method and related apparatus (and stack of (parallel) systems), which allow obtaining (or producing) (valuable) cellular substances from suspended biomass material, with a high throughput (compared to prior art methods and apparatuses).

**[0019]** Aspects of the present invention envisage providing such method for disrupting suspended biomass material and related apparatus (and stack of (parallel) systems), which allow for integration with a solubilization and/or subsequent selective extraction or separation of the obtained (valuable) cellular substances.

**[0020]** More particularly, it is envisaged to provide a subsequent solubilization of cellular substances from suspended biomass material and minimization of contaminating load by tuning the extraction ratio of the preferred product or biochemical. After solubilization, a subsequent selective extraction or separation of the obtained cellular substances is also envisaged.

**[0021]** Aspects of the present invention envisage providing such method (and related apparatus and stack of (parallel) systems) for disrupting suspended biomass material with (optionally) a solubilization and/or subsequent selective extraction or separation of the obtained cellular substances, which allow decreasing the total operational expenditure (OPEX) (or total cost) (compared to prior art methods and apparatuses) and being applicable on industrial scale.

## Summary of the Invention

**[0022]** According to one aspect of the invention, there is therefore provided a method for disrupting suspended biomass material, comprising the steps of: providing a suspension of biomass material in a first vessel (1), said suspension having a dry cell weight being comprised between 0.1% and 15% (w/w); pressurizing said suspension to a first pressure (p1) being comprised between 7500 kPa and 450000 kPa by using a first high-pressure pump (10); providing a pressurized gas disruption agent and/or a pressurized liquid disruption agent in a second vessel (2); pressurizing said gas and/or liquid disruption agent to a second pressure (p2) being comprised between 7500 kPa and 450000 kPa by using a second high-pressure pump (20), said second pressure (p2) being higher than said first pressure (p1); injecting a stream of said pressurized disruption agent into a stream of said pressurized suspension of biomass material and forming a micro-emulsion; passing said micro-emulsion wherein the stream of said pressurized disruption agent is injected into the stream of said pressurized suspension through openings (29,30,31,32) of a diameter (d3) comprised between 0.01 mm and 1 mm; passing said micro-emulsion sequentially through a hydrodynamic agitation system (11) and a relief valve (13).

**[0023]** According to another aspect of the invention, there is provided an apparatus for disrupting suspended biomass material, comprising: at least one first vessel (1) for holding a suspension of biomass material connected to at least one first high-pressure pump (10), said first high-pressure pump (10) being adapted for pressurizing the suspension of biomass material supplied by said first vessel (1); at least one second vessel (2) for holding a gas disruption agent and/or liquid disruption agent connected to at least one second high-pressure pump (20), said second high-pressure pump (20) being adapted for pressurizing the gas and/or liquid disruption agent supplied by said second vessel (2); wherein each of said first and second high-pressure pump (10,20) is connected to at least one sparger system (7), said sparger system (7) being adapted for injecting a first stream of the pressurized disruption agent into a second stream of

the pressurized suspension of biomass material and for forming a micro-emulsion, wherein said sparger system (7) comprises a first sparger tube (21) having a first diameter (d1) and having two inlets (24,34) and one outlet (28), said first sparger tube (21) comprising a second sparger tube (22) placed along the direction formed by one of the inlets (34) and the outlet (28), said second sparger tube (22) having a second diameter (d2) being two times smaller than the first diameter (d1) of the first sparger tube (21), said second sparger tube (22) comprising a distribution of sets (23) of first openings through a wall of the second sparger tube (22) along a width (w), said first openings having a third diameter (d3) comprised between 0.01 mm and 1 mm, wherein said sparger system (7) is further connected to a tubing system (8) for mixing said first and second streams, said tubing system (8) comprising a primary tube (15) and at least one hydrodynamic agitation system (11), and said tubing system (8) being further connected to a relief valve (13). According to another aspect of the invention, there is provided a sparger system, comprising: a first tube (21) comprising a first inlet (24) for supplying a stream of disruption agent into the tube (21); a second tube (22) comprising a second inlet (34) for supplying a stream of a suspension of biomass material through the tube (22), the second tube (22) comprising an outlet (28), wherein the second tube (22) traverses the first tube (21) and comprises at least one set (23) of first openings (29-32) through a wall of the tube (22), the first openings (29-32) being in fluid communication with the first tube (21) such that an emulsion is formed by forcing the stream of disruption agent through the first openings (29-32) into the second tube (22), wherein the first openings (29-32) have a third diameter (d3) between 0.01 mm and 1 mm.

**[0024]** According to another example of the disclosure, there is provided a stack of (parallel) systems for disrupting suspended biomass material, based on the apparatus of the present invention.

**[0025]** According to other examples of the diclosure, there is provided the use of the apparatus of the invention.

**[0026]** Advantageous aspects of the present invention are set out in the dependent claims.

## Short Description of the Drawings

**[0027]** Aspects of the invention will be described in more detail with reference to the appended drawings, wherein same reference numerals illustrate same features. In the drawings, not all alternatives and options are shown and therefore the invention is not limited to the content of the given drawings.

**[0028]** **Figure 1a** schematically represents an apparatus according to an aspect of the present invention, for disrupting suspended biomass material and, optionally, solubilizing the (valuable) cellular substances.

**[0029]** **Figure 1b** schematically represents an apparatus according to an aspect of the present invention, for disrupting suspended biomass material and, optionally, solubilizing the (valuable) cellular substances, said apparatus further comprising a pressure oscillation system (9).

**[0030]** **Figure 2** schematically represents a sparger system (7) used in the apparatus of **figures 1a** and **1b**.

**[0031]** **Figure 3** depicts the comparison of the solubilization ratios (%) of different

## CLAIMS

**1.** A method for disrupting suspended biomass material comprising the steps of:

- providing a suspension of biomass material in a first vessel (1), said suspension having a dry cell weight being comprised between 0.1% and 15% (w/w);
- pressurizing said suspension to a first pressure (p1) being comprised between 7500 kPa and 450000 kPa by using a first high-pressure pump (10);
- providing a pressurized gas disruption agent and/or a pressurized liquid disruption agent in a second vessel (2);
- pressurizing said gas and/or liquid disruption agent to a second pressure (p2) being comprised between 7500 kPa and 450000 kPa by using a second high-pressure pump (20), said second pressure (p2) being higher than said first pressure (p1);
- injecting a stream of said pressurized disruption agent into a stream of said pressurized suspension of biomass material and forming a micro-emulsion, wherein the stream of said pressurized disruption agent is injected into the stream of said pressurized suspension through openings (29,30,31,32) of a diameter (d3) comprised between 0.01 mm and 1 mm;
- passing said micro-emulsion sequentially through a hydrodynamic agitation system (11) and a relief valve (13).

**2.** The method of claim 1, wherein after the step of forming the micro-emulsion and before the step of passing sequentially through the hydrodynamic agitation system (11) and the relief valve (13), said micro-emulsion is passing through a pressure oscillation system (9).

**3.** The method of claim 1 or 2, wherein simultaneously with the step of passing the micro-emulsion through the relief valve (13), the temperature of the micro-emulsion is increased to the subcritical or critical point of the disruption agent and the process stream coming out of the relief valve (13) is transferred to a third vessel (35) at reduced or atmospheric pressure, thereby solubilizing cellular substances.

**4.** The method of any one of the preceding claims, wherein the steps of the method before the step of increasing the temperature to the subcritical or critical point of the disruption agent are performed at a temperature range between 2°C up to 70°C.

**5.** The method of claim 4, wherein the treated suspension of biomass material is separated by centrifugation and/or filtration, so as to obtain soluble and insoluble phases.

**6.** The method of any one of the preceding claims, wherein the disruption agent is selected from the group consisting of ethane, propane, butane, ethylene, propylene, butylene, other saturated or unsaturated hydrocarbons, carbon dioxide, nitrous oxide, dimethyl ether, sulfur hexafluoride, freon, and mixtures thereof.

**7.** The method of any one of the preceding claims, wherein the ratio of the disruption agent to the suspension of biomass material is comprised between 0.001 and 0.95 kg/kg.

**8.** An apparatus for disrupting suspended biomass material comprising:
at least one first vessel (1) for holding a suspension of biomass material connected to at least one first high-pressure pump (10), said first high-pressure pump (10) being adapted for pressurizing the suspension of biomass material supplied by said first vessel (1); at least one second vessel (2) for holding a gas disruption agent and/or liquid disruption agent connected to at least one second high-pressure pump (20), said second high-pressure pump (20) being adapted for pressurizing the gas and/or liquid disruption agent supplied by said second vessel (2); wherein each of said first and second high-pressure pump (10,20) is connected to at least one sparger system (7), said sparger system (7) being adapted for injecting a first stream of the pressurized disruption agent into a second stream of the pressurized suspension of biomass material and for forming a micro-emulsion, wherein said sparger system (7) comprises a first sparger tube (21) having a first diameter (d1) and having two inlets (24,34) and one outlet (28), said first sparger tube (21) comprising a second sparger tube (22) placed along the direction formed by one of the inlets (34) and the outlet (28), said second sparger tube (22) having a second diameter (d2) being two times smaller than the first diameter (d1) of the first sparger tube (21), said second sparger tube (22) comprising a distribution of sets (23) of first openings through a wall of the second sparger tube (22) along a width (w), said first openings having a third diameter (d3) comprised between 0.01 mm and 1 mm, wherein said sparger system (7) is further connected to a tubing system (8) for mixing said first and second streams, said tubing system (8) comprising a primary tube (15) and at least one hydrodynamic agitation system (11), and said tubing system (8) being further connected to a relief valve (13).

**9.** The apparatus of claim 8, wherein the tubing system (8) comprises at least one pressure oscillation system (9) upstream from the at least one hydrodynamic agitation system (11).

**10.** The apparatus of claim 9, wherein said pressure oscillation system (9) comprises at least two orifices in primary tube (15) connected in series, each orifice having a diameter (d8), said diameter (d8) being four to sixty-four times smaller than a diameter (d7) of the primary tube (15).

**11.** The apparatus of any one of claim 8 to 10, wherein said hydrodynamic agitation system (11) comprises at least one subsidiary tubing system (16) connected to the primary tube (15), wherein the tubes of the subsidiary tubing system (16) have diameters (d9) at least two times smaller than the diameter (d7) of the primary tube (15).

**12.** The apparatus of any one of claim 8 to 11, wherein the relief valve (13) comprises a means (14) for heating a stream coming out of the tubing system (8), said relief valve being further connected to a third vessel (35).

**13.** A sparger system, comprising:
a first tube (21) comprising a first inlet (24) for supplying a stream of disruption agent into the tube (21);
a second tube (22) comprising a second inlet (34) for supplying a stream of a suspension of biomass material through the tube (22), the second tube (22) comprising an outlet (28),
wherein the second tube (22) traverses the first tube (21) and comprises at least one set (23) of first openings (29-32) through a wall of the tube (22), the first openings (29-32) being in fluid communication with the first tube (21) such that an emulsion is formed by forcing the stream of disruption agent through the first openings (29-32) into the second tube (22),
wherein the first openings (29-32) have a third diameter (d3) between 0.01 mm and 1 mm.

**14.** The sparger system of claim 13, wherein the first tube (21) is dead ending on the second tube (22).

**15.** The sparger system of claim 13 or 14, wherein the first tube (21) has a first diameter (d1) comprised between 1.6 mm and 25.4 mm.

**16.** The sparger system of any of claim 13 to 15, wherein the second tube (22) has a second diameter (d2) comprised between 0.8 mm and 12.7 mm.

**17.** The sparger system of claim 13 or 16, wherein the ratio of the second diameter (d2) of the second tube (22) to the first diameter (d1) of the first tube (21) is 0.5.

**18.** The sparger system of any of claim 13 to 17, wherein the second tube (22) comprises a second opening (27) at the outlet (28) of the second tube (22), said second opening (27) having a diameter (d4) comprised between 0.01 mm and 0.6 mm.

**19.** The sparger system of any of claim 13 to 18, wherein each set (23) of first openings in second tube (22) comprises at least two first openings (29,31), said openings being located opposite each other in one plane, said plane being perpendicular to the second tube (22), the first openings (29,31) being located through the wall of the second tube (22) at a distance D1 satisfying the equation D1=(diameter (d2) of second tube x π)/2 from the next opening of the set.

**20.** The sparger system of any of claim 13 to 19, wherein each set (23) of first openings in second tube (22) comprises at least four first openings (29-32), said openings being located two by two opposite each other in one plane, said plane being perpendicular to the second tube (22), each of the first openings (29-32) being located through the wall of the second tube (22) at a distance D1 satisfying the equation D1=(diameter (d2) of second tube x π)/4 from the next opening of the set.