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Mets

(54) SYSTEM FOR THE PRODUCTION OF METHANE FROM CO2

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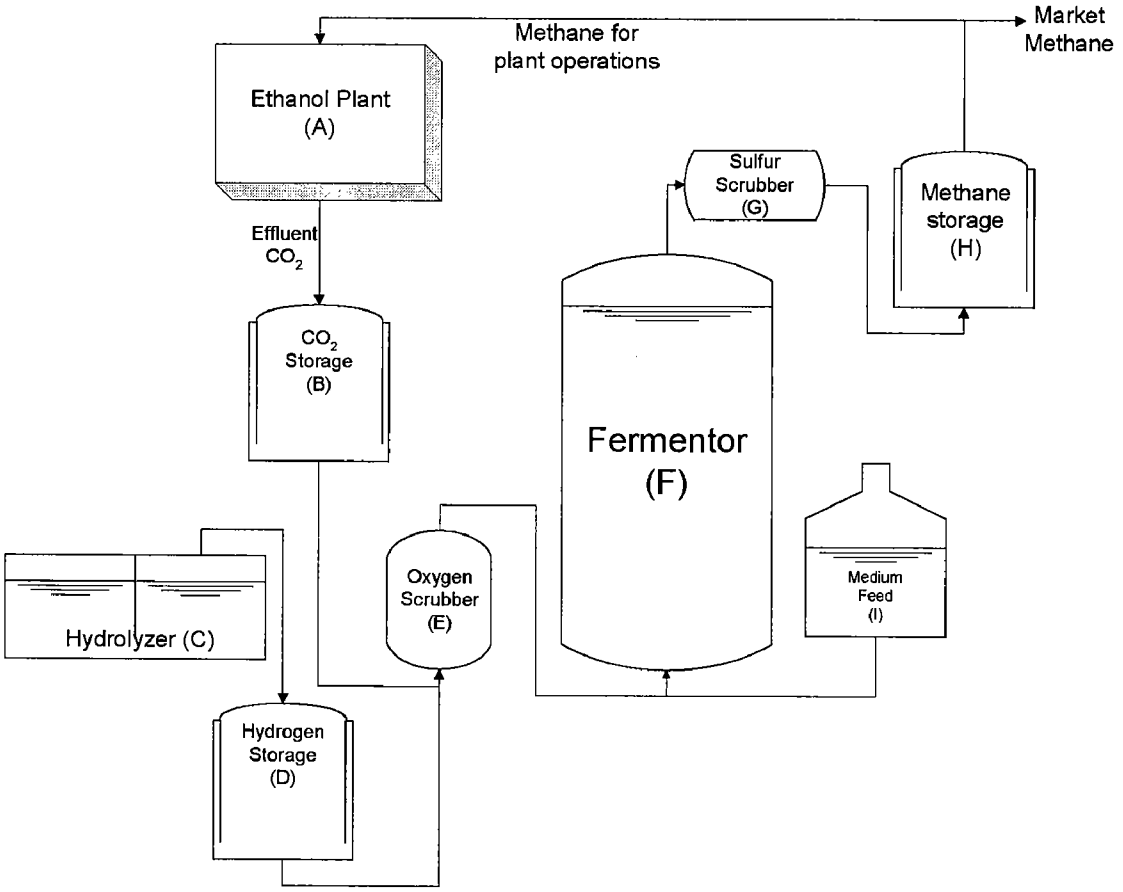
(52) U.S. Cl. **435/167**; 435/300.1

(57) **ABSTRACT**

A method of converting CO_2 gas produced during industrial processes comprising contacting methanogenic archaea with the CO_2 gas under suitable conditions to produce methane.



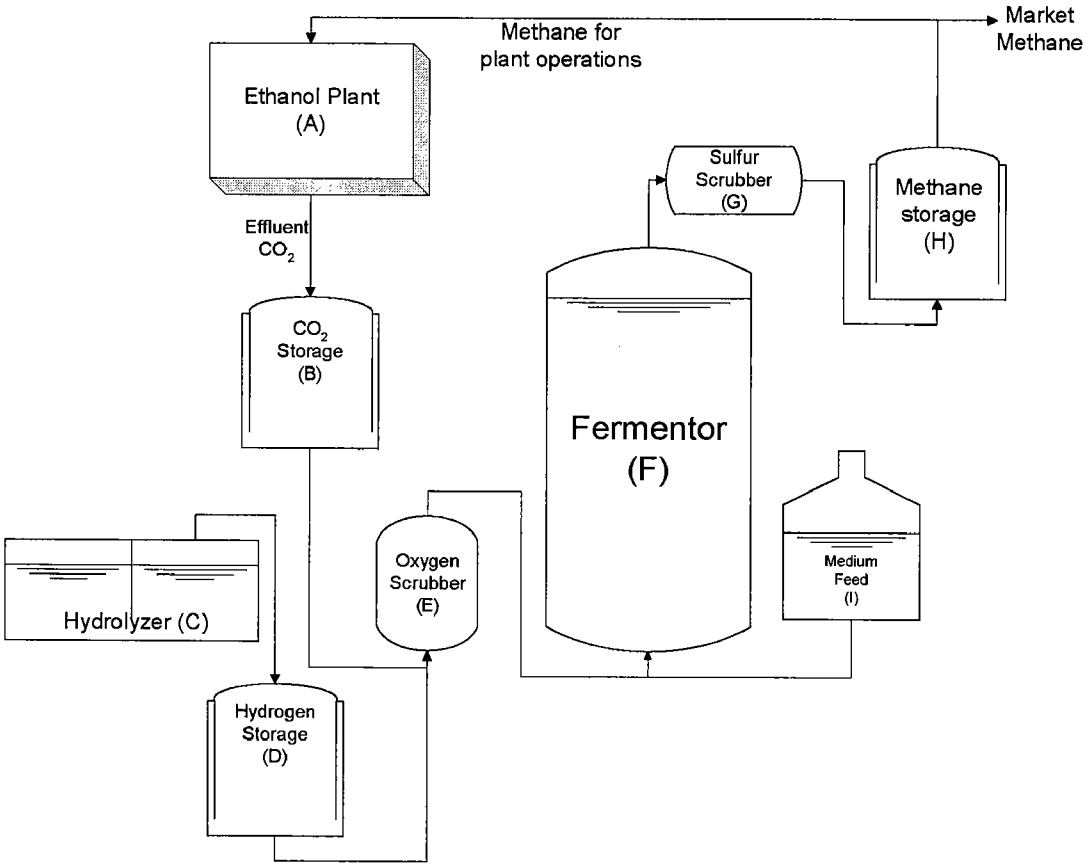

Methane for
plant operations
Market
Methane
Ethanol Plant
(A)
Sulfur
Scrubber
(G)
Methane
storage
(H)
Effluent
CO_2
CO_2
Storage
(B)
Fermentor
(F)
Oxygen
Scrubber
(E)
Medium
Feed
(I)
Hydrolyzer (C)
Hydrogen
Storage
(D)

FIG. 1

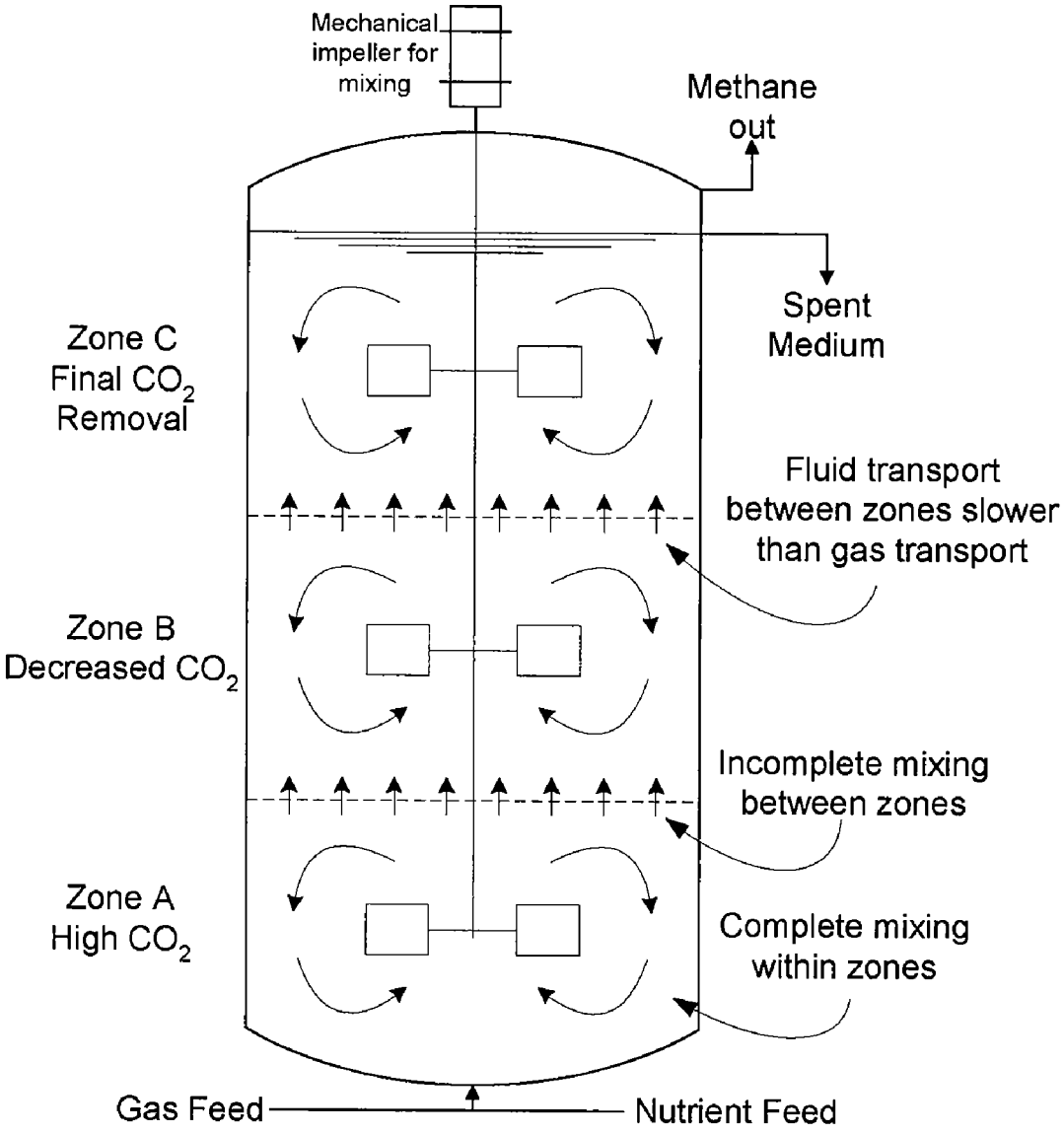

Mechanical impeller for mixing
Methane out
Spent Medium
Zone C Final CO_2 Removal
Fluid transport between zones slower than gas transport
Zone B Decreased CO_2
Incomplete mixing between zones
Zone A High CO_2
Complete mixing within zones
Gas Feed
Nutrient Feed

FIG. 2

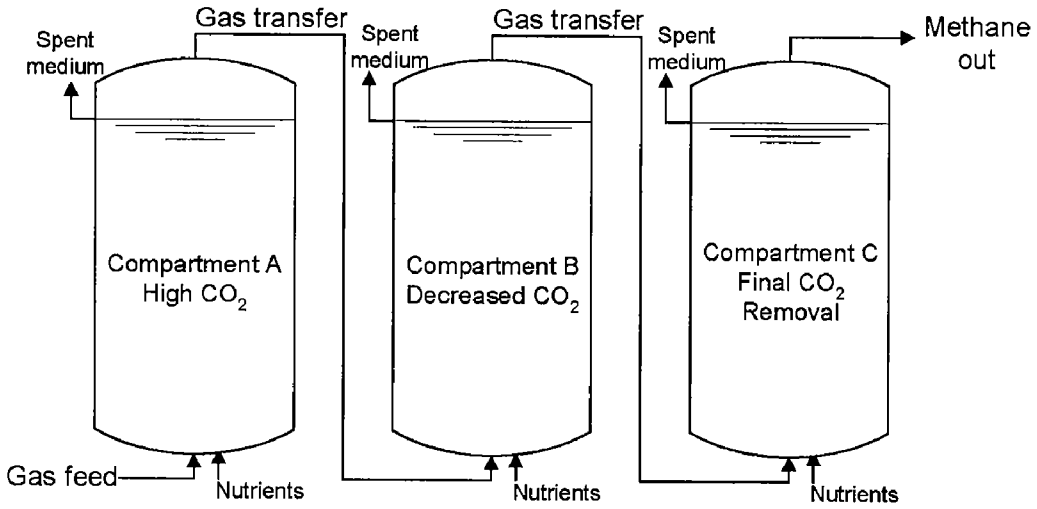

Gas transfer
Spent medium
Gas transfer
Spent medium
Spent medium
Methane out
Compartment A High CO_2
Compartment B Decreased CO_2
Compartment C Final CO_2 Removal
Gas feed
Nutrients
Nutrients
Nutrients

FIG. 3

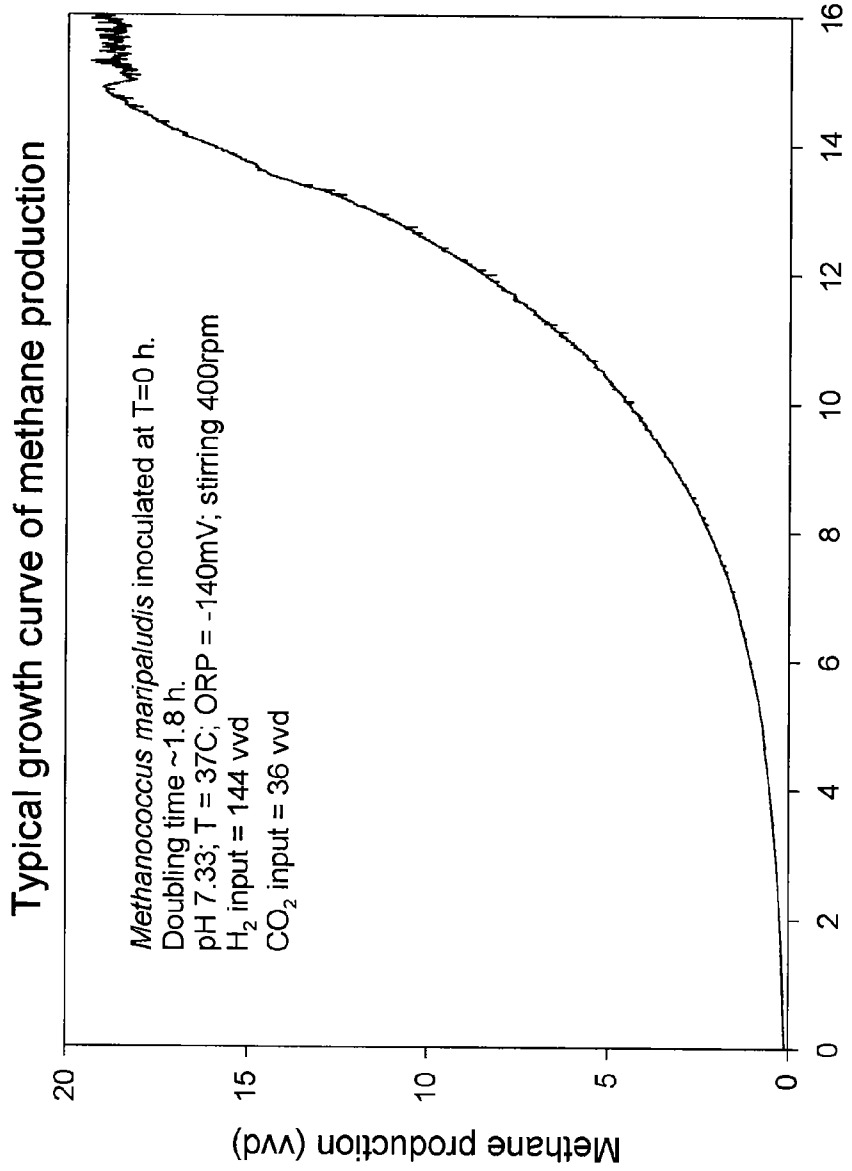

Typical growth curve of methane production
Methanococcus maripaludis inoculated at T=0 h.
Doubling time ~1.8 h.
pH 7.33; T = 37C; ORP = -140mV; stirring 400rpm
H_2 input = 144 vvd
CO_2 input = 36 vvd
Methane production (vvd)
0
5
10
15
20
0
2
4
6
8
10
12
14
16
time (h)

FIG. 4

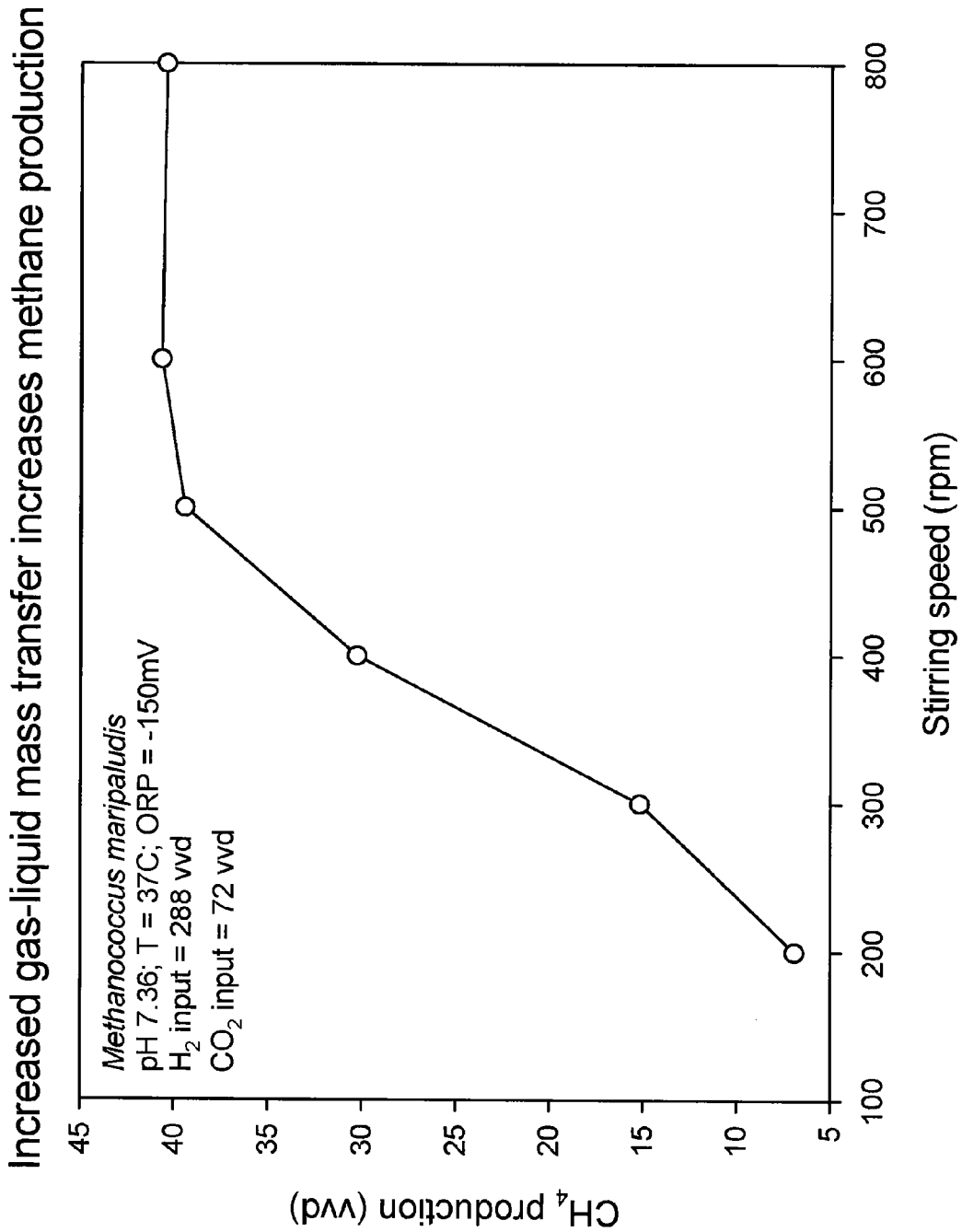

Increased gas-liquid mass transfer increases methane production
Methanococcus maripaludis
pH 7.36; T = 37C; ORP = -150mV
H2 input = 288 vvd
CO2 input = 72 vvd
CH4 production (vvd)
45
40
35
30
25
20
15
10
5
100
200
300
400
500
600
700
800
Stirring speed (rpm)

FIG. 5

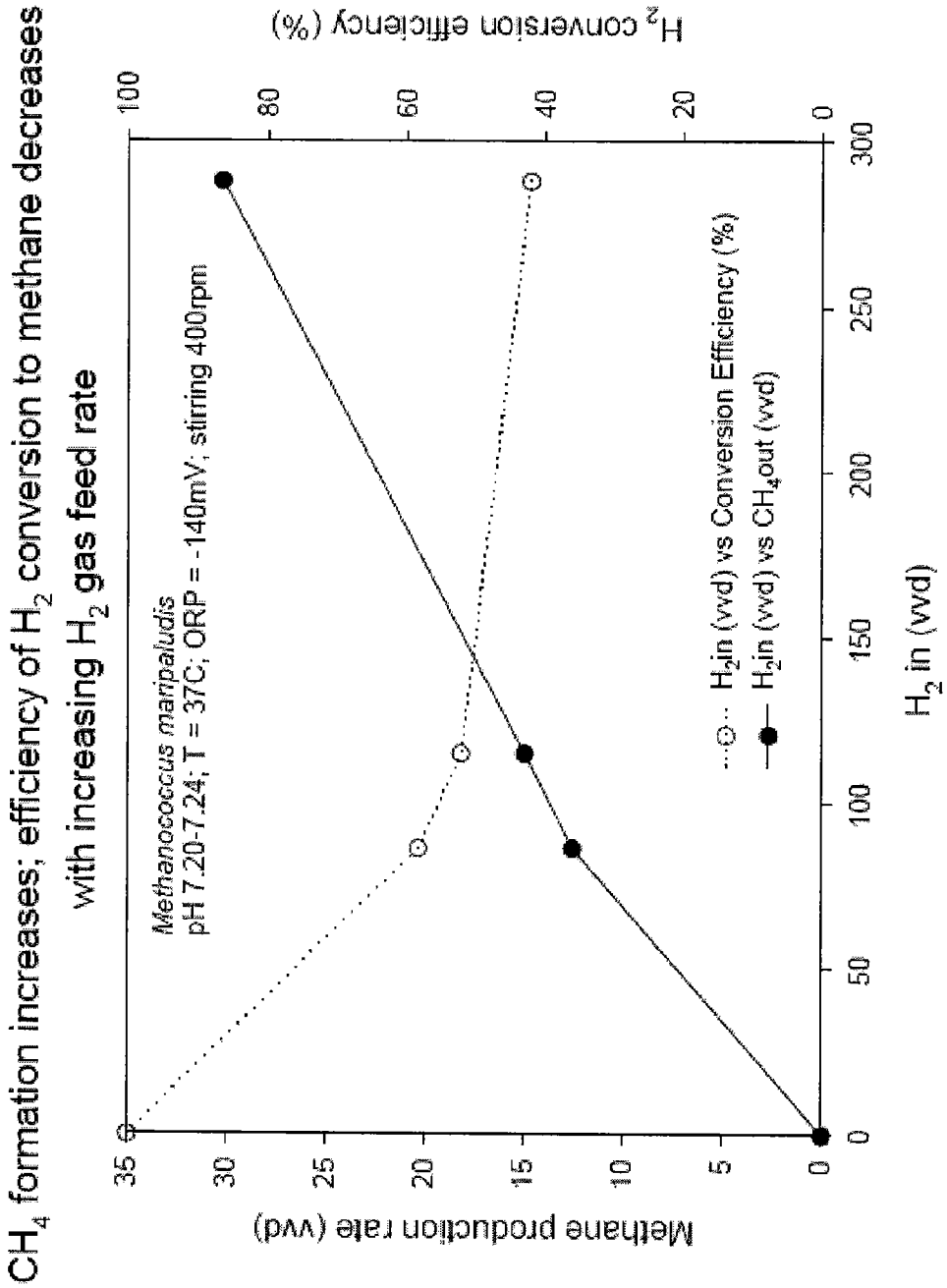

CH_4 formation increases; efficiency of H_2 conversion to methane decreases with increasing H_2 gas feed rate
Methanococcus maripaludis
pH 7.20-7.24; T = 37C; ORP = -140mV; stirring 400rpm
Methane production rate (vvd)
H_2 conversion efficiency (%)
H_2 in (vvd)
H_2in (vvd) vs Conversion Efficiency (%)
H_2in (vvd) vs CH_4out (vvd)
0
5
10
15
20
25
30
35
0
20
40
60
80
100
0
50
100
150
200
250
300

FIG. 6

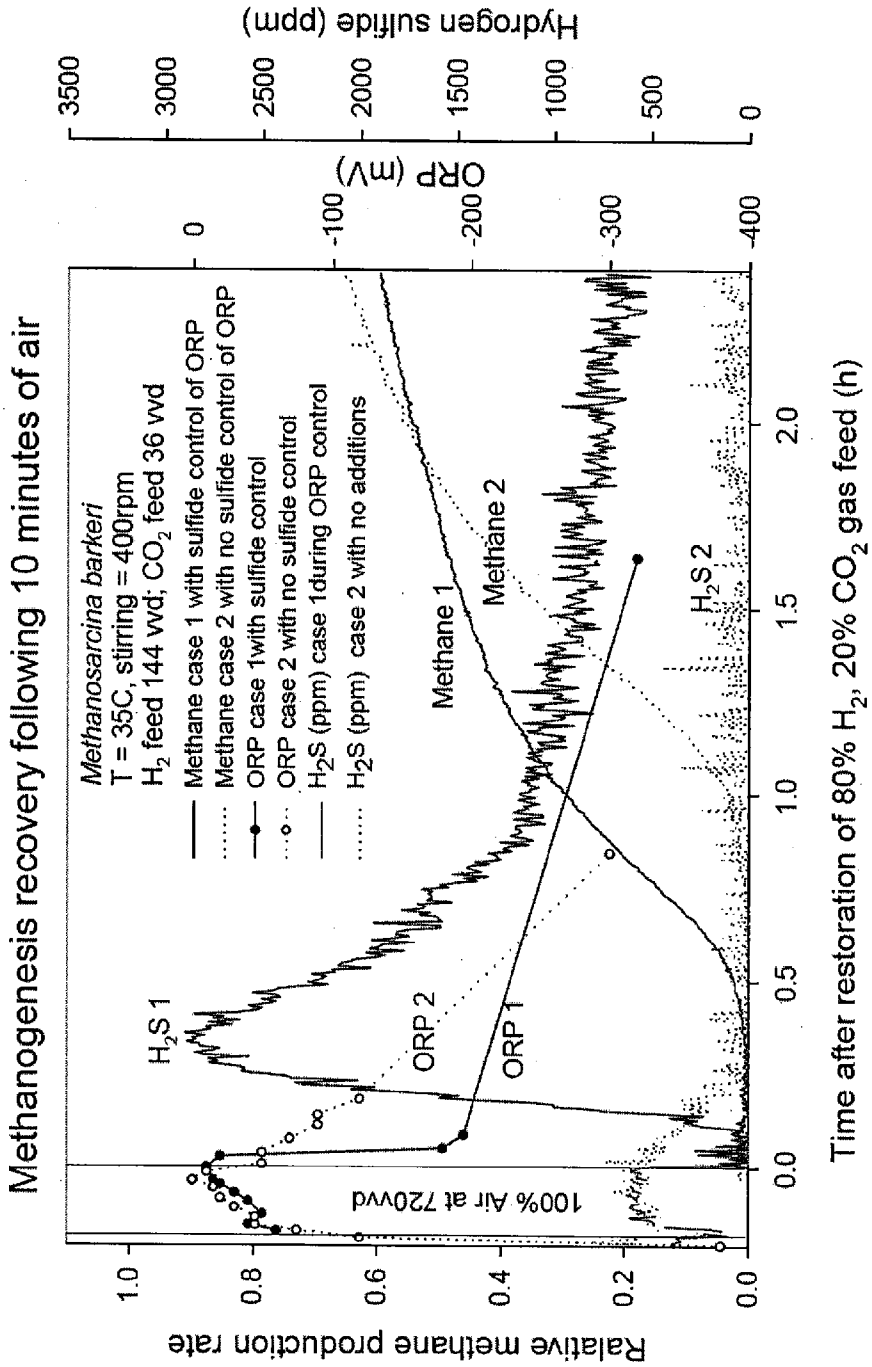

Methanogenesis recovery following 10 minutes of air
Methanosarcina barkeri
T = 35C, stirring = 400rpm
H2 feed 144 vvd; CO2 feed 36 vvd
Methane case 1 with sulfide control of ORP
Methane case 2 with no sulfide control of ORP
ORP case 1with sulfide control
ORP case 2 with no sulfide control
H2S (ppm) case 1during ORP control
H2S (ppm) case 2 with no additions
H2S 1
ORP 2
ORP 1
Methane 1
Methane 2
H2S 2
100% Air at 720vvd
Relative methane production rate
ORP (mV)
Hydrogen sulfide (ppm)
Time after restoration of 80% H2, 20% CO2 gas feed (h)

FIG. 7

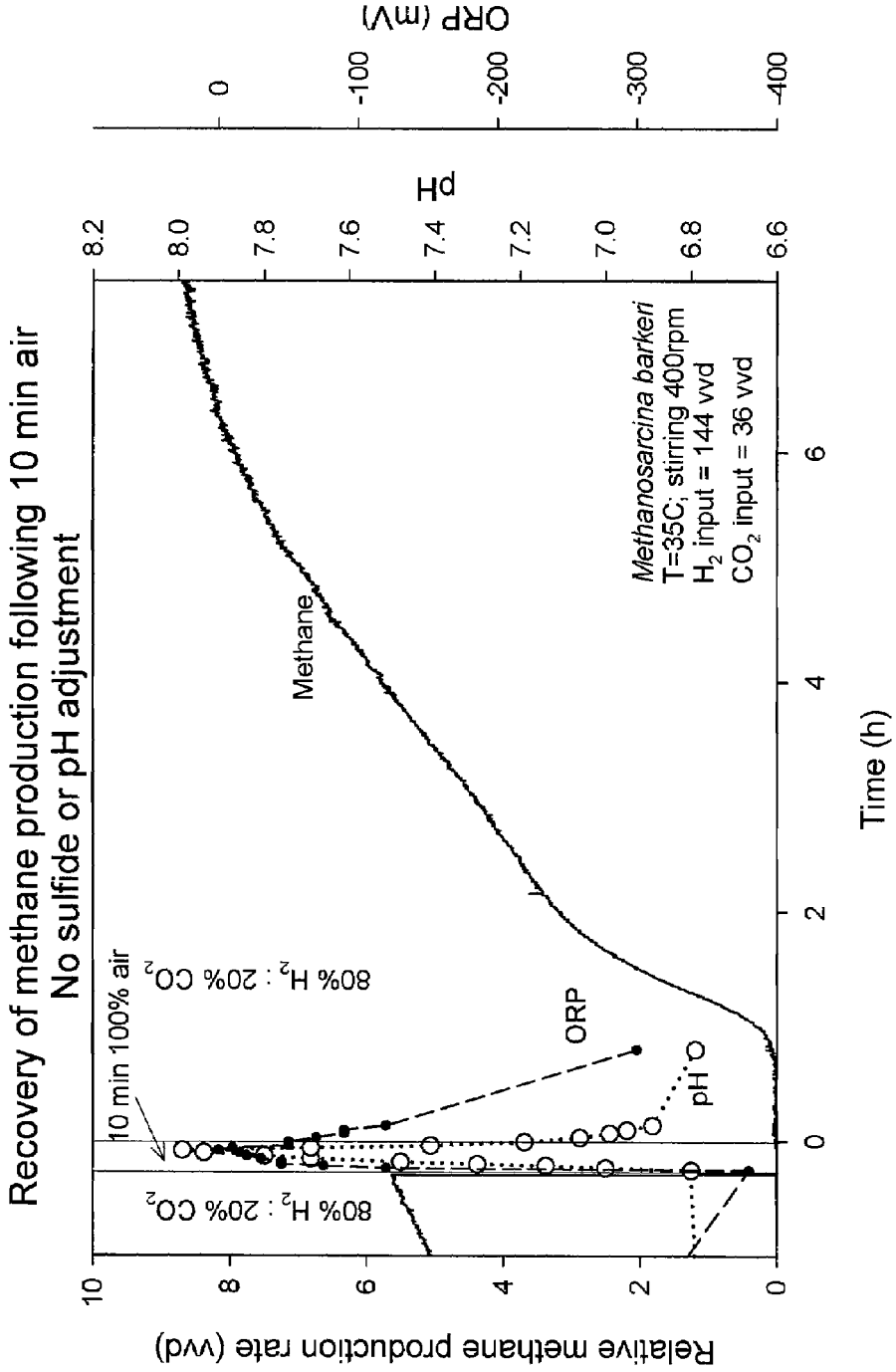

Recovery of methane production following 10 min air
No sulfide or pH adjustment
10 min 100% air
80% H2 : 20% CO2
80% H2 : 20% CO2
Methane
ORP
pH
Methanosarcina barkeri
T=35C; stirring 400rpm
H2 input = 144 vvd
CO2 input = 36 vvd
Relative methane production rate (vvd)
pH
ORP (mV)
Time (h)
0
2
4
6
10
8
6
4
2
0
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
0
-100
-200
-300
-400

FIG. 8

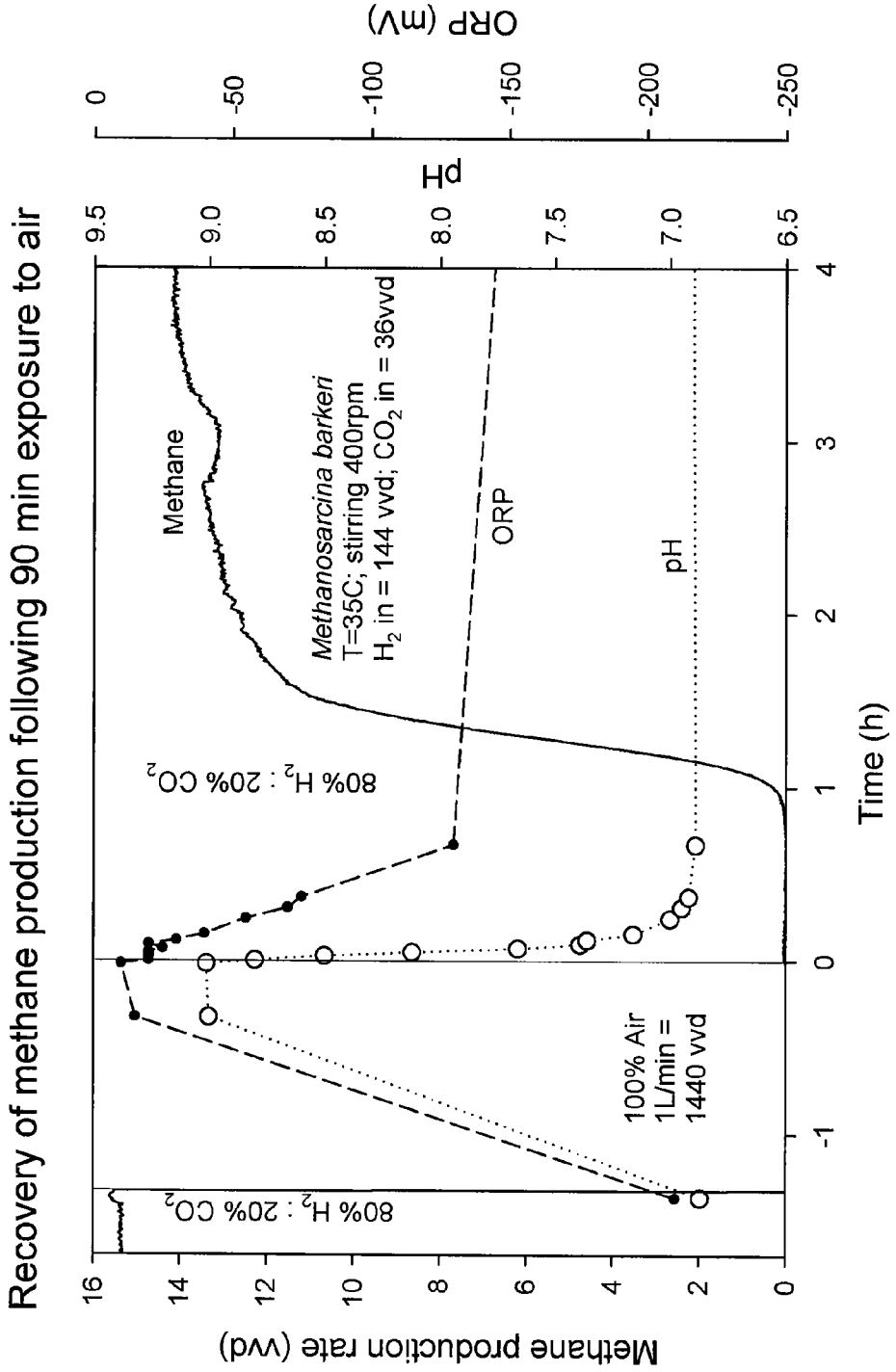

Recovery of methane production following 90 min exposure to air
Methane production rate (vvd)
16
14
12
10
8
6
4
2
0
80% H2 : 20% CO2
Methane
Methanosarcina barkeri
T=35C; stirring 400rpm
H2 in = 144 vvd; CO2 in = 36vvd
ORP
pH
100% Air
1L/min =
1440 vvd
80% H2 : 20% CO2
-1
0
1
2
3
4
Time (h)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
pH
0
-50
-100
-150
-200
-250
ORP (mV)

FIG. 9

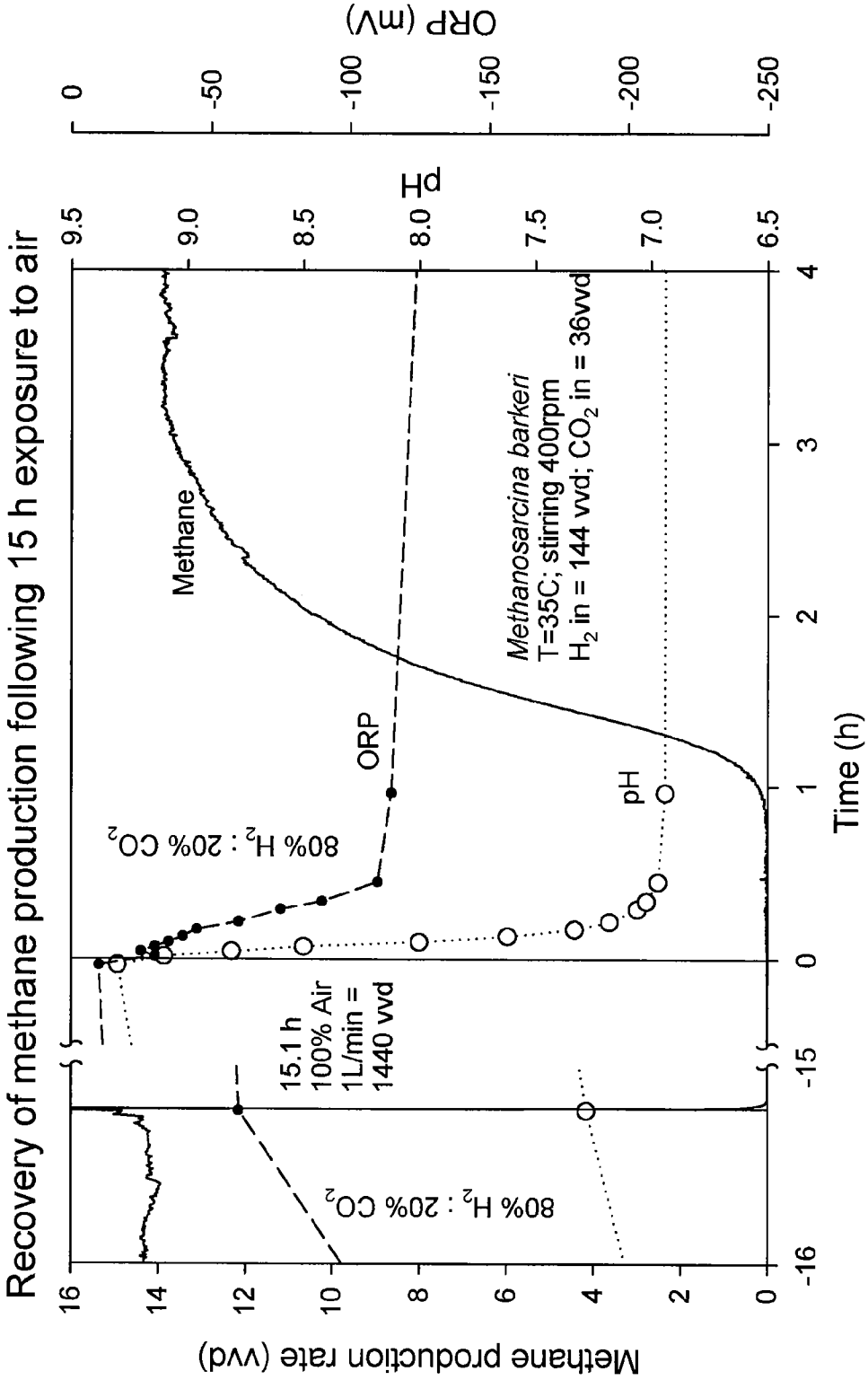

Recovery of methane production following 15 h exposure to air
Methane production rate (vvd)
16
14
12
10
8
6
4
2
0
-16
-15
0
1
2
3
4
Time (h)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
pH
0
-50
-100
-150
-200
-250
ORP (mV)
Methane
ORP
pH
80% H2 : 20% CO2
80% H2 : 20% CO2
15.1 h
100% Air
1L/min =
1440 vvd
Methanosarcina barkeri
T=35C; stirring 400rpm
H2 in = 144 vvd; CO2 in = 36vvd

FIG. 10

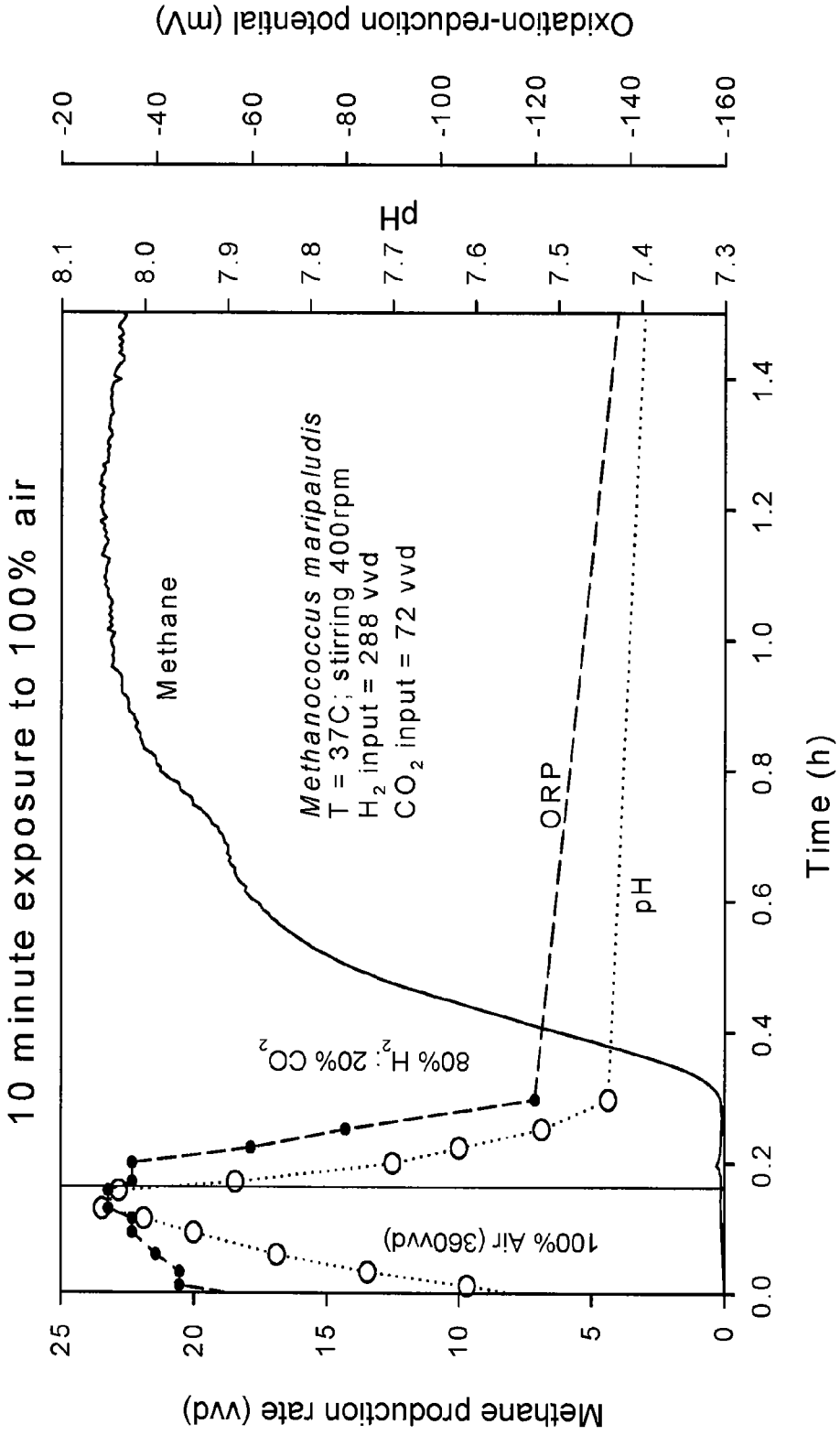

10 minute exposure to 100% air
Methane
Methanococcus maripaludis
T = 37C; stirring 400rpm
H2 input = 288 vvd
CO2 input = 72 vvd
ORP
pH
80% H2: 20% CO2
100% Air (360vvd)
Methane production rate (vvd)
pH
Oxidation-reduction potential (mV)
Time (h)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
5
10
15
20
25
7.3
7.4
7.5
7.6
7.7
7.8
7.9
8.0
8.1
-20
-40
-60
-80
-100
-120
-140
-160

FIG. 11

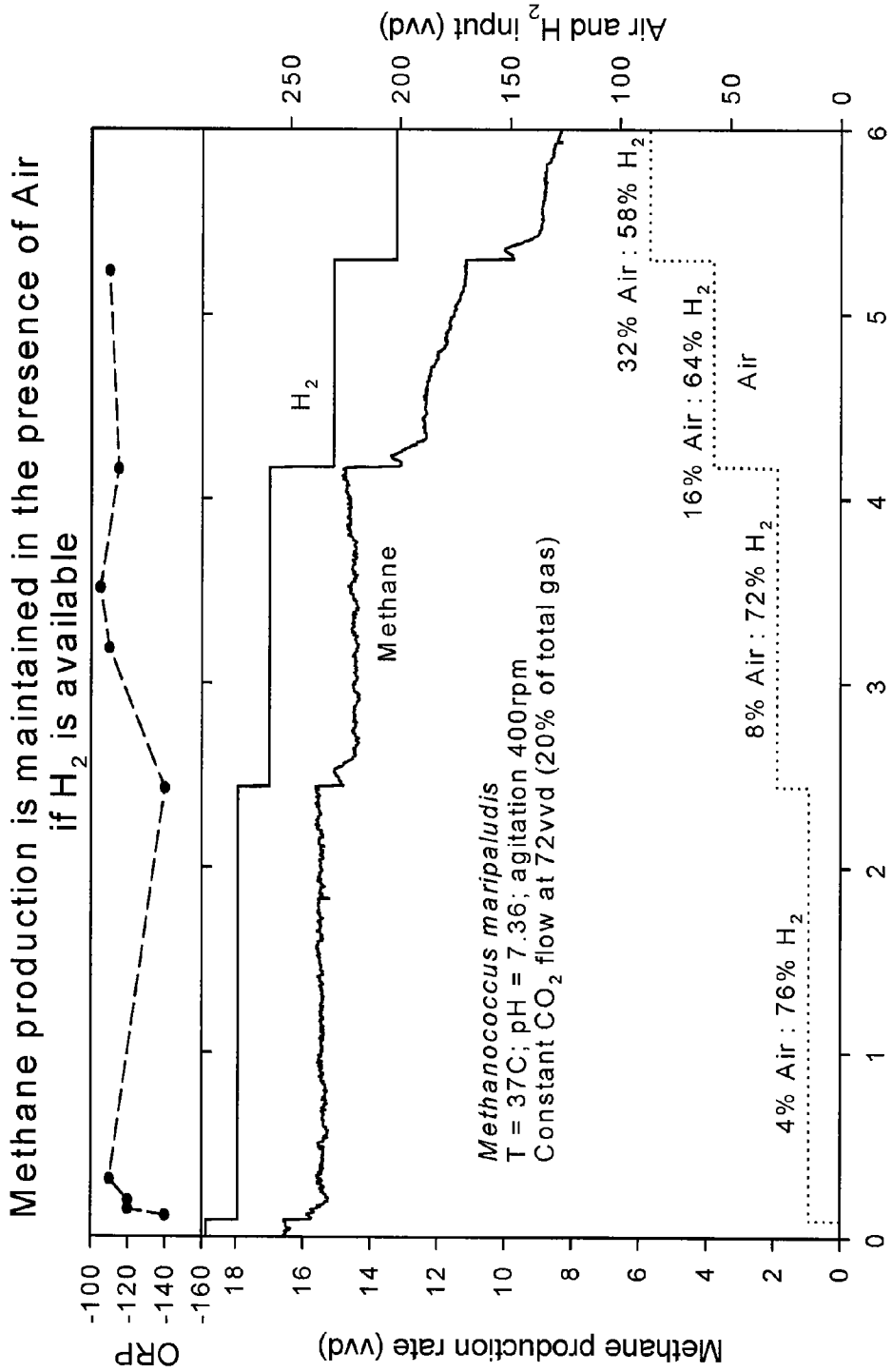

Methane production is maintained in the presence of Air if H_2 is available
ORP
-100
-120
-140
-160
Methane production rate (vvd)
18
16
14
12
10
8
6
4
2
0
Air and H_2 input (vvd)
250
200
150
100
50
0
H_2
Methane
Methanococcus maripaludis
T = 37C; pH = 7.36; agitation 400rpm
Constant CO_2 flow at 72vvd (20% of total gas)
4% Air : 76% H_2
8% Air : 72% H_2
16% Air : 64% H_2
32% Air : 58% H_2
Air
0
1
2
3
4
5
6
Time (h)

FIG. 12

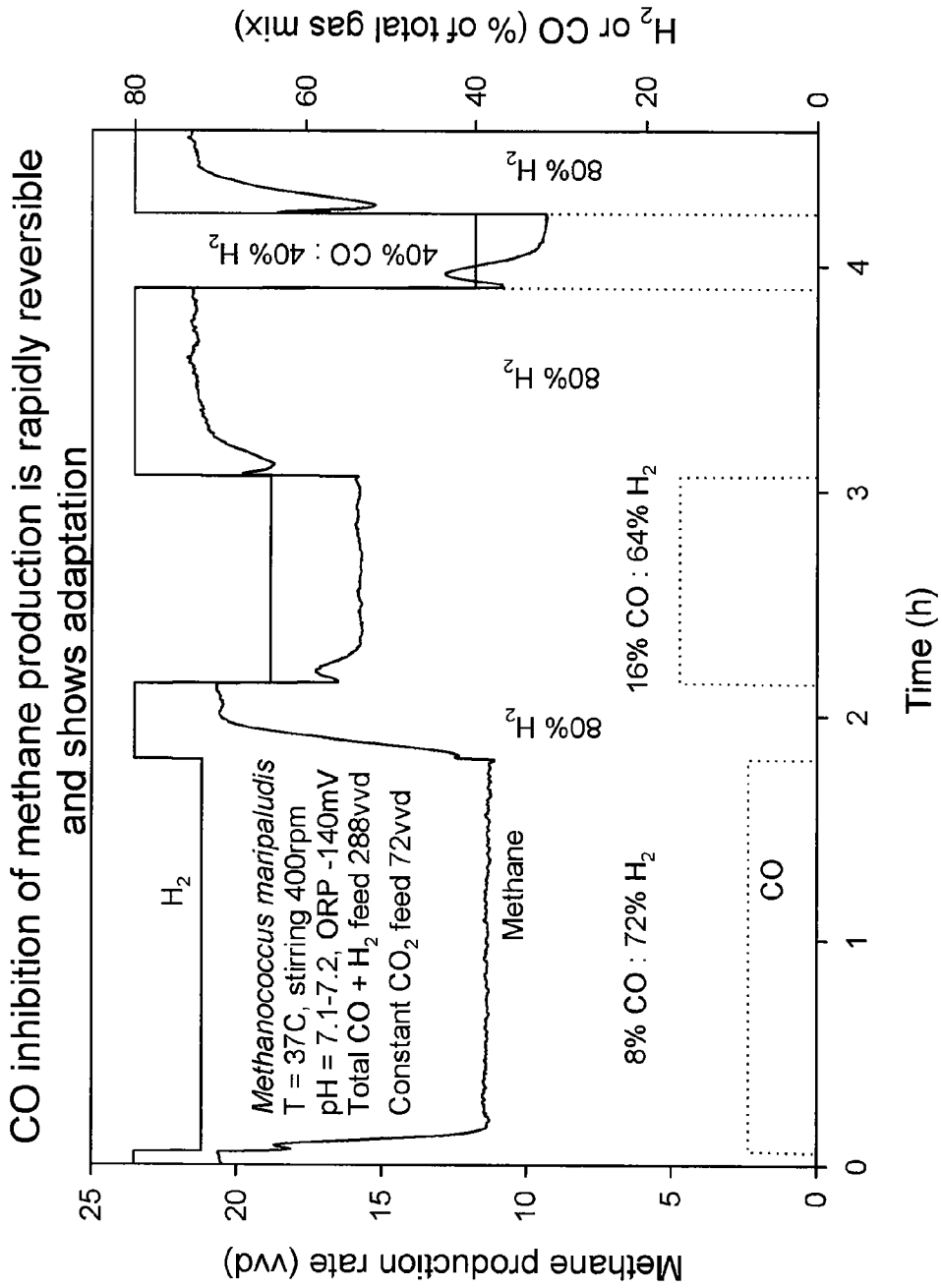

CO inhibition of methane production is rapidly reversible and shows adaptation
Methanococcus maripaludis
T = 37C, stirring 400rpm
pH = 7.1-7.2, ORP -140mV
Total CO + H2 feed 288vvd
Constant CO2 feed 72vvd
H2
Methane
CO
8% CO : 72% H2
16% CO : 64% H2
80% H2
80% H2
40% CO : 40% H2
80% H2
Methane production rate (vvd)
H2 or CO (% of total gas mix)
Time (h)
0
5
10
15
20
25
0
20
40
60
80
0
1
2
3
4

FIG. 13

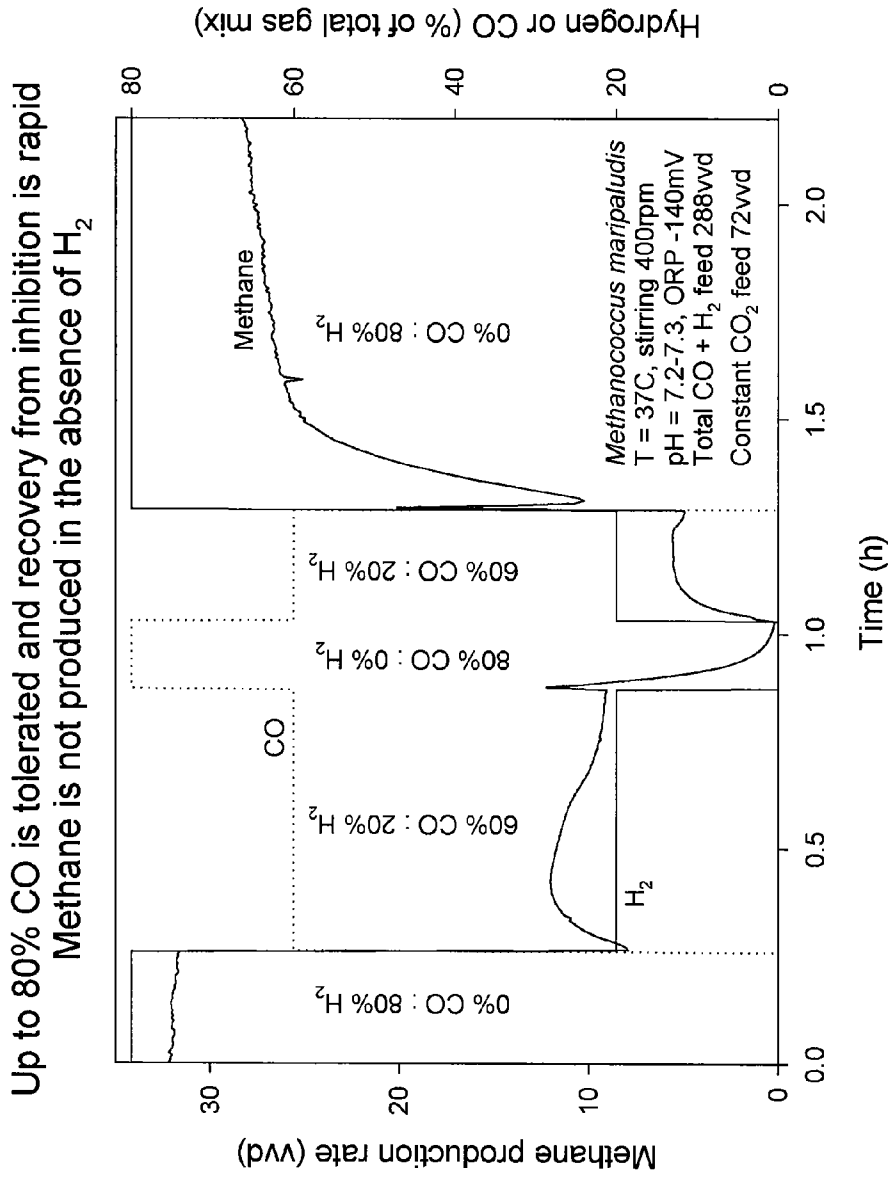

Up to 80% CO is tolerated and recovery from inhibition is rapid
Methane is not produced in the absence of H_2
Methane production rate (vvd)
Hydrogen or CO (% of total gas mix)
Time (h)
0% CO : 80% H_2
60% CO : 20% H_2
80% CO : 0% H_2
60% CO : 20% H_2
0% CO : 80% H_2
CO
H_2
Methane
Methanococcus maripaludis
T = 37C, stirring 400rpm
pH = 7.2-7.3, ORP -140mV
Total CO + H_2 feed 288vvd
Constant CO_2 feed 72vvd
0
10
20
30
0.0
0.5
1.0
1.5
2.0
0
20
40
60
80

FIG. 14

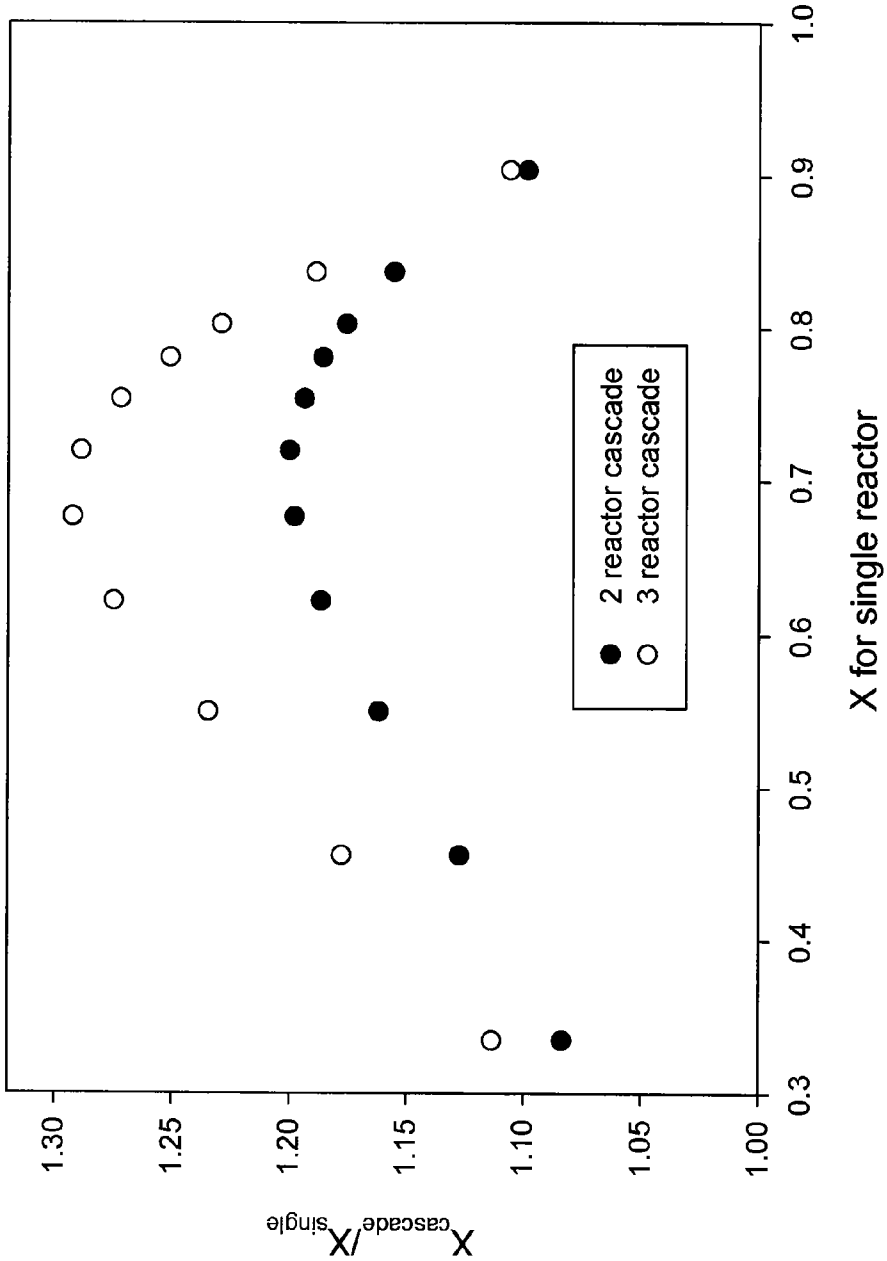

1.30
1.25
1.20
1.15
1.10
1.05
1.00
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
X for single reactor
$X_{cascade}/X_{single}$
2 reactor cascade
3 reactor cascade

FIG. 15

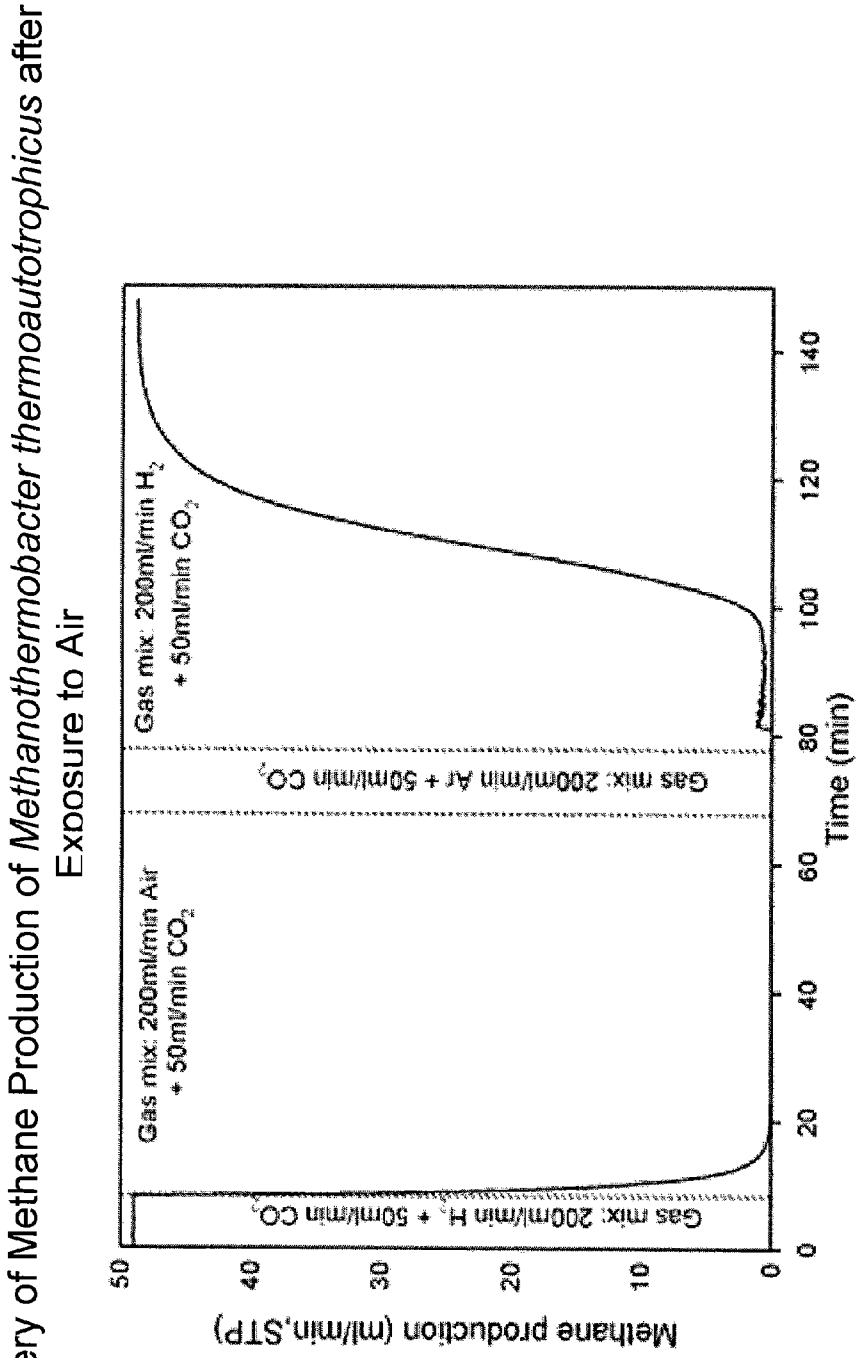

Recovery of Methane Production of *Methanothermobacter thermoautotrophicus* after Exposure to Air
Gas mix: 200ml/min Air + 50ml/min CO_2
Gas mix: 200ml/min H_2 + 50ml/min CO_2
Gas mix: 200ml/min Ar + 50ml/min CO_2
Gas mix: 200ml/min H_2 + 50ml/min CO_2
Methane production (ml/min,STP)
Time (min)
0
10
20
30
40
50
0
20
40
60
80
100
120
140

FIG. 16

SYSTEM FOR THE PRODUCTION OF METHANE FROM CO2

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part under 35 U.S.C. 1.111 of International Application No. PCT/US2007/071138, filed Jun. 13, 2007, which claims the benefit of priority to U.S. Provisional Application No. 60/813,020, filed Jun. 13, 2006. This application also claims the benefit of priority to U.S. Provisional Application No. 61/028,413, filed Feb. 13, 2008, all of which are incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

[0002] Energy self-sufficiency and sustainable energy systems with lower environmental impacts are critical national goals. Increased use of biomass-derived ethanol as a fuel is advantageous because it uses solar energy, rather than fossil fuel energy, as a portion of its energy input and CO_2 obtained by photosynthesis from the environment as a portion of its material requirement for energy carriers. At present ethanol production from corn requires significant energy input from fossil fuels for distillation of the final product and for drying of fermentation residues for use in animal feed. Present domestic ethanol production methods, therefore, are not energetically or economically competitive with ethanol produced abroad from sugar cane. In addition, one third of the carbon in the corn starch is released as a concentrated CO_2 stream during ethanol production. The U.S. Department of Energy has identified that increasing the energy efficiency and reducing the CO_2 emissions of the fuel ethanol production process is essential for increasing the role of ethanol in meeting our energy needs. Currently, fuel ethanol production relies on federal subsidies for its economic viability. Therefore, it will be important to achieve greater economic efficiency in the ethanol production process if the industry is to be viable and self-sustaining.

[0003] The present invention provides a system that reduces the CO_2 emissions from industrial processes, including ethanol production, by using a bioreactor system that uses the emissions to produce methane (natural gas).

SUMMARY OF THE INVENTION

[0004] The present invention provides a system that converts the CO_2 into methane (natural gas). The present invention utilizes CO_2 produced by industrial processes. Examples of processes that that produce CO_2 are biomass fermentation to produce liquid fuels and coal and biomass gasification processes. Gasification is a process that converts carbonaceous materials, such as coal, petroleum, petroleum coke or biomass (living or dead biological material), into carbon monoxide, hydrogen and carbon dioxide. In the system of the present invention, CO_2 industrial waste-gas streams, such as those formed during the production of ethanol or those produced by combined cycle coal fired energy plants, is combined with hydrogen and undergoes a microbial fermentation process catalyzed by methanogenic archaea, producing methane and water. Hydrogen gas may be produced from a variety of sources. In one embodiment, inexpensive electric power can be used to produce hydrogen from water via electrolysis. The integrated electrolysis/methane fermentation system can be viewed as converting an intermittent energy source (e.g. inexpensive off-peak electricity from power plants) to a stable chemical energy store, using hydrogen as an intermediate and methane as the final energy carrier.

[0005] The present invention uses a bioreactor containing methanogenic archaea to catalyze the following chemical reaction:

$$CO_2+4H_2 \rightarrow CH_4+2H_2O$$

[0006] This reaction occurs with high efficiency with >95% conversion of CO_2 to methane at moderate temperatures. Suitably the bioreactor conditions will allow a reaction vessel that is 1/10 or less the volume of the ethanol fermentation system to handle all of the CO_2 stream.

[0007] In one embodiment, the present invention provides a method of converting carbon dioxide produced during an industrial process to methane comprising contacting a culture comprising methanogenic archaea with H_2 gas and an output gas from an industrial process comprising CO_2 gas in a bioreactor under suitable conditions to produce methane. The industrial process can be coal gasification, biomass gasification, or liquid fuel production by biomass fermentation, suitably ethanol production from a biomass such as corn.

[0008] Any suitable methanogenic archaea can be used, and a suitable temperature and pressure for the bioreactor condition can be selected depending at least in part on the methanogenic archaea selected. In some embodiments, suitably pressures within the bioreactor range from about 0.5 atmospheres to about 500 atmospheres. The bioreactor can also contain a source of intermittent agitation of the culture.

[0009] The culture conditions should suitably maintaining a redox potential of about −100 mV or less. In one embodiment, this redox potential is maintained by supplying a suitable amount of hydrogen gas.

[0010] Also in one embodiment, the methane gas removed from the bioreactor suitably comprises less than about 450 ppm hydrogen sulfide, or alternatively less than about 400 ppm, 300 ppm, 200 ppm, 150 ppm, 100 ppm, 50 ppm or 20 ppm of hydrogen sulfide.

[0011] Further, in certain embodiments the industrial output gas at least intermittently further comprises air and/or carbon monoxide. Suitably the industrial output gas comprises about 32% or less air by volume, or between from about 0.1% to about 32% air by volume, or less than about 4% air by volume, or at least about 4%, 8% or 16% air by volume. Suitably the industrial output gas can also comprise less than about 40% carbon monoxide by volume, or less than about 8% carbon monoxide by volume, or at least about 8% or 16% carbon monoxide by volume.

[0012] An another embodiment, the bioreactor comprises a culture of methanogenic archaea, a source of an output gas from an industrial process comprising CO_2 that feeds into the bioreactor, a source of hydrogen gas that feeds into the bioreactor, a gas feed from the bioreactor for removing gas from the bioreactor, a feed for providing fresh medium, and a feed for removing the culture.

DRAWINGS

[0013] FIG. **1** shows a design schematic of one embodiment of a CO_2 recapture and methane production plant.

[0014] FIG. **2** shows a design schematic of one embodiment of a stratified bioreactor.

[0015] FIG. **3** shows a design schematic of a system of bioreactors set up in cascaded serial arrangement.

[0016] FIG. **4** is a chart showing the growth curve of methane production of *Methanococcus maripaludis.*

[0017] FIG. **5** is a chart demonstrating the effects of agitation of a culture of *Methanococcus maripaludis* with respect to methane production.

[0018] FIG. **6** is a chart showing the changes in hydrogen conversion to methane catalyzed by *Methanococcus maripaludis* with respect to changes in the feed rate hydrogen gas into the bioreactor.

[0019] FIG. **7** is a chart showing the recovery of methanogenesis in a culture *Methanosarcina barkeri* after exposure to 10 minutes of 100% air.

[0020] FIG. **8** is a chart showing the recovery of methanogenesis in a culture *Methanosarcina barkeri* after exposure to 10 minutes of 100% air.

[0021] FIG. **9** is a chart showing the recovery of methanogenesis in a culture *Methanosarcina barkeri* after exposure to 90 minutes of 100% air.

[0022] FIG. **10** is a chart showing the recovery of methanogenesis in a culture *Methanosarcina barkeri* after exposure to 15 hours of 100% air.

[0023] FIG. **11** is a chart showing the recovery of methanogenesis in a culture *Methanococcus maripaludis* after exposure to 10 minutes of 100% air.

[0024] FIG. **12** is a chart showing the recovery of methanogenesis in a culture *Methanococcus maripaludis* during exposure of a mixture of air and hydrogen gas.

[0025] FIG. **13** is a chart showing the recovery of methanogenesis in a culture *Methanococcus maripaludis* during exposure of a mixture of carbon monoxide and hydrogen gas.

[0026] FIG. **14** is a chart showing the recovery of methanogenesis in a culture *Methanococcus maripaludis* during exposure of a mixture of carbon monoxide and hydrogen gas.

[0027] FIG. **15** is a chart showing a projected model of the extent of the conversion of hydrogen to methane in a two and three reactor cascade (Xcascade) divided by the extent of the conversion of hydrogen to methane in an equivalent total volume single reactor (Xsingle), the ratio plotted against Xsingle. Each of the single reactor cascade, 2 reactor cascade and 3 reactor cascade have the same total volume and same hydrogen input rate.

[0028] FIG. **16** is a chart showing the recovery of *Methanothermobacter thermoautotrophicus* after exposure to air.

DETAILED DESCRIPTION OF THE INVENTION

[0029] The present invention comprises a bioreactor system that can be integrated with industrial processes that produce CO_2 gas as a byproduct. In one embodiment such a process is the production of ethanol from biomass. The invention comprises a bioreactor containing a microbial culture capable of hydrogenotrophic methanogenesis (i.e. the conversion of CO_2 gas plus hydrogen gas to methane gas). The bioreactor is coupled to a hydrogen source and a CO_2 gas source. Suitably the CO_2 gas source is the CO_2 gas stream that is emitted by the production of ethanol. The hydrogen source is suitably hydrogen produced by the electrolysis of water. Suitably this hydrolysis is powered by electricity used in off peak times. The methane produced by the system can be fed back into the ethanol production facility to power various processes, and/or can be stored and sold as fuel.

[0030] Microbial cultures suitable for practice of the invention are readily obtainable from public collections of organisms or can be isolated from a variety of environmental sources. Such environmental sources include anaerobic soils and sands, bogs, swamps, marshes, estuaries, dense algal mats, both terrestrial and marine mud and sediments, deep ocean and deep well sites, sewage and organic waste sites and treatment facilities, and animal intestinal tracts and feces. Many pure cultures of single species are suitable. Classified pure cultures are all members of the Archaeal domain [Woese et al. Proc Natl Acad Sci USA 87:4576-4579 (1990) "Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucharya.", incorporated herein by reference] and fall within 4 different classes of the Euryarchaea kingdom. Examples of suitable organisms have been classified into 4 different genera within the Methanobacteria class (e.g. *Methanobacterium alcaliphilum, Methanobacterium bryantii, Methanobacterium congolense, Methanobacterium defluvii, Methanobacterium espanolae, Methanobacterium formicicum, Methanobacterium ivanovii, Methanobacterium palustre, Methanobacterium thermaggregans, Methanobacterium uliginosum, Methanobrevibacter acididurans, Methanobrevibacter arboriphilicus, Methanobrevibacter gottschalkii, Methanobrevibacter olleyae, Methanobrevibacter rum inantium, Methanobrevibacter smithii, Methanobrevibacter woesei, Methanobrevibacter wolinii, Methanothermobacter marburgensis, Methanothermobacter thermautotrophicum* (also known as *Methanothermobacter thermoautotroiphicus*), *Methanothermobacter thermoflexus, Methanothermobacter thermophilus, Methanothermobacter wolfeii, Methanothermus sociabilis*), 5 different genera within the Methanomicrobia class (e.g. *Methanocorpusculum bavaricum, Methanocorpusculum parvum, Methanoculleus chikuoensis, Methanoculleus submarinus, Methanogenium frigidum, Methanogenium liminatans, Methanogenium marinum, Methanosarcina acetivorans, Methanosarcina barkeri, Methanosarcina mazei, Methanosarcina thermophila, Methanomicrobium mobile*), 7 different genera within the Methanococci class (e.g. *Methanocaldococcus jannaschii, Methanococcus aeolicus, Methanococcus maripaludis, Methanococcus vannielii, Methanococcus voltaei, Methanothermococcus thermolithotrophicus, Methanocaldococcus fervens, Methanocaldococcus indicus, Methanocaldococcus infernus, Methanocaldococcus vulcanius*), and one genus within the Methanopyri class (e.g. *Methanopyrus kandleri*). Suitable cultures are available from public culture collections (e.g. the American Type Culture Collection, the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, and the Oregon Collection of Methanogens). Many suitable hydrogenotrophic methanogens, isolated in pure culture and available in public culture collections, have not yet been fully classified. Preferred pure culture organisms include *Methanosarcinia barkeri, Methanococcus maripaludis*, and *Methanothermobacter thermoautotrophicus.*

[0031] Suitable cultures of mixtures of two or more microbes are also readily isolated from the specified environmental sources [Bryant et al. Archiv Microbiol 59:20-31 (1967) "*Methanobacillus omelianskii*, a symbiotic association of two species of bacteria.", incorporated herein by reference]. Suitable mixtures may be consortia in which cells of two or more species are physically associated or they may be syntrophic mixtures in which two or more species cooperate metabolically without physical association. Mixed cultures may have useful properties beyond those available from pure cultures of known hydrogenotrophic methanogens. These properties may include, for instance, resistance to contaminants in the gas feed stream, such as oxygen, ethanol or other

trace components, or aggregated growth, which may increase the culture density and volumetric gas processing capacity of the culture.

[0032] Suitable cultures of mixed organisms may also be obtained by combining cultures isolated from two or more sources. One or more of the species in a suitable mixed culture should be an Archaeal methanogen. Any non-Archael species may be bacterial or eukaryotic.

[0033] Suitable cultures may also be obtained by genetic modification of non-methanogenic organisms in which genes essential for supporting hydrogenotrophic methanogenesis are transferred from a methanogenic microbe or from a combination of microbes that may or may not be methanogenic on their own. Suitable genetic modification may also be obtained by enzymatic or chemical synthesis of the necessary genes.

[0034] The bioreactor system may provide continuous or discontinuous methane production using a continuous hydrogenotrophic methanogenic culture operating under stable conditions. An example of such suitable conditions is set forth below in the examples and is also provided in Schill, N., van Gulik, M., Voisard, D., & von Stockar, U. (1996) Biotechnol & Bioeng 51:645-658. "Continuous cultures limited by a gaseous substrate: development of a simple, unstructured mathematical model and experimental verification with *Methanobacterium thermoautotrophicum*", incorporated herein by reference. Culture media may be comprised of dilute mineral salts, and should be adapted to the particular culture in use.

[0035] The medium should be replenished at a rate suitable to maintain a useful concentration of essential minerals and to eliminate any metabolic products that may inhibit methanogenesis. Dilution rates below 0.1 culture volume per hour are suitable, since they yield high volumetric concentrations of active methane generation capacity. Surprisingly, dilution rates of less than 0.001 volumes was found to provide active methane generating capacity.

[0036] Total gas delivery rates (CO_2 plus H_2) in the range of 0.2 to 4 volume of gas (STP) per volume of culture per minute are suitable, since they both maintain and exploit high volumetric concentrations of active methane generation capacity.

[0037] In one embodiment, the redox potential is maintained below −100 mV or lower during methanogenesis. The method of the present invention encompasses conditions in which the redox potential is transiently increased to above −100 MV, as for example when air is added to the system.

[0038] In the examples below the temperature of the culture was maintained near the optimum for growth of the organism used in the culture (e.g. about 35° C. to about 37° C. for mesophilic organisms such as *Methanosarcinia barkeri* and *Methanococcus maripaludis* or about 60°-65° C. for thermophiles such as *Methanothermobacter thermoautotrophicus*, and about 85° C.-90° C. for organisms such as*Methanocaldococcus jannaschii, Methanocaldococcus fervens, Methanocaldococcus indicus, Methanocaldococcus infernus*, and *Methanocaldococcus vulcanius*.). However, it is envisioned that temperatures above or below the temperatures for optimal growth may be used. In fact, higher conversion rates of methane may be obtained at temperatures above the optimal growth rate temperature.

[0039] In one embodiment of the invention, a reducing agent is introduced into the fermentation process along with CO_2 and hydrogen, this reducing agent can suitably be hydrogen sulfide or sodium sulfide. In one embodiment, a 4:1 mixture of H_2 and CO_2 gases can be provided at a total gassing rate (vvm) of from 0.1 L gas per L culture per minute [L/(L-min)] to >1.0 L/(L-min), with greater than 95% of the CO_2 converted to methane and the rest of the CO_2 in the input being converted to cellular biomass.

[0040] In another embodiment, hydrogen itself can be used as a reductant to maintain the redox potential of the culture in the range (<−100 mV) necessary for optimum performance of hydrogenotrophic methanogenesis. Generally, hydrogen gas is provided in the method in concentrations effective in allowing for at least a portion of the carbon dioxide in the bioreactor to be converted into methane.

[0041] In another embodiment, the redox potential of the culture can be maintained at <−100 mV via an electrochemical cell immersed in the medium.

[0042] In another embodiment, the system comprises various methods and/or features that reduce the presence of oxygen in the CO_2 stream that is fed into the bioreactor. When obligate anaerobic methanogenic microorganisms are used to catalyze methane formation, the presence of oxygen may be detrimental to the performance of the process and contaminates the product gas. Therefore the reduction of the presence of oxygen in the CO_2 stream is helpful for improving the process. In one embodiment, the oxygen level is reduced prior to entry of the gas into the fermentation vessel by passing the mixed H_2/CO_2 stream over a palladium catalyst, which converts any trace oxygen to water. In this embodiment, H_2 is provided in an amount above the amount needed in the culture by a 2:1 ratio relative to the contaminating oxygen. In another embodiment, the oxygen is removed by pre-treatment of the gas stream in a bioreactor. In this embodiment, the reductant may be provided either by provision of a source of organic material (e.g. glucose, starch, cellulose, fermentation residue from an ethanol plant, whey residue, etc.) that can serve as substrate for an oxidative fermentation. The microbial biological catalyst is chosen to oxidatively ferment the chosen organic source, yielding CO_2 from the contaminant oxygen. In this embodiment, additional H_2 would be provided to enable conversion in the anaerobic fermentor of this additional CO_2 to methane. In another embodiment, oxygen removal is accomplished in the main fermentation vessel via a mixed culture of microbes that includes one capable of oxidative fermentation of an added organic source in addition to the hydrogenotrophic methanogen necessary for methane production. An example of a suitable mixed culture was originally isolated as "*Methanobacillus omelianskii*" and is readily obtained from environmental sources [Bryant et al. Archiv Microbiol 59:20-31 (1967) "*Methanobacillus omelianskii*, a symbiotic association of two species of bacteria.", incorporated herein by reference]. In another embodiment, an oxygen tolerant methanogen is used in the bioreactor to improve the stability of the methane formation process in the presence of contaminating oxygen. Both *Methanosarcinia barkeri* and *Methanococcus maripaludis* are sufficiently oxygen tolerant in the presence of contaminating oxygen.

[0043] FIG. **1** depicts one embodiment of a CO_2 recapture and methane production plant using the methods set forth above. An industrial CO_2 source (A)—e.g. fuel ethanol plant—with CO_2 effluent and natural gas demand, vents CO_2 to a CO_2 collection and storage tank (B). A hydrolyzer (C) produces hydrogen, suitably from electrolysis. Hydrogen produce by the hydrolyzer (C) is collected in a hydrogen storage tank (D). The hydrogen and CO_2 from their respective storage tanks are fed through an oxygen scrubber (E) for

removal of oxygen from the CO_2 effluent stream. After passing through the oxygen scrubber (E), the hydrogen and CO_2 are feed into a fermentor/bioreactor system (F) for conversion of CO_2+H_2 to methane. A storage tank providing medium (I) is also connected to the fermentor/bioreactor system (F) to provide for replenishment of nutrients in the fermentor. The methane gas vented from the fermentor/bioreactor (F) passes through a sulfur scrubber (G) for recovering sulfur from the product methane stream. The methane gas can then be stored in a methane storage tank (H).

[0044] A bioreactor, also known as a fermentor vessel, as set forth in the invention is any suitable vessel in which methanogenesis can take place. Suitable bioreactors to be used in the present invention should be sized relative to the volume of the CO_2 source. Typical streams of 2,200,000 lb CO_2/day from a 100,000,000 gal/yr ethanol plant would require a CO_2 recovery/methane production fermentor of about 750,000 gal total capacity. Fermentor vessels similar to the 750,000 gal individual fermentor units installed in such an ethanol plant would be suitable.

[0045] FIG. **2** depicts one embodiment of a stratified bioreactor that can be used in the present invention. In this embodiment, the bioreactor has the CO_2 and hydrogen entering into the bottom of the bioreactor along with the nutrients for the bioreactor. A mechanical impeller is positioned on the top of the bioreactor and is used to move a mixing apparatus within the bioreactor. The bioreactor has three zones, A, B and C. Zone A at the bottom of the reactor is a high CO_2 zone. Zone B, in the middle of the bioreactor has a decreased CO_2 presence, and Zone C at the top end of the reactor has little if any CO_2. The methane produced, and the spent medium is removed from the top of the bioreactor.

[0046] FIG. **3** depicts one embodiment of a cascaded bioreactor that can be used in the present invention. In this embodiment, the hydrogen, CO_2 and cell nutrients are fed into the bottom of a first compartment (A). In this compartment (A), even after fermentation, there is still a high level of CO_2. The gas produced by the fermentation reaction in the first compartment (A) is then transferred from the top of the first compartment to the bottom of a second compartment (B) along with cell nutrients. In this second compartment (B) the CO_2 level is decreased from the levels found in the first compartment (A). The gas produced by the fermentation reaction in the second compartment (B) is transferred from the top of the second compartment (B) to the bottom of a third compartment (C) along with cell nutrients. In this third compartment (C) most if not all of the CO_2 has been removed and only the methane gas is left to be removed from the top of the compartment. In each of the compartments, spent medium can be removed from the compartments.

EXAMPLE 1

General Setup for Bench Scale Bioreactor

[0047] A bench-scale bioreactor was used to test a series of variables important in the design and operation of an industrial scale bioreactor. A 1.3 L fermenter vessel (bioreactor) (BioFlo 110, New Brunswick), fitted with an Ingold autoclavable pH electrode for measuring pH in the medium and a Lazar Labs double junction platinum band autoclavable ORP electrode for measuring the oxidation-reduction potential (ORP) of the medium was used in the following experiments. The bioreactor contained 1 L culture medium and was stirred at 400 rpm with a Rushton impeller. With 1 L of medium, the bioreactor has a headspace of 300 cc of gas. The chamber was also fitted with a peristaltic pump that could control the addition of a chemical reductant, such as Na_2S. A second peristaltic pump controlled the constant addition of fresh culture medium to the vessel to enable continuous culture operation. A third peristaltic pump was used to remove excess liquid from the culture vessel, maintaining a constant volume of 1 L. The excess liquid included the metabolic water generated during methanogenesis as well as increased medium volume from continuous culture operation. The temperature of the culture was controlled by a heating blanket. Gas mixtures were introduced via a sparger at the bottom of the vessel. The composition of the gas mixture was controlled by three mass flow controllers, one for H_2, one for CO_2, and a third that could be used for controlling addition of air, CO, or N_2. Generally, a gas composition of 1 volume CO_2 to 4 volumes of H_2 was used and was passed over a palladium catalyst (Alfa AESAR) prior to introduction to the culture. The culture in the bioreactor was maintained at about 1 atmosphere of pressure. The gas exiting the culture vessel at ambient atmospheric pressure was passed through a condenser at 4° C. to reduce water vapor content. The composition of the effluent gas stream was analyzed by a Cirrus quadrupole mass spectrometer continually scanning the mass range of 1 to 50 atomic mass units. To correct for variations in ambient pressure over time, each scan was normalized to the sum of detected masses. Composition of individual gasses was determined by comparison with mixtures of various composition generated with the mass flow control system. Measurements were made of the amount of methane produced by a given volume of culture per unit time, as well as the efficiency of conversion of input CO_2 and H_2 to methane.

EXAMPLE 2

Bench Scale Bioreactor Using *Methanococcus maripaludis*

[0048] The general setup of Example 1 was used with the organism *Methanococcus maripaludis*. *Methanococcus maripaludis* is grown at 37° C. in modified McCas medium containing the following components per L of medium: KCl 0.335 g, $MgCl_2.6H_2O$ 2.75 g, $MgSO_4$.7H2O 3.45 g, $CaCl_2.2H_2O$ 0.14 g, NH_4Cl 0.5 g, $NaHCO_3$ 8.4 g, NaCl 22 g, K_2HPO_4 0.14 g, $FeSO_4.7H_2O$ 9.5 mg, Resazurin 1 mg, Casamino acids 2 g, cysteine.H_2O.HCl 0.5 g, Na_3Citrate.$2H_2O$ 21 mg, $MnSO_4.2H_2O$ 5 mg, $CoCl_2(0.6H_2O)$ 1 mg, $ZnSO_4(0.7H_2O)$ 1 mg, $CuSO_4.5H_2O$ 0.1 mg, $AlK(SO_4)_2$ 0.1 mg, H_3BO_4 0.1 mg, $Na_2MoO_4.2H_2O$ 1 mg, $NiCl_2.6H_2O$ 0.25 mg, Na_2SeO_3 2 mg, V(III)Cl 0.1 mg, $Na_2WO_4.2H_2O$ 1 mg, biotin 0.02 mg, folic acid 0.02 mg, pyridoxine HCl 0.10 mg, thiamine HCl 0.05 mg, riboflavin 0.05 mg, nicotinic acid 0.05 mg, DL-calcium pantothenate 0.05 mg, vitamin B12 0.001 mg, p-aminobenzoic acid 0.05 mg, lipoic acid 0.05 mg. After autoclaving and before inoculation, the medium was reduced by the addition of 0.5 g/L Na_2S from a 50× anaerobic, sterile stock solution, yielding an ORP of the medium below −100 mV. The medium was equilibrated prior to inoculation with a gas phase containing 0.2 atmosphere partial pressure of CO_2 to yield a pH in the range of 7.2-7.3. The initial medium used to start the culture contained, in addition to the above components, 1.4 g/L NaAcetate.$3H_2O$, but the medium reservoir used in continuous culture conditions lacked the addition of acetate.

[0049] 1 L of the fresh medium was initially inoculated with 5 mL of *Methanococcus maripaludis* in a stationary phase, and methane production was monitored over time. As shown in FIG. **4**, a gas feed of 4:1 H_2:CO_2 (125 cc/min or 180 volumes of gas (STP) per volume of culture per day (VVD), 144 vvd H_2 and 36 vvd CO_2) was provided at a culture of pH 7.33 and an ORP of −140 mV. During the growth phase, the rate of methane production is limited by the available biological catalysts for the reaction. The methane production rate stabilized once the dissolved hydrogen in the medium was depleted. The stabilized rate is limited by the physical process of gas-to-liquid mass transfer of hydrogen, rather than by biological factors. Once this transition to stable methane production was observed, the culture was switched to continuous culture conditions in which fresh medium was added at a constant rate of 0.94 ml/h, or 22.5 ml/day. It was found that a slower input of fresh culture medium led to a denser culture and hence better volumetric performance. At the limit of no fresh medium, however, it was found that the culture ultimately dies.

EXAMPLE 3

Effect of Agitation on Methane Production of *Methanococcus maripaludis*

[0050] The turbidity of the culture obtained in Example 2 continued to increase after the gas-to-liquid mass transfer-limited rate of methane production was reached, providing an excess of biological catalytic capacity. This additional catalytic capacity can be accessed by changing physical parameters that increase the gas-to-liquid mass transfer rate. As shown in FIG. **5**, the mixing rate in the culture was varied from the standard 400 rpm. A total feed rate of a 4:1 H_2:CO_2 gas mixture (250 cc/min (288 vvd H_2; 72 vvd CO_2)) was used. At this gas feed rate, the conversion efficiency of both CO_2 and hydrogen reaches 55-56% at higher mixing speeds demonstrating that higher stirring rates increases the gas-to-liquid mass transfer and therefore higher methane production. Other abiotic methods that may be used to increase the gas-to-liquid mass transfer and hence the methane production rate include 1) increased gas pressure and 2) increased temperature. Some methanognic archaea can thrive at pressures over 500 atmospheres. With respect to different temperature conditions, thermophilic methanogens, such as *Methanothermobacter thermoautotrophicus* (at about 60° C.-65° C.) or *Methanocaldococcus jannaschii* (at about 85°-90° C.), can be used as the biological catalyst.

EXAMPLE 4

Conversion Efficiency of H_2 by *Methanococcus maripaludis*

[0051] A culture of *Methanococcus maripaludis* was setup in a bioreactor as set forth in Example 2. As shown in FIG. **6**, gas was fed to a mature culture at varying rates, maintaining a 4:1 hydrogen to carbon dioxide ratio. Once the culture was above the gas-liquid mass transfer-limited cell density, it was found that methane production could be increased by increasing the H_2 gas feed rate. However, at higher H_2 gas feed rates, it was found that a decreasing proportion of the H_2 gas was converted to methane. The converse was also found to be true, that at lower gas feed rates, hydrogen was converted more efficiently to methane. Because the volume of feed gas (4 volumes of hydrogen plus 1 volume of CO_2) decreases as it is converted to methane (1 volume of methane product), a cascade or stratified bioreactor system as shown in FIG. **2** and FIG. **3** is advantageous. In a serial bioreactor system as shown in FIG. **3**, the residual hydrogen in the effluent gas from a first fermenter would provide a lower feed rate to a second fermenter and would therefore be converted at higher efficiency in the second fermenter. This phenomenon can be used to obtain a highly efficient conversion in a cascaded fermenter design

EXAMPLE 5

Bench Scale Bioreactor Using *Methanosarcina barkeri*

[0052] The general setup of Example 1 was used with the organism *Methanosarcina barkeri*. *Methanosarcina barkeri* (strain DSM 804 obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) was grown at 35° C. in MS enriched medium containing the following components per L of medium: $NaHCO_3$ 8.4 g, yeast extract 2.0 g, trypticase peptones 2.0 g, mercaptoethanesulfonic acid 0.5 g, NH_4Cl 1.0 g, $K_2HPO_4.7H_2O$ 0.4 g, $MgCl_2.7H_2O$ 1.0 g, $CaCl_2.2H_2O$ 0.4 g, Resazurin 1 mg, cysteine.H_2O.HCl 0.25 g, $Na_2EDTA.2H_2O$ 5 mg, $MnCl_2.4H_2O$ 1 mg, $CoCl_2$(0.6H_2O) 1.5 mg, $FeSO_4.7H_2O$ 1 mg, $ZnCl_2$ 1 mg, $AlCl_3.6H_2O$ 0.4 mg, $Na_2WO_4.2H_2O$ 0.3 mg, CuCl 0.2 mg, $NiSO_4.6H_2O$ 0.2 mg, H_2SeO_3 0.1 mg, H_3BO_4 0.1 mg, $Na_2MoO_4.2H_2O$ 0.1 mg, biotin 0.02 mg, folic acid 0.02 mg, pyridoxine HCl 0.10 mg, thiamine HCl 0.05 mg, riboflavin 0.05 mg, nicotinic acid 0.05 mg, DL-calcium pantothenate 0.05 mg, vitamin B12 0.001 mg, p-aminobenzoic acid 0.05 mg, lipoic acid 0.05 mg. After autoclaving and before inoculation, the medium was reduced by the addition of 0.5 g/L Na_2S from a 50× anaerobic, sterile stock solution, yielding an ORP of the medium below −100 mV. The medium was equilibrated prior to inoculation with a gas phase containing 0.2 atmosphere partial pressure of CO_2 to yield a pH in the range of 6.8-7.0.

[0053] 1 L of the fresh medium was initially inoculated with 20 mL *Methanosarcina barkeri* in a stationary phase, and methane production was monitored over time. Once the transition to stable methane production was observed, the culture was switched to continuous culture conditions in which fresh medium was added at a constant rate of 0.94 ml/h, or 22.5 ml/day. It was found that a slower input of fresh culture medium led to a denser culture and hence better volumetric performance. At the limit of no fresh medium, however, it was found that the culture ultimately dies.

EXAMPLE 6

Recovery from Oxygen Exposure

Recovery of *Methanosarcina barkeri* with Exposure of 10 Minutes of Air

[0054] Methanogenic organisms are regarded as extremely strict anaerobes. Oxygen is known as an inhibitor of the enzyme catalysts of both hydrogen uptake and methanogenesis. A low oxidation-reduction potential (ORP) in the growth medium is regarded as important to methanogenesis. Air is a possible contaminant of carbon dioxide streams that could be used to support energy storage in the form of methane and so the effects of air on the capacity of the cultures to produce methane was examined.

[0055] FIG. **7** shows the recovery of the methanogenic activity of *Methanosarcina barkeri* after exposure to air. A

bench bioreactor containing *Methanosarcina barkeri* was prepared as set forth in Example 5. Two experiments were performed involving exposing the culture to 100% air for 10 minutes at a flow rate of 500 cc/min. Ambient air comprises approximately (by molar content/volume) 78% nitrogen, 21% oxygen, 1% argon, 0.04% carbon dioxide, trace amounts of other gases, and a variable amount (average around 1%) of water vapor. During exposure to 100% air, methanogenesis stopped and the ORP of the culture medium rose. The air used in the experiment also displaces CO_2 dissolved in the medium, causing the pH to rise (not shown in this figure). Following the 10 minute exposure to 100% air, gas flows of H_2 and CO_2 were restored (100 cc/min H_2, 25 cc/min CO_2).

[0056] In the first experiment, 1.5 ml of a 2.5% solution of sulfide ($Na_2S.7H_2O$) was added within 4 minutes of terminating air feed and restoring the H_2/CO_2 gas feed. Sulfide is widely used to control the ORP of the cultures, control that is regarded as essential. In another experiment, so sulfide was added. The dotted curves show a case in which no sulfide was added and the solid line shows the recovery of the culture where sulfide was added. In both cases, methanogenesis recovers. The figure shows that addition of sulfide for ORP control in case 1 causes the emitted hydrogen sulfide to rise to 3000 ppm. In the case of ORP control with hydrogen (no sulfide addition), the hydrogen sulfide level is at or below the limit of detection of the mass spectrometer under these operating conditions (50-100 ppm). Methanogenesis begins to recover more quickly in the case of ORP control with sulfide, but the experiment shows that sulfide is not essential for recovery. The presence of the hydrogen in the gas phase is sufficient to reduce the ORP of the culture to enable methanogenesis, no additional control of the ORP of the culture is required. The lack of necessity of sulfide is of note in that methanogenic cultures are typically maintained at 10,000 ppm hydrogen sulfide in the gas phase. Such high levels of sulfide are not tolerated in certain industrial process, for instance, natural gas pipeline tariffs in the United States set maximum levels of hydrogen sulfide content of natural gas ranging from 4-16 ppm, depending upon the pipeline system.

[0057] FIG. **8** also shows the recovery of *Methanosarcina barkeri* after the air exposure above (absent the addition of sulfide) after 7 hours.

EXAMPLE 7

Recovery from Oxygen Exposure

Recovery of *Methanosarcina barkeri* with Exposure of 90 Minutes of Air

[0058] FIG. **9** shows the recovery of *Methanosarcina barkeri* after 15 hours of exposure to air. A bench bioreactor containing *Methanosarcina barkeri* was prepared as set forth in Example 5. The culture was exposed to 100% air for 90 minutes introduced at IL/min (1440 vvd). During exposure to 100% air, methanogenesis stopped and the ORP of the culture medium rose. The air used in the experiment also displaced CO_2 dissolved in the medium, causing the pH to rise. Following the 90 minute exposure to 100% air, gas flows of H_2 and CO_2 were restored (100 cc/min H_2 (144 vvd) and 25 cc/min CO_2 (36 vvd). Restoration of hydrogen as a reductant was sufficient to reduce the ORP to levels that favor methanogenesis. Methane production began within 1 hr of restoring 4:1 H_2:CO_2 gas phase (100 cc/min H_2, 25 cc/min CO_2). Full recovery of methane production was achieved within 3-4 hrs.

EXAMPLE 8

Recovery from Oxygen Exposure

Recovery of *Methanosarcina barkeri* with Exposure of 15 Hours of Air

[0059] FIG. **10** shows the recovery of *Methanosarcina barkeri* after 15 hours of exposure to air. A bench bioreactor containing *Methanosarcina barkeri* was prepared as set forth in Example 5. The culture was exposed to 100% air for 15.1 hours at a flow rate of 1 L/min. During exposure to 100% air, methanogenesis stopped and the ORP of the culture medium rose to about −10 mV. The air used in the experiment also displaced CO_2 dissolved in the medium, causing the pH to rise to 9.3. Following the 15.1 hour exposure to 100% air, gas flows of H_2 and CO_2 were restored (100 cc/min H_2, 25 cc/min CO_2). Restoration of hydrogen as a reductant was sufficient to reduce the ORP to levels that favor methanogenesis. Methane production began within 1.1 hr of restoring 4:1 H_2:CO_2 gas phase (100 cc/min H_2, 25 cc/min CO_2). Full recovery of methane production was achieved within 3 hrs.

EXAMPLE 9

Recovery from Oxygen Exposure

Recovery of *Methanococcus maripaludis* with Exposure of 10 Minutes of Air

[0060] FIG. **11** shows the recovery of the methanogenic activity of *Methanococcus maripaludis* after exposure to air. A bench bioreactor containing *Methanococcus maripaludis* was prepared as set forth in Example 2. The culture was exposed to 100% air for 10 minutes at 360 vvd. During exposure to 100% air, methanogenesis stopped and the ORP of the culture medium rose. The air used in the experiment also displaces CO_2 dissolved in the medium, causing the pH to rise. Following the 10 minute exposure to 100% air, gas flows of H_2 and CO_2 were restored (288 vvd H_2, 72 vvd CO_2). Methanogenesis in was shown to have recovered within 10 min, with full recovery of methane production rate occurring within 1.5 hours.

EXAMPLE 10

Maintained Methane Production by *Methanococcus maripaludis* in the Presence of Air

[0061] FIG. **12** shows that methane production can continue even in the presence of air provided that hydrogen is present to maintain the ORP of the culture at productive levels. A bench bioreactor containing *Methanococcus maripaludis* was prepared as set forth in Example 2. The culture was exposed to a mixture of 4% air and 76% hydrogen at a flow rate of 100 cc/min and CO_2 at 25 cc/min for a period of 2.3 hours. The percentage of air was then increased to 8%, providing a mixture of 8% air and 72% hydrogen at a flow rate of 100 cc/min and CO_2 at 25 cc/min for a period of 1.7 hours. The percentage of air was then increased to 16%, providing a mixture of 16% air and 64% hydrogen at a flow rate of 100 cc/min and CO_2 at 25 cc/min for a period of 1.1 hours. Finally, the percentage of air was then increased to 32%, providing a mixture of 32% air and 58% hydrogen at a flow rate of 100 cc/min and CO_2 at 25 cc/min for a period of 0.6 hours. Meth-

ane production continues even in the presence of air provided that hydrogen is present to maintain the ORP of the culture at productive levels. Up to 4% air (0.8% oxygen) is tolerated without a persistent reduction in methane production. The efficiency of conversion of the input hydrogen to methane remains unaffected at 22-23% under the conditions of the experiment until the air concentration rises from 8% to 16% of the total gas mix.

[0062] Note that the gas mixtures produced by the culture under these conditions could be explosive, since the oxygen is not consumed by the organisms and appears in the effluent gas stream. A potentiostat culture system provides a method for maintaining the ORP of the culture during air exposure without introducing hydrogen or generating methane.

EXAMPLE 11

Recovery from Carbon Monoxide Exposure by *Methanococcus maripaludis*

[0063] Carbon monoxide is another known inhibitor of enzymes involved in both hydrogen uptake and methanogenesis. CO is a potential contaminant of CO_2 and hydrogen streams derived from gasification of coal or biomass resources. The effect CO on methane formation by methanogen cultures was examined. FIG. **13** shows the recovery of the methanogenic activity of *Methanococcus maripaludis* after exposure to carbon monoxide. A bench bioreactor containing *Methanococcus maripaludis* was prepared as set forth in Example 2. In this experiment, the pH of the culture was maintained constant by keeping CO_2 at 20% of the gas mix and changing only the composition of the other 80% of the gas. The ORP of the culture remained relatively constant between −140 and −150 mV.

[0064] The culture was exposed to a mixture of 8% CO and 72% hydrogen at a flow rate of 100 cc/min and CO_2 at 25 cc/min for a period of 1.7 hours. Then the culture was restored to a flow of 80% hydrogen at a flow rate of 100 cc/min and CO_2 at 25 cc/min. Upon removal of the CO and restoration of 50% H_2, methanogenesis recovered completely within a 5 minutes (within the mixing time of the gas phase in the culture). This rapid recovery suggests that the primary effect of CO under these experimental conditions is as a reversible inhibitor of the methanogenesis process.

[0065] The culture was then exposed to a mixture of 16% CO and 64% hydrogen at a flow rate of 100 cc/min and CO_2 at 25 cc/min for a period of 1 hour. This higher exposure of CO showed only a 25% inhibition of methane formation rates. This suggests that the initial exposure caused an adaptation in the culture that reduced its sensitivity to CO inhibition. The culture was then restored to a flow of 80% hydrogen at a flow rate of 100 cc/min and CO_2 at 25 cc/min. Recovery of methanogenesis following CO removal was again immediate.

[0066] Finally, the culture was exposed to a mixture of 40% CO and 40% hydrogen at a flow rate of 100 cc/min and CO_2 at 25 cc/min for a period of 20 minutes. This CO exposure showed almost as much inhibition of the adapted process as occurred in the initial low level exposure of the un-adapted organisms. The culture was then restored to a flow of 80% hydrogen at a flow rate of 100 cc/min and CO_2 at 25 cc/min. Recovery from this level of CO was also immediate.

[0067] Another experiment was performed showing the effects of even higher concentration of CO on methanogenesis. A bench bioreactor containing *Methanococcus maripaludis* was prepared and maintained as set forth above. FIG. **14** shows that when the culture was given a doses of a mixture of 60% CO and 20% hydrogen at a flow rate of 100 cc/min and CO_2 at 25 cc/min, this led to a complete loss of methane formation in this species. Recovery from this level of CO has an immediate phase but full recovery requires several hours. These experiments show that methanogenesis achieved by methanogen cultures is tolerant to levels of CO likely to be found in industrial CO_2 streams derived from coal or biomass gasification, but that higher levels may be poorly tolerated.

EXAMPLE 12

Efficiency of Methane Production from a Cascaded Bioreactor

[0068] A mathematical projection was performed to determine the projected efficiencies of methane production according to the methods of the present invention in a cascaded bioreactor system similar to what is depicted in FIG. **3**. The performance of a methanogen culture in converting hydrogen and carbon dioxide to methane was modeled as a gas-phase continuously stirred tank reactor (CSTR) in which the gas is homogeneously mixed with a liquid phase in which the reaction occurs, catalyzed by the organisms. Under mass-transfer limited conditions, the reaction is first order in hydrogen with a rate that is governed by the gas-liquid transfer of hydrogen. The consumption of hydrogen by the organisms is sufficiently rapid to keep the dissolved concentration of hydrogen well below the saturating concentration. Under this model, the product of the first order rate constant, k, and the residence time of gas in the gas phase, τ, govern the extent of the reaction of the hydrogen:

$$k\tau = \frac{X}{1-X},$$

where x is the fraction of hydrogen converted to methane. It is assumed that there is at least enough CO_2 present to consume all of the hydrogen in methane formation.

[0069] The extent of the reaction, X, is determined by the input flow of hydrogen gas, F_o (in units of moles/time), the mass balance for the conversion to methane, and the first order rate constant, assuming constant volume, pressure and temperature:

$$F_o = \frac{kV_gP_g(1-X)}{RTX\left(\frac{5}{4}-X+D\right)}$$

[0070] where V_g is the volume of the gas phase that is efficiently mixed with the liquid phase, P_g is the operating pressure, R is the universal gas constant, T is the chamber temperature in ° K, and D is the total mole fraction in the gas phase of non-reactant gases, including any excess CO_2 as well as water vapor. As input reactant gas flow is increased, more product methane is produced, but at the expense of lower extent of reaction because of a lower retention time.

[0071] During the reaction, 4 volumes of hydrogen and 1 volume of carbon dioxide are consumed to produce 1 volume of methane. Hence, as the reaction proceeds, the flow of gas out of the chamber is less than the input flow. This strong reduction in flow during the reaction provides a uniquely

valuable advantage to a system of cascaded reactors in which the exit gas from one reaction chamber becomes the inlet for a successive chamber. Under steady state conditions, the exit flow of hydrogen, F, is given by $F=F_o(1-X)$ in a given chamber. For a cascade of two identical chambers, the flow rate in the second chamber will be less than that in the first, and hence the retention time and the extent of conversion will be greater. The advantage of the cascade approach can be appreciated by comparing the extent of conversion in a single chamber of a fixed volume with the total extent of conversion of the same inlet gas flow by two cascaded chambers, each half the volume of the single chamber and with that of three cascaded chambers, each one third the volume of the single chamber, as shown in FIG. **15**. For this illustration, the initial hydrogen:CO_2 ratio is 4:1.

TABLE 1

Conversion efficiency		
Single	Cascade	
Reactor	2 reactor	3 reactor
0.3350	0.3626	0.3729
0.4562	0.5134	0.5368
0.5502	0.6389	0.6788
0.6222	0.7376	0.7927
0.6775	0.8110	0.8750
0.7204	0.8636	0.9280
0.7542	0.9004	0.9593
0.7813	0.9262	0.9770
0.8034	0.9443	0.9868
0.8369	0.9667	0.9954
0.9042	0.9928	0.9998

[0072] In this simulation, the input flow rate and the conversion rate constant were adjusted to give a range of extents of reaction in a single tank reactor. The extent of reaction, X, for this single reactor is listed in the first column of Table 1. The same initial flow rate was then fed to 2 or three cascaded reactors of ½ or ⅓ the volume of the single tank reactor, respectively, keeping the other reaction conditions constant. The extent of reaction measured at the exit of the final reactor of the cascade is listed in the second and third columns of the table. The same data are also presented graphically in FIG. **15**. Here, the final extent of reaction in the cascade, Xcascade, is divided by the extent of reaction of the equivalent total volume single reactor, Xsingle, and the ratio is plotted against Xsingle. Conditions near the peaks of these graphs would optimize the conversion gain of the cascade arrangement, while conditions to the right of the peaks would exploit the cascade for efficiently reducing the final residual unreacted hydrogen in the product gas stream.

[0073] This model assumes a homogeneous residence time for the gas. In practice, the bubbles suspended in the liquid act as independent mini-reactors for some period of time, a behavior that causes a dispersion of net conversion in different bubbles and in apparent residence times. This dispersion of residence times can be determined under operating conditions by injecting an inert gas tracer into the inlet gas stream and monitoring the time that it takes to exit. Depending on the exact shape of the residence time distribution, this phenomenon could degrade the performance of the single tank reactor. However, the use of cascading reactors sharpens the residence time distribution, which favors the cascaded reactor performance over that of a single reactor of the same total volume.

EXAMPLE 13

Maintenance of a Methane Producing Culture of *Methanothermobacter thermoautotrophicus* in Stationary Phase

[0074] *Methanothermobacter thermoautotrophicus* (DSMZ 3590) was grown in a culture medium comprising: NaCl 10 mM, NH_4Cl 120 mM, nitrilotriacetic acid (NTA) 1.2 mM, $MgCl_2.7H_2O$ 1 mM, KH_2PO_4 10 mM, $CoCl_2$ 2.5 μM, Na_2MoO_4 2.5 μM, $NiCl_2$ 5 μM, $FeSO_4.7H_2O$ 0.2 mM, Na_2SeO_3 1 μM, Na_2WO_4 10 μM, at 60° C. in a 1.3 L BioFlo 110 fermenter vessel containing 700 ml medium and agitated at 1000 RPM until it reached stationary phase. During initial growth of the culture, sodium sulfide was added at a rate that maintained hydrogen sulfide in the output gas stream at 10 ppm. The culture was sparged with a 4:1 H_2:CO_2 gas mixture at a total rate of 0.25 SLPM. In the stationary phase, a culture gassed at this rate produces 49 ml/min methane (101 vvd; 98% conversion of the input hydrogen). During methanogenesis, the culture produced two moles of metabolic water per mole of methane, which is a significant fraction of the medium volume. Medium, containing cells, was removed from the fermenter to keep the liquid volume constant. Medium components removed along with the liquid were replaced with concentrated stock solutions. During stationary phase, sulfide addition was necessary only for maintaining cell replacement and was maintained at a level below 1 ppm in the output gas stream.

EXAMPLE 14

Recovery of Methane Production by *M. thermoautotrophicus* Following Exposure to Air

[0075] A stationary phase culture of *M. thermoautotrophicus* 3590 producing methane at 49 ml/min (101 vvd) from a 0.25 SLPM input gas stream of 4:1 H_2:CO_2 was exposed to air by replacing the hydrogen in the input gas mixture with air. The composition of the output gas was analyzed by mass spectrometry and the output rates of the various gases were computed in SLPM. As shown in FIG. **16**, the output gas methane production declined immediately. After 1 hr, the air in the input gas was replaced with argon for 10 min and then with hydrogen, restoring the original 4:1 H_2:CO_2 gas mixture. After a lag of 22 min, methane production recovered quickly, reaching 50% of the original production rate within 32 min and full 98% conversion efficiency by 57 min.

EXAMPLE 15

Methane Production by a Stationary Phase Culture of *M. thermoautotrophicus* at Different Gassing Rates

[0076] A stationary phase culture of *M. thermoautotrophicus* was grown as in Example 13, except with an initial hydrogen gassing rate in a 4:1 H_2:CO_2 mixture of 200 ml/min (450 vvd) and a liquid volume of 650 ml. This arrangement provides a 1:1 ratio of liquid culture to gaseous headspace and the agitation of 1000 RPM is adequate to maintain thorough mixing of the headspace with the liquid medium. The culture was then gassed at various rates with 4:1 H_2:CO_2 until the output gas composition stabilized. A pulse of argon gas was introduced into the gas feed stream as a tracer to measure the average residence time of the gas, τ. These data showed simple exponential decay of the gas tracer, indicating thor-

ough mixing of the gas and liquid phases. The performance of the culture at each gassing rate is given in Table 2.

TABLE 2

H2 in (vvd)	CH4 out (vvd)	X (fraction complete)	τ (s)
450	111	0.987	299
900	215	0.955	98.8
1350	311	0.922	61.6
1800	399	0.886	47.8
2250	475	0.845	47
3150	595	0.755	35.9
4050	673	0.664	37.4
5400	758	0.562	33.3
6750	840	0.498	ND

[0077] A completely efficient catalyst (X=1.0) would yield 1012 vvd of methane from an input flow of 4050 vvd hydrogen. In practice, X at 4050 vvd hydrogen (1 atmosphere reactor pressure) is 0.664, corresponding to 673 vvd methane production in a single-pass continuously stirred tank reactor operating under the conditions specified. As projected in Example 12, a three-stage cascade reactor with the same total volume as the single reactor would operate with a final conversion efficiency improved to 0.86 and a corresponding increase in methane production to 860 vvd. A single reactor producing the same purity of product (same value of X) would produce only about 380 vvd methane from an input of 1700 vvd of hydrogen. If the volume of this same reactor operating at X=0.86 and 1700 vvd hydrogen input were divided into a three-tank cascade, it would produce 425 vvd methane at X=0.99.

[0078] While the present invention has now been described and exemplified with some specificity, those skilled in the art will appreciate the various modifications, including variations, additions, and omissions that may be made in what has been described. It is to be understood that the invention is not limited in its application to the details of construction and the arrangements of the components set forth in the previous description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or being carried out in various ways. Accordingly, it is intended that these modifications also be encompassed by the present invention and that the scope of the present invention be limited solely by the broadest interpretation that lawfully can be accorded the appended claims.

[0079] Also, it is understood that the phraseology and terminology used herein are for the purpose of description and should not be regarded as limiting. The use of "including", "having" and "comprising" and variations thereof herein is meant to encompass the items listed thereafter and equivalents thereof as well as additional items and equivalents thereof. It also is understood that any numerical value recited herein includes all values from the lower value to the upper value. For example, if a concentration range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3%, etc., are expressly enumerated in this specification. These are only examples of what is specifically intended, and all possible combinations of numerical values between the lowest value and the highest value enumerated are to be considered to be expressly stated in this application.

1. A method of converting carbon dioxide produced during an industrial process to methane comprising:

a) preparing a culture of hydrogenotrophic methanogenic archaea in a bioreactor;

b) supplying an output gas from an industrial process to the bioreactor; wherein the output gas comprises CO_2 and between 0.02% and 6.7% oxygen (moles/volume of output gas); and

c) wherein the hydrogenotrophic methanogenic archaea converts the output gas to continuously produce methane.

2. The method of claim **1** wherein the culture is a substantially pure culture of one hydrogenotrophic methanogenic archaea species.

3. The method of claim **1** wherein H_2 is supplied in an amount to maintain a redox potential in the bioreactor under −100 mV or less, and wherein no additional constituent other than the H_2 gas is added to the bioreactor to maintain the redox potential in the bioreactor under −100 mV or less.

4. The method of claim **1** wherein the industrial process is coal gasification, biomass gasification, or liquid fuel production by biomass fermentation.

5. The method of claim **2** wherein the hydrogenotrophic methanogenic archea species is selected from the group consisting of *Methanobacterium alcaliphilum, Methanobacterium bryantii, Methanobacterium congolense, Methanobacterium defluvii, Methanobacterium espanolae, Methanobacterium formicicum, Methanobacterium ivanovii, Methanobacterium palustre, Methanobacterium thermaggregans, Methanobacterium uliginosum, Methanobrevibacter acididurans, Methanobrevibacter arboriphilicus, Methanobrevibacter gottschalkii, Methanobrevibacter olleyae, Methanobrevibacter ruminantium, Methanobrevibacter smithii, Methanobrevibacter woesei, Methanobrevibacter wolinii, Methanothermobacter marburgensis, Methanothermobacter thermautotrophicum, Methanothermobacter thermoflexus, Methanothermobacter thermophilus, Methanothermobacter wolfeii, Methanothermus sociabilis, Methanocorpusculum bavaricum, Methanocorpusculum parvum, Methanoculleus chikuoensis, Methanoculleus submarinus, Methanogenium frigidum, Methanogenium liminatans, Methanogenium marinum, Methanosarcina acetivorans, Methanosarcina barkeri, Methanosarcina mazei, Methanosarcina thermophila, Methanomicrobium mobile, Methanocaldococcus jannaschii, Methanococcus aeolicus, Methanococcus maripaludis, Methanococcus vannielii, Methanococcus voltaei, Methanothermococcus thermolithotrophicus, Methanopyrus kandleri, Methanothermobacter thermoautotroiphicus, Methanocaldococcus fervens, Methanocaldococcus indicus, Methanocaldococcus infernus*, and *Methanocaldococcus vulcanius.*

6. The method of claim **5** wherein the hydrogenotrophic methanogenic archea is selected from the group consisting of *Methanosarcina barkeri* and *Methanococcus maripaludis.*

7. The method of claim **6** wherein the conditions include a temperature of about 35° C. to about 37° C.

8. The method of claim **5** wherein the hydrogenotrophic methanogenic archea is *Methanothermobacter thermoautotroiphicus.*

9. The method of claim **8** wherein the conditions include a temperature of about 60° C. to about 65° C.

10. The method of claim **5** wherein the hydrogenotrophic methanogenic archea is selected from the group consisting of *Methanocaldococcus fervens, Methanocaldococcus indicus, Methanocaldococcus infernus*, and *Methanocaldococcus vulcanius*.

11. The method of claim **10** wherein the conditions include a temperature of about 80° C. to about 100° C.

12. The method of claim **1** wherein the industrial output gas comprises at least 0.8% oxygen (moles/volume of output gas).

13. The method of claim **1** wherein the industrial output gas comprises at least 1.7% oxygen (moles/volume of output gas).

14. The method of claim **1** wherein the industrial output gas comprises at least 3.4% oxygen (moles/volume of output gas).

15. The method of claim **1** wherein the industrial output gas comprises between 3.4%-6.7% oxygen (moles/volume of output gas).

16. The method of claim **1** wherein the industrial output gas further comprises carbon monoxide.

17. The method of claim **16**, wherein the industrial output gas comprises at least about 8% carbon monoxide by volume.

18. The method of claim **16**, wherein the industrial output gas comprises at least about 16% carbon monoxide by volume.

19. The method of claim **16**, wherein the industrial output gas comprises at least about 60% carbon monoxide by volume.

20. A method of converting carbon dioxide produced during an industrial process to methane comprising:

a) contacting a culture comprising hydrogenotrophic methanogenic archaea with H_2 gas and an output gas from an industrial process comprising CO_2 gas in a bioreactor;

b) supplying an amount of H_2 gas to maintain a redox potential in the bioreactor under −100 mV or less, wherein no additional constituent other than the H_2 gas is added to the bioreactor to maintain the redox potential in the bioreactor under −100 mV or less; and

c) wherein the hydrogenotrophic methanogenic archaea converts the H_2 gas and the CO_2 gas to methane.

21. A method of converting carbon dioxide produced during an industrial process to methane comprising:

a) preparing an initial culture of hydrogenotrophic methanogenic archaea and placing the culture in a bioreactor;

b) supplying an output gas from an industrial process to the bioreactor; wherein the output gas comprises CO_2;

c) supplying fresh medium to the culture;

d) wherein the hydrogenotrophic methanogenic archaea converts the output gas to continuously produce methane; and

e) wherein no sulfur is added in the method, other than the presence of sulfur in the initial culture.

22. A method of converting carbon dioxide produced during an industrial process to methane using a cascaded bioreactor process, the process comprising:

a) preparing a culture of hydrogenotrophic methanogenic archaea, wherein the culture is present in a first and second reactor vessel;

b) supplying fresh medium to the first and second reactor vessels through a first medium feed line attached to the first reactor vessel, and a second medium feed line attached to the second reactor vessel;

c) supplying H_2 gas to the first reactor vessel through hydrogen gas feed line;

d) supplying an output gas from an industrial process to the first reactor vessel; wherein the output gas comprises CO_2, through an output gas feed line;

e) wherein the hydrogenotrophic methanogenic archaea culture in the first reactor converts H_2 gas and CO_2 gas to produce methane;

f) transferring at least a portion of gas in the first reactor vessel to the second reactor vessel by a first gas feed line;

g) wherein the hydrogenotrophic methanogenic archaea culture in the second reactor converts H_2 gas and CO_2 gas to produce methane.

23. The method of claim **22** further comprising:

a) preparing a culture of hydrogenotrophic methanogenic archaea, wherein the culture is present in a third reactor vessel;

b) supplying fresh medium to the third reactor vessels through a third medium feed line attached to the third reactor vessel;

c) transferring at least a portion of gas in the second reactor vessel to the third reactor vessel by a second gas feed line;

d) wherein the hydrogenotrophic methanogenic archaea culture in the third reactor converts H_2 gas and CO_2 gas to produce methane.

24. The method of claim **22** wherein the output gas further comprises between 0.02% and 6.7% oxygen (moles/volume of output gas) and wherein the hydrogenotrophic methanogenic archaea in the first and second reactor vessels continuously produce methane.

25. The method of claim **22** wherein H_2 is supplied in an amount to maintain a redox potential in the first reactor vessel under −100 mV or less, and wherein no additional constituent other than the H_2 gas is added to the first reactor vessel to maintain the redox potential in the first reactor vessel under −100 mV or less.

26. The method of claim **22** wherein no sulfur is in the process other than sulfur in the culture medium.

27. The method of claim **22** wherein the hydrogenotrophic methanogenic archea comprises one or more species selected from the group consisting of *Methanobacterium alcaliphilum, Methanobacterium bryantii, Methanobacterium congolense, Methanobacterium defluvii, Methanobacterium espanolae, Methanobacterium formicicum, Methanobacterium ivanovii, Methanobacterium palustre, Methanobacterium thermaggregans, Methanobacterium uliginosum, Methanobrevibacter acididurans, Methanobrevibacter arboriphilicus, Methanobrevibacter gottschalkii, Methanobrevibacter olleyae, Methanobrevibacter ruminantium, Methanobrevibacter smithii, Methanobrevibacter woesei, Methanobrevibacter wolinii, Methanothermobacter marburgensis, Methanothermobacter thermautotrophicum, Methanothermobacter thermoflexus, Methanothermobacter thermophilus, Methanothermobacter wolfeii, Methanothermus sociabilis, Methanocorpusculum bavaricum, Methanocorpusculum parvum, Methanoculleus chikuoensis, Methanoculleus submarinus, Methanogenium frigidum, Methanogenium liminatans, Methanogenium marinum, Methanosarcina acetivorans, Methanosarcina barkeri, Methanosarcina mazei, Methanosarcina thermophila, Methanomicrobium mobile, Methanocaldococcus jannaschii, Methanococcus aeolicus, Methanococcus maripaludis, Methanococcus vannielii, Methanococcus voltaei, Methanothermococcus therm-*

olithotrophicus, Methanopyrus kandleri, Methanothermobacter thermoautotroiphicus, Methanocaldococcus fervens, Methanocaldococcus indicus, Methanocaldococcus infernus, and *Methanocaldococcus vulcanius*.

28. A cascaded bioreactor comprising:

a first reactor vessel

a second reactor vessel

a culture of hydrogenotrophic methanogenic archaea which is present in the first and second reactor vessels;

a source of an output gas from an industrial process comprising CO2 that feeds into the first reactor vessel;

a source of hydrogen gas that feeds into the first reactor vessel;

a gas feed from the first reactor vessel to the second reactor vessel;

a feed to the first reactor vessel for providing fresh medium;

a feed to the second reactor vessel for providing fresh medium;

a feed to the first reactor vessel for removing the hydrogenotrophic methanogenic archaea culture; and

a feed to the second reactor vessel for removing the hydrogenotrophic methanogenic archaea culture.

29. The cascaded bioreactor of claim **28** further comprising:

a third reactor vessel;

a culture of hydrogenotrophic methanogenic archaea which is present in the third reactor vessel;

a gas feed from the second reactor vessel to the third reactor vessel;

a feed to the third reactor vessel for providing fresh medium; and

a feed to the third reactor vessel for removing the hydrogenotrophic methanogenic archaea culture.

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