Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 3093857 A1 2019/09/19

(21) 3 093 857

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2019/03/08
(87) Date publication PCT/PCT Publication Date: 2019/09/19
(85) Entrée phase nationale/National Entry: 2020/09/14
(86) N° demande PCT/PCT Application No.: BR 2019/050074
(87) N° publication PCT/PCT Publication No.: 2019/173888
(30) Priorité/Priority: 2018/03/13 (BR BR 10 2018 004973 9)

(51) Cl.Int./Int.Cl. *C12M 1/33* (2006.01),
C11B 1/04 (2006.01)

(71) Demandeur/Applicant:
PETROLEO BRASILEIRO S.A.-PETROBRAS, BR

(72) Inventeurs/Inventors:
AREDES MARTINS, MARCIO, BR;
NOVAIS ROCHA, DILSON, BR;
DE OLIVEIRA LEITE, MAURICIO, BR;
BEZERRA VIEIRA, BRUNO, BR;
RICHARD JOAO, RAFAEL, BR;
BRANTES BACELLAR MENDES, LEONARDO, BR

(74) Agent: SMART & BIGGAR LLP

(54) Titre : DISPOSITIF ET PROCEDE POUR LA RUPTURE DE CELLULES DE MICRO-ORGANISMES PAR EXTRUSION

(54) Title: DEVICE AND METHOD FOR DISRUPTION BY MICROORGANISM CELLS BY EXTRUSION

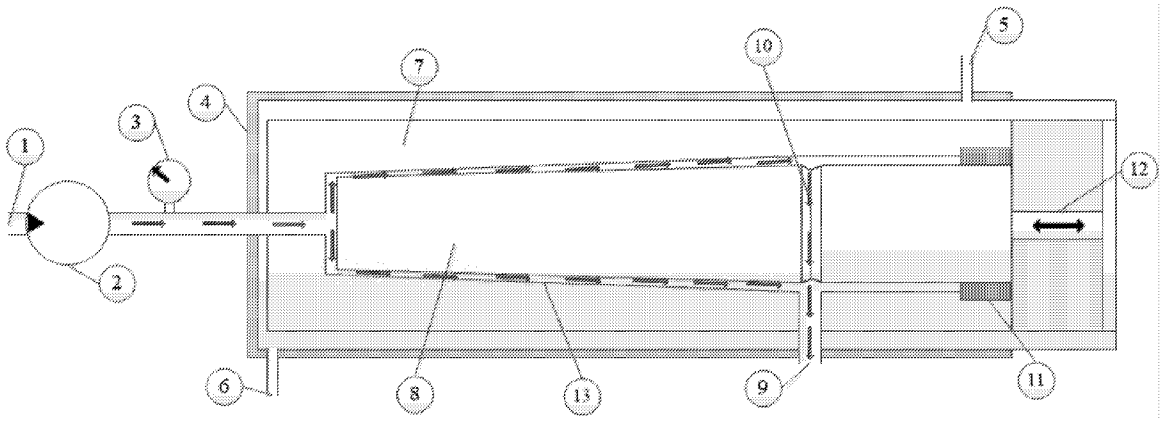

FIG. 1

(57) Abrégé/Abstract:
The present invention relates to devices and systems for microorganism cell wall disruption. Within this scenario, the present invention provides a device for the disruption of cells in a microorganism suspension, comprising (i) an inlet duct (1) for microorganisms, (ii) an annular canal (13) downstream from the inlet duct (1) and in communication therewith, tailored for microorganism cell disruption, the annular canal (13) being formed of an external component (7) and an internal component (8), the internal component being located on the inside of the cavity formed by the external component (7), and (iii) an outlet duct (9) downstream from the annular canal (13) and in communication therewith, for the removal of ruptured microorganisms. The invention also provides a method associated with the above-described device.

ABSTRACT

"DEVICE AND METHOD FOR DISRUPTION OF MICROORGANISM CELLS BY EXTRUSION"

The present invention is related to devices and systems for microorganism cell wall disruption. In this scenario, the present invention provides a device for cell disruption of a microorganism suspension comprising (i) an inlet duct (1) of microorganisms, (ii) an annular channel (13) downstream of inlet duct (1) and in communication therewith, adapted for disruption of microorganism cells, the annular channel (13) being formed by an external part (7) and an internal part (8), the internal part being positioned inside the cavity formed by the external part (7) and (iii) an outlet duct (9) downstream of annular channel (13) and in communication therewith, for output of the ruptured microorganisms. The invention further provides a method associated with the device described above.

Date Reçue/Date Received 2020-09-14

"DEVICE AND METHOD FOR DISRUPTION OF MICROORGANISM CELLS BY EXTRUSION"

FIELD OF THE INVENTION

[1] The present invention is related to devices and systems for the disruption of the cell wall of microorganisms. In particular, the present invention is related to devices and systems for the disruption of the cell wall of microalgae.

FUNDAMENTALS OF THE INVENTION

[2] Microalgae are recognized as an excellent source of proteins, lipids, polyunsaturated fatty acids, carotenoids, pigments and vitamins and can be used in the food, feed, cosmetics, pharmaceutical and biofuel industries. As a source of energy, they are a promising alternative for the production of biofuels, when compared with other conventional energy crops. Their photosynthetic efficiency, associated with rapid growth and the production of lipids, makes their use possible in the production of biofuels, such as ethanol, hydrogen and biodiesel.

[3] Microalgae, like cyanobacteria, are competitive organisms for use in industrial applications, since they exhibit rapid cell growth and have basic nutritional needs (sunlight, water and CO_2) and elevated mutation rates, thus ultimately presenting great potential for genetic modification. Because of their natural diversity and ability to grow in a variety of habitats, there is a growing need to exploit these microorganisms in the production of biofuels and food, especially in areas of low agricultural value.

[4] Rupture of the cell wall of the microalgae is necessary to extract the intracellular metabolites of interest. Several methods of cell disruption for extraction of these compounds of interest are disclosed in scientific articles, such as the use of ultrasound, microwaves, mechanical processes (use of high-pressure homogenizers and mills), chemical processes (solvents and acids), high temperatures (autoclave), freezing and thawing cycles, and extraction by supercritical fluids and ionic liquids.

[5] Mechanical disruption using homogenizers with pressures from 305.9 to 1529.5 kgf/cm^2 (300 to 1500 bar) has been successful in large-

Date Reçue/Date Received 2020-09-14

scale applications due to greater extraction yield when compared to other methods. However, high energy consumption is a limitation in terms of use of this technology to extract products with low added value.

[6] The document "Show, K. Y., Lee, D. J., Tay, J. H., Lee, T. M., Chang, J. S., Microalgal drying and cell disruption" presents a comparison of cell disruption methods utilizing:

(i) a high-pressure press, which is efficient in cell disruption but requires considerable energy, making its use impracticable on a large scale due to the high operating costs;

(ii) a ball mill, which consists of a practical method for large-scale mechanical cell disruption, but the degree of cell disruption depends upon the characteristics of the grinding elements, and its large-scale application requires a large amount of energy;

(iii) the ultrasound technique, which favors extraction in a short time and reduces the use of solvents, but the high energy consumption and the difficulty of large-scale use are negative factors;

(iv) extraction with supercritical fluids, which does not produce toxic waste and employs solvents from renewable sources, but the high energy consumption, high cost of implementation and difficulties in scaling up make the technology impracticable in the biofuel scenario; and

(v) enzymatic extraction, which is used in combination with other cell disruption methods for greater extraction efficiency and for more resistant organisms, but has high operating costs due to the cost of the enzymes.

[7] Also cited is the cryogenic process, which is easy to use and does not require a solvent, but large-scale use results in high operating costs, making the process impracticable.

[8] The microalgae oil extraction system documented in US patent 8,043,496 B1 proposes rupturing the microalgal cell wall after pumping and impact against deflectors. Following this stage, the liquid phase flows into a tank where three phases will presumably be formed: oils, wastewater and biomass. However, damage to the cell structure depends on the diameter and

Date Reçue/Date Received 2020-09-14

species of the microalgae. However, there is no information in this document on the working pressure level.

[9] The high-pressure homogenizers used in the dairy industry can be adapted for cell disruption of microalgae and have as advantages the possibility of working with algal biomass with high solids content and continuously. The mechanisms of cell disruption by the homogenizer are not completely understood but have been attributed to pressure variation, shear stress, inertial forces, shock, turbulence and cavitation.

[10] High pressures from 305.9 to 1529.5 kgf/cm^2 (300 to 1500 bar) are required in the homogenizers with a hydraulic dwell time of 30 minutes to 3 hours. Because of high energy consumption, large scale use of cell disruption with a homogenizer is of questionable economic viability for production of biofuels. Moreover, the temperature increase occurring in homogenizers can interfere with the physicochemical quality of the compounds of interest, such as proteins and unsaturated oils. In addition, adjustment is made according to the required pressure level and not according to the size of the cells of the microalgae species being processed.

[11] As will be detailed below, the present invention seeks to solve the problems of the prior art described above in a practical and efficient manner.

SUMMARY OF THE INVENTION

[12] The primary object of the present invention is to provide a device and method for mechanical disruption of cells of microorganisms by extrusion, using low pressures from 76.5 to 153.0 kgf/cm^2 (75 to 150 bar), and therefore reduced energy consumption.

[13] The present invention has the secondary object of producing a device and method for cell disruption allowing regulation of the system, depending on the species and size of the microorganisms.

[14] The present invention has the tertiary object of providing a device and method for cell disruption of microorganisms, comprising a cooling system to avoid loss of physicochemical properties of the material extracted by cell disruption.

Date Reçue/Date Received 2020-09-14

[15] To realize the aforementioned objects, the present invention provides a device for disruption of microorganism cells by extrusion, comprising (i) an inlet duct of a suspension of microorganisms, (ii) an annular channel downstream of the inlet duct and in communication therewith, adapted for disruption of microorganism cells, the annular channel being formed by an external part and an internal part, the internal part being positioned inside the cavity formed by the external part and (iii) an outlet duct downstream of the annular channel and in communication therewith for output of the ruptured cells.

[16] The present invention also provides a method for disruption of microorganism cells by extrusion, comprising the steps of (i) promoting the forced flow of a suspension of microorganisms through an annular channel downstream of an inlet duct and in communication therewith, the annular channel being adapted for disruption of the microorganism cells and being formed by an external part and an internal part, the internal part being positioned inside the cavity formed by the external part; and (ii) driving the ruptured cells through an outlet duct downstream of the annular channel and in communication therewith.

BRIEF DESCRIPTION OF THE FIGURES

[17] The detailed description presented below refers to the attached figure and its respective reference numbers.

[18] Figure 1 shows a cross section of the device according to a preferred embodiment of the present invention.

[19] Figure 2 shows results of application of the device and method of the present invention, more specifically a result of analysis of flow cytometry in the microalgae *Scenedesmus obliquus* BR003 without rupture (0) and with a number of passes of 1, 5, 10, 20 and 40.

DETAILED DESCRIPTION OF THE INVENTION

[20] Preliminarily, it is emphasized that the description that follows starts from a preferred embodiment of the invention. However, the invention is not limited to this particular embodiment.

Date Reçue/Date Received 2020-09-14

[21] Figure 1 shows a cross section of the device according to a preferred embodiment of the present invention. The cell disruption device of the present invention comprises an inlet duct 1 of a suspension of microorganisms. An annular channel 13 is provided downstream of the inlet duct 1 and in communication therewith, whose size can be adjusted according to the species and diameter of the microorganism, for disruption or cells by extrusion, as will be seen below.

[22] The stream of the microorganisms is forced into the annular channel 13 so that the cell walls are ruptured by extrusion. The forced flow into the annular channel 13 is preferably promoted by means of a positive displacement pump 2, preferably positioned in inlet duct 1.

[23] A pressure gauge 3 is preferably provided at any point between point 2 and annular channel 13 to measure the inlet pressure of the device.

[24] Alternatively, a negative displacement pump (not shown) is used in the device downstream of the annular channel 13 to draw the suspension of microorganisms into the device.

[25] The annular channel 13 is formed by an external part 7 and an internal part 8, the internal part 8 being positioned inside the cavity formed by the external part 7. Internal part 8 preferably has precisely the same cavity-shaped shape formed by external part 7 so that the annular channel 13 has essentially parallel walls. More preferably, external part 7 has a female truncated cone shape. The internal part 8 in this embodiment has the same truncated cone shape but with a male fitting.

[26] The internal part 8 is optionally adjustable with respect to the inner cavity formed by the external part 7 by means of an adjustment mechanism 12 to regulate the diameter of annular channel 13. Moreover, the adjustment mechanism 12 can be a pneumatic, hydraulic, mechanical, electric or manual adjustment mechanism. Activation of the adjustment mechanism 12 can also be formed by a combination of at least two types of drive.

[27] An automated control system is optionally provided to control adjustment mechanism 12.

Date Reçue/Date Received 2020-09-14

[28] The device of the present invention further comprises an outlet duct 19 downstream of annular channel 13 and in communication therewith for the output of ruptured cells.

[29] Internal part 8 preferably comprises a cavity 10 positioned near outlet duct 9. This cavity 10 has the function of generating a low-pressure zone at this point and directing the flow of ruptured material to outlet duct 9. More preferably, cavity 10 is aligned with the outlet duct, as shown in Figure 1.

[30] The device of the present invention preferably also includes a sealing element 11 positioned at the end of the annular channel 13 opposite inlet duct 1 in the vicinity of adjustment mechanism 12. Sealing element 11 is preferably a gasket made of flexible material.

[31] To avoid an excessive increase in temperature of the microorganism suspension, a cooling system is preferably provided in the device of the present invention. The system comprises a cooling jacket 4 positioned around the external part 7.

[32] The cooling system also includes a coolant input 5 to inject coolant into cooling jacket 4 and a coolant output 6 to remove coolant from cooling jacket 4. The coolant inlet 5 is preferably positioned longitudinally and transversely away from the coolant output 6 to promote coolant flow throughout virtually the entire cooling jacket 4 and external part 7.

[33] The external part 7 is preferably made from a heat-conductive material, such as metal, permitting efficient heat exchange between the coolant and the microorganism suspension.

[34] The present invention also provides a method for disruption of microorganism cells, comprising the steps of:

(i) promoting a forced flow of a suspension of microorganisms through an annular channel 13 downstream of an inlet duct 1 and in communication therewith, the diameter of the annular channel 13 being adapted for cell disruption and formed by an external part 7 and internal part 8, the internal part 8 being positioned inside the cavity formed by external part 7; and

Date Reçue/Date Received 2020-09-14

(ii) passing the ruptured microorganisms through an outlet duct 9 located downstream of annular channel 13 and in communication therewith.

[35] The method of the present invention also preferably includes the step of adjusting the position of the internal part 8 in relation to the cavity formed by the external part 7 by an adjustment mechanism 12 in order to regulate the diameter of annular channel 13.

[36] The method of the present invention preferably comprises the additional step of cooling the microorganism suspension inside annular channel 13 by means of a cooling system. More preferably, the step of cooling the microorganism suspension includes the circulation of a coolant through cooling jacket 4.

[37] The present invention therefore provides a device and method for cell disruption of a microorganism suspension by extrusion, using low pressures from 76.5 to 153.0 kgf/cm^2 (75 to 150 bar) and thus reduced energy consumption. The device of the present invention even permits the regulation of the diameter of the annular channel as a function of the diameter and width of the cells of the microorganisms, making it fully and efficiently adapted to disruption of the species of interest.

[38] The cooling system also prevents the loss of physicochemical properties of the extracted material.

[39] To demonstrate the efficiency of the proposed device and method, the disruption of cells of the species *Scenedesmus obliquus* BR003 was carried out with the present invention. Flow cytometry (BD Facsverse, BD Biosciences) analysis was used to check cell disruption. The analysis revealed that a cycle of five passes at a pressure of 127.5 kgf/cm^2 (125 bar) was sufficient to cause damage to the cell structure, reducing the relative size of the cells (FSC - forward scatter, Figure 2) by about 50% when compared to the control (without disruption).

[40] In addition to the relative size of the cells, there was also a reduction in granulosity, observed by the parameter SSC (SSC - side scatter, Figure 2). This parameter depends on the internal complexity of the particle, for

Date Reçue/Date Received 2020-09-14

example, shape of the nucleus, number and type of cytoplasmic granules and roughness of the membrane. Thus, the reduction of this parameter indicates that there was a reduction in the number of intact cells, producing cell fragments (debris). The statistical analyses shown in Table 1 indicated that the variation in number of passes through the device, between 10 and 40, did not result in a significant difference in relative cell size.

Table 1

Number of passes	SSC-A average	FSC-A average	FSC-A SD	FSC-A VC (%)	FSC-A median
0	34,806	111,871	50,149	44.8	109,368
1	27,016	73,560	39,442	53.6	66,187
5	23,658	62,318	31,500	50.6	55,724
10	22,739	59,217	29,629	50.0	52,950
20	21,507	57,099	27,496	48.2	51,431
40	21,840	57,063	27,858	48.8	51,025

[41] It should be noted that the FSC index shown in Table 1 is related to cell size. SSC is related to internal complexity of the cells and SD and VC correspond to standard deviation and variation coefficient (%), respectively.

[42] Numerous variations on the scope of protection of this application are possible. This reinforces the fact that the present invention is not limited to the particular configurations/embodiments described above.

Date Reçue/Date Received 2020-09-14

CLAIMS

1. Device for cell disruption of microorganisms by extrusion, characterized in that it comprises:

an inlet duct (1) of a microorganism suspension;

an annular channel (13) downstream of the inlet duct (1) and in communication therewith, adapted for disruption of microorganism cells, the annular channel (13) being formed by an external part (7) and an internal part (8), the internal part (8) being positioned inside the cavity formed by the external part (7); and

an outlet duct (9) downstream of annular channel (13) and in communication therewith, for output of ruptured cells.

2. Device according to claim 1, characterized in that it also includes a pump (2) adapted to pump a suspension of microorganisms through annular channel (13).

3. Device according to claim 2, characterized in that pump (2) is a positive displacement pump positioned in inlet duct (1).

4. Device according to any of the claims 1 to 3, characterized in that the position of the internal part (8) is adjustable with respect to the cavity formed by the external part (7) by means of an adjustment mechanism (12) in order to regulate the diameter of annular channel (13).

5. Device according to claim 4, characterized in that the adjustment mechanism (12) is at least one pneumatic, hydraulic, mechanical, electric and manual device.

6. Device according to any of the claims 1 to 5, characterized in that it also includes one pressure gauge (3) adapted to check the inlet pressure of the device.

7. Device according to any of the claims 4 to 6, characterized in that it also includes an automated control system adapted to control the adjustment mechanism (12).

Date Reçue/Date Received 2020-09-14

8. Device according to any of the claims 1 to 7, characterized in that the internal part (8) includes a cavity (10) positioned near outlet duct (9).

9. Device according to claim 8, characterized in that it includes a sealing element (11) adapted to seal the end of annular channel (13).

10. Device according to claim 9, characterized in that the sealing element (11) is positioned at the end of annular channel (13) opposite inlet duct (1).

11. Device according to any of the claims 1 to 10, characterized in that it additionally includes a coolant system, comprising:

a cooling jacket (4) positioned around external part (7);

a coolant input (5) adapted to inject coolant into cooling jacket (4); and

a coolant outlet (6) adapted to remove coolant from cooling jacket (4).

12. Method for cell disruption of microorganisms by extrusion, characterized in that it includes the steps of:

promoting a forced flow of a suspension of microorganisms through an annular channel (13) downstream of an inlet duct (1) and in communication therewith, the annular channel (13) being adapted for disruption of microorganism cells and formed by an external part (7) and an internal part (8), the internal part being positioned inside the cavity formed by the external part (7); and

passing the ruptured cells through an outlet duct (9) located downstream of annular channel (13) and in communication therewith.

13. Method according to claim 12, characterized in that it includes the additional step of adjusting the position of the internal part (8) in relation to the cavity of the external part (7) by means of an adjustment mechanism (12) in order to regulate the diameter of the annular channel (13) as a function of the species and diameter of the microorganism.

Date Reçue/Date Received 2020-09-14

14. Method according to claim 12 or 13, characterized in that it includes the additional step of cooling the suspension of microorganisms inside annular channel (13) by means of a cooling system.

15. Method according to claim 14, characterized in that the cooling step of the microorganism suspension includes circulation of coolant through a cooling jacket (4).

Date Reçue/Date Received 2020-09-14

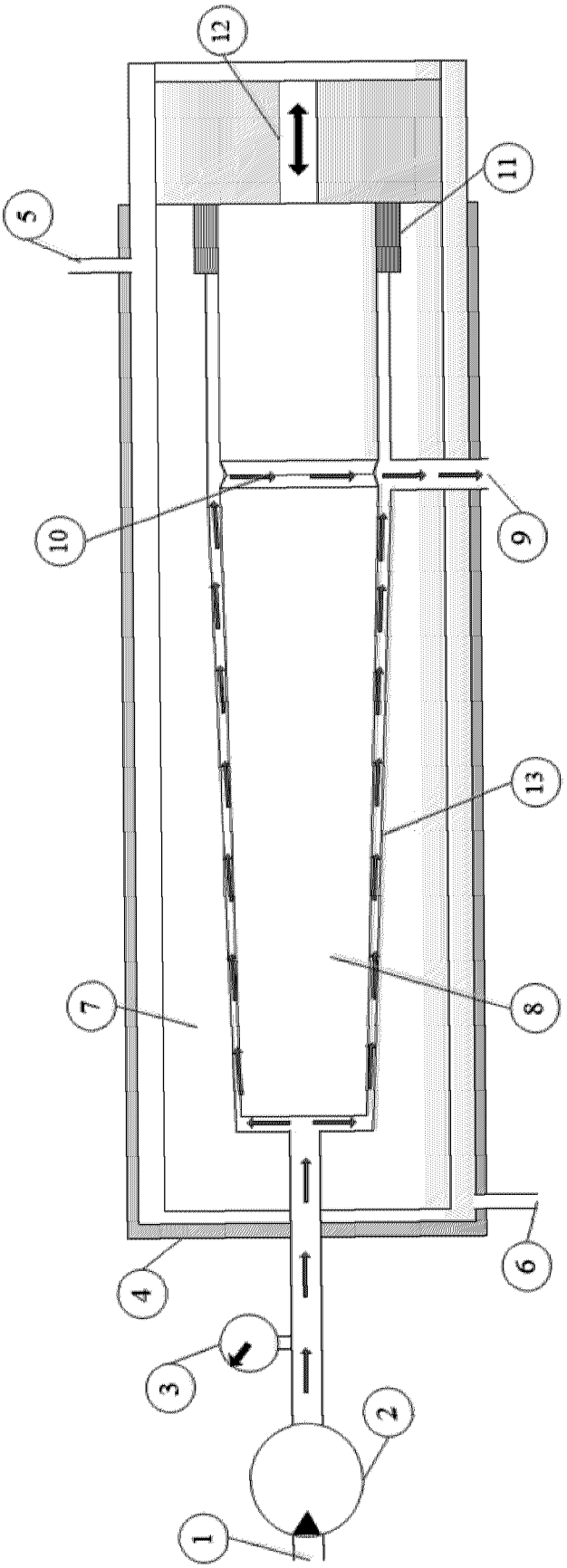

FIG. 1

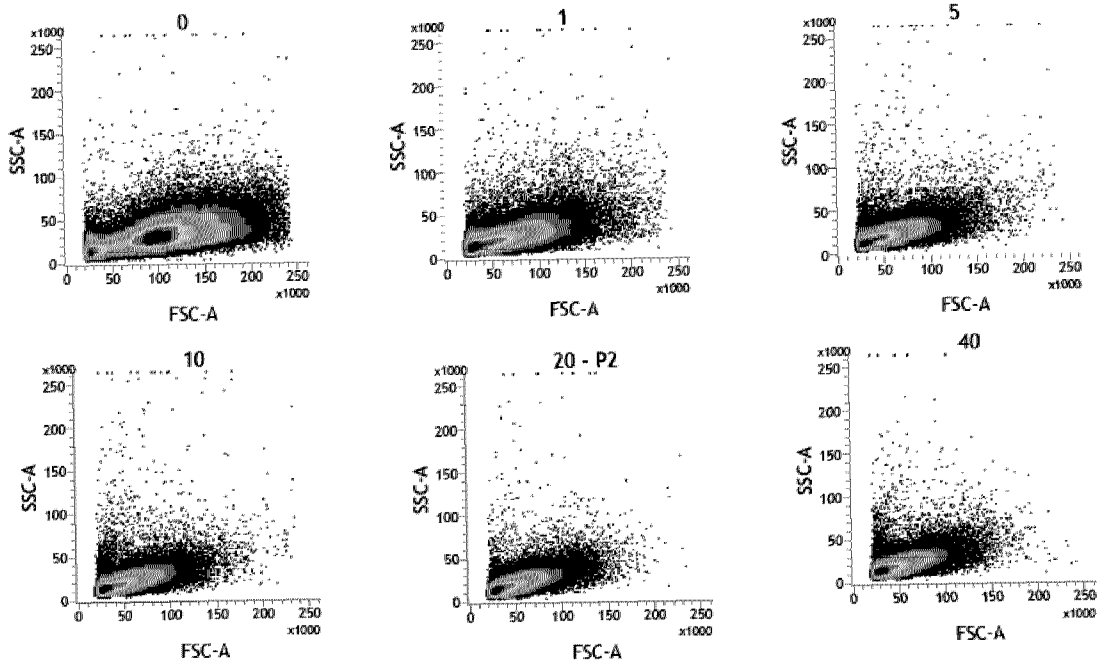

FIG. 2

Date Reçue/Date Received 2020-09-14

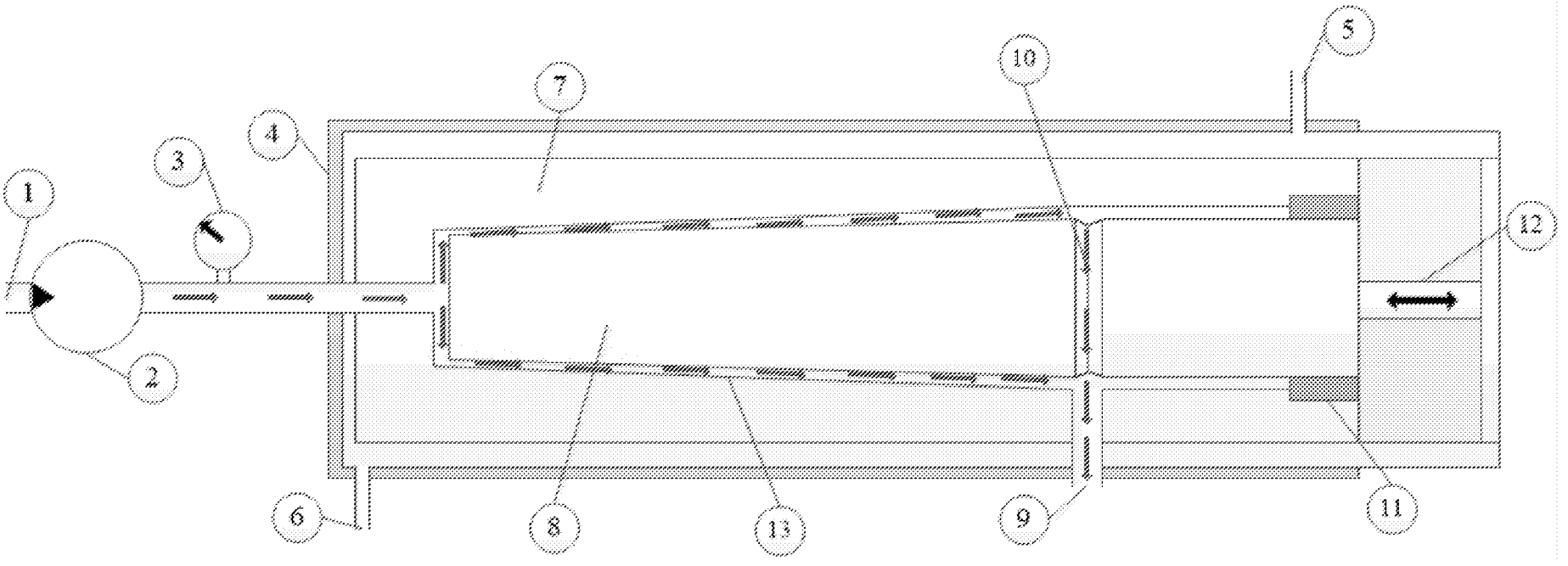

FIG. 1