CERAMIC MEMBRANE CONTACTOR SYSTEM FOR INSOLUBLE GAS DISSOLUTION

Field of Invention

The current invention relates to methods of biogas upgrading and systems suitable for biogas upgrading.

Background

The listing or discussion of a prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

Anaerobic digestion (AD) of organic matter is an important biochemical process which generates energy (biogas) through the digestion of solids. The produced biogas comprised approximately 60% methane (CH_4), 40% carbon dioxide (CO_2) with minor concentrations of hydrogen (H_2), ammonia (NH_3), hydrogen sulfide (H_2S) and others. While it is becoming a valuable source of renewable energy, its utilization is restricted by the low calorific energy (20–25 MJ/m^3) from the high CO_2 content (Fu et al., *Trends Biotechnol.*, **2021**, *39*, 336). Biogas upgrading – the process where transformed into biomethane with the same characteristics as natural gas (>95% methane content) can be done via physical, chemical, or biological means, or a combination of these approaches. It is necessary to expand the use of biogas upgrading as it can be injected into natural gas grid.

Physico-chemical biogas upgrading approaches such as pressure swing adsorption, water scrubbing, chemical absorption, and membrane separation are some of the longest-standing methods used to remove impurities and carbon dioxide from biogas to improve the content of methane. However, it is plagued with high investment and operational costs related to energy inputs and in some cases toxic solvents. In addition, the carbon dioxide removed from the physical upgrading processes is wasted and cannot be utilized as a carbon source.

In response to urging concerns to reduce greenhouse gas emissions, biological method of biogas upgrading can be used. In recent years, biological biogas upgrading using microbes has emerged as a promising alternative to physico-chemical techniques due to their simple configuration and environmentally sustainable nature. This is achieved through the direct injection of H_2 into AD for in-situ biogas upgrading, which enables production with methane content of 90% and higher. Hydrogenotrophic methanogenesis is the dominant pathway given

in Eq. (1), where CO_2 is directly reduced into CH_4 by methanogens using H_2 as an electron donor (Fu et al., *Trends Biotechnol.*, **2021**, *39*, 336), thus reducing the CO_2 emission while increasing CH_4 production during anaerobic digestion process. This can be performed at moderate conditions (room temperature and atmospheric pressure) resulting in desirable advantages such as reduced carbon and energy footprints.

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \Delta G^0 = -130.7 kJ/mol \quad \text{Eq. (1)}$$

However, exogeneous H_2 injection provides external stress to the reactor due to the complex build-up of the communities in the AD. Excessive hydrogen supply in dissolution leads to excess partial pressure of hydrogen. High H_2 partial pressure is known to inhibit microbial activity and suppress anaerobic digestion, as the oxidation of volatile fatty acids (VFA) is endergonic under standard conditions and is thermodynamically feasible only when the H_2 partial pressure is kept low (Fu et al., *Trends Biotechnol.*, **2021**, *39*, 336; Mulat et al., *Waste Manag.*, **2017**, *68*, 146). This could lead to lower biogas yield, VFA accumulation, or more severely a whole process breakdown (Wahid et al., *Biotechnology and Biofuels*, **2019**, *12*, 104). At the same time, an insufficient H_2 supply leads to incomplete conversion of CO_2 into CH_4 and energy wastage.

Another key limiting factor, however, is the dissolution of hydrogen in the sludge media. Hydrogen as most non-polar gases is known to have very little solubility in water. The low solubility of H_2 in water (1.6 mg/L at 20 °C and 101 kPa) is a significant limitation in biological biogas upgrading (Fu et al., *Trends Biotechnol.*, **2021**, *39*, 336), as only dissolved H_2 can be utilized by the methanogens. Jensen et al. (*Appl. Energy*, **2018**, *222*, 840) testing a direct injection of H_2 using a venturi-based injection system achieved only 10% – 26% H_2 conversion. Hence, the mass transfer of hydrogen into the liquid is a key limiting factor for hydrogen conversion in biogas upgrading.

As H_2 has a very low diffusion efficiency in water, improving H_2 dissolution in the liquid is the most critical rate limiting factor for biogas upgrading, yet supplying too much hydrogen will lead to gas wastage (cost) and high hydrogen partial pressure in the liquid phase which inhibits the microbe's activity. It is important to ensure that hydrogen gas can not only be efficiently dissolved in the liquid phase, the hydrogen supplied can also be immediately utilized by the microbes without accumulation. This can be achieved from a high efficiency dissolution set-up which optimize the contact between the gaseous and aqueous phase to ensure full dissolution of the gas.

It is apparent that there is also a need to reduce the size of the gas bubbles to improve the mass transfer efficiency. As a high hydrogen partial pressure also inhibit microbial activity, it is critical to supply a specific amount of hydrogen to the system with improved dissolution such that it can be fully utilized by the microbes without accumulation in the digestor.

An example of such a dissolution concept is the bubbleless diffusion system. These are largely performed for aeration studies and uses polymeric hollow fiber membranes directly submerged within the reactor system. Bubbleless diffusion of gas can achieve almost 100% gas dissolution efficiency. Despite this advantage, it is less used industrially for normal aeration due to the higher pressure requirement to push the gas through the membrane modules compared to conventional diffusers or aerators. However, due to the low solubility of H_2, this concept of a pressurized dissolution system could be the technically advantaged solution for H_2 dissolution in biogas upgrading.

While many studies investigated methods to improve H_2 gas–liquid transfer rate, membrane-based technology has demonstrated some of the highest gas dissolution efficiencies, with Luo and Angelidaki (*Appl. Microbiol. Biotechnol.*, **2013**, *97*, 3739) achieving as high as 96% CH_4 yield using submerged hollow fiber membranes (HFM). This is similar to the concept of the Membrane Aerated Biofilm Reactor (MABR). While polymeric membranes have been studied, it is noteworthy that, to date, limited amount of study has explored the use of ceramic membranes for biogas upgrading. Alfaro et al. (*Bioresour. Technol.*, **2018**, *258*, 142) used a submerged ceramic membrane module with gas recirculation for thermophilic ex-situ biogas upgrading to achieve a yield of 88% CH_4. Deschamps et al., (*Bioresour. Technol.*, **2021**, *337*, 125444; *Membranes*, **2022**, *12*, 1220) investigated a hydrophobic-coated ceramic membrane as an external module connected to a "bubble column" anaerobic membrane bioreactor (AnMBR), acting as a microbubble sparger achieving up to 97.9 % CH_4 yield. However, their technology has restrictions on the type of applicable ADs.

Ceramic membranes are another possible membrane option due to its much longer lifespan (>20 years) and resistance to tough conditions, making the hydrogen dissolution using membrane system a cost-effective technology for long term operations. The limited number of studies for ceramic membrane-based gas dissolution could be attributed to perceived limitations in the surface area available compared to polymeric membranes, as well as greater pressure requirements than diffuser systems. However, the numerous advantages of ceramic membrane system, including an extended membrane lifespan and higher chemical stability, make the hydrogen dissolution using ceramic membrane system an attractive option for AD

that typically operate under more hazardous conditions since it could be a cost-effective technology for long term operations. Furthermore, ceramic membranes can be operated at high temperatures up to 90 °C and can therefore be useful for both mesophilic and thermophilic AD applications, compared to polymeric membranes that have a typical working temperature below 50 °C (Mashhadikhan et al., *Renew. Sustain. Energy Rev.*, **2024**, *189*, 113902).

It is apparent that successful utilisation of a ceramic membrane-based system for gas dissolution in a biogas upgrading setup remains a highly desirable yet unachieved target in the development of biogas upgrading.

Summary of Invention

The current invention relates to an external ceramic membrane contactor module for H_2 gas to liquid transfer designed for in-situ biogas upgrading. It has been surprisingly found that by having the configuration worked as an externally connected module and gas-liquid contact achieved at the membrane's inner surface through sludge recirculation, the limitations of low specific membrane area and ensure the ease of maintenance were successfully circumvented.

Commercially available ceramic membrane that is hydrophilic in nature was also tested and compared. This minimized the fouling risks associated with hydrophobic membranes, and also eliminated the need of an extra coating which constitutes additional cost and complexities in industrial applications. Additionally, the current invention evaluated the efficiency of the designed membrane contactor through examining the changes in the biogas quality, VFA composition, and shift in microbial community structure. A comparative analysis with diffuser-type gas sparging systems was also performed to evaluate the feasibility of current ceramic membrane over conventional H_2 injection methods.

The current invention demonstrated the successful utilization of an external ceramic membrane contactor module for in-situ H_2-assisted biogas upgrading. The membrane system facilitates dissolution and diffusion of H_2 with minimal bubble formation and can be externally connected to typical AD. The resulting biogas had a CH_4 content at an average 98.8 % and H_2 below 1.0 %, with a significant increase of CH_4 production rate by 108 %. Propionic acid accumulation towards the end of the operation did not impact the upgrading efficiencies of the system. The prevalence of hydrogenotrophic methanogens contributed to the efficient uptake and conversion of H_2.

The current invention presents an innovative system for applying bubbleless gas diffusion via ceramic membranes with the following unique features.

1. Ceramic membrane module is used to improve the lifespan of the system due to the high durability of ceramic membrane. The ceramic membrane module is much easier to maintain and can withstand a variety of conditions.
2. Utilizing an externally connected ceramic membrane module for outward-in gas diffusion with constant recirculation of sludge through the internal diameter of the ceramic membrane module. This improves mixing within the reactor, gas uptake and utilization, and ease of future scale up using the ceramic membrane module.
3. Compared to normal membrane gas diffusion or aeration device, this system allows for enhanced recirculation of the sludge while (1) reduce the likelihood of membrane fouling due to the pressure difference between the external gas phase and the internal liquid phase of the membrane, and (2) improved sludge solubilization due to enhanced mixing leading to higher CH_4 production rate.

Aspects and embodiments of the invention will now be described by reference to the following numbered clauses.

1. A method of biogas upgrading, the method comprising the steps of:

(a) providing:

a system comprising:

a bioreactor comprising a liquid inlet and a liquid outlet; and

an external ceramic membrane module comprising a hollow ceramic membrane and a gas-tight housing surrounding the hollow ceramic membrane,

where the hollow ceramic membrane has an interior lumen, a first end fluidly connected to the liquid outlet of the bioreactor and a second end fluidly connected to the liquid inlet of the bioreactor, and

the gas-tight housing has a gaseous inlet suitable to provide a gas to an interior of the gas-tight housing; and

a wastewater mixture situated within the bioreactor comprising a wastewater to be treated and a microbial population comprising anaerobic bacteria; and

(b) operating the system under anaerobic conditions and recirculating the wastewater mixture from the bioreactor through the liquid outlet into the hollow ceramic membrane and back to the bioreactor via the liquid inlet, wherein:

hydrogen gas is provided through the gaseous inlet of the gas-tight housing at a suitable pressure to enable the hydrogen gas to transit into the interior lumen of the hollow ceramic membrane and be substantially dissolved in the wastewater mixture; and

the hydrogen gas is reacted by the anaerobic bacteria with CO_2 generated by microbial action on the wastewater to generate a biogas comprising methane.

2. The method according to Clause 1, wherein the first and second ends of the hollow ceramic membrane are treated to prevent fluid ingress or egress from an interior surface of the hollow ceramic membrane to an exterior surface of the hollow ceramic membrane and *vice versa*, optionally wherein the first and second ends of the hollow ceramic membrane are glass sealed.

3. The method according to Clause 1 or Clause 2, where substantially no hydrogen gas bubbles are produced.

4. The method according to any one of the preceding clauses, wherein the anaerobic bacteria include acetoclastic methanogens and, more particularly, hydrogenotrophic methanogens.

5. The method according to any one of the preceding clauses, wherein the hydrogen gas dissolution efficiency is greater than or equal to 99%, such as about 100%.

6. The method according to any one of the preceding clauses, wherein the hollow ceramic membrane has a pore size of from 70 to 800 nm.

7. The method according to Clause 6, wherein the hollow ceramic membrane has a pore size of from 100 to 600 nm.

8. The method according to any one of the preceding clauses, wherein the hollow ceramic membrane has an area of from 0.5 to 3 m^2/m^3 volume of the bioreactor.

9. The method according to Clause 8, wherein the hollow ceramic membrane has an area of from 1 to 2 m^2/m^3 volume of the bioreactor.

10. The method according to any one of the preceding clauses, wherein the pressure of the hydrogen gas in the external ceramic membrane module is from 50 kPa to 800 kPa, such as from 250 kPa to 500 kPa, such as about 300 kPa.

11. The method according to any one of the preceding clauses, wherein the method employs a total suspended solids in the wastewater mixture of from 0 to 30 g/L, such as from 1 to 20 g/L, such as from 3 to 5 g/L.

12. The method according to any one of the preceding clauses, wherein the bioreactor further comprises a means or apparatus for agitation of the wastewater mixture therein, optionally wherein the means or apparatus is a mechanical stirrer, further optionally wherein the mechanical stirrer operates at a speed of from 100 to 500 rpm, such as from 125 to 200 rpm, such as about 150 rpm.

13. The method according to any one of the preceding clauses, wherein the wastewater mixture has a recirculation flow rate of from 0.03 to 0.3 of the total volume of the wastewater mixture/minute, such as from 0.15 to 0.17 of the total volume of the wastewater mixture/minute.

14. The method according to any one of the preceding clauses, wherein, when operating in a steady state, the biogas is from 97 to 99.5%, such as from 98 to 99% methane.

15. The method according to any one of the preceding clauses, wherein, the wastewater mixture is selected from one or more of the group consisting of an anaerobic sludge, a liquid stream comprising glucose, a pre-treated cardboard waste liquor, a pre-treated lignocellulosic liquor, a food wastewater/waste, a manure leachate/waste, a waste activated sludge, and a thermal hydrolysis sludge.

16. The method according to any one of the preceding clauses, wherein the external ceramic membrane module comprises a plurality of hollow ceramic membranes, such as from 2 to 20 hollow ceramic membranes.

17. A system suitable for biogas upgrading, the system comprising:

a bioreactor comprising a liquid inlet and a liquid outlet; and

an external ceramic membrane module comprising a hollow ceramic membrane and a gas-tight housing surrounding the hollow ceramic membrane,

where the ceramic membrane has an interior lumen, a first end fluidly connected to the liquid outlet of the bioreactor and a second end fluidly connected to the liquid inlet of the bioreactor, and

the gas-tight housing has a gaseous inlet suitable to provide a gas to an interior of the gas-tight housing.

18. The system according to Clause 17, wherein the first and second ends of the hollow ceramic membrane are treated to prevent fluid ingress or egress from an interior surface of the hollow ceramic membrane to an exterior surface of the hollow ceramic membrane and *vice versa*, optionally wherein the first and second ends of the hollow ceramic membrane are glass sealed.

19. The system according to Clause 17 or Clause 18, wherein the hollow ceramic membrane has a pore size of from 70 to 800 nm.

20. The system according to Clause 19, wherein the hollow ceramic membrane has a pore size of from 100 to 600 nm.

21. The system according to any one of Clauses 17 to 20, wherein the hollow ceramic membrane has an area of from 0.5 to 3 m^2/m^3 volume of the bioreactor.

22. The system according to Clause 21, wherein the hollow ceramic membrane has an area of from 1 to 2 m^2/m^3 volume of the bioreactor.

23. The system according to any one of Clauses 17 to 22, wherein the system is configured to provide a gas to the external ceramic membrane module at a pressure of from 50 kPa to 800 kPa, such as from 250 kPa to 500 kPa, such as about 300 kPa.

24. The system according to any one of Clauses 17 to 23, wherein the bioreactor further comprises a means or apparatus for agitation, optionally wherein the means or apparatus is a mechanical stirrer.

25. The system according to any one of Clauses 17 to 24, wherein system further comprises a means or apparatus to effect recirculation of a wastewater mixture from the bioreactor through the hollow ceramic membrane and back into the bioreactor, optionally wherein the means or apparatus to effect recirculation is a pump.

26. The system according to any one of Clauses 17 to 25, wherein the external ceramic membrane module comprises a plurality of hollow ceramic membranes, such as from 2 to 20 hollow ceramic membranes.

Drawings

FIG. 1 depicts the schematic diagram of the diffuser based H_2 injection system.

FIG. 2 depicts the schematic of the ceramic membrane contactor set-up attached to a continuous stirred tank reactor (CSTR) for in-situ H_2-assisted biogas upgrading.

FIG. 3 depicts the schematic of the ceramic membrane contactor set-up when sludge saturated with dissolved H_2 is returned back to the reactor and used for biogas upgrading. The sludge re-enters the reactor at the headspace of the reactor near the top of the sludge working volume. Dissolution of H_2 is irrespective of the type of reactor configuration, as it occurs in the membrane contactor beforehand.

FIG. 4 depicts the schematic of the ceramic membrane contactor set-up with sludge recirculation. The dissolution of H_2 does not occur from bubbling along the height of the reactor, but through the ceramic membrane set-up. If the H_2 is not dissolved effectively for microbe consumption, it will escape directly into the headspace instead of being utilised as there is no gas recirculation present.

FIG. 5 depicts the relationship between membrane pore size and estimated gas transfer rate. The solid line represents the average gas transfer at different pore size, the dotted line represents the estimated maximum value, and the dash-dotted line represents the minimum value.

FIG. 6 depicts the (a) gas composition profiles, and (b) CH_4 production rate over 170 days of in-situ H_2-assisted biogas upgrading with diffuser (I) and membrane (II and III).

FIG. 7 depicts the total VFA profiles and individual VFA profiles for acetic acid and propionic acid over 170 days of in-situ H2-assisted biogas upgrading with diffuser (I) and membrane (II and III).

FIG. 8 depicts the percent abundance of the main phylum in samples collected on day 1, 41, 51, 161, and 170.

FIG. 9 depicts the percent abundance of the main genus of (a) bacteria and (b) archaea in samples collected on collected on day 1, 41, 51, 161, and 170.

FIG. 10 depicts the substrate composition of (a) pretreated cardboard waste; and (b) pretreated poplar wood waste.

FIG. 11 provides a summary of the advantage of the current invention compared to existing methods, along with its potential applications.

Description

The current invention provides a method of biogas upgrading, where it has been surprisingly found that an external ceramic membrane can be used as a means for introducing hydrogen gas into a bioreactor system in a manner that enables almost complete dissolution and utilisation of the hydrogen gas within the reactor. Thus, in a first aspect of the invention, there is provided a method of biogas upgrading, the method comprising the steps of:

(a) providing:

a system comprising:

a bioreactor comprising a liquid inlet and a liquid outlet; and

an external ceramic membrane module comprising a hollow ceramic membrane and a gas-tight housing surrounding the hollow ceramic membrane,

where the hollow ceramic membrane has an interior lumen, a first end fluidly connected to the liquid outlet of the bioreactor and a second end fluidly connected to the liquid inlet of the bioreactor, and

the gas-tight housing has a gaseous inlet suitable to provide a gas to an interior of the gas-tight housing; and

a wastewater mixture situated within the bioreactor comprising a wastewater to be treated and a microbial population comprising anaerobic bacteria; and

(b) operating the system under anaerobic conditions and recirculating the wastewater mixture from the bioreactor through the liquid outlet into the hollow ceramic membrane and back to the bioreactor via the liquid inlet, wherein:

hydrogen gas is provided through the gaseous inlet of the gas-tight housing at a suitable pressure to enable the hydrogen gas to transit into the interior lumen of the hollow ceramic membrane and be substantially dissolved in the wastewater mixture; and

the hydrogen gas is reacted by the anaerobic bacteria with CO_2 generated by microbial action on the wastewater to generate a biogas comprising methane.

The word "comprising" refers herein may be interpreted as requiring the features mentioned, but not limiting the presence of other features. Alternatively, the word "comprising" may also relate to the situation where only the components/features listed are intended to be present (e.g. the word "comprising" may be replaced by the phrases "consists of" or "consists essentially of"). It is explicitly contemplated that both the broader and narrower interpretations can be applied to all aspects and embodiments of the present invention. In other words, the word "comprising" and synonyms thereof may be replaced by the phrase "consisting of" or the phrase "consists essentially of" or synonyms thereof and *vice versa*.

The phrase, "consists essentially of" and its pseudonyms may be interpreted herein to refer to a material where minor impurities may be present. For example, the material may be greater than or equal to 90% pure, such as greater than 95% pure, such as greater than 97% pure, such as greater than 99% pure, such as greater than 99.9% pure, such as greater than 99.99% pure, such as greater than 99.999% pure, such as 100% pure.

As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a composition" includes mixtures of two or more such compositions, reference to "an oxygen carrier" includes mixtures of two or more such oxygen carriers, reference to "the catalyst" includes mixtures of two or more such catalysts, and the like.

The term "bioreactor" as used herein is intended to take its normal meaning in the art. As the method and systems disclosed herein are intended to make use of an anaerobic atmosphere, the bioreactor may be a vessel capable of being sealed against the ambient environment so that an anaerobic atmosphere may be encouraged within it. As noted, the vessel may have at least an inlet and an outlet so that a wastewater may be circulated back and forth to the external ceramic membrane module, where hydrogen gas may be dissolved and hence be fed into the main bioreactor vessel. The bioreactor may also contain suitable means or apparatus to siphon off produced gas from the system (e.g. biogas or methane, or a mixture of methane and other gases that may accumulate, as described in more detail in the examples section hereinbelow). Preferably, the gas collected may be substantially pure methane. The term "substantially pure" when used herein may refer to a material that is greater than or equal to 95% pure, such as greater than 97% pure, such as greater than 99% pure, such as greater than 99.9% pure, such as greater than 99.99% pure, such as greater than 99.999% pure, such as 100% pure.

The external ceramic membrane module includes a hollow ceramic membrane and a gas-tight housing surrounding the hollow ceramic membrane. The purpose of this arrangement is to enable the uptake of hydrogen gas into a wastewater in a manner that enables its almost complete dissolution and use by the anaerobic bacterial species within the wastewater mixture. It will be appreciated that the gas-tight housing has a gaseous inlet suitable to provide a gas to an interior of the gas-tight housing so as to effect the transfer of hydrogen gas into the wastewater mixture through the ceramic membrane. This may be accomplished by providing hydrogen gas through the gaseous inlet of the gas-tight housing at a suitable pressure to enable the hydrogen gas to transit into the interior lumen of the hollow ceramic membrane and be substantially dissolved in the wastewater mixture. The operating parameters of the recirculation speed of the wastewater mixture and pressure of the hydrogen gas may be optimised for any particular arrangement/volume of the system by a skilled person, so as to ensure the maximal diffusion and dissolution of the hydrogen gas into the wastewater mixture.

The hollow ceramic membrane has an interior lumen, a first end fluidly connected to the liquid outlet of the bioreactor and a second end fluidly connected to the liquid inlet of the bioreactor. As will be appreciated, the first and second ends of the hollow ceramic membrane should be arranged to allow for the flow of the wastewater mixture through the interior lumen. This may be arranged using any suitable connection methods that enables a suitable fluid connection to be created between the first and second ends of the hollow ceramic membrane and the liquid inlet and outlet, respectively, of the bioreactor. This may be achieved through the use of suitable fluid connection apparatus, such as tubing line(s) and the like. It will be appreciated that a pump may be connected at some point to the fluid connection so as to enable the recirculation of the wastewater from the bioreactor to the hollow ceramic membrane and back again. While the first and second ends of the hollow ceramic membrane may be made of the same material as the rest of the membrane, as these may still be covered by tubing so as to create a gas-tight seal, preferred arrangements may be ones in which the first and second ends of the hollow ceramic membrane are treated to prevent fluid ingress or egress from an interior surface of the hollow ceramic membrane to an exterior surface of the hollow ceramic membrane and *vice versa*. For example, the first and second ends of the hollow ceramic membrane may be glass sealed. That is, the ends are coated in glass so as to prevent egress of any fluid (particularly gas) through these end sections of the hollow ceramic membrane.

As an example, a suitable membrane with glass end seals may be provided by Inopor™. Such membranes may end-sealed using a silica glass.

It will be appreciated that the first and second ends may be positioned within the gas-tight housing of the external ceramic membrane module or external thereto. The latter may be more convenient, as it enables an easy fluid connection to be established between the bioreactor and the external ceramic membrane module (with appropriate seals/gaskets to ensure a gas-tight fit at the point of egress of the first and second ends from the gas-tight housing of the external ceramic membrane module). However, the fluid connection may be made internally within the external ceramic membrane housing, in which case there may be seals or gaskets etc. used to ensure that a gas-tight environment is maintained within the gas-tight housing of the external ceramic membrane module.

In particular embodiments of the invention that may be mentioned herein, the method may be one in which substantially no hydrogen gas bubbles are produced. That is, substantially no hydrogen gas bubbles may be produced within the wastewater mixture, meaning that substantially all of the hydrogen gas is instead dissolved and consumed within the wastewater mixture. As discussed hereinbelow, this is a particular advantage of the current method and associated system, which enables the superior biogas/methane generation disclosed herein. For example, the hydrogen gas dissolution efficiency may be greater than or equal to 99%, such as about greater than or equal to 99.5%, such as about less than or equal to 100% (e.g. from 99% to less than about 100% (e.g. 99.95%)). Without wishing to be bound by theory, it is believed that such as high dissolution efficiency enables a number of advantages associated with the currently claimed method and system.

It will be appreciated that reference to the dissolution of the hydrogen gas in the wastewater mixture may refer to the dissolution of the hydrogen gas in a liquid phase of the wastewater mixture.

The hollow ceramic membrane is porous, such that it allows for the ingress of a gas into the interior lumen, while preventing the egress of the wastewater mixture. This is in part due to the pressure difference between the gas and the wastewater mixture, but also due to the selection of a suitable pore size to enable this to occur. While any suitable pore size may be used, in certain embodiments mentioned herein, the hollow ceramic membrane may have a pore size of from 70 to 800 nm, such as from 100 to 600 nm.

The hollow ceramic membrane may be formed from one or more hollow ceramic membranes, and the hollow ceramic membrane(s) may have any suitable (internal/interior) surface area relative to the volume of the reactor. For example, in certain embodiments of the invention, the hollow ceramic membrane may have an area of from 0.5 to 3 m^2/m^3 volume of the

bioreactor, such as from 1 to 2 m^2/m^3 volume of the bioreactor. The surface area of the membrane may be calculated as being based on the interior surface area of the membrane.

As noted above, the pressure of the hydrogen gas may be selected to ensure that the gas enters the interior lumen of the external ceramic membrane, while egress of the wastewater mixture is substantially zero. Any suitable pressure for the hydrogen gas may be used herein according to the selection of the skilled person. However, in certain embodiments of the invention that may be mentioned herein, the pressure of the hydrogen gas in the external ceramic membrane module may be from 50 kPa to 800 kPa, such as from 250 kPa to 500 kPa, such as about 300 kPa.

The wastewater mixture may have any suitable total suspended solids value. In certain particular embodiments of the invention that may be mentioned herein, the method may employ a total suspended solids in the wastewater mixture of from 0 to 30 g/L, such as from 1 to 20 g/L, such as from 3 to 5 g/L.

In certain embodiments of the invention, it may be preferred to provide some mixing to the contents of the bioreactor. Thus, in certain embodiments of the invention, the bioreactor may further comprise a means or apparatus for agitation of the wastewater mixture therein. For example, the means or apparatus for agitation may be a mechanical stirrer. When a mechanical stirrer is used, it may be operated at any suitable revolution per minute (rpm). For example, the mechanical stirrer may operate at a speed of from 100 to 500 rpm, such as from 125 to 200 rpm, such as about 150 rpm.

The wastewater mixture may have a recirculation flow rate that is selected to provide a pressure differential with the hydrogen gas that enables the ingress of the hydrogen gas into the interior lumen of the external ceramic membrane, while preventing egress of the wastewater mixture. Any suitable flow rate may be selected, for example, the wastewater mixture may have a recirculation flow rate of from 0.03 to 0.3 (e.g. from 0.1 to 0.2) of the total volume of the wastewater mixture/minute, such as from 0.15 to 0.17 of the total volume of the wastewater mixture/minute. It will be appreciated that the flow rates mentioned here may be suitable for use in combination with the pressures mentioned above for the hydrogen gas.

The method disclosed herein may be able to provide a biogas that is substantially methane when the method has reached a steady state. More particularly, the method disclosed herein, when operating in a steady state, may provide a biogas that is from 97 to 99.5%, such as from 98 to 99% methane. When used herein, the term “steady state” means when the system has

been operating for a sufficient amount of time under optimal conditions. An example of such conditions is discussed in the examples section below. It will be appreciated that the optimal conditions may differ for different set-ups and constituent components and so a degree of variability in the optimal conditions will apply. For example, the exact conditions to achieve maximal methane production from a wastewater sludge may differ from those to achieve a similar result from a food wastewater or food waste/slurry. Similarly, if the reactor set-up is different, for example the external membrane module contains one or three external ceramic membranes, then the optimal conditions may differ and be adjusted accordingly by the skilled person.

As noted above, the method makes use of a wastewater mixture that includes a microbial population comprising anaerobic bacteria. In other words, the wastewater mixture may also be referred to as a waste and microbial mixture or a mixed liquor. While any anaerobic bacteria may be used herein, it is particularly preferred that the anaerobic bacteria include acetoclastic methanogens and, more particularly, hydrogenotrophic methanogens. The microbial population may be any suitable anaerobic bacterial population obtained from a wastewater itself or from a suitable seed population (e.g. a sludge from an anaerobic wastewater treatment plant). The method may make use of an initiation stage, where the system is subjected to conditions that apply a selection pressure onto the microbial population to increase the relative amount of hydrogenotrophic methanogens in the microbial population. The method may make use of any suitable wastewater or waste. For example, the wastewater mixture or source to be treated may be selected from one or more of the group consisting of an anaerobic sludge, a liquid stream comprising glucose, a pre-treated cardboard waste liquor, a pre-treated lignocellulosic liquor, a food wastewater/waste, a manure leachate/waste, a waste activated sludge, and a thermal hydrolysis sludge.

As intimated above, while the method has been described by reference to a system that makes use of a single external ceramic membrane, the external ceramic membrane module may comprise a plurality of hollow ceramic membranes. For example, the external ceramic membrane module may comprise from 2 to 20 hollow ceramic membranes. It will be appreciated that the total surface area of the plurality of hollow ceramic membranes to the volume of the bioreactor may still fall within the ratios mentioned hereinbefore. That is, the hollow ceramic membranes may have a total area of from 0.5 to 3 m^2/m^3 volume of the bioreactor, such as from 1 to 2 m^2/m^3 volume of the bioreactor.

In a second aspect of the invention, there is also provided a system suitable for biogas upgrading, the system comprising:

a bioreactor comprising a liquid inlet and a liquid outlet; and

an external ceramic membrane module comprising a hollow ceramic membrane and a gas-tight housing surrounding the hollow ceramic membrane,

where the ceramic membrane has an interior lumen, a first end fluidly connected to the liquid outlet of the bioreactor and a second end fluidly connected to the liquid inlet of the bioreactor, and

the gas-tight housing has a gaseous inlet suitable to provide a gas to an interior of the gas-tight housing.

As the system has been described in tandem with the method above, it is not described again here for brevity. However, it will be appreciated that the system is configured to accept a wastewater mixture and the system may be configured to provide a gas to the external ceramic membrane module. For example, the system may be configured to provide a gas to the external ceramic membrane module at a pressure of from 50 kPa to 800 kPa, such as from 250 kPa to 500 kPa, such as about 300 kPa (i.e. about 3 bar).

A schematic of an example system that may be used in the currently disclosed method is provided by Figure 2. As depicted in Figure 2, the system **100** includes a bioreactor **110** comprising a liquid inlet **111**, a liquid outlet **112** and a gaseous outlet **113**. The bioreactor **110** is fluidly connected to an external ceramic membrane module **120** that comprises a hollow ceramic membrane **125** and a gas-tight housing **130** surrounding the hollow ceramic membrane. The fluid connections are accomplished by a first set of tubing **121** from the liquid outlet **112** to a first end **126** of the hollow ceramic membrane **125**, *via* a pump **122**, and a second set of tubing **123** connecting the liquid inlet **111** to a second end **127** of the hollow ceramic membrane **125**. The gas-tight housing **130** incorporates a gaseous inlet **135** that enables hydrogen gas **136** to be pumped into the interior of the gas-tight housing **130** and hence through the exterior surface of the hollow ceramic membrane **125** into an interior lumen (not shown explicitly). As will be appreciated, the hydrogen gas may be provided by any suitable hydrogen gas source, such as a hydrogen gas vessel or a biological or electrochemical source of hydrogen that may be generated in situ. The bioreactor **110** may also include a mechanical stirrer **114** to cause agitation to a wastewater within the bioreactor while it is in operation. In operation, a wastewater mixture **140** in the bioreactor **110** may be circulated from the bioreactor through the hollow ceramic membrane **125** and back again. This circulation allows hydrogen gas to pass into the wastewater for utilisation by the anaerobic bacteria communities (i.e. methanogenic bacteria (when used herein the term "methanogenic bacteria" may also refer to "methanogenic archaea") in the wastewater mixture

140, thereby enabling the generation of methane and the upgrading of the gas produced by the system.

It is noted that FIG. 2 depicts a reactor set-up where the wastewater/sludge returns to a top portion of the reactor vessel. It will be noted that the return flow can be at any position, including the bottom of the reactor vessel. However, the experimental set-up of FIG. 2 was chosen to demonstrate that the system enables rapid H_2 dissolution and consumption, even when sludge is reintroduced from the top. It is believed that this shows the connection point in the current set-up has less impact compared to the membrane contactor on biomethane conversion efficiency. Nevertheless, it is noted that should the sludge return point be located at a bottom portion of the reactor vessel, then the contact time of the hydrogen gas with the microbes will be longer, given the water pressure in the reactor vessel.

As noted herein, the current invention relates to a system suitable for biogas upgrading and methods for in-situ biogas upgrading. Further aspects and embodiments of the invention are described in the following numbered statements.

1. A system comprising:
    a) a bioreactor configured to house anaerobic sludge;
    b) a first channel connecting the bioreactor to an external ceramic membrane module;
    c) a gas inlet to the ceramic membrane module;
    d) a second channel connecting the external ceramic membrane module to the bioreactor.

2. A method comprising:
    e) providing a mixture of anaerobic sludge in the above-mentioned bioreactor;
    f) pumping the mixture through the first channel to the external ceramic membrane module;
    g) pumping hydrogen into the external ceramic membrane module through the gas inlet to obtain a hydrogen-rich mixture;
    h) pumping the hydrogen-rich mixture through the second channel to the bioreactor;

wherein the anaerobic microbes in the anaerobic sludge use hydrogen to convert the CO_2 originally present in the biogas to CH_4 to produce biomethane.

In the above system and method, hydrogen gas is substantially (> 99%) dissolved in the anaerobic sludge mixture. Minimal hydrogen gas bubbles are produced with this system.

The current invention may be applied in processes that include, but are not limited to, the following:

(i) existing or new anaerobic digestion systems with in-situ biogas production and biogas upgrading; and

(ii) industrial processes which require dissolution of inert and low solubility gases in the liquid phase.

Some advantages of the ceramic membrane configuration for H_2 delivery over existing systems may include on or more of the following.

(1) Use of ceramic membrane over conventional polymeric membranes or diffusers for higher chemical stability and longer membrane lifespan (>20 years) which allows applications at wider range of conditions, making it a more cost-effective solution for long term operation. As anaerobic digestion systems generally deal with systems of high total suspended solid (TSS) (>5g/L), the greater TSS tolerance of ceramic membranes also enables the use of more aggressive chemical and hydraulic cleaning methods which reduces the risk of irreversible fouling is less compared with polymeric membranes.

(2) Uniform mixing in the AD has always been a challenge. In this specific configuration of the membrane contactor module, the gas phase is introduced outside of the membrane, while the sludge was introduced at the inside of the membrane, and the gas-liquid contacting was taking place at the membrane inner surface without bubble creation. Sludge circulation via the inner diameter of the ceramic membrane aims to improve the mixing within the reactor and while enhancing the liquid surface area in contact with the gas.

(3) Compared to conventional type of diffusers or submerged hollow fiber systems (e.g. MABR) which injects H_2 in the gaseous form into the reactor, this system can deliver dissolved H_2 into the sludge media for immediate uptake by the methanogens, hence further enhancing the H_2 conversion rate.

(4) Lastly, instead of having the membrane fully submerged within the reactor system which poses difficulty in retrofitting and maintenance of existing AD, a separate membrane module external of the reactor is used.

Further aspects and embodiments of the invention will now be described by reference to the following non-limiting examples.

Examples

Materials

Chemicals used in the following examples were purchased from Sigma Aldrich. The sludge used herein were locally-maintained anaerobic sludge sourced from Advanced Environmental Biotechnology Centre (AEBC) lab in Singapore. IKA bioreactors and Inopor ceramic membrane were used in the disclosed Examples below.

Analytical Methods

Total suspended solid (TSS) and volatile suspended solid (VSS) were analysed based on standard method 2540B and 2540E respectively (APHA, 2012, *Standard methods for the examination of water and wastewater*, 22nd edn. Washington DC). Samples were centrifuged at 8500 rpm for 10 mins and the supernatant was filtered through a 0.22 µm nylon syringe filter for further analysis of soluble fraction of sludge samples. Total organic carbon (TOC) and total inorganic carbon (TIC) in the soluble fraction was analysed using Shimadzu (Japan) TOC 5000A analyser at a furnace temperature of 680 °C. Soluble chemical oxygen demand (sCOD) was measured with high range chemical oxygen demand (COD) digestion vials (HACH, United States) in accordance with the standard method 5220D (USEPA, 2018). VFA compositions were analysed weekly with Agilent 8890B Gas Chromatography (GC) equipped with flame ionization detection (FID) system. DB-FFAP column (30 m) was used with nitrogen as the carrier gas at 205 °C. 0.5 µL of sample was injected. The total run time was 16 mins and C_2 to C_7 VFA were quantified. Biogas composition was measured using Agilent 7890A GC equipped with dual thermal conductivity detectors (TCD), with columns MolSieve 13x (3 m), MolSieve 5 Å (3 m) and HaySep C (3 m). Helium and nitrogen were used as carrier gases for separation at 220 °C. Gas peaks of H_2, CO_2, CH_4, O_2, N_2 and CO were identified, and the percent gas composition was determined by plotting the peak area against the calibration curve of individual gases.

Example 1. Feedstock Preparation, Reactor Setup, and Ceramic Membrane Contactor Configuration

Feedstock Preparation and Reactor Setup

Inoculation was performed using laboratory-maintained sludge that was enriched with hydrogenotrophic methanogens at a total suspended solid (TSS) concentration of 5.4 g/L with a volatile suspended solid (VSS)/TSS ratio of 0.8. The experiment was conducted in a commercial fermenter (IKA Habitat, Germany) operated as a continuous stirred tank reactor (CSTR) with 4 L working volume. The overhead mixer was controlled at 150 rpm throughout

the entire fermentation period, and the temperature was kept at mesophilic condition of 37 °C. The synthetic wastewater used in the current invention contained glucose (50 g/L) as the sole carbon source, $(NH_4)_2SO_4$ (5.5 g/L), KH_2PO_4 (2.3 g/L), $MgCl_2$ (0.2 g/L), $CaCl_2$ (0.2 g/L), and trace mineral solution, as described in Xu et al. (*Water Res.*, **2024**, *250*, 121022). A constant Organic Loading Rate (OLR) of 0.2 gCOD/(L·d) was fed throughout the entire experiment. No discard of the biomass was performed through the experiment except during sampling to prevent the washout of the essential H_2 utilizing bacteria due to the low growth rate of the anaerobic communities.

External Ceramic Membrane Contactor

A unique ceramic membrane contactor configuration was employed for H_2 dissolution. FIG. 2 shows a schematic of the membrane contactor connected to the bioreactor. A single tubular ceramic membrane with glass end sealing (1 x 25 cm length, 7 mm inner diameter, 600 nm pore size) (inopor®, Germany) of total area 0.0049 m^2 was used. The selected membrane area is within the range of 1 – 2 m^2 per m^3 volume of the reactor. The vessels housing the ceramic membrane were constructed from stainless steel, ensuring air- and water-tight conditions within the module. The membrane module was externally connected to the reactor tank through inflow and outflow connections. H_2 gas was introduced outside of the membrane, while the sludge was recirculated through membrane. Gas-liquid contacting took place at the membrane inner surface. Masterflex 77200–50 peristaltic pump (Masterflex®, Germany) was used for recirculation flow of the sludge within the membrane module at 165 mL/(L·min). H_2 was supplied with HK Plus 100 hydrogen generator (VICI DBS, Italy) at pressure between 2.5–3.0 bar. The pH in the reactor was controlled below 8.0 through the addition of hydrochloric acid (HCl).

Diffuser with Biogas Recirculation

Diffuser systems remains the most common method used in wastewater treatment for gases injection, including aeration in aerobic treatment facilities and biogas upgrading in anaerobic systems (Voelklein et al., *Appl. Energy.*, **2019**, *235*, 1061). Before using the membrane contactor, diffuser sparging was first tested for the bioreactor to determine the baseline efficiency of conventional gas transfer methods. The set-up was developed based on typical gas introduction methodology used in various studies and commercial settings (Voelklein et al., *Appl. Energy.*, **2019**, *235*, 1061). An air stone diffuser was inserted at the bottom of the reactor, and H_2 gas from an externally connected gas bag was injected into the reactor using a Watson Marlow 120U peristaltic pump (Watson Marlow Fluid Technology, UK) at a continuous flow rate. The volume of H_2 injected into the system was measured by monitoring the gas bag's volume before and after a fixed duration of time. Biogas recirculation using a

Watson Marlow 120U pump was performed at a rate ranging from 30 – 50 mL/min to recycle the unreacted H_2 and CO_2 back into the liquid phase. The schematic diagram of the diffuser configuration is as depicted in FIG. 1.

Results and Discussions

FIG. 2 shows a schematic of the reactor and housing membrane contactor as well as a picture of the full reactor set-up. Vessels housing the ceramic membrane are made of stainless steel and designed to ensure air- and water-tight conditions within the module.

The gas dissolution process involves supplying gas from the permeate side of the membrane module at specified pressure to maintain the desired gas flow rate into the system at 100% gas dissolution efficiency. Dissolution of H_2 gas is irrespective of the type of reactor configuration as it occurred in the membrane contactor before the sludge entered the reactor (FIG. 3). The gas phase is introduced outside of the membrane, while the sludge was introduced at the inside of the membrane, and the gas-liquid contacting was taking place at the membrane inner surface without bubble creation. Upon saturated with dissolved H_2, the sludge was returned back to the reactor and used for biogas upgrading. The sludge re-enters the reactor at the headspace near the top of the sludge working volume (FIG. 3). The dissolution of H_2 does not occur from bubbling along the height of the reactor, but through the ceramic membrane set-up. If the H_2 is not dissolved effectively for microbe consumption, it will escape directly into the headspace instead of being utilised as there is no gas recirculation present (FIG. 4).

The membrane module is externally connected to the reactor tank via an inflow and outflow connection. The sludge is pumped through the internal diameter of the ceramic membrane module at a specified flow rate based on the required scale of the system. The liquid flow rate through the internal membrane module is at 660 ml/min for a 4L working volume in the reactor vessel. A constant H_2 pressure of 3 bar is maintained at the permeate injection port.

To explore the effectiveness of such a system configuration, a long-term experiment was conducted with anaerobic sludge for in-situ biogas upgrading. The sludge TSS used for the study is 5.4 g/L at 4L working volume, with a constant stirring rate of 150 rpm. A ceramic membrane with end sealings made of glass (1 x 25cm (length), 7mm (id), 600nm pore size) was used. Recirculation flow of the sludge within the membrane module is 660 mL/min for a 4 L working volume and the H_2 pressure tested on the permeate side is between 2.5 – 3 bar.

The membrane pore size was derived based on the equations provided by Chan et al. (*Bioresource Technology*, **2024**, *406*, 130981). FIG. 5 relates to the optimal gas transfer efficiency based on the membrane properties and operating conditions (e.g. membrane pore size, porosity, gas pressure) based on equations provided in Example 2. Theoretical mass transfer efficiency calculations were made based on 1pc ceramic membrane used in the experiment. The membrane porosity is 40 – 55% when the pore size is 70nm – 800nm. When the pore size is <70 nm, the membrane porosity is 30 – 55%. However, the above calculation did not assume pore wetting. With larger pore size, there will be a higher chance of pore wetting due to a lower liquid entry pressure, thus reducing the actual gas transfer rate. Hence from literature, a maximum pore size of 600nm is recommended to minimize pore wetting (Alkhudhiri et al., *Desalination*, **2012**, *287*, 2; Schneider et al., *Journal of Membrane Science*, **1988**, *39*, 25). It was concluded the recommended pore size to be 100nm – 600nm.

A comparison study using the reactor is performed with conventional diffuser method of gas delivery in water treatment plant, coupled with a biogas recirculation rate of up to 50 mL/min. The results were presented in Table 1, which shows the summary of the operating conditions for both the conventional diffuser set-up and the current membrane contactor configuration. The initial CH_4 production from the influent wastewater before H_2 injection was measured at 66 ± 3 mL/(L·day). Initial start-up when H_2 was added at 200 mL/(L·day), calculated from initial CO_2 content in the headspace according to the 4:1 H_2:CO_2 stoichiometric ratio, overwhelmed the reactor with high H_2 accumulation. Stepwise acclimatization of H_2 from day 15 was employed to recover the reactor by introducing H_2 at a lower flow rate and gradually increased to 255 mL/(L·day), on day 39 to target a higher CH_4 content. A biogas recirculation of 30 mL/min was also employed to recycle the excess H_2 back to the reactor. The CH_4 content improved from 67.0 ± 3.0 % to 84.0 ± 1.4 %, reaching a maximum value of 86.4 % on day 49 (FIG. 6a). To further enhance the utilization of unused H_2 in the reactor headspace, the biogas recirculation rate was further increased from 30 mL/min to 50 mL/min on day 45. Despite the adjustment of a higher gas recirculation rate, the CH_4 content remains similar with a slight drop to 83.4 % while excess H_2 was detected at 5.8 % in the resultant biogas on day 51. The overall CH_4 production rate increased to 105 ± 2 mL/(L·day) at the end of phase I (FIG. 6b). The conventional diffuser system could only achieve a maximum of 86.4% CH_4 content with a wastage of 4.9% - 5.8% H_2 (FIG. 6).

Given that the CO_2 content remains relatively high at 10.1 ± 1.1 % at the end of phase I, the disproportionate H_2 (5.6 ± 0.4 %) remaining suggests the prevalence of alternative H_2 utilization pathways, such as the conversion of H_2 and CO_2 to acetate by homoacetogens

driven by high H_2 partial pressure. Other potential pathways include sulfate and nitrate reductions (Dar et al., *Appl. Microbiol. Biotechnol.*, **2008**, *78*, 1045) and chain elongation reactions (Baleeiro et al., *Front. Bioeng. Biotechnol.*, **2021**, *9*, 650631).

A H_2 accumulation of 5.8 % in the headspace also suggests critical limitations of H_2 transfer in the diffuser/gas recirculation systems. The system was unable to achieve higher dissolution due to low H_2 solubility, leading to significant gas wastage over prolonged operation. Further increasing biogas recirculation beyond 30 mL/min did not enhance biogas quality, consistent with findings by Hafuka et al (*Sci. Total Environ.*, **2022**, *828*, 154573) where biogas recirculation showed no significant impact when CH_4 content exceeded 85 %. Thus, relying on diffusers in a typical AD may pose challenges in achieving more than 95 % CH_4 in the upgraded biogas.

The designed membrane contactor underwent subsequent testing following the purging of the reactor with N_2 and re-acclimatization with H_2 (Table 1 and FIG. 6). On day 80 (phase II), the H_2 flow rate was determined at 124 mL/ (L·day) at a H_2 pressure of 2.5 bar, with a sludge recirculation flow rate of 165 mL/(L·min). The H_2 flow rate was calculated based on the volume reduction of CO_2 content in the biogas using the 4:1 ratio. The CH_4 content increased to 77.5 ± 0.8 % at the end of phase II. H_2 was almost undetected in the headspace (< 0.1 %), indicating that the H_2 gas transfer with current supplied gas pressure was insufficient for achieving complete biogas upgrading.

In a stepwise manner, the H_2 supply was further increased to 284 mL/(L·day) at a H_2 gas pressure of 3 bar starting from day 98 (phase III). Following this adjustment, there was a steady increase in biogas quality, with the CH_4 content at 98.2 % on day 124 on a rising trend. The excess H_2 remained below 1.0 % in the headspace, indicating the effectiveness of microbial uptake and H_2 utilization through the membrane contactor system. A leakage within the setup led to a transient accumulation of H_2 from day 124–136 that was quickly rectified. For the final 21 days in phase III (day 149 – day 170), the CH_4 content in the biogas reached a maximum value of 99.1 % and sustained at 98.8 ± 0.2 %. The membrane contactor was able to achieve a vastly better biogas quality with a CH_4 content of 99% in the biogas at the stable state (FIG. 6). Only 0.4 ± 0.3 % H_2 remained in the resultant biogas. The results demonstrated near 100% H_2 utilization efficiency and suggested significant advantage in cost and energy wastage minimization with supplying H_2 using the membrane contactor to the AD.

Table 1. Summary of the operational parameters and reactor performance using diffuser injection and membrane contactor system.

Phase	Duration	H_2 Pressure	Sludge Recirculation Rate	Biogas Recirculation Rate	H_2 Supply	CH_4 Production Rate	Final Biogas Content (%)			H_2 Utilization Efficiency
	(d)	(bar)	(mL/(L·min))	(mL/min)	(mL/(L·d))	(mL/(L·d))	CH_4	CO_2	H_2	(%)
Baseline	-	-	-	-		66 ± 3	67.0 ± 3.0	33.0 ± 3.0	0	-
Diffuser										
I	15 - 51	-	-	30 - 50	180 - 255	105 ± 2	84.0 ± 1.4	10.1 ± 1.1	5.6 ± 0.4	94.4 ± 0.4
Membrane Contactor										
II	80 - 97	2.5	165	-	125 ± 3[a]	87 ± 1	77.5 ± 0.8	22.4 ± 0.8	<0.1	99.9 ± 0.1
III	98 - 170	3.0	165	-	276 ± 18[a]	137 ± 10	98.8 ± 0.3	0.8 ± 0.3	0.4 ± 0.3	99.6 ± 0.3

[a] calculated with the assumption that the increase in CH_4 production rate is due to full conversion of CO_2 into CH_4 based on the 4:1 stoichiometric ratio of H_2/CO_2.

The average CH_4 production rate increased by 108 % to 137 ± 10 mL/L/day at the stable upgrading phase. It is also a 30 % improvement from the diffuser injection system. This was potentially attributed to the (1) enhanced mixing from the sludge recirculation which led to greater nutrient movement and substrate transfer within the reactor, and (2) the greater rate of conversion of H_2 and CO_2 into CH_4 by hydrogenotrophic methanogens.

Example 2. Theoretical Mass Transfer Efficiency

As there was no buildup of H_2 in the headspace during the membrane contactor operation, gas diffusion was hypothesized to be the dominant mode of gas transfer with minimal bubble formation. The H_2 supply was calculated from the stochiometric conversion of CO_2 to CH_4 in the system. The calculation on the theoretical gas supply through diffusion attainable with the ceramic membrane contactor was performed to identify potential optimization parameters for system performance enhancement. The detailed parameters used for calculations can be found in Table 2.

Mass Transfer and Utilization Efficiencies

The rate of H_2 consumption is related to the efficiency of the conversion of CO_2 into CH_4. During steady state, assuming low solubility of H_2 in the liquid phase, the H_2 consumption rate (R_{H_2}) and the efficiency of H_2 utilization η_{H_2} was calculated based on Eqs. (2) and (3) (Xu et al., *Bioresour. Technol.*, **2020**, *299*, 122598) as follows:

$$Q_{G,in}\, C_{G,in} - Q_{G,out}\, C_{G,out} = R_{H_2} V_E 0 \qquad \text{Eq. (2)}$$

$$\eta_{H_2} = \frac{R_{H_2} V_E}{Q_{G,in} C_{G,in}} \cdot 100\% \qquad \text{Eq. (3)}$$

Where $Q_{G,in}$ is the flow rate of the inlet H_2 feed gas (mL/(L·day)), QG,out is the flow rate of the collected biogas (mL/(L·day)), $C_{G,in}$ is H_2 concentration in the inlet gas (%), $C_{G,out}$ is H_2 concentration in the outlet gas (%) and V_E is the effective liquid volume (L) of the reactor.

The theoretical mass transfer efficiency for the system was evaluated to estimate the theoretical gas supply that can be achieved with the membrane system. For diffusion based gas transfer through the membrane, the mass transfer coefficient can be estimated from Eq. (4) given in Mansourizadeh and Ismail (*J. Hazard. Mater.*, **2009**, *171*, 38).

$$k_m = \frac{D_{g,eff} \varepsilon_m}{\tau_m\, l_m} \qquad \text{Eq. (4)}$$

Where k_m is the mass transfer coefficient (m/s), which is affected the geometrical characteristics of the membrane (i.e. thickness, l_m, porosity, ε_m and tortuosity, τ_m). The membrane thickness and porosity were based on supplier's membrane specifications. The membrane tortuosity was estimated from Tanko (*Int. J. Sci. Eng. Res.*, **2018**, *9*, 2163) who measured a range of alumina and silica membrane materials, and Graczyk and Matyka (*Sci. Rep.*, **2020**, *10*, 21488) to calculate the tortuosity of the membrane based on the membrane porosity. The effective diffusion coefficient, $D_{g,eff}$ (m^2/s), can be estimated from Eq. (5).

$$D_{g,eff} = \left(\frac{1}{D_{g,m}} + \frac{1}{D_{g,kn}}\right)^{-1} \quad \text{Eq. (5)}$$

Where $D_{g,Kn}$ (m^2/s) is the Knudson diffusion coefficient given by Eq. (6):

$$D_{g,Kn} = 97 r_p \left(\frac{T}{M}\right)^{0.5} \quad \text{Eq. (6)}$$

Where r_p is the pore radius (m) and T is the temperature (K).

$D_{g,m}$ (m^2/s) is the molecular self-diffusion coefficient calculated from the kinetic gas theory, given by Eq. (7):

$$D_{g,m} = 1200\mu\left(\frac{RT\Omega_\mu}{MP\Omega_D}\right) \quad \text{Eq. (7)}$$

Where μ is the dynamic viscosity (Pa·s), M is the gas molecular weight, P is the gas pressure (Pa), R is the gas constant ($Pa \cdot m^3/(mol \cdot K)$). Ω_μ and Ω_D are collision integrals used for diffusion, which are dimensionless function of temperature derived from the Neufeld empirical equations. The value for H_2 was obtained from Appendix K in Welty et al. (*Fundamentals of momentum, heat and mass transfer*, 5th ed. John Wiley & Sons Ltd, New York., 2007).

An overall mass transfer efficiency from gas to liquid phase was calculated from Eq. (8) (Karimi et al., *Iranian Journal of Environmental Health Science & Engineering*, **2013**, *10*, 6):

$$k_m L t = \ln\left(\frac{C_{sat}}{C_{sat} - C}\right) \quad \text{Eq. (8)}$$

Where t is the liquid retention time (s) within the membrane, L is the membrane length (m), and C_{sat} is the H_2 concentration at saturation. Based on Henry's Law, the calculated saturation concentration at 3 bar pressure and 298 K is 0.00462 g/L.

C is the concentration of H_2 at the membrane outlet, and can be estimated using a mass balance of hydrogen on the membrane based on the theoretical gas transfer GT (m^3/s) and

sludge recirculation rate R_s (m^3/s) similar to Deschamps et al. (*Membranes*, **2022**, *12*, 1220) (Eq. (9)).

$$G_T = R_s\ (C - C(0))$$ Eq. (9)

C(0) is the concentration of H_2 at the membrane inlet and was taken to be zero due to the rapid consumption of the supplied H_2 in the reactor.

Hence, the following equations Eq. (10) and Eq. (11) can be established to calculate the theoretical gas supply based on k_m.

$$k_m Lt = \ln\left(\frac{C_{sat}}{C_{sat} - \frac{G_T}{R_s}}\right)$$ Eq. (10)

$$G_T = C_{sat}\left(1 - \frac{1}{\exp(k_m Lt)}\right)R_s$$ Eq. (11)

Table 2. Detailed parameter values to calculate theoretical mass transfer efficiency (MTE) and gas flow rate.

Parameter	**Values**
Membrane area (m^2)	0.0049
Membrane thickness (m)	0.0015
Porosity	0.40 - 0.55
Tortuosity	1.20 - 2.58
Liquid recirculation rate (m^3/s)	0.000011
Henry constant for H_2 (mol/(L·bar))	0.00077
Input pressure (Pa)	30,000
Collision integral Ω_μ	0.82
Diffusion collision integral Ω_d	0.74
Gas constant ($Pa \cdot m^3/(mol \cdot K)$)	8.31
Dynamic viscosity of H_2 (Pa·s)	0.0000090
Density of H_2 (kg/m^3)	0.084
Knudson diffusion coefficient $D_{g,Kn}$ (m^2/s)	0.00036
Molecular self-diffusion coefficient $D_{g,m}$ (m^2/s)	5.01×10^{-5}
k_m (calculated)	0.0046 - 0.013
Theoretical gas supply (L/d)	1.9 – 2.2

Results and Discussions

The calculated theoretical achievable gas diffusion supply based on the membrane characteristics was 1.90 L – 2.18 L H_2 /day. However, the H_2 supply estimated based on the full conversion of CO_2 to CH_4 in the current reactor system was 1.14 L/day, which was only 52 – 60% of the theoretical achievable gas transferred through the membrane. Underestimation of the actual gas supply from full CO_2 conversion could be a potential reason as H2 might be utilized for side reactions (e.g. VFA formation or chain elongation). Another possibility for the lower gas transfer rate could be attributed to the partial pore wetting for hydrophilic membrane which increases the distance of diffusion through the wetted pores, thus lowering the actual H_2 mass transfer efficiency (Mansourizadeh and Ismail, *J. Hazard. Mater.*, **2009**, *171*, 38). A potential remediation could be to reduce the membrane pore size, which correspondingly reduces the liquid entry pressure and could enhance wetting resistance (McGaughey et al., *ACS Applied Polymer Materials*, **2020**, *2*, 1256). The improvement of H_2 dissolution across membrane can be further enhanced through optimization of the parameters affecting the gas transfer efficiency such as the gas pressure, liquid recirculation rate, and membrane characteristics.

Example 3. Effect of H_2 on pH and Volatile Fatty Acids

The consumption of the CO_2 within the liquid phase resulted in a rise in the pH of the reactor from 7.1 ± 0.1 in phase II to a maximum of 8.0 on day 129 in phase III aligning with the high methane conversion (Table 3). Similarly, the TIC reduced from 293.4 ± 0.1 mg/L in phase II to 114.5 ± 1.4 mg/L in phase III as the dissolved CO_2 species including the carbonates and bicarbonates were consumed. As the main goal of the current invention is to develop the membrane configuration for H_2 transfer, maintaining stable substrate degradation and biogas production was crucial. The high pH, while optimal for hydrogenotrophic methanogenesis, may not be suitable for microbes involved in the initial stages of the AD. Therefore, the pH was controlled below 8.0 with the addition of HCl and maintained at 7.5 ± 0.3 in phase III.

The total VFA was monitored over the three phases (FIG. 7). No significant fluctuations were observed during the phase I, and no obvious acetic acid accumulation was observed throughout the experiment, indicating the reactor's efficiency in acetic acid utilization.

The total VFA observed an increasing trend in the later stage of the membrane contactor operation and accumulated to 184.7 mg/L on day 170 (end of phase III). The main VFA accumulation for the membrane system was the accumulation of propionic acid in the reactor.

Table 3. Detailed data for each phase of the reactor.

Phase	I	II	III
OLR (g/(L·d))	0.2	0.2	0.2
TSS (g/L)	5.4 ± 0.5	5.1 ± 0.8	4.1 ± 0.7
VSS/TSS	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
NH_4 – N (mg/L)	503 ± 15	515 ± 25	561 ± 19
Total Alkalinity (mg/L)	2282 ± 59	2067 ± 33	2121 ± 124
H_2 supply (mL/(L·d))	255	125 ± 3	276 ± 18
CH_4 production rate (mL/(L·d))	105 ± 2	87 ± 1	137 ± 10
pH (mg/L)	7.3 ± 0.1	7.1 ± 0.1	7.5 ± 0.3
TOC (mg/L)	200.7 ± 2.1	217.1 ± 1.8	256.8 ± 5.6
TIC (mg/L)	327.5 ± 0.2	293.4 ± 0.1	114.5 ± 1.4
Resultant biogas composition (%)			
CH_4	84.0 ± 1.4	77.5 ± 0.8	98.8 ± 0.3
CO_2	10.1 ± 1.1	22.4 ± 0.8	0.8 ± 0.3
H_2	5.6 ± 0.4	< 0.1	0.4 ± 0.3
H_2 utilization (%)	94.4 ± 0.4	99.9 ± 0.1	99.6 ± 0.3

The main source of VFA accumulation was propionic acid, whose concentration started to increase from day 150 onwards from 3.2 mg/L to a maximum value of 120.9 mg/L on day 170, contributing to 65 % of the total VFA in the system. Propionate cannot be directly used by the methanogens and must be converted first to acetate and H_2 based on Eq. (12), which requires the highest Gibbs free energy (+73.7 kJ/mol) compared to other VFA conversion. When dissolved H_2 is high in the system, the oxidation of propionate becomes energetically unfavorable and thermodynamic inhibition of propionate degradation occurs, leading to its accumulation (Han et al., *Chemosphere*, **2020**, *255*, 126840; Fukuzaki et al., *Appl. Environ. Microbiol.*, **1990**, *56*, 719).

$$C_3H_5O_2^- + 2H_2O \rightarrow C_2H_3O_2^- + CO_2 + 3H_2 \left(\Delta G^0 = +73.7 kJ/mol\right)$$ Eq. (12)

As the accumulation only occurred beyond day 150 corresponding to a high biogas quality of more than 98 % CH_4, the system instabilities might be brought forth by the reduction of dissolved CO_2 in the sludge and observed from increasing pH in the reactor (controlled with acid dosage). While CO_2 was still being produced by the system, both CO_2 and H_2 should be provided at an equivalent rate for complete reaction to prevent the accumulation of either chemical species. In this scenario, CO_2 may emerge as the limiting factor, as its production was reliant on the rate of substrate conversion. This could result in the transient buildup of H_2 which may inhibit the propionate reducing species and contribute to propionate accumulation.

Since the VFA accumulation only occurred at high CH_4 content in the reactor, it may be crucial to target a trade-off between the biogas quality and potential system instabilities. Maintaining the biogas quality at an acceptable CH_4 percentage, such as 95 % to 97 % CH_4 in the upgraded biogas, can be considered. The presence of 3–5 % CO_2 in the headspace translates to a higher buffer of dissolved CO_2 in the liquid phase. Furthermore, this level of CH_4 content aligns with the standard requirements for biomethane that can be injected into the natural gas grid (Khan et al., *Bioresour. Technol.*, **2021**, *345*, 126219). This was also demonstrated in Table 5, which presents the biogas upgrading results for various organic-rich waste substrates at similar feeding rates. The CH_4 content was kept at around 97% at steady state, and the VFA consistently remained at a very low level (<5 mg/L) throughout the steady upgrading phase. Maintaining a CH_4 percentage within this range thus helps to ensure that the biogas composition is optimized for downstream applications while also mitigating the risk of VFA accumulation and other operational challenges associated with excess H_2 input, thus enhancing the viability of the biogas upgrading system.

Example 4. Comparison with Existing Technologies

Comparison with various literatures for biogas upgrading studies also found the current membrane contactor system is able to perform vastly better in terms of the produced biogas quality compared to other reactor configurations for in-situ biogas upgrading (Table 4).

The studied membrane contactor system could achieve an excellent CH_4 content in the biogas up to 99.1 %, coupled with the near 100 % H_2 utilization efficiency. This performance surpasses alternative methods for in-situ systems, such as HFM (Alfaro et al., *Bioresour. Technol.*, **2019**, *280*, 1-8; Luo and Angelidaki, *Appl. Microbiol. Biotechnol.*, **2013**, *97*, 3739),

two stage up-flow anaerobic sludge blanket (Xu et al., *Bioresour. Technol.*, **2020**, *299*, 122598), microbial electrolysis cell (Ning et al., *Energ. Conver. Manage.*, **2024**, *304*, 118245), biogas recirculation (Hafuka et al., *Sci. Total Environ.*, **2022**, *828*, 154573) etc. in both mesophilic and thermophilic systems, which produces biogas with CH_4 content between 73 –96 %. The potential reason for the high CH_4 conversion efficiency could be attributed to diffusion being the dominant mode of gaseous transfer. H_2 gas was dissolved directly into the liquid phase in contact with the hydrophilic membrane, reducing the tendency for gas to form bubbles (Li et al., *J. Membr. Sci.*, **2010**, *362*, 47). The higher gas pressure of 3 bar also promoted the formation of smaller bubbles that quickly dissolved within the sludge media (Luo et al., *AIChE J*, **1999**, *45*, 665), minimizing H_2 escape into the reactor's headspace. Since only dissolved H_2 were used by the methanogens, immediate uptake and conversion occurred, proving more efficient than conventional diffusers and polymeric membrane configurations that inject H_2 in gaseous form. With full H_2 utilization, biogas recirculation mechanisms to recycle unused H_2/CO_2 back to the liquid phase was not required.

The ceramic membrane contactor was also designed as an externally coupled module to anaerobic reactors without the need to change reactor configurations. This is crucial for existing AD systems managing waste degradation, as it enables rapid initiation of biogas upgrading without risking process damage or delays. As CSTR is one of the more common reactor type for AD (Voelklein et al., *Appl. Energy.*, **2019**, *235*, 1061), alternative methods generally necessitate significant modifications or even complete reconstruction of the AD, which may not be feasible for existing plants. Sludge liquid recirculation in the proposed system can also partially replace the function of mixers or stirrers (Deschamps et al., *Bioresour. Technol.*, **2021**, *337*, 125444), which are major energy consumers in bioreactors. The improved substrate and nutrients transfer from sludge recirculation also facilitated a 108% increase in the CH_4 production. The current membrane prototype can upgrade at least 165 mL/L/day biogas while achieving CH_4 content of 98.8 ± 0.3 % at the stable phase. H_2 supply and biogas conversion rates can be further increased at the current scale through adjustment of the gas pressure, liquid recirculation rate, membrane pore size, and membrane thickness. In phase II and III, pressure adjustment was performed, and an increase of 0.5 bar doubled the H_2 supply due to the prevention of pore wetting at higher pressures. Nevertheless, since the membrane system focuses on gas transfer, biomethane from complex wastes may require further purification to remove contaminants such as H_2S, siloxanes and VOCs (Chin et al., *Sci. Total Environ.*, **2020**, *729*, 138702) before gas grid injection.

Other biological methods include technologies for ex-situ biological biogas upgrading, which due to the simpler biochemical reactions involved, can typically achieve good product gas

Table 4. Comparison of the biogas upgrading quality in various technologies tested by other studies.

Bioreactor Configuration	Working Volume (L)	Substrate	H_2 Utilization Efficiency (%)	Maximum Methane Content (%)	pH	Reference
CSTR with ceramic membrane contactor	4	Synthetic wastewater	99.6	99.1	7.5	Current invention
CSTR with diffuser and biogas recirculation	4	Synthetic wastewater	96.6	86.4	7.5	Current invention
Thermophilic CSTR with hollow fiber membrane (HFM)	1	Synthetic wastewater	96.0	96.0	8.0	Luo and Angelidaki (*Appl. Microbiol. Biotechnol.*, **2013**, *97*, 3739)
Two-stage CSTR	3.5	Cattle manure	93.0	89.0	7.8	Bassani et al. (*Environmental Science & Technology*, **2015**, *49*, 12585)
Batch reactor	0.075	Food waste	72.0	77.0	8.5	Okoro-Shekwaga et al. (*Applied Energy*, **2019**, *254*, 113629)
CSTR	11.2	Swine manure	18.0	70.0	7.7	Zhu et al. (*Journal of Industrial Microbiology and Biotechnology*, **2019**, *46*, 1569)
CSTR with HFM and biogas recirculation	20	Sewage sludge	94.0	73.1	7.3 – 8.0	Alfaro et al. (*Bioresour. Technol.*, **2019**, *280*, 1-8)
Batch reactor	0.389	Cattle manure	98.0	94.5	7.6	Wahid et al. (*Biotechnology and Biofuels*, **2019**, *12*, 104)
Modified two-stage up-flow anaerobic sludge blanket (UASB) reactor	0.7	Anaerobic granular sludge	95.0	92.0	7.8 – 8.0	Xu et al. (*Bioresour. Technol.*, **2020**, *299*, 122598)
Anaerobic membrane bioreactor (AnMBR)	2.37	Waste activated Sludge	n.d.	92.0	7.0 – 7.4	Hafuka et al (*Sci. Total Environ.*, **2022**, *828*, 154573)
AnMBR with external hydrophobic microporous membrane	150	Distillery Wastewater	99.2	97.9	7.0	Deschamps et al., (*Bioresour. Technol.*, **2021**, *337*, 125444)

quality with high CH_4 content between 79–98% (Angelidaki et al., *Biotechnol. Adv.*, **2018**, *36*, 452). These include trickle bed reactors and microalgae photobioreactor, although the latter is also affected by O_2 contamination and CH_4 inhibition (Marín et al., *Bioresour. Technol.*, **2019**, *280*, 112). A dedicated secondary reactor for H_2/CO_2 conversion is required for ex-situ upgrading, while in contrast the proposed ceramic membrane system can be adapted for both in-situ and ex-situ applications, as its primary function is to enhance H_2 dissolution.

Compared to well-established physico-chemical techniques for CH_4 separation such as pressure swing adsorption, water scrubbing, chemical absorption, and membrane separation, the current system for biological biogas upgrading enables the use of endogenously produced CO_2 from biomass to achieve a net negative CO_2 emissions (Al-Wahaibi et al., *Sci. Rep.*, **2020**, *10*, 15719; Fu et al., *Trends Biotechnol.*, **2021**, *39*, 336). While catalytic methanation also converts H_2/CO_2 to CH_4 via the Sabatier reaction, it requires high temperatures of around 600 °C, unlike the use of mesophilic conditions in the current method. Although physico-chemical methods could also reach methane purity of > 96 %, they often entail high investment, operational and maintenance costs, high energy requirements, and the production of toxic waste chemicals (Fu et al., *Trends Biotechnol.*, **2021**, *39*, 336). Thus, the overall benefit of the current technology is in its flexibility for enhanced integration into existing AD operations, while also ensuring a lower energy and carbon footprint in its adoption.

Example 5. Microbial Community Analysis

Identification of the microorganisms involved in biogas production and biogas upgrading was performed to determine the shift in the microbial community in response to the H_2 injection methods.

DNA extraction and high-throughput sequencing analysis

A total of 5 sludge samples were collected in duplicates on days 0, 44, 51, 161, 170 for 16S rRNA high-throughput sequencing analysis. DNA extraction was performed using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer's instructions. Modified 515F/806R primer pair was used to amplify the V4 hypervariable region of the bacterial and archaeal 16S rRNA gene via polymerase chain reaction (PCR). Amplifications were performed in triplicate using the ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). The PCR product was extracted from 2 % agarose gel and purified with the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega, USA). Purified amplicons were pooled in equimolar amounts and sequenced using paired-end

sequencing on an Illumina MiSeq PE300 platform (Illumina Inc., CA, US) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). All sequenced sample libraries were quality-filtered by fastp version 0.19.6 (Chen et al., *Bioinformatics*, **2018**, *34*, i884) and merged by FLASH version 1.2.7 (Mago˘c and Salzberg, *Bioinformatics*, **2011**, *27*, 2957). The obtained sequences were grouped into operational taxonomic units (OTUs) using UPARSE 7.1 with a 97 % similarity threshold. Subsequently, the taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 (Wang et al., *Appl. Environ. Microbiol.*, **2007**, *73*, 5261) against the 16S rRNA gene database (Silva v138) using confidence threshold of 0.7 to classify them into their respective species. The raw sequencing reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA1117760).

Results and Discussions

Based on the Shannon Weiner index, the overall diversity remained similar within the reactor. The bacteria diversity remained within a small range of 2.7 ± 0.1 and 2.9 ± 0.1, while the archaea diversity fluctuated slightly more between 0.3 ± 0.1 to 0.9 ± 0.2.

FIG. 8 shows the abundance of bacterial and archaeal community at phylum level. At the start of the study (day 0), *Firmicutes, Synergistota* and *Bacteroidota* were the three most common phyla present in the reactor. *Firmicutes* and *Bacteroidota* contains many acidogenic bacteria community which help in the fermentation of the hydrolysate monomers to alcohol, acetate, propionate, butyrate, H_2, CO_2, and other solvents (Lim et al., *Advances in Bioenergy*, **2020**, *5*, 1). *Bacteroidetes* abundance remained similar at the start and at end of the study (11.4 ± 1.0 % – 12.7 ± 1.0 %), while *Firmicutes,* which initially accounted for the greatest proportion 41.4 ± 0.2 % of the microbial community, dropped to 19.3 ± 1.2 % at the end of the membrane operation in phase III (day 170). Replacing the *Firmicutes* was *Spirochaetota* whose abundance increased significantly, from 3.4 ± 0.6 % to 7.2 ± 1.1 % at the end of the diffuser operation in phase I (day 51), and further to 23.3 ± 10.3 % at the end of the membrane operation to become the most dominant phyla in the reactor. The *Euryarchaeota* phyla, which consists of the mesophilic archaea responsible for biogas production, also increased from 2.7 ± 1.0 % to 5.5 ± 0.1 % at the end of the membrane operation. *Synergistota* phyla abundance increased from 21.7 ± 0.4 % to 28.4 ± 0.6 % during the diffuser operation, but dropped to 15.8 ± 1.0 % during the membrane operation. This phylum contains syntrophic bacteria species that reduces propionate and butyrate accumulation, while also converting amino acids and sugars to VFAs through syntrophic interactions with methanogenic archaea (Zhu et al. 2022, *Fuel*, **2022**, *318*, 123604).

The genus distribution of the bacterial and the archaeal communities are illustrated in FIG. 9. A total of 5 archaea genera and 20 dominant bacteria genera was presented the samples. While some bacteria could not be classified into a certain genus unit (listed as unclassified *Planococcaceae, Anaerolineaceae*, no rank *Spirochateceae, ST-12 K33* and *D8A-2*), they were still dominant in the bacteria community. Unidentified members of the *Spirochaetaceae* family increased from 3.4 ± 0.6 % on day 1 to 23.3 ± 10.3 % on day 170. Certain members within this family have the function to perform homoacetogenesis as identified in various studies (Wang et al., *Bioresour. Technol.*, **2013**, *146*, 234; Zhang et al., *Fuel*, **2011**, *90*, 324) which is a potential pathway in the utilization of H_2. The reactor conditions generally supported spirochaetes growth, including the maintenance of a weakly basic pH (between 7.5 and 7.8) and the external addition of H_2 (Kligler & Robertson, *J. Exp. Med.*, **1922**, *35*, 303; Lee et al., *Bioresour. Technol.*, **2013**, *145*, 25). The disproportionate amount of unutilized H_2 remaining in the reactor during phase I (diffuser operation) could be attributed to its usage in homoacetogenesis pathways. Lee et al. (*Bioresour. Technol.*, **2013**, *145*, 25) also found that reactor abundant in spirochaetes metabolize acetate better than other types of substrates. Therefore, the increase in spirochaetes abundance could also explain the observed lack of acetate accumulation in the reactor.

Among the genus within *Synergistota*, *Thermovirga* was one of the most dominant in the reactor throughout study. However, while its abundance increased from 15.0 ± 0.4 % to 17.8 ± 1.6 % for samples collected on day 0 and 51 (phase I), it dropped to 12.6 ± 0.4 % for sample collected on day 170 (phase III). *Thermovirga* spp. has been found to dominate with methanogens in multiple AD experiments with the suggestion that this genus plays an important role in propionate degradation and biogas production pathways (Puengrang et al., *Microorganisms*, **2020**, *8*, 277). Another *Synergistota* genus, *Acetomicrobium*, also contains species known to be involved in acetate and H_2 production from VFA degradation including propionate (De Bernardini et al., *Microbiome*, **2022**, *10*, 117). It showed a decrease in abundance only during the membrane operation stage from 2.4 ± 0.1 % from day 51 to 0.7 ± 0.1 % on day 170, possibly affected by the external H_2 addition. The reduction in members that promote propionate utilization could have also contributed to the propionate accumulation towards the later part of phase III.

For the archaea genus identified, *Methanobacterium* remained the largest proportion in the reactor, with an abundance between 69.0 ± 12.0 % to 95.2 ± 0.4 % throughout the experiment. *Methanospirillum*, which was initially not detected, also increased in abundance to 1.9 ± 0.4 % at day 170. Both *Methanobacterium and Methanospirillum* spp. utilize H_2 and CO_2 as substrate to produce CH_4 via the hydrogenotrophic methanogenesis pathway. Conversely,

Methanosaeta spp. as strict acetoclastic methanogen that feeds predominantly on acetate for CH_4 production, found its abundance reduced from 7.2 ± 0.2 % on day 1 to 1.9 ± 1.0 % on day 170. The change in the relative proportion of the methanogenic archaea utilizing acetate and H_2 indicated that hydrogenotrophic methanogenesis was the most dominant biogas production pathway for the efficient uptake of H_2. Nonetheless, the lack of acetate accumulation indicated that despite the lower levels of acetoclastic methanogens (e.g. *Methanosaeta* spp.) within the reactor, they still performed a strong functional role in the efficient conversion of acetate to CH_4.

Example 6. Biogas upgrading on Organic-rich Waste Substrates

The biogas upgrading ceramic membrane module has been further tested on various organic-rich waste substrates by following the protocols disclosed in the earlier Examples.

Results and Discussions

Beyond synthetic wastewater streams, other organic waste feeds with greater industrial relevance were also tested. These included lignocellulosic poplar wood waste and cardboard waste, both of which were acid-pretreated to release simpler sugars. This process generated

Table 5. Biogas upgrading using different organic-rich waste substrates.

	Substrate		
	Synthetic wastewater (Glucose)	**Pre-treated cardboard waste**	**Pre-treated poplar wood waste**
OLR (gCOD/L/day)	0.2	0.2	0.2
H_2 pressure (bar)	3.0	3.0	2.8
Final biogas composition (%)			
CH_4	98.8 ± 0.3	97.3 ± 0.1	97.7 ± 0.1
CO_2	0.8 ± 0.3	2.1 ± 0.03	1.8 ± 0.2
H_2	0.4 ± 0.3	0.6 ± 0.1	0.5 ± 0.1
H_2 utilization (%)	99.6	99.4	99.5
CH_4 production rate (mL/L/day)	137 ± 10	142 ± 8	122 ± 1
Total VFA (mg/L)	104 ± 44	4.6 ± 4.4	3.9 ± 2.2

a mixture of hydrolyzed compounds from the hemicellulose and cellulose fractions, including acetate and furfural—compounds known to inhibit fermentation. Such pretreatment is commonly employed in commercial biorefineries to valorize cellulosic waste into organic chemicals or biofuels. The composition of the two substrates was illustrated (FIG. 10). Biogas upgrading using the current invention on these organic-rich waste substrates yielded similarly positive results, achieving CH_4 concentrations of 97–99% under stable conditions (Table 5). The experiment on these substrates highlights the viability of this process to convert different waste streams into high-value bioenergy.

Claims

1. A method of biogas upgrading, the method comprising the steps of:

(a) providing:

a system comprising:

a bioreactor comprising a liquid inlet and a liquid outlet; and

an external ceramic membrane module comprising a hollow ceramic membrane and a gas-tight housing surrounding the hollow ceramic membrane,

where the hollow ceramic membrane has an interior lumen, a first end fluidly connected to the liquid outlet of the bioreactor and a second end fluidly connected to the liquid inlet of the bioreactor, and

the gas-tight housing has a gaseous inlet suitable to provide a gas to an interior of the gas-tight housing; and

a wastewater mixture situated within the bioreactor comprising a wastewater to be treated and a microbial population comprising anaerobic bacteria; and

(b) operating the system under anaerobic conditions and recirculating the wastewater mixture from the bioreactor through the liquid outlet into the hollow ceramic membrane and back to the bioreactor via the liquid inlet, wherein:

hydrogen gas is provided through the gaseous inlet of the gas-tight housing at a suitable pressure to enable the hydrogen gas to transit into the interior lumen of the hollow ceramic membrane and be substantially dissolved in the wastewater mixture; and

the hydrogen gas is reacted by the anaerobic bacteria with CO_2 generated by microbial action on the wastewater to generate a biogas comprising methane.

2. The method according to Claim 1, wherein the first and second ends of the hollow ceramic membrane are treated to prevent fluid ingress or egress from an interior surface of the hollow ceramic membrane to an exterior surface of the hollow ceramic membrane and *vice versa*, optionally wherein the first and second ends of the hollow ceramic membrane are glass sealed.

3. The method according to Claim 1 or Claim 2, where substantially no hydrogen gas bubbles are produced.

4. The method according to any one of the preceding claims, wherein the anaerobic bacteria include acetoclastic methanogens and hydrogenotrophic methanogens.

5. The method according to any one of the preceding claims, wherein the hydrogen gas dissolution efficiency is greater than or equal to 99%, such as about 100%.

6. The method according to any one of the preceding claims, wherein the hollow ceramic membrane has a pore size of from 70 to 800 nm.

7. The method according to Claim 6, wherein the hollow ceramic membrane has a pore size of from 100 to 600 nm.

8. The method according to any one of the preceding claims, wherein the hollow ceramic membrane has an area of from 0.5 to 3 m^2/m^3 volume of the bioreactor.

9. The method according to Claim 8, wherein the hollow ceramic membrane has an area of from 1 to 2 m^2/m^3 volume of the bioreactor.

10. The method according to any one of the preceding claims, wherein the pressure of the hydrogen gas in the external ceramic membrane module is from 50 kPa to 800 kPa, such as from 250 kPa to 500 kPa, such as about 300 kPa.

11. The method according to any one of the preceding claims, wherein the method employs a total suspended solids in the wastewater mixture of from 0 to 30 g/L, such as from 1 to 20 g/L, such as from 3 to 5 g/L.

12. The method according to any one of the preceding claims, wherein the bioreactor further comprises a means or apparatus for agitation of the wastewater mixture therein, optionally wherein the means or apparatus is a mechanical stirrer, further optionally wherein the mechanical stirrer operates at a speed of from 100 to 500 rpm, such as from 125 to 200 rpm, such as about 150 rpm.

13. The method according to any one of the preceding claims, wherein the wastewater mixture has a recirculation flow rate of from 0.03 to 0.3 of the total volume of the wastewater mixture/minute, such as from 0.15 to 0.17 of the total volume of the wastewater mixture/minute.

14. The method according to any one of the preceding claims, wherein, when operating in a steady state, the biogas is from 97 to 99.5%, such as from 98 to 99% methane.

15. The method according to any one of the preceding claims, wherein, the wastewater mixture is selected from one or more of the group consisting of an anaerobic sludge, a liquid stream comprising glucose, a pre-treated cardboard waste liquor, a pre-treated lignocellulosic liquor, a food wastewater/waste, a manure leachate/waste, a waste activated sludge, and a thermal hydrolysis sludge.

16. The method according to any one of the preceding claims, wherein the external ceramic membrane module comprises a plurality of hollow ceramic membranes, such as from 2 to 20 hollow ceramic membranes.

17. A system suitable for biogas upgrading, the system comprising:

a bioreactor comprising a liquid inlet and a liquid outlet; and

an external ceramic membrane module comprising a hollow ceramic membrane and a gas-tight housing surrounding the hollow ceramic membrane,

where the ceramic membrane has an interior lumen, a first end fluidly connected to the liquid outlet of the bioreactor and a second end fluidly connected to the liquid inlet of the bioreactor, and

the gas-tight housing has a gaseous inlet suitable to provide a gas to an interior of the gas-tight housing.

18. The system according to Claim 17, wherein the first and second ends of the hollow ceramic membrane are treated to prevent fluid ingress or egress from an interior surface of the hollow ceramic membrane to an exterior surface of the hollow ceramic membrane and *vice versa*, optionally wherein the first and second ends of the hollow ceramic membrane are glass sealed.

19. The system according to Claim 17 or Claim 18, wherein the hollow ceramic membrane has a pore size of from 70 to 800 nm.

20. The system according to Claim 19, wherein the hollow ceramic membrane has a pore size of from 100 to 600 nm.

21. The system according to any one of Claims 17 to 20, wherein the hollow ceramic membrane has an area of from 0.5 to 3 m^2/m^3 volume of the bioreactor.

22. The system according to Claim 21, wherein the hollow ceramic membrane has an area of from 1 to 2 m^2/m^3 volume of the bioreactor.

23. The system according to any one of Claims 17 to 22, wherein the system is configured to provide a gas to the external ceramic membrane module at a pressure of from 50 kPa to 800 kPa, such as from 250 kPa to 500 kPa, such as about 300 kPa.

24. The system according to any one of Claims 17 to 23, wherein the bioreactor further comprises a means or apparatus for agitation, optionally wherein the means or apparatus is a mechanical stirrer.

25. The system according to any one of Claims 17 to 24, wherein system further comprises a means or apparatus to effect recirculation of a wastewater mixture from the bioreactor through the hollow ceramic membrane and back into the bioreactor, optionally wherein the means or apparatus to effect recirculation is a pump.

26. The system according to any one of Claims 17 to 25, wherein the external ceramic membrane module comprises a plurality of hollow ceramic membranes, such as from 2 to 20 hollow ceramic membranes.