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Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))
- of inventorship (Rule 4.17(iv))

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(54) Title: PRODUCTION OF ALGAE USING CO₂-CONTAINING GAS

(57) Abstract: The invention is directed to a method for using a gaseous feed stream comprising CO₂ as a feed stream for growing algae, said method comprising a) contacting said gaseous feed stream with an absorption liquid comprising carbonic anhydrase in an absorption module to produce a lean gas stream and a liquid feed stream comprising bicarbonate; b) contacting part of the liquid feed stream and/or part of the gaseous feed stream comprising CO₂ with microorganisms to generate carbonic anhydrase and c) contacting part of the liquid feed stream with an algae culture to produce algae-containing biomass. Another aspect of the invention is directed to an apparatus for sequestration of CO₂ by using algae, said apparatus comprising two coupled circulation systems that each comprise at least one bioreactor.



Title: Production of algae using CO₂-containing gas

The invention is in the field of CO₂ capture and sequestration, as
5 well as in the field of producing algae. The invention is in particular
directed to using CO₂-containing gas to grow algae for biomass production
as well as to an apparatus suitable for carrying out this production process.

To reduce the amount of CO₂ emission to the atmosphere, CO₂
contained in gaseous streams, such as flue gases, can be captured and
10 sequestered from these streams. A number of different methods are known
to achieve this. Particular methods of interests are those that use algae for
CO₂ sequestration. These methods have the concomitant advantage that the
algae proliferate on CO₂ and can serve as a biomass source for the
production of valuable products. Conventional methods include directly
15 contacting an algae culture with gaseous CO₂. This however has a limited
efficiency, in particular when the process is carried out at large scale. This
issue has been addressed by a combined enzymatic CO₂ capture and
subsequent sequestration by algae.

WO 2013/022348 describes the capture of CO₂ by carbonic
20 anhydrase by conversion of the CO₂ into bicarbonate. The bicarbonate is
subsequently fed to algae. To limit the waste of enzymes, a filtering system
and recycling of the enzymes are provided. However, this has only a limited
efficiency and although carbonic anhydrase is an enzyme and acting as a
catalyst theoretically not consumed, the carbonic anhydrase in practice loses
25 reactivity (efficiency) due to decomposition. It is therefore required to
replenish the carbonic anhydrase in the absorption unit. Conventionally,
this is achieved by adding carbonic anhydrase that is processed in a
separate process but this is overall less efficient. It is desired to improve the
efficiency of the process.

A first aspect of the present invention is accordingly directed to a method for producing algae-containing biomass with CO₂, said method comprising:

- 5 a) contacting at least part of an acidic gaseous stream comprising CO₂ with an aqueous absorption liquid comprising carbonic anhydrase in an absorption module to produce a lean gaseous stream that has a lower CO₂ concentration than the acidic gaseous stream and to produce a liquid feed stream that comprises bicarbonate;
- 10 b) contacting part of the liquid feed stream and/or part of the acidic gaseous stream comprising CO₂ with microorganisms to generate carbonic anhydrase and to produce an aqueous stream comprising carbonic anhydrase that is fed to the absorption module;
- c) contacting at least part of the liquid feed stream with an algae culture to produce algae-containing biomass
- 15 wherein step b) and c) are carried out in separate bioreactors.

Figure 1 is a schematic representation of a particular embodiment of the present invention.

The absorption module as such is known in the art and could be any device that facilitates mass transfer between the gaseous feed stream and the absorption liquid.

A particular advantage of the method of the present invention is that the integrated production of the carbonic anhydrase reduces the necessity for further carbonic anhydrase addition which may be produced off-site. This greatly simplifies the overall process and increases its efficiency. An additional advantage of the present method is that the acidic gaseous stream is either directly or indirectly used to produce the carbonic anhydrase. This increases efficiency and reduces the amount of CO₂ emission as well.

The gaseous feed according to the present invention typically comprises flue gas, *e.g.* flue gas originating from a fossil-fuel electrical

power plant or a biogas production plant, preferably flue gas comprising between 4-25 wt% CO₂ and 70-95 wt% nitrogen or flue gas comprising between 20-50 wt% CO₂ and 45-75 wt% methane. Flue gas generally comprises, besides CO₂, gaseous NO_x. NO_x includes nitrogen oxides (*e.g.* nitrogen monoxide NO) that are considered toxic and harmful for the environment. Gaseous NO_x is therefore preferably removed from the acidic gaseous stream as well. It has surprisingly been found that by oxidization the NO_x to NO₂, this NO_x can however be advantageously used in the present invention. Therefore, in a preferred embodiment of the present invention, the acidic gaseous stream further comprises NO_x and said method further comprises oxidation of said NO_x with a catalyst to form an oxidized acidic gaseous stream comprising NO₂ and the CO₂. NO_x can be oxidized by known catalysts. Examples thereof include palladium, platina, titanate (TiO₂), cobalt, zinc, nickel, manganese, and combinations thereof. Suitable catalysts are well known in the art, see for example the catalyst materials described in US8557203, which is incorporated herein. Instead of, or in addition to using the oxidation catalyst, the oxidation can be carried out with oxidation agents such as ozone (see *e.g.* US5985223)

In this particular embodiment, the oxidized acidic gaseous stream is subsequently used in step a) of the present invention.

In case the acidic gaseous stream comprises CO₂ and NO_x and is oxidized to the oxidized acidic gaseous stream, an oxidation catalyst that is capable of forming nitrite (NO₂⁻) and/or nitrate (NO₃⁻) from NO₂ is preferably present in the absorption liquid. Examples of oxidation catalysts known for the conversion of NO₂ into NO₃⁻ and NO₂⁻ are for instance Fe²⁺. Nagase *et al.* (Characteristics of Biological NO_x Removal from Flue Gas in a *Dunaliella Tertiolecta* Culture System. J. Ferment. Bioeng. **83** (1997)461–465) reported 60% NO_x (25 to 500 ppm) removal in presence of the algae *D. tertiolecta* and 30% when only led through cell free medium. It was assumed that photochemical oxidation took place, catalyzed by Fe²⁺.

Accordingly, the liquid feed stream may further comprise nitrite and/or nitrate which can be used to feed and culture the algae. Thus, the conventionally undesired NO_x may advantageously be used for the present invention to in fact improve growth of the algae.

5 In accordance with the present invention, part of the liquid feed stream is contacted with microorganisms to generate carbonic anhydrase and to produce the aqueous stream comprising carbonic anhydrase that is fed to the absorption module, while also at least part of the liquid feed stream comprising bicarbonate is contacted with an algae culture to produce
10 algae-containing biomass. This means that the liquid feed stream that is enriched with bicarbonate can be divided over the carbonic anhydrase production unit and the algae-containing biomass production unit. In a preferred embodiment, part of the liquid feed stream is contacted with said microorganisms while the remainder of the liquid feed stream is contacted
15 with said algae culture.

 In a particular embodiment of the invention, the carbonic anhydrase production unit could potentially be directly fed with at least part of the acidic gaseous stream. In this context, directly feeding means that the acidic gaseous stream does not contact the aqueous absorption
20 liquid but that the acidic gaseous stream may for instance be bubbled through the culture medium comprising the microorganisms in the first bioreactor. Accordingly, at least part of said acidic gaseous stream is contacted with the aqueous absorption liquid, while also part of the acidic gaseous stream comprising CO₂ is directly contacted with said
25 microorganisms in the production unit. Preferably all of the acidic gaseous stream is contacted with the aqueous absorption liquid in the absorption module.

 Carbonic anhydrase (also referred to as carbonate anhydrase; EC 4.2.1.1) is known to catalyze the hydration of CO₂ (see for instance
30 WO2013/022348 which is incorporated herein in its entirety). The

generation of the carbonic anhydrase in the present invention is achieved by contacting part of the liquid feed stream and/or part of the acidic gaseous stream comprising CO₂ with microorganisms. Examples of microorganisms that can be used to generate carbonic anhydrase are for instance bacteria as described by A. Di Fiori *et al.* (Int. J. Mol. Sci. **16** (2015) 15456-15480) and the references cited therein. It may be appreciated that the microorganism are contacted with part of the liquid feed stream and/or part of the acidic gaseous stream in the presence of a culture medium.

Carbonic anhydrase is widespread among the entire prokaryotic and eukaryotic domain (see Shekh *et al.*; Recent Advancements in Carbonic Anhydrase-Driven Processes for CO₂ Sequestration: Minireview; Crit. Rev. Environ. Sci. Technol. 2012, **42** (14) 1419-1440). Carbonic anhydrase is either localized inside the cell, or can be secreted outside. External carbonic anhydrase is located outside or associated with the periplasmic cell membrane. External carbonic anhydrase is expressed under certain conditions, especially when CO₂ supplements are limited to the cell (see Lam *et al.*; Current Status and Challenges on Microalgae-Based Carbon Capture. Int. J. Greenh. Gas Control **10** (2012) 456-469). A stream of the carbonic anhydrase generated in step b) of the invention is typically directly fed to the absorption module. Alternatively, the stream of carbonic anhydrase can first be combined with a basic liquid stream that is additionally produced in step c), after which the combined stream comprising carbonic anhydrase and the basic liquid are fed to the absorption module.

The liquid feed stream is an aqueous liquid that comprises the bicarbonate which the algae can use to proliferate. Further nutrients required for algae growth such as vitamins, nitrous and phosphorus components, and microelements may also be present in the liquid feed stream. These can be added before or after the absorption of CO₂ in the absorption module. After the liquid feed stream has contacted the algae

culture sufficiently for the algae to absorb at least part of the bicarbonate and optionally further nutrients from the liquid feed stream, the liquid feed stream is separated from the algae culture and a basic liquid stream is produced. The basic liquid stream is thus the result of contacting the algae
5 and the liquid feed stream comprising the bicarbonate.

The algae process the bicarbonate present in the liquid feed stream which results in an increase of the pH of the culture, typically due to the production of hydroxide. The pH of the algae culture is preferably maintained at constant levels, preferably at pH values in the range from 7
10 to 10. This can be achieved by a selective (essentially cell-free) outflow of a basic liquid stream from the algae culture. A filter may be used to prevent the outflow of algae together with the basic liquid. Due to the outflow of the basic liquid and subsequent replenishment with the relatively acidic liquid feed stream, the pH value in the algae culture may be kept constant. In
15 other words, the algae have a optimum pH for growth, *e.g.* a pH in the range of 7-8. However, upon formation of biomass bicarbonate is taken up from the liquid feed stream which results in an increase of pH, to *e.g.* pH >8. By replacing spent liquid feed solution (*i.e.* basic liquid stream) with high pH by fresh liquid feed stream having for instance a pH of 7-8, optimal growth
20 conditions are maintained for the algae.

An example of a suitable medium for the algae culture is Bold 3N Medium, commercially available from UTEX Culture Collection of Algae, The University of Texas at Austin, USA.

The basic liquid stream is preferably recycled to the absorption
25 module where it is used as the absorption liquid. Due to the absorption of CO₂, the pH of the absorption liquid decreases in the absorption module. However, the pH value of the absorption liquid is preferably maintained at a pH value ranging from 6-8. Hence, by providing an outflow and recycling of the basic liquid stream, the pH values of both the algae culture and the
30 absorption liquid can be controlled. This is particularly beneficial for the

overall efficiency of the process since it prevents the requirement of further addition of base and/or acid.

Steps b) and c) are carried out in separate bioreactors. Step b) may for instance be carried out in a first bioreactor and step c) is carried out
5 in a second bioreactor. Each bioreactor generally comprises a separation module (*e.g.* a filter or a membrane) to separate the microorganisms and/or algae from the outflowing stream, *e.g.* the stream of carbonic anhydrase and/or the liquid basic stream. The bioreactor used for each step can comprises a plurality of parallel bioreactors. This greatly facilitates flexible
10 scaling of the process, depending on the amount of the acidic gaseous stream to be processed.

The culture of algae can further be fed with water and nutrients besides with the bicarbonate and optional nitrates and/or nitrites. For instance the following nutrients may be present: sodium nitrate, calcium
15 chloride, magnesium sulfate, potassium phosphate (mono- and dibasic), sodium chloride, sodium EDTA, iron (III) chloride, manganese (II) chloride, zinc (II) chloride, cobalt (II) chloride, sodium molybdate, vitamin B12, biotin, thiamine, and combinations thereof. These additional water and nutrients can be supplied to the liquid feed stream before this stream is
20 contacted with the algae or can be supplied as a separate stream to the algae culture.

The algae culture preferably comprises *Spirulina platensi* and/or *Neochloris oleoabundans*. Other examples of strains are Chlorella, Chlamydomonas, Nannochloropsis, Botryococcus, *etc.* The culture is capable
25 of converting the bicarbonate into valuable compounds such as β -carotene, anti-oxidants, lipids and fatty acids can be produced by the algae. The produced algae-containing biomass thus comprises a number of valuable compounds. In a preferred embodiment of the present invention, the method further comprises a step of harvesting the algae and subsequent extraction

of the valuable compounds. These valuable compounds can be used in applications such as biofuel and cosmetics.

A further aspect of the present invention is an apparatus that is especially adapted for the method according to the present invention.

5 As is illustrated in figure 1, the apparatus according to the present invention comprises a first fluid circulation system wherein a fluid can be circulated comprising an absorption module (1) and a first bioreactor (2) downstream of the absorption module. The absorption module comprises a gas inlet through which the acidic gaseous stream can be let into the
10 module.

The apparatus is preferably adapted such that the fluid that can be circulated can be free of cells. Therefore, the first bioreactor is adapted to culture microorganisms producing carbonic anhydrase and comprises a filter (21) positioned downstream in the reactor such that the microorganisms are
15 essentially retained in the first bioreactor and an outflow stream that comprises carbonic anhydrase can be obtained that is essentially cell-free.

The apparatus further comprises a second fluid circulation system, that is connected to the first circulation system and extends directly from the absorption module (1) or downstream of the absorption module and
20 upstream of the first bioreactor (2), to the absorption module or to the first circulation system downstream of the first bioreactor and upstream of the absorption module. The second fluid circulation system comprises a second bioreactor (4) that is adapted to culture algae. The second bioreactor (4) comprises a filter (41) positioned downstream such that the algae are
25 essentially retained in the second bioreactor and a basic outflow stream that is essentially cell-free can be obtained and the taken volume be replaced with relatively acidic feed stream with the aim to maintain a desired pH value in the bioreactor.

The second fluid circulation system is adapted to be connected to the first fluid circulation such that the circulating fluid flows from the absorption module to the second bioreactor, as indicated in figure 1.

In the case that a plurality of parallel bioreactors are used for the
5 algae culture, the apparatus further comprises additional fluid circulation systems connected parallel to the second fluid circulation system. Each additional fluid circulation system comprises an additional bioreactor (5) similar to the second bioreactor (4) which additional bioreactor (5) accordingly comprises an additional filter (51). The additional fluid
10 circulation system preferably comprises a valve upstream of the additional bioreactor such that the additional fluid circulation system can flexibly be used depending on the demand of capacity.

In addition, the system may comprise an algae recovery unit (6) that is connected upstream to the output of the bioreactors to recover the
15 produced algae from the bioreactors. As such, the optional additional filter (51), if present, can be circumvented such that the algae are allowed to exit the bioreactors and enter the algae recovery unit (6). The algae recovery unit (6) typically comprises a filtration system to separate the algae from the culture medium and/or the circulation fluid.

20 In a preferred embodiment of the apparatus, the apparatus further comprises a catalyst module (3) adapted for oxidizing NO_x. The catalyst module is generally connected to the absorption module via a gas transport system such as a tube.

Claims

1. Method for producing algae-containing biomass with CO₂, said method comprising:

- 5 a) contacting at least part of an acidic gaseous stream comprising CO₂ with an aqueous absorption liquid comprising carbonic anhydrase in an absorption module to produce a lean gaseous stream that has a lower CO₂ concentration than the acidic gaseous stream and a liquid feed stream that comprises bicarbonate;
- 10 b) contacting part of the liquid feed stream and/or part of the acidic gaseous stream comprising CO₂ with microorganisms to generate carbonic anhydrase and to produce an aqueous stream comprising carbonic anhydrase that is fed to the absorption module;
- c) contacting at least part of the liquid feed stream with an algae culture to produce algae-containing biomass

wherein step b) and c) are carried out in separate bioreactors.

15

2. Method according to claim 1, wherein said acidic gaseous stream further comprises NO_x and said method further comprises oxidation of NO_x, preferably oxidation with a catalyst, to form an oxidized acidic gaseous stream comprising NO₂ and CO₂ that is contacted with the absorption liquid
- 20 in step a); such that the liquid feed stream further comprises NO₂⁻ and NO₃⁻.

3. Method according to any of the previous claims, wherein the aqueous absorption liquid comprises chemical components required for algae growth, carbonic anhydrase, and preferably compounds that facilitate
- 25 absorption of bicarbonate into the absorption liquid.

4. Method according to any of the previous claims, wherein step b) is essentially free from producing algae-containing biomass, preferably wherein the microorganisms in step b) are essentially free from algae.
- 5 5. Method according to any of the previous claims, wherein the pH of the absorption liquid is controlled by the addition of a basic liquid stream that is additionally produced in step c), wherein said basic liquid stream has a higher pH than the liquid feed stream.
- 10 6. Method according to the previous claim, wherein the pH of the algae culture has a pH that is controlled by the outflow of the basic liquid stream.
7. Method according to any of the previous claims, wherein the
15 microorganisms comprise cyanobacteria.
8. Method according to any of the previous claims, wherein step c) is carried out in a plurality of parallel bioreactors comprising algae.
- 20 9. Method according to any of the previous claims, wherein additional water and nutrients are added to the liquid feed stream before it is contacted with the algae.
10. Method according to any of the previous claims, wherein the
25 acidic gaseous stream comprises flue gas originating from a fossil-fuel electrical power plant or a biogas production plant, preferably flue gas comprising between 4-25 wt% CO₂ and 70-95 wt% nitrogen or flue gas comprising between 20-50 wt% CO₂ and 45-75 wt% methane.

11. Apparatus for sequestration of CO₂ by using algae, preferably by using a method according to any of the previous claims, said apparatus comprising:

- a first fluid circulation system comprising
- 5 - an absorption module comprising a gas inlet and;
- downstream of the absorption module a first bioreactor adapted to produce carbonic anhydrase comprising a filter adapted to retain microorganisms in the first bioreactor;
- a second fluid circulation system, that is connected to the first
- 10 circulation system and extends from the absorption modules or downstream of the absorption module and upstream of the first bioreactor to upstream of the absorption module or to the first circulation system downstream of the first bioreactor and upstream of the absorption module, said second fluid circulation system
- 15 comprising a second bioreactor that is adapted to culture algae, said second bioreactor comprises an outlet comprising a filter adapted to enable a liquid outflow while retaining the algae in the second bioreactor.

- 20 12. Apparatus according to claim 11, further comprising an oxidation module comprises a catalyst module adapted for oxidizing NO_x connected to the gas inlet of the absorption module via a gas transport system such as a tube.

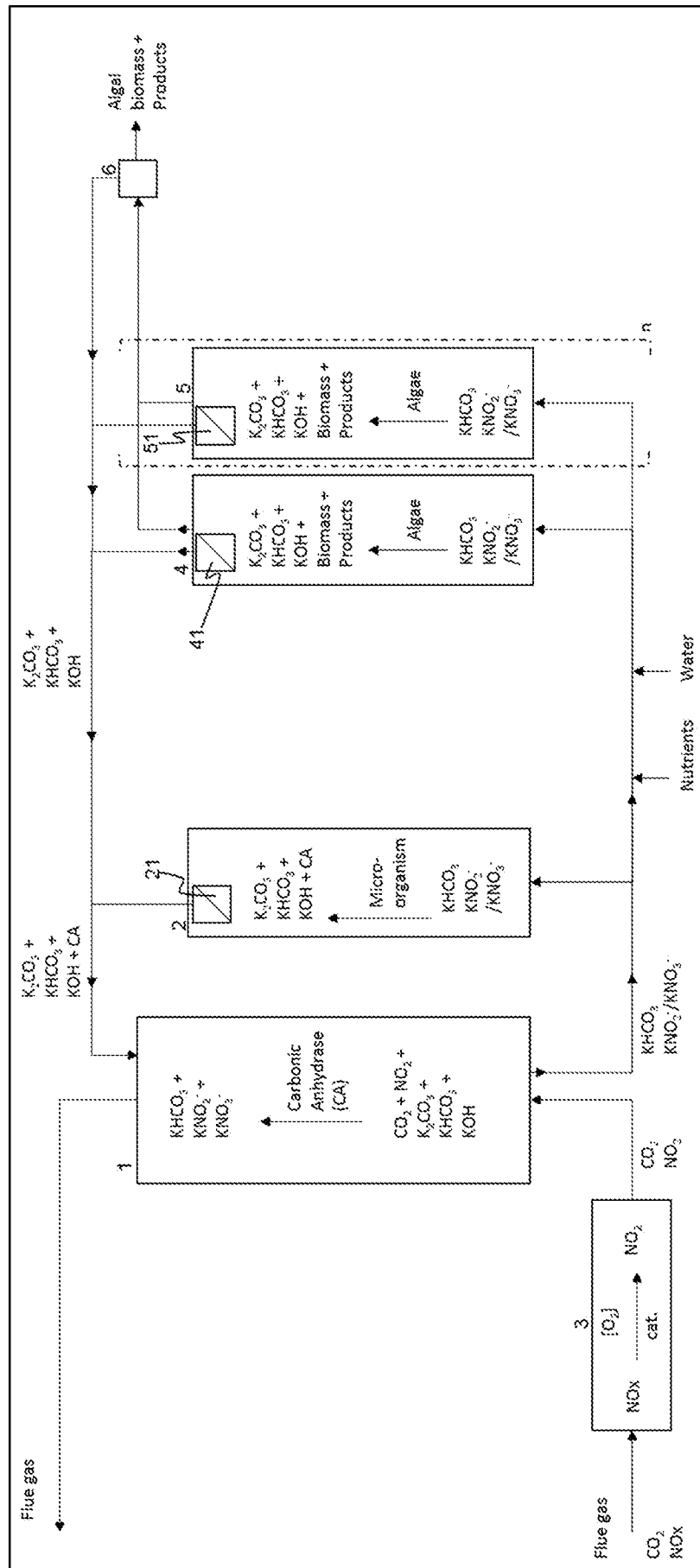


Fig. 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2017/050537

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12P3/00 B01D53/75 B01D53/78 B01D53/84 C12M1/00
C12M1/40 C12N1/12 C12P7/64

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 B01D C12M C12N

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2013/022348 A1 (TNO [NL]; GOETHEER EARL LAWRENCE VINCENT [NL]; GEERDINK PETER [NL]; NG) 14 February 2013 (2013-02-14) cited in the application the whole document	1-12
X	WO 2013/106932 A1 (CO2 SOLUTIONS INC [CA]) 25 July 2013 (2013-07-25) page 27, line 14 - page 28, line 23; claims; figures page 29, line 19 - page 30, line 8 page 12, lines 6-20	1-12
X	EP 2 556 881 A1 (TNO [NL]) 13 February 2013 (2013-02-13) paragraph [0016]; claims	1,2,7-12
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☒ 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

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"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

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26/10/2017

Name and mailing address of the ISA/

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Boeker, Ruth

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2017/050537

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KIRA SCHIPPER ET AL: "New Methodologies for the Integration of Power Plants with Algae Ponds", ENERGY PROCEDIA, vol. 37, 2013, pages 6687-6695, XP055416502, NL ISSN: 1876-6102, DOI: 10.1016/j.egypro.2013.06.601 the whole document -----	1-12

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/NL2017/050537

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