The continuous fermentation apparatus of claim 1,
wherein the source includes one selected from the group consisting of marine algae, cellulosic biomass, and the combination thereof.

[Claim 4] The continuous fermentation apparatus of claim 1,
wherein the source includes a sugar mixture selected from the group consisting of glucose, galactose, sucrose, xylose, and the combinations thereof.

[Claim 5] The continuous fermentation apparatus of claim 1,
further including a collection unit for collecting microbial cells and precipitates on a lower part of the downward fermentation tube.

[Claim 6] The continuous fermentation apparatus of claim 1,
wherein the downward fermentation tube and the upward fermentation tube further includes at least one impeller and at least one screen.

[Claim 7] The continuous fermentation apparatus of claim 2,
wherein the microbial cells collected by the microbial cell collection unit of the upward fermentation tube are supplied into the downward fermentation tube to be recycled.

[Claim 8] A multi-step continuous fermentation process of sugar mixture using a continuous fermentation apparatus as claimed in any one of claims 1 to 7.

[Claim 9] The multi-step continuous fermentation process of sugar mixture of claim 8,
wherein the process comprising the steps of:
supplying the sugar mixture into a source input unit; and
fermenting the sugar mixture in a fermentation unit including a downward fermentation tube and an upward fermentation tube.

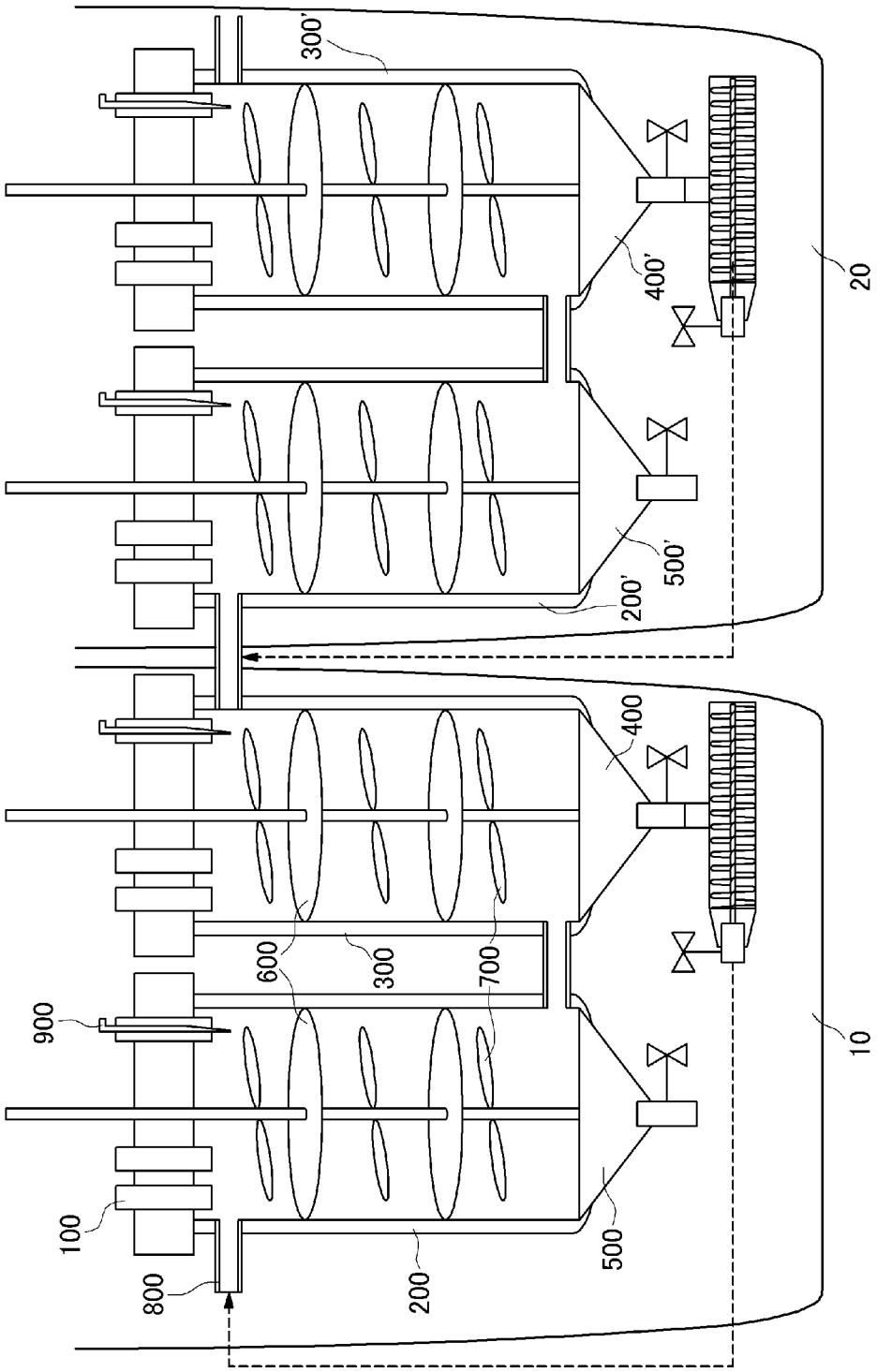
[Claim 10] The multi-step continuous fermentation process of sugar mixture of claim 9,

wherein microbial cells are collected from the upward fermentation tube and supplied into the downward fermentation tube to be recycled.

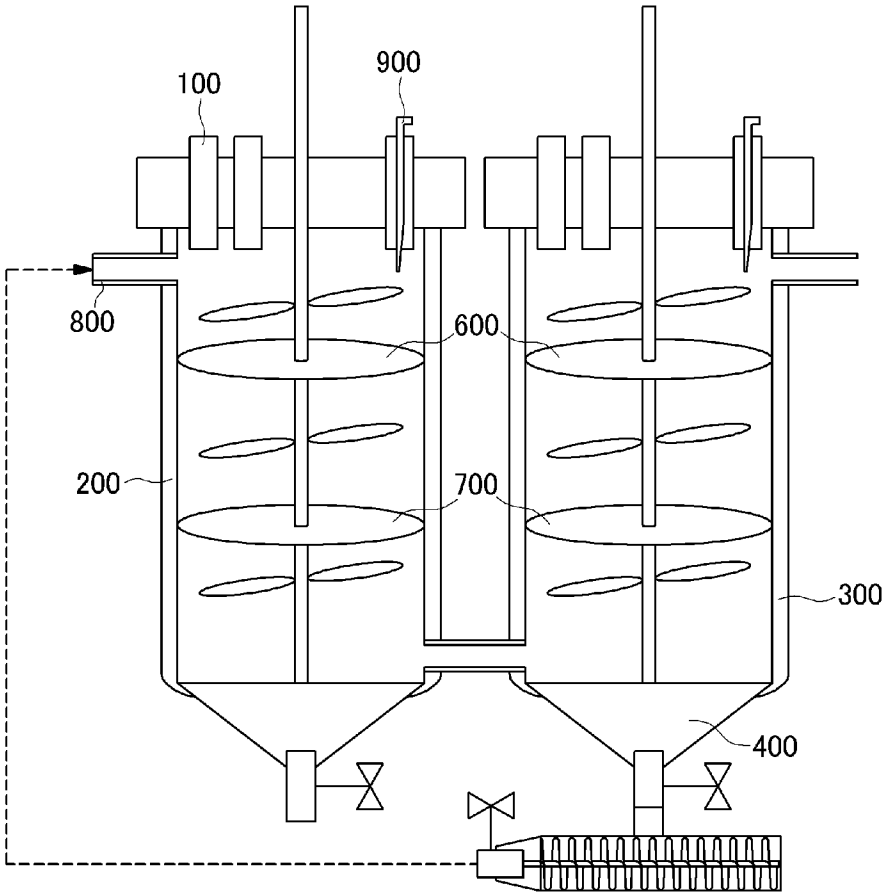
[Claim 11] The multi-step continuous fermentation process of sugar mixture of claim 9,

wherein the step of fermenting is carried out at least one time according to the number of sugar contained in the sugar mixture.

[Fig. 1]

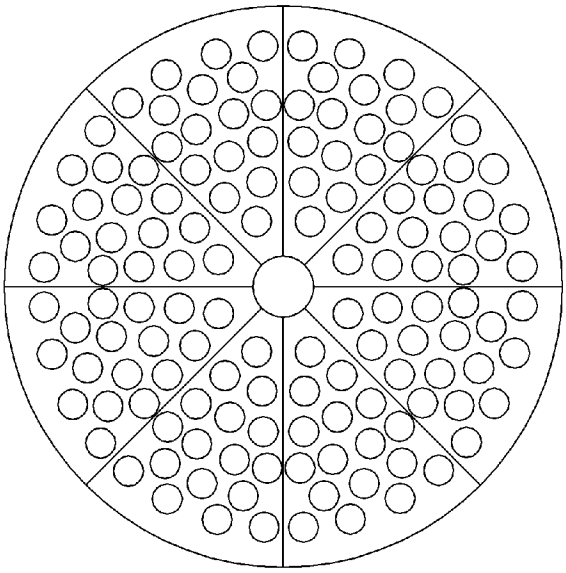


[Fig. 2]

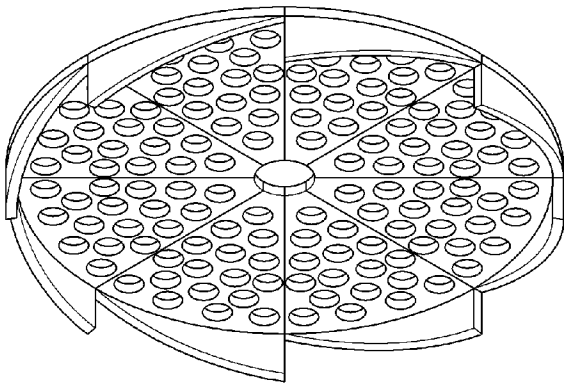


[Fig. 3]

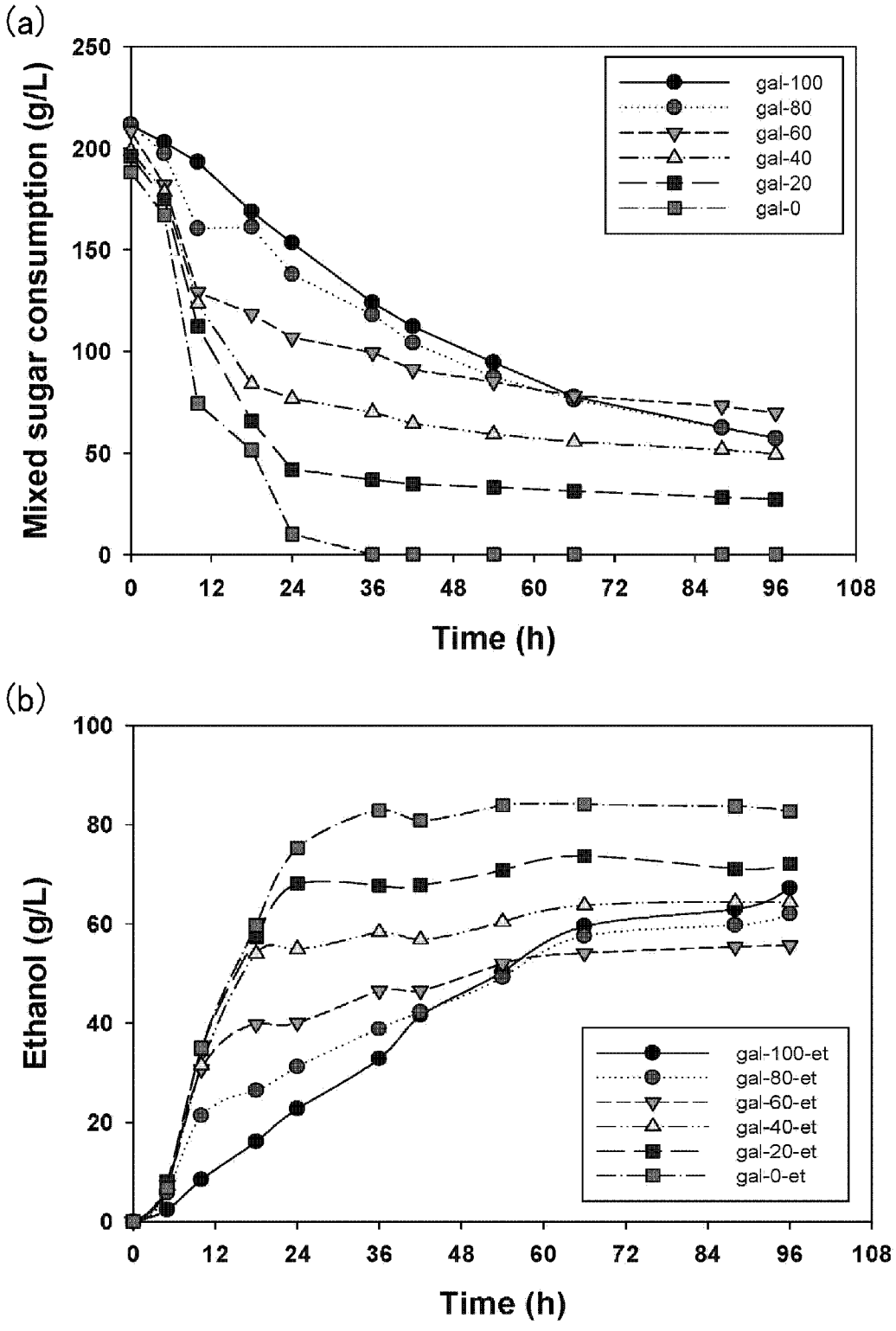
(a)



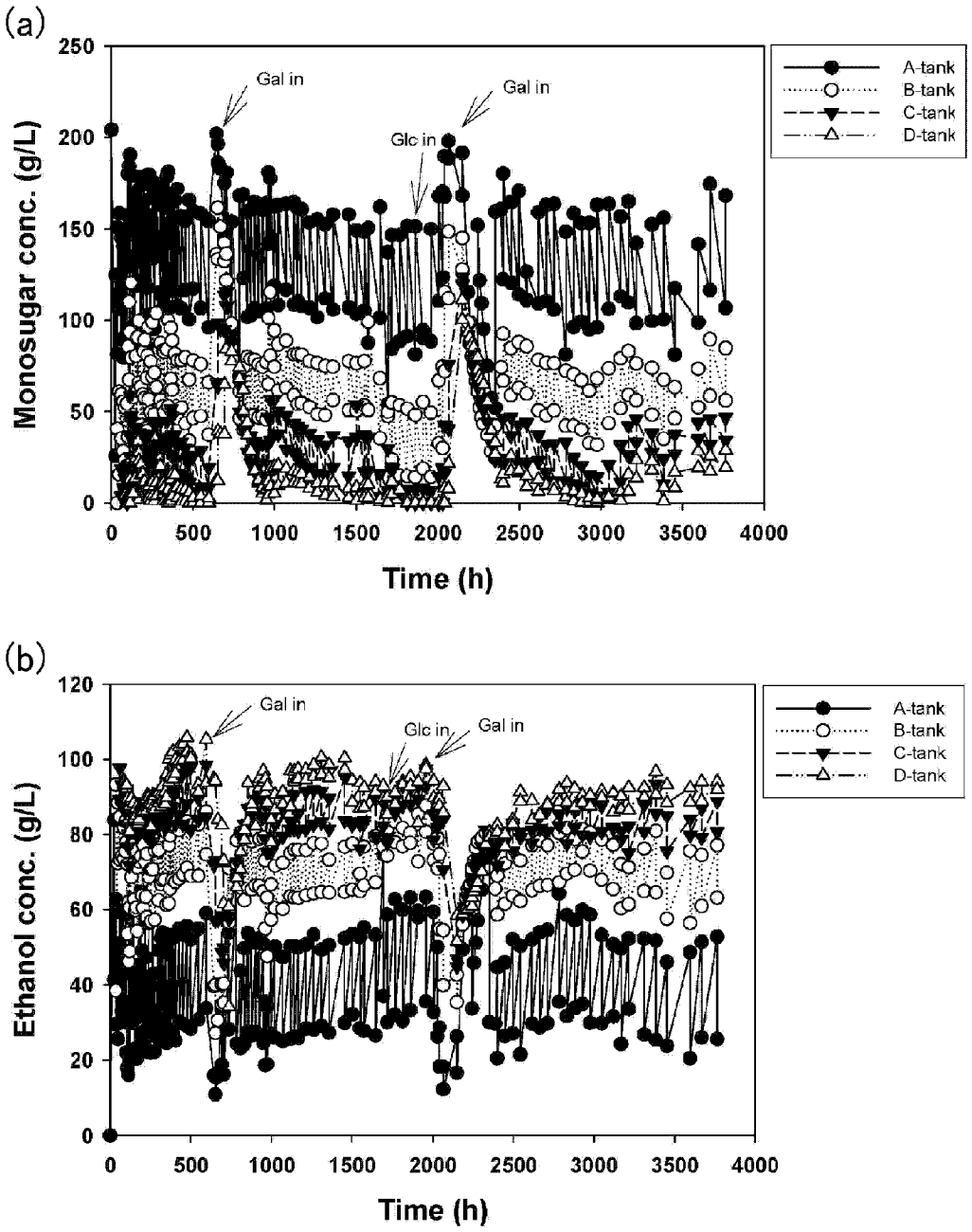
(b)



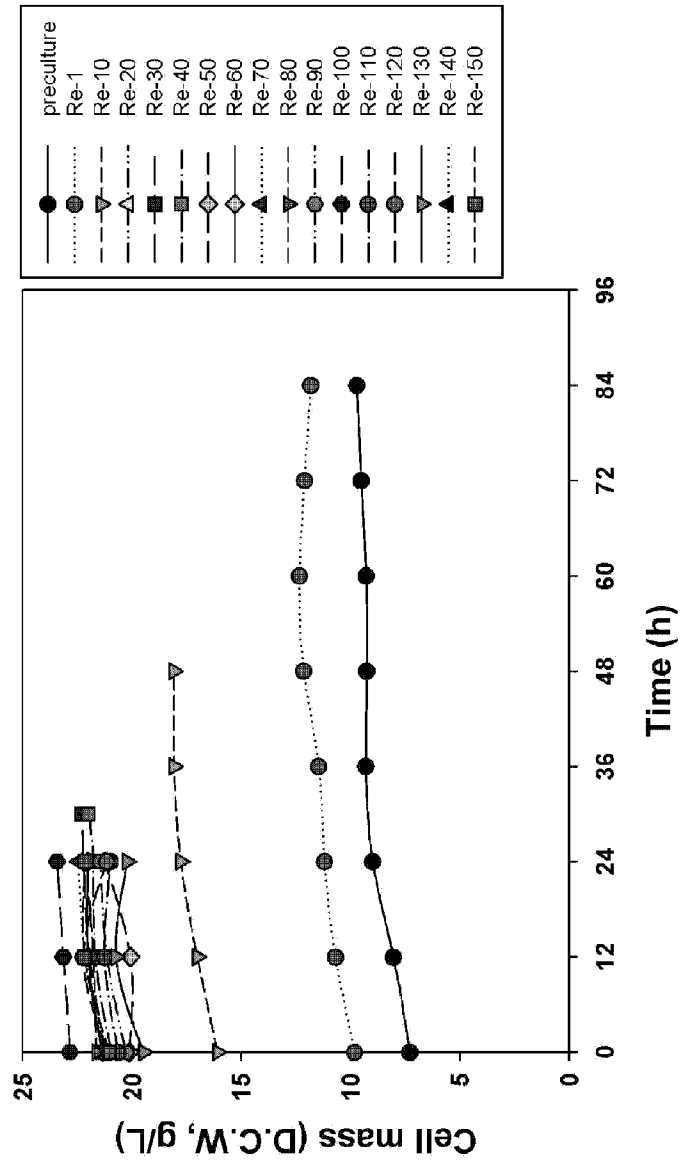
[Fig. 4]


(a)
Mixed sugar consumption (g/L)
250
200
150
100
50
0
0
12
24
36
48
60
72
84
96
108
Time (h)
gal-100
gal-80
gal-60
gal-40
gal-20
gal-0
(b)
Ethanol (g/L)
100
80
60
40
20
0
0
12
24
36
48
60
72
84
96
108
Time (h)
gal-100-et
gal-80-et
gal-60-et
gal-40-et
gal-20-et
gal-0-et

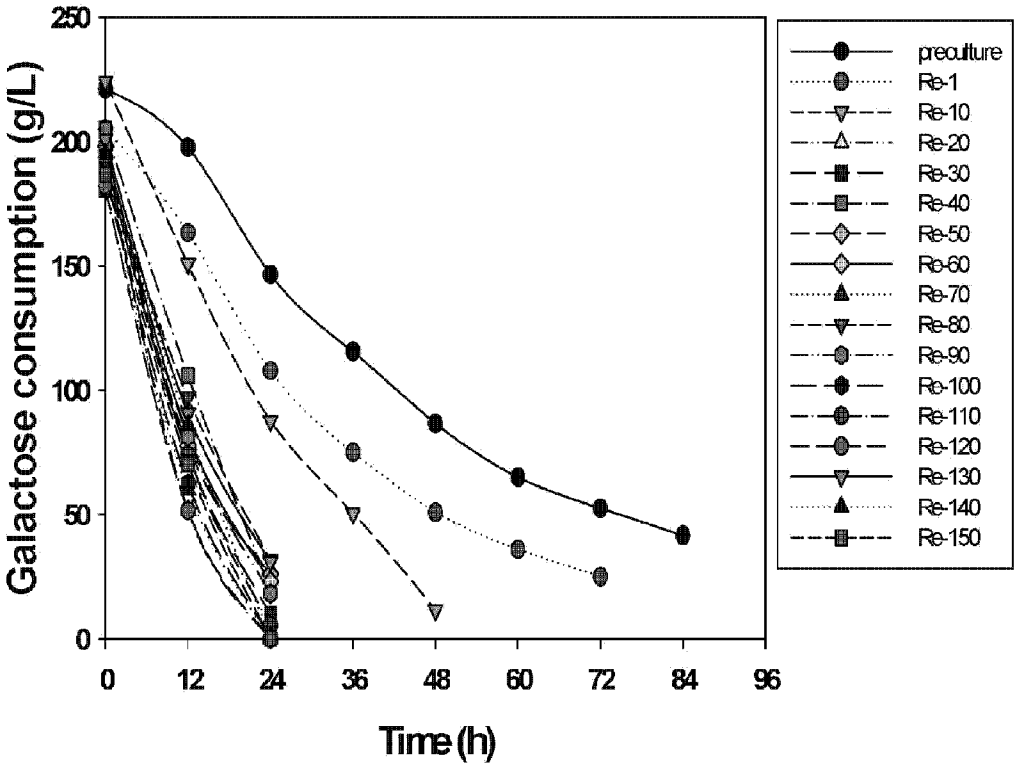
[Fig. 5]


(a)
Monosugar conc. (g/L)
Time (h)
Gal in
Glc in
Gal in
A-tank
B-tank
C-tank
D-tank
(b)
Ethanol conc. (g/L)
Time (h)
Gal in
Glc in
Gal in
A-tank
B-tank
C-tank
D-tank

[Fig. 6]


preculture
Re-1
Re-10
Re-20
Re-30
Re-40
Re-50
Re-60
Re-70
Re-80
Re-90
Re-100
Re-110
Re-120
Re-130
Re-140
Re-150
Cell mass (D.C.W, g/L)
0
5
10
15
20
25
Time (h)
0
12
24
36
48
60
72
84
96

[Fig. 7]


Galactose consumption (g/L)
250
200
150
100
50
0
0
12
24
36
48
60
72
84
96
Time (h)
precultured
Re-1
Re-10
Re-20
Re-30
Re-40
Re-50
Re-60
Re-70
Re-80
Re-90
Re-100
Re-110
Re-120
Re-130
Re-140
Re-150

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2012/008897

A. CLASSIFICATION OF SUBJECT MATTER

C12M 1/06(2006.01)i, C12M 1/24(2006.01)i, C12P 7/10(2006.01)i, C10L 1/02(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)

C12M 1/06; C12M 1/00; C02F 3/28; C05F 11/08; C12N 1/12; C02F 11/04; H01M 8/16; C12P 7/06

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & keywords: fermentation, microbial cell collection unit, sugar mixture

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010-0068791 A1 (MERIMON, TOMMIE et al.) 18 March 2010 See abstract; claims 1-12; figures 1 and 2.	1-11
A	JP 61-038698 A (COMP GENERALE ELECTRICITE et al.) 24 February 1986 See claim 1; figure 1.	1-11
A	US 2010-0144017 A1 (SHEPHERD, SAMUEL L.) 10 June 2010 See abstract; claim 1; figure 1.	1-11
A	WO 2008-105618 A1 (KOREA INSTITUTE OF INDUSTRIAL TECHNOLOGY) 4 September 2008 See abstract; p.18, lines 9-23; claim 1; figure 1.	1-11
A	US 8017366 B1 (SCHUH, ALLEN JOHN et al.) 13 September 2011 See abstract; claim 1; figure 1.	1-11

☐ 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 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
15 MARCH 2013 (15.03.2013)	**18 MARCH 2013 (18.03.2013)**

Name and mailing address of the ISA/KR

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2012/008897

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		US 2012-064617 A1	15.03.2012
JP 61-038698 A	24.02.1986	CA 1251749 A1	28.03.1989
		DE 3562021 D1	05.05.1988
		EP 0167984 A1	15.01.1986
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US 2010-0144017 A1	10.06.2010	US 8372631 B2	12.02.2013
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		EP 2129784 A1	09.12.2009
		JP 2010-518850 A	03.06.2010
		KR 10-1216426 B1	28.12.2012
		KR 10-2009-0025221 A	10.03.2009
		US 2010-0124774 A1	20.05.2010
US 8017366 B1	13.09.2011	None	