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Publisher's version / Version de l'éditeur:

<https://doi.org/10.1021/es102728m>

Environmental science & technology, 45, 5, pp. 2006-2012, 2011-02-03

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Potential of Wastewater-Treating Anaerobic Granules for Biomethanation of Synthesis Gas

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S Supporting Information

ABSTRACT: Gasification of biomass produces a mixture of gas (mainly carbon monoxide (CO), carbon dioxide (CO₂), and hydrogen (H₂)) called synthesis gas, or syngas, by thermal degradation without combustion. Syngas can be used for heat or electricity production by thermochemical processes. This project aims at developing an alternative way to bioupgrade syngas into biogas (mainly methane), via anaerobic fermentation. Nonacclimated industrial granular sludge to be used as reactor inoculum was initially evaluated for mesophilic carboxydutrophic methanogenesis potential in batch tests at 4 and 8 mmol CO/g VSS.d, in the absence and presence of H₂ and CO₂, respectively. Granular sludge was then introduced into a 30 L gas-lift reactor and supplied with CO, to study the production of methane and other metabolites, at different gas dilutions as well as feeding and recirculation rates. A maximal CO conversion efficiency of 75%, which was gas–liquid mass transfer limited, occurred at a CO partial pressure of 0.6 atm combined with a gas recirculation ratio of 20:1. The anaerobic granule potential for methanogenesis from CO was likely hydrogenotrophic, combined with CO-dependent H₂ formation, either under mesophilic or thermophilic conditions. Thermophilic conditions provide the anaerobic granules with a CO-bioconversion potential significantly larger (5-fold) than under mesophilic conditions, so long as the gas–liquid transfer is alleviated.

INTRODUCTION

Biomass-based energy demand is increasing. Several bioconversion routes exist to turn biomass into gas or liquid fuels. A significant portion of biomass is however difficult and/or slowly biodegradable by microorganisms, due to its refractory and polymeric nature. When the organic residue is relatively dry (e.g., woodchips, bug wood, etc.) or nonreadily biodegradable (bark, plastics, rubber, etc.), it might be more appropriate to use thermo-chemical conversion techniques such as gasification. Gasification transforms biomass at high temperature (500–1500 °C) and pressure (1–80 atm) with limited amounts of water and oxygen into synthesis gas (syngas) mainly composed of carbon monoxide (CO), carbon dioxide (CO₂), and hydrogen (H₂). Minor syngas components include water vapor, methane, light hydrocarbons (ethylene (C₂H₄), ethane (C₂H₆)), and some volatile impurities. Syngas can be used directly to power industrial boilers, gas turbines, or fuel cells (e.g., solid oxide fuel cells¹) to make electricity. Syngas can also be upgraded into methane with chemical catalysts. This includes the water-gas shift (WGS) reaction for increasing the H₂/CO ratio followed by nickel-catalyzed methanation of CO and CO₂ into methane and water (reactions of Fischer–Tropsch ($n\text{CO} + (2n+1)\text{H}_2 \rightarrow \text{C}_n\text{H}_{(2n+2)} + n\text{H}_2\text{O}$) and Sabatier ($\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$)).² After separation, methane could be used locally for energy needs or reinjected in the natural gas grid. Catalyzed chemical processes are well established. They normally involve high pressure and/or temperature, may be problematic when impurities are present, and tend to have low product specificity.³

To circumvent these disadvantages and use milder treatments with minimal chemical and energy, the power of microorganisms can be harnessed to convert the syngas compounds into biogas (biomethanation). A small number of microbes can reduce the CO from syngas into methane (*Methanobacterium thermoautotrophicum*,

Methanothermobacter wolfeii, *Methanobrevibacter arboriphilicus*, *Methanocaldococcus jannaschii*, *Methanopyrus kandleri*, *Methanosaeta thermophila*, *Methanosarcina acetivorans*, *M. barkeri*).^{4–10} In short, the syngas can be a substrate for those carboxydutrophic (CO-oxidizing) methanogenic archaea that grow chemolithoautotrophically on CO and H₂.¹¹ Two direct reactions are possible: (I) $4\text{CO} + 2\text{H}_2\text{O} \rightarrow \text{CH}_4 + 3\text{CO}_2$ ($\Delta G^\circ = -53 \text{ kJ/mol CO}$, but growth might be slow); (II) $\text{CO} + 3\text{H}_2 \rightarrow \text{CH}_4 + \text{H}_2\text{O}$ ($\Delta G^\circ = -150 \text{ kJ/mol CO}$, faster growth). In theory, as long as H₂ is not limiting, the second reaction, thermodynamically more favorable, should prevail, and the first one should take over, once H₂ becomes deficient. Indirect reactions are also possible, with a mixed anaerobic consortium. For instance: carboxydutrophic hydrogenogenesis (enzymatic WGS reaction,¹² $\text{CO} + \text{H}_2\text{O} \rightarrow \text{H}_2 + \text{CO}_2$, $\Delta G^\circ = -20 \text{ kJ/mol CO}$) followed by either the methanogenic reduction of CO₂, or carboxydutrophic methanogenesis (in either case, net result: $4\text{CO} + 2\text{H}_2\text{O} \rightarrow \text{CH}_4 + 3\text{CO}_2$); either CO-homoacetogenesis ($2\text{CO} + 2\text{H}_2 \rightarrow \text{CH}_3\text{COOH}$, $\Delta G^\circ = -67 \text{ kJ/mol CO}$) or acetogenesis ($4\text{CO} + 2\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + 2\text{CO}_2$, $\Delta G^\circ = -44 \text{ kJ/mol CO}$) followed by acetoclastic methanogenesis; or carboxydutrophic production of methanol ($\text{CO} + 2\text{H}_2 \rightarrow \text{CH}_3\text{OH}$, $\Delta G^\circ = -39 \text{ kJ/mol CO}$) followed by methylotrophic methanogenesis ($\text{CH}_3\text{OH} \rightarrow 3/4\text{CH}_4 + 1/4\text{CO}_2 + 1/2\text{H}_2\text{O}$); or oxidation of CO into formic acid ($\text{CO} + \text{H}_2\text{O} \rightarrow \text{HCOOH}$), followed by its reduction into CH₄. Furthermore, Clostridia produce, besides acetate, significant amounts of ethanol, butyrate, and butanol that can then be converted into acetate and then methane. Whatever the set of

Received: August 10, 2010

Accepted: January 13, 2011

Revised: December 20, 2010

Published: February 03, 2011

reactions, the final CH_4 yield is identical, i.e., 0.25 mol of CH_4 per mol of CO, plus 0.25 mol of CH_4 per mol of H_2 . This would result within a treatment chain made of gasification followed by biomethanation of syngas, into a practical CH_4 yield from biomass varying between 0.2 and 0.4 m^3 STP CH_4 /kg VS gasified, depending on the syngas composition (i.e., CO between 30 and 60% (vol./vol.), H_2 between 60 and 25%, and CO_2 between 35 and 3%).

Industrial wastewater-treating anaerobic granules could be used for achieving those transformations, providing that they have a carboxydutrophic methanogenic potential that is already significant or which could be enhanced. In this case, the microbial constituent would not be an issue, which should allow for focusing on the reactor design and operation optimization. Since these organisms are massively available at free or low cost, and already adapted to harsh conditions that would prevail with crude syngas, scale-up later would be facilitated as well. However, because the aqueous solubility of CO and H_2 is low, syngas fermentations are typically limited by the gas–liquid mass transfer rate.¹³ This will represent the major engineering challenge for development of large-scale syngas fermentation facilities cost-effectively compatible with upstream gasifier productivity. The reactor design development will have to aim at achieving a high microbial density and minimizing gas–liquid mass transfer limitations. Hence the mass transfer driving force has to be intensified through various means, e.g., pressurizing the reactor gas phase, increasing the interfacial area by gas dispersion (e.g., using microspargers or bubble-free sparging), and high-rate gas recirculation.

The objective of this work is to assess whether carboxydutrophic methanogenic potential is existing and can be enriched within industrial anaerobic granules, using a closed-loop gas-lift reactor continuously fed with a gas stream of N_2 -diluted CO, under varying operational conditions, and their sensitivity to high levels of CO in the gas phase.

MATERIAL AND METHODS

Reactor Setup. The closed-loop gas lift reactor (internal diameter 0.2 m; height 1.13 m; working liquid volume 30 L; headspace 4 L) used in this study enclosed an internal draft-tube of 0.6 m height and 0.1 m diameter (Figure S1, Supporting Information). The reactor temperature and pH were controlled at 35 °C and at 6.9 to 7.8, respectively. The CO content of feeding gas was adjusted using nitrogen (N_2) gas. The gas phase was recycled at a flow rate varying between 0.6 and 1.15 L/min. Other details can be found in the Supporting Information.

The reactor was inoculated with anaerobic granules from a full-scale UASB plant treating fruit processing wastewater (Lassonde Inc., Rougemont, QC, Canada). Dilution water (in mg/L: Na_2HCO_3 , 2700; NaH_2PO_4 , 1100; $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 1442; K_2HPO_4 , 4000) was supplied at an average flow rate of 0.2 L/d. The nutrient (in mg/L: KH_2PO_4 , 4040; K_2HPO_4 , 5160; NH_4HCO_3 , 19521) and trace metal solutions (in mg/L: $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 856; H_3BO_3 , 5; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 13; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 60; $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 33; CuSO_4 , 45; $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$, 9; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 273; $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$, 2; $\text{Na}_2\text{-EDTA}$, 33; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1630; Na_2SeO_4 , 6; $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$, 6) were pumped into the dilution water stream according to a volume ratio of 45/47/100, respectively.

Specific Activity Tests. Acetoclastic, hydrogenotrophic, and carboxydutrophic (CO-oxidizing) specific activity tests were

performed in triplicates on the granular anaerobic inoculum as well as on granules sampled intermittently during the reactor operation (i.e., essentially at the end of main phases i.e., precisely at day 29, 51, 72, 96). Anaerobic activities for acetate were determined in 120 mL serum bottles by measuring the rate of methane production and/or substrate depletion, individually and under nonlimiting conditions, as described previously.¹⁴ Briefly after filled with the microbial biomass and buffer solution, bottles were capped, sealed, and flushed with N_2/CO_2 gas (80/20% vol./vol.) to establish anaerobic conditions and placed in a rotary shaker (New Brunswick, Edison, NJ) in a dark, thermostatically controlled environment (35 ± 1 °C) and gyrated at 100 rpm. The acetate solution was injected into all bottles to obtain an initial concentration of 3 g/L, except the bottles for endogenous control. The hydrogenotrophic activity tests were performed similarly, using as substrate instead of acetate, headspace filled with CO_2/H_2 (80/20% vol./vol.) pressurized at 2.5 atm and by shaking at 400 rpm to ensure the gas transfer between the headspace and liquid. The carboxydutrophic activity was assessed similar to the hydrogenotrophic activity, however using 0.20 atm CO as substrate. Conditions tested were mesophilic (35 °C) and thermophilic (60 °C), shaking at 100 and 400 rpm, absence of hydrogen (balance made of N_2 , 0.8 atm) and presence of hydrogen (0.64 atm, balance made of 0.16 atm CO_2).

Analytical Methods. Measurements of chemical oxygen demand (COD), total and suspended solids (TS and SS), and total and suspended volatile solid (VS and VSS) were made according to the standard methods.¹⁵ Volatile fatty acids (VFA, i.e., acetate, propionate and butyrate), solvents (methanol, ethanol, 2-propanol, n-propanol, tert-butanol, sec-butanol, n-butanol, acetone), and gas components (O_2 , H_2 , CH_4 , N_2 , CO, CO_2) were measured by gas chromatography. Details can be found in the Supporting Information.

RESULTS AND DISCUSSION

Methanogenic Potential of Inoculum. The fruit processing wastewater-treating anaerobic granules were first characterized for their carboxydutrophic and methanogenic potential. Table 1 shows specific activities of the granular inoculum under various conditions. Acetoclastic and hydrogenotrophic methanogenic specific activities, at ca. 360 and 200 mg substrate consumed per g VSS per day, respectively, were in a range as expected. The carboxydutrophic activity was assessed for various conditions: lower and higher mass transfer conditions; with or without H_2/CO_2 as cosubstrate; CO at 0.2 atm. A typical time course of CO and product (H_2 and CH_4) amount is shown in Figure 1. Anaerobic granules presented a promising carboxydutrophic potential, although they were nonadapted to grow solely on CO. The specific activity was around 4 mmol CO/g VSS · d in the absence of H_2 and CO_2 , and 5 to 8 mmol CO/g VSS · d, in the presence of H_2 and CO_2 (Table 1). No lag time was observed; however, for the activity to be fully expressed, CO partial pressure (p_{CO}) had to decrease to 0.15 atm (Figure 1). Such a CO utilization potential by anaerobic sludge was expected as CO is a catabolic intermediate of the acetate dismutation into CH_4 and CO_2 by methanogens. As well CO is a primary substrate for a large number of acetogenic bacteria while it is a true intermediate in acetyl-CoA synthesis by acetogenic bacteria whether they grow on inorganic substrates such as H_2/CO_2 or organic substrates.^{16–18} The detection of H_2 , although at a much lower level than CH_4 , indicated that this apparent carboxydutrophic

Table 1. Specific Mesophilic Activities of the Inoculum (35 °C)

activity type	substrate	mixing regime	specific activity		
			mmol substrate ^d /g VSS · d	mmol CH ₄ /g VSS · d	CH ₄ yield % stoichiometric yield ^e
acetoclastic	acetate	100 rpm	6 ± 0.3	7 ± 0.6	102
hydrogenotrophic	H ₂ /CO ₂ ^a	400 rpm	105 ± 21	27 ± 6	102
carboxydutrophic	CO/N ₂ ^b	100 rpm	4 ± 1	1.2 ± 0.4	97 ± 8
		400 rpm	4 ± 1.5	1 ± 0.2	100 ± 4
	CO/H ₂ /CO ₂ ^c	100 rpm	5 ± 0.7	5 ± 0.8	94 ± 4
		400 rpm	8 ± 1.4	11 ± 1.3	130 ± 12

^a 20%/80% vol./vol.; total pressure 2.5 atm. ^b 20%/80% vol./vol.; total pressure 1 atm. ^c 20%/64%/16% vol./vol.; total pressure 1 atm. ^d Acetate for acetoclastic; H₂ for hydrogenotrophic; CO for carboxydutrophic. ^e 1 mol of CH₄ per mol of acetate; 1/4 mol of CH₄ per mol of H₂; 1/4 mol of CH₄ per mol of CO.

Figure 1. Time course of measured gases (carbon monoxide, methane, hydrogen) in carboxydutrophic activity tests (35 °C and 100 rpm agitation) along with the CO partial pressure evolution.

methanogenesis could have been hydrogenotrophic, combined with CO-dependent H₂ formation. VFAs (predominantly acetate, at 90%) were also detected, although in trace amounts (0.5% of the CO input, when no H₂/CO₂ was added). Thus one cannot either exclude that part of the methane came from acetate, especially as acetogenesis from CO is more thermodynamically favorable than hydrogenogenesis (ΔG° , -44 and -20 kJ/mol CO, respectively). Thus direct conversion of CO into CH₄ might be negligible, as already reported in the literature.^{6,17,19} Under mesophilic conditions, the CO transformation would be the limiting reaction in such a two-step process irrespective of the methane precursor. The CO conversion was minimally or not limited by gas–liquid mass transfer, while methanogenic activity significantly increased at higher agitation (Table 1). The VFA production was higher with the addition of CO₂/H₂ as cosubstrates and accordingly increased with the mass transfer. The measured CH₄ yields were in all cases close to 100% of stoichiometric yield.

Reactor Performance. The carboxydutrophic methanogenic potential of anaerobic granules were then tested in the 30 L closed-loop gas-lift reactor continuously fed with a gas stream of N₂-diluted CO. Different experimental phases were carried out under various operational conditions as shown in Table 2. Actual temperature fluctuated between 34.0 and 36.9 °C, with an overall average at 35.2 °C ± 2.0, while actual pH was 7.1 ± 0.2, except in

the first phase (pH 7.7). Three days after the inoculation the CO loading was increased, then kept relatively constant, at 0.35–0.41 L_{CO} at standard temperature and pressure (STP) or 15–18 mmol per g VSS per day. Without gas recirculation the insufficient gas holdup limited the CO mass transfer and as a result, the CO conversion efficiency at 4%. At day 10, a low gas recirculation was set on which increased the gaseous CO conversion efficiency to 51%. At day 30, the gas recirculation-to-feeding ratio was doubled from 9:1 to 18:1. This increased the gaseous CO conversion efficiency to 70%. However some oxygen accidentally contaminated the gas recirculation, probably due to a leak amplified by the pressure and high flow rate. Therefore to make sure this would not have impaired the bioconversion process, conditions of phase II were resumed, and the gas recirculation ratio was returned back to 10:1 (IIbis in Table 2). This resulted in an even higher conversion efficiency, 62%, as compared to phase II. Thus even if the O₂ contamination could have impaired the carboxydutrophic activity, this better efficiency may also be partially due to some carboxydutrophic enrichment of the biomass since phase II, twenty-five days earlier. Such higher specific conversion potential may have allowed for a higher volumetric conversion potential as well, which may have resulted in a faster depletion of the dissolved CO, and in turn improved the gas–liquid mass transfer rate. Then at day 57 while the gas recirculation ratio was kept at 10:1, the p_{CO} in the feeding stream was augmented from 0.4 to 0.6 atm. While as compared to phase IIbis, the CO conversion efficiency increased to 67%, this allowed only for a relative conservation of the CO conversion as compared to phase III. Then a combination of higher partial pressure and gas recirculation ratio, i.e., 0.6 atm and 20:1 respectively, resulted into a gas conversion further increased to 75% (phase V). This indicates that higher gas recirculation seems to be more effective in enhancing the CO gas–liquid mass transfer than higher p_{CO}. This also seemed to indicate that the CO transfer was the limiting step in the CO conversion process. To corroborate this as well as to quantify the limit of the technology a final experimental phase was performed at high CO loading rate (p_{CO} ≈ 1 atm) and feeding flow rate of ca. 6 L_{STP} CO/g VSS · d (as opposed to ca. 0.4 L_{STP} CO/g VSS · d in the previous stages). As shown in Table 2, this resulted in a drastic decrease of the efficiency although the in-reactor specific bioconversion rate increased from ca. 13 to 18 mmol CO/g VSS · d. Given that one cannot exclude that some inhibition took place at such a high p_{CO}, it is likely that previously the biomass specific CO loading rate hardly exceeded its specific potential for

Table 2. Performance of Reactor, As a Function of the Operational Time and Conditions

phase period (days)	I 3–9	II 10–29	III 30–51	IIbis 52–56	IV 57–72	V 73–96	VI 97–100
CO loading rate (mmol/g VSS·d)	17.2 ± 0.4	16.8 ± 0.6	15.0 ± 1.6	16.7 ± 3.7	18.3 ± 1.6	17.4 ± 1.6	122 ± 5.9
gas recirculation ratio	0	9.4 ± 0.3:1	18 ± 0.7:1	11 ± 1.5:1	10 ± 1:1	20 ± 0.9:1	4 ± 2:1
gas recirculation flow (L/h)	0	36	69	36	36	69	69
gas RT (d)	0.36	0.32	0.32	0.36	0.36	0.37	0.07
oxygen (%)	0	0.3 ± 0.3	1.6 ± 1.3	0	0	0	0.2 ± 0.0
p _{CO} in gas feeding (atm)	0.42	0.43	0.42	0.42	0.61	0.62	0.96 ± 0.6
CO in gas feeding (%)	30 ± 0.5	29 ± 0.5	29 ± 0.7	29 ± 0.3	41 ± 0.2	41 ± 0.3	60 ± 33
CO in reactor headspace (%)	nd	19 ± 5.6	11 ± 2.2	11 ± 0.2	13 ± 0.6	8 ± 0.6	57 ± 26
CO consumed (%)	4 ± 0.4	51 ± 1.1	70 ± 0.9	62 ± 1.1	67 ± 1.1	75 ± 0.8	17 ± 3.8
CO consumed (mmol/g VSS·d)	0.7 ± 0.1	9.2 ± 1.4	10 ± 1.9	10.8 ± 2.8	12.3 ± 1.5	12.8 ± 0.3	18.0 ± 0.1
CH ₄ produced (mmol/gVSS·d)	0.49 ± 0.1	2.43 ± 0.1	2.55 ± 0.4	2.08 ± 0.03	3.03 ± 0.13	2.92 ± 0.09	4.77 ± 1.21
yield CH ₄ /CO (% theor.) ^a	299 ± 36	114 ± 4	98 ± 11	105 ± 13	95 ± 4	95 ± 3	87 ± 1
H ₂ produced (mmol/gVSS·d)	0	0.02	0.10	0.07	0.05	0.01	0.72 ± 0.2
yield H ₂ /CO (% theor.) ^a	0	0.2 ± 0.1	0.8 ± 0.8	0.9 ± 0.1	0.4 ± 0.1	0.1 ± 0	3.6 ± 1.9
acetate produced (μmol/gVSS·d)	0.7 ± 27	−40 ± 122	4.1 ± 23	−9.5	−22 ± 65	nd ^b	60
propionate produced (μmol/gVSS·d)	0.1 ± 10	5.7 ± 19	0.4 ± 5	4.8	−1 ± 2	nd ^b	8.6
methanol produced (μmol/gVSS·d)	37 ± 385	64 ± 219	−49 ± 83	13.4	−4.1 ± 10	nd ^b	−2.6
ethanol produced (μmol/gVSS·d)	0.2 ± 19	3.4 ± 5	−0.7 ± 1	0	0	nd ^b	0

^a Stoichiometric yield: H₂/CO=1 ; CH₄/CO =1/4 ; acetate/CO = 1/4. ^b nd: not determined.

CO conversion, hence that the CO gas–liquid transfer rate was the limitation.

Those performances corresponded to methane yields of 24 to 29% (vol./vol.), i.e., a CO conversion efficiency close to 100%. The excess of methane production noted during the early days (first phase) could readily be explained by the degradation of the organic matter brought in the reactor with the inoculum. The above reactor performance, expressed in terms of methane specific carboxydutrophic productivity, showed an increase over phases II to V from 1.7 ± 0.9 to 3 ± 0.3 mmol CH₄/gVSS·d. In parallel, specific activity tests performed in batch bottles on granules sampled intermittently during the reactor operation provided values tending to slightly increase from 1.2 ± 0.4 mmol CH₄/g VSS·d at inoculation (Table 1) to 1.3 ± 0.2 at phase II and to 1.7 ± 0.4 at phase V (not shown). There was thus a disparity between the in-reactor and bottle activities. Higher specific values found in the reactor as compared to the bottle batch tests for the same granules, likely indicated that the reactor conditions were less limiting as compared to the conditions in the test bottles: notably the gas recirculation in the reactor as well as the feeding continuous mode allowed for a higher nutrients and C-source availability than the batch mode and agitation at 100 rpm in the bottles.

Metabolites other than methane were observed, although in trace amounts. The concentrations accumulated in the liquid are given in Table 2. Methanol was the main cometabolite. This was unexpected because its formation from CO depends on H₂ and is less exergonic than for other liquid metabolites such as acetate or ethanol.²⁰ However as significant concentrations of methanol only occurred until day 30, it is presumed that it is formed from residual intragranular organics introduced together with the inoculum, namely pectins, since granules were originated from a fruit juice wastewater plant. Clostridia have been reported to produce methanol as a major end-product during growth on pectin.²¹ Based on the production rate of each cometabolite, their yield assessed with respect to CO (not shown) appeared

negligible as compared to the CH₄ yield, except for H₂ at phase VI. As already noticed in batch activity tests, higher concentrations of H₂ augmented the homoacetogenic conversion of CO, although minimally with an acetate/CO yield of 1.3% (mol/mol). This was expected as chemolithoautotrophic homoacetogens usually derive the reducing equivalents from H₂.²⁰

Sensitivity to CO. CO is both a substrate and inhibitor. To investigate CO concentration optima, an activity test-based kinetic study was performed, using the nonadapted anaerobic sludge (inoculum) diluted within anaerobic phosphate buffer under different initial p_{CO} varying from 0.09 to 0.91 atm. Those p_{CO} values corresponded to dissolved CO concentrations from 0.09 to 0.79 mM, using a value of 1148 atm·L/mol for the Henry constant at 35 °C.²² Carboxydutrophic and methanogenic specific activity results are presented as a function of dissolved CO concentration in Figure 2A. The other CO fermentation products accumulated in the bottle were also measured at the end of the test, i.e., hydrogen in the gas phase as well as the liquid-phase products, such as acetate, propionate, butyrate, methanol, ethanol, propanol, butanol, and acetone. Acetate was largely the predominant liquid metabolite, representing alone between 72 and 86% of all liquid products on molar basis. Thus only the H₂ and acetate yields (% stoichiometric ratio, i.e., 1 mol H₂ and 1/4 mol acetate per mol CO) are shown in parallel in Figure 2B. The nonacclimated sludge showed a maximum activity of 8.15 mmol CO/g VSS·d at a p_{CO} of 0.19 atm (0.25 atm initial p_{CO}). The p_{CO} for maximal carboxydutrophic activity corresponded to a dissolved CO concentration of 0.17 mmol/L. This is consistent with the two-phase time profile of carboxydutrophic activity i.e., CO oxidation was effective but slow as long as p_{CO} was above 0.15 atm (Figure 1). This also may explain the disparity between the specific activity measured in batch bottles and in the continuous reactor on the same granules, as in the reactor the constant gas recirculation ensured a dilution of the gas at an homogeneous concentration

Figure 2. Specific activity rates measured as a function of the dissolved CO concentration (initial value) (A). Hydrogen and methane yields (% of the stoichiometric yield, i.e., 1 mol H₂ and 1/4 mol acetate per mol CO) measured at the end of the activity test for various dissolved CO concentrations (B).

lower than in the bottles (CO in the reactor headspace, between 8 and 13% (v/v.) during phase III to V, Table 2).

Above the maximal point the specific activity dropped rapidly but CO-inhibition stayed partial: a residual carboxydutrophic activity of 2–3 mmol CO/g VSS·d remained at the highest CO concentrations employed (between 0.3 and 0.82 atm of CO), while methanogenesis inhibition was complete (Figure 2A). This is consistent with literature which typically reports that methanogenesis is very sensitive to CO, while both hydrogenogenesis and acetogenesis seem more tolerant to CO.^{5,16,23,24} This is also consistent with the much lower methanogenic activity in the presence of CO/H₂/CO₂ than in the presence of only H₂/CO₂ when gas–liquid mass transfer is not limiting (400 rpm agitation) (Table 1).

The CO-activity profile exhibited a close similarity with that of enzymatic acetyl-CoA synthesis rates as measured at various CO concentrations by Maynard and Coll,²⁵ with a typical residual steady activity for the same CO range. This residual activity of the acetyl-CoA-synthase (ACS) along with the high CO-sensitivity of hydrogenases¹⁶ likely explained the accumulation of methane precursors, predominantly acetate, at higher CO concentrations (Figure 2B). Many acetogens indeed can grow at high CO concentrations.^{20,26}

Thermophilic Potential. Since synthesis gas is produced at high temperature (≥ 500 °C), it might be more appropriate to operate a syngas-processing bioreactor under thermophilic conditions. To preliminarily investigate such a possibility, specific CO-oxidizing activity tests were performed on the granular anaerobic inoculum at 60 °C, under low and high mass transfer conditions (i.e., agitation at 100 and 400 rpm, respectively) (Table 3). As opposed to mesophilic conditions (Table 1), the thermophilic metabolic pathway and accordingly the methane yield depended on gas–liquid mass transfer. At low agitation (100 rpm), only 0.03 mol CH₄ was obtained for each mol CO consumed and high quantities of acetate were generated (acetate/CO 25% mol/mol on average). At higher mass transfer

(agitation at 400 rpm), the specific activity increased 5-fold and final CH₄ yields were maximal. However, markedly the process was found to be biphasic where hydrogen was produced first by the biological WGS conversion of CO which was followed by hydrogenotrophic methanogenesis once CO was depleted (Figure 3). This two-step form of the thermophilic biomethanation of CO has been confirmed with another nonadapted anaerobic sludge incubated at 400 rpm, although the activity rates were slower (3.4 mmol CH₄/g VSS·d). This suggests that at low gas–liquid mass transfer rate (i.e., low CO available in liquid) CO-homoacetogenesis was favored over hydrogenogenic carboxydutrophy, while at a higher dissolved CO concentration (resulting from higher gas–liquid mass transfer) hydrogenogenesis became more competitive. Consistent with literature findings, these results also confirm the low sensitivity of thermophilic hydrogenogenic carboxydutrophs to CO,²⁰ while thermophilic methanogens seemed as or more sensitive than their mesophilic counterparts.^{4,23} Overall, the CO-bioconversion into CH₄ was significantly faster under thermophilic conditions as compared to mesophilic operation.

Prospects. Industrial anaerobic granule sludge clearly has a significant carboxydutrophic methanogenesis potential and does not require special acclimation. Enrichment under thermophilic conditions should provide the anaerobic granules with a CO-bioconversion potential significantly larger than under mesophilic conditions, providing that the gas–liquid transfer limitation is minimized. However could not this advantage be mitigated by lower gas–liquid mass transfer rate at higher temperature? The volumetric gas–liquid mass transfer rate (k_La) may vary broadly, depending on the reactor configurations and hydrodynamic conditions: k_La values reported for syngas varied from 2 to 3670 h⁻¹ in a temperature range of 20–35 °C.²⁷ However there are no k_La data for the thermophilic range. One often anticipates that a decrease of the gas solubility at higher reactor temperature will decrease the transfer rate accordingly. The CO solubility decreases by 35% from 35 to 60 °C.²⁸ On the other hand, the CO gas diffusivity almost doubles when temperature is raised from 35 to 60 °C.²⁹ This means that for the same reactor configurations and hydrodynamic conditions, the transfer rate at 60 °C would become equal to that at 35 °C at dissolved CO concentration of 6.2 mg/kg H₂O (i.e., 0.22 mM) and exceed it below 6.2 mg/kg H₂O by up to 24% at dissolved CO concentration approaching 0 (Figure S2, Supporting Information). Interestingly the concentration range at 60 °C for higher gas–liquid mass transfer corresponds also to CO concentrations that are not inhibitory to microorganisms.

After enrichment, one might assume that the anaerobic sludge specific activity potential could reach a level of 20 mmol CH₄/g VSS·d, especially under thermophilic conditions. If a typical UASB-type reactor containing active granular biomass at 40 g VSS/L reactor is retrofitted for syngas treatment, one could expect a volumetric productivity in the range of 20 m³ STP CH₄/m³ reactor·d, so long as the mass transfer limitation is completely alleviated. However in order to achieve this, further improvement of the gas–liquid mass transfer rate is needed, which will represent a major engineering challenge.

Municipal solid wastes (MSW) in North America³⁰ are typically composed of a compostable or digestible organic fraction (food, yard, garden) at 17% (ca. 30% dryness), a nonreadily digestible organic fraction (paper, board, wood, plastics, non-compostable organics) at 54% (ca. 45% dryness), and an inorganic fraction (metals, glass, and other inerts) at 29%.

Table 3. Carboxydrotrophic Specific Activities of the Inoculum at Thermophilic Conditions (60 °C)^c

mixing regime	specific activity		acetate concentration, ^a mmol/L	% stoichiometric yield ^b	
	mmol CO/g VSS · d	mmol CH ₄ /g VSS · d		CH ₄ yield	acetate yield
100 rpm	4 ± 0.0	0.1 ± 0.01	5.3 ± 1.2	11 ± 5	100 ± 35
400 rpm	20.9 ± 3.2	5.6	1.9 ± 0.1	104	16 ± 2

^a Measured in bottle at end of the test. ^b 1/4 mol CH₄ per mol CO; 1/4 mol acetate per mol CO. ^c Headspace: CO/N₂ 20%/80% vol./vol.; total pressure 2.5 atm.

Figure 3. Comparison of the time courses of measured gases (carbon monoxide, methane, hydrogen) in carboxydrotrophic activity tests under mesophilic and thermophilic conditions. Open symbols: 60 °C and 400 rpm agitation; filled symbols: 35 °C and 400 rpm agitation.

Anaerobic digestion of the first fraction, at a degradation efficiency of 60% and a yield of 0.5 m³ STP CH₄/kg solid degraded, could generate an overall CH₄ production of 15 m³ STP CH₄ per metric ton of MSW. If this is complemented by organics gasification of the second fraction and biomethanation of the syngas produced, at a conservative integrated yield of 0.3 m³ STP CH₄/kg solid gasified, an overall CH₄ production of up to 73 m³ STP CH₄ could be expected for this fraction, resulting hence in a total of about 88 m³ STP CH₄ per metric ton of MSW i.e., almost six times the potential by anaerobic digestion alone. However the energy-intensiveness of gasification may mitigate the net energy output.³¹

■ ASSOCIATED CONTENT

Supporting Information. Details on the reactor setup, the analytical methods, and a graphics-based comparison of relative CO gas–liquid mass transfer rates as a function of [CO] at 35 and 60 °C. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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■ ACKNOWLEDGMENT

The authors wish to thank A. Corriveau, C. Beaulieu, and S. Deschamps for analytical assistance. The study was partially

funded by the AAFC-NRCan-NRC National Bioproducts Program. NRC paper # 53340.

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