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BIOTECHNOLOGY METHODS

Rapid enrichment of (homo)acetogenic consortia from animal feces using a high mass-transfer gas-lift reactor fed with syngas

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Abstract A gas-lift reactor having a high mass transfer coefficient ($k_La = 80.28\ h^{-1}$) for a relatively insoluble gas (carbon monoxide; CO) was used to enrich (homo)acetogens from animal feces. Samples of fecal matter from cow, rabbit, chicken, and goat were used as sources of inoculum for the enrichment of CO and H_2 utilizing microbial consortia. To confirm the successful enrichment, the Hungate roll tube technique was employed to count and then isolate putative CO utilizers. The results of this work showed that CO and H_2 utilizing consortia were established for each inoculum source after 8 days. The number of colony-forming units in cow, rabbit, chicken, and goat fecal samples were 3.83×10^9, 1.03×10^9, 8.3×10^8, and 3.25×10^8 cells/ml, respectively. Forty-two colonies from the animal fecal samples were screened for the ability to utilize CO/H_2. Ten of these 42 colonies were capable of utilizing CO/H_2. Five isolates from cow feces (samples 5, 6, 8, 16, and 22) were highly similar to previously unknown (homo)acetogen, while cow-7 has shown 99 % similarity with *Acetobacterium* sp. as acetogens. On the other hand, four isolates from chicken feces (samples 3, 8, 10, and 11) have also shown high CO/H_2 utilizing activity. Hence, it is expected that this research could be used as the basis for the rapid enrichment of (homo)acetogenic consortia from various environmental sources.

Keywords Gas-lift reactor · Mass transfer · (Homo)acetogen · Synthesis gas

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Introduction

Global energy spending has rapidly escalated over the last century because of increases in the world population and rapid industrialization, particularly in developed countries. During this period of growth, crude oil has been the main resource used to cater to the energy demand. However, the expected depletion of crude oil reserves and environmental issues that have been attributed to the use of crude oil such as global warming, climate change, and atmospheric pollution have triggered the need to develop sustainable alternative energy resources. Researchers are now studying various forms of renewable energy including biomass and bioenergy, which are considered particularly advantageous, to satisfy the growing demand for energy sustainability. It is commonly thought that biofuels produced from renewable resources such as plants would help to reduce the use

of fossil fuels and CO_2 production [23], though concerns exist about competition with edible crops and land use [12]. For these reasons, there is a need for biofuels to be made from a non-edible biomass. Lignocellulosic biomass is often regarded as the most promising alternative [23] and can be converted to biofuels either simultaneously through saccharification and fermentation or sequentially by gasification and microbial processes. Simultaneous saccharification and fermentation schemes are often plagued by the fact that lignin, which has 30 % more energy content than the cellulosic biomass, is not efficiently utilized [24]. This limitation can be overcome by microbial processes that convert syngas to biofuels [14].

Biomass gasification is a process that can be used to convert diverse types of biomass into synthesis gas, which is comprised mainly of CO, H_2, and CO_2. This process is carried out at high temperature and atmospheric pressure using a controlled amount of gasifying agent (oxygen and/or steam) [11]. Non-renewable materials such as coal, coke, industrial/urban solid (organic) wastes [11], natural gas [4], and pyrolysis oil [28] can also be used to produce syngas via gasification [3]. Syngas produced in this manner could then be used as a promising feedstock for the manufacturing of biofuels. Syngas can be converted into sustainable energy or chemicals through chemical or biological conversion processes. Examples of chemical conversion processes include methanation, Fischer-Tropsch chemistry, synthesis of oxygenates, embrace carbonylation, and methanol methyl formate chemistry [16]. The chemical conversion of syngas involves specific H_2/CO ratios and purity requirements for the reactions, which are often costly, in order to avoid the irreversible poisoning of noble metal catalysts [29]. In contrast, biological conversions using microbial catalysts have certain inherent advantages over chemical routes, as they can utilize a variety of biomass feedstocks regardless of their quality, and do not usually require a specific H_2/CO ratio [14]. Although several microbial catalysts capable of converting syngas to biofuels and biochemicals have been reported, there is still a need to secure highly productive and robust strains for the wider commercialization of syngas microbial processes. Conventional attempts to obtain CO or H_2 utilizing strains have typically involved time intensive cultivation in serum vials, which requires several transfers that can lengthen the enrichment time up to 1 month [1, 19]. Hence, the development of a capable method of providing high selectivity within a short time is strongly desired.

Mass transfer limitations due to the low solubility of syngas components are another big hurdle for obtaining a high syngas conversion rate [25]. Previously, Chang et al. [9] demonstrated that the mass transfer rate is a key factor for cultivating *Eubacterium limosum* KIST612 using a gas-lift reactor. However, operating the reactor at high k_La conditions can overcome the CO mass transfer limitations, even at high cell density and low CO partial pressure. Thus, a high k_La is significant for enriching (homo)acetogens, which apply a pathway for reducing CO_2 to acetyl-CoA and to assimilate CO_2 into cell carbon [13]. Even though (homo)acetogens produce acetate as a main end product, it has also been reported that some acetogens can produce butyrate (*Butyribacterium methylotrophicum* and *E. limosum*) [7, 26] and ethanol (*Clostridium autoethanogenum*, *C. ljungdahlii*, and *C. carboxidivorans*) [1, 20, 27] as end products. Since ethanol and butyrate have a greater energy-generating potential compared to acetate, microbiologists are quite interested in finding specific (homo)acetogens that produce ethanol and butyrate as major products. To date, (homo)acetogens have been isolated from anaerobic habitats such as animal feces, agricultural settling lagoons, and sewage digesters [13]. Among these environmental habitats, a number of (homo)acetogens have been isolated from animal feces, especially feces from herbivores. For example, *C. ljungdahlii* DSM13528^T and *C. autoethanogenum* DSM10061^T have been isolated from chicken yard waste [27] and from rabbit feces [1], respectively.

In this study, a gas-lift reactor with a high mass transfer coefficient was employed for the enrichment of (homo)acetogens from animal feces; animal feces are prominent sources of (homo)acetogens, which are prominent sources of these microorganisms. It was expected that the successful enrichment of CO-utilizing microbial consortia would occur rapidly.

Materials and methods

Inoculum sources

Each animal fecal sample (cow, rabbit, chicken, and goat) was collected from a local livestock farm (35°17′N 126°44′E, South Korea). Wet fecal samples were collected from pens, and the samples were immediately put into plastic bags before being transported to the laboratory. For each experiment, approximately 0.5 g of the fecal sample and 0.1 ml of a sodium sulfide solution (2.5 %) were mixed with 10 ml of saline solution (under anoxic and aseptic conditions) in an anaerobic glove box (Coy Lab. Products, Inc., Grass Lake, MI, USA). An aliquot (4 ml) of each mixture was then used as the inoculum in the gas-lift reactor.

Media composition

The composition of carbonate-buffered basal medium (CBBM) used for the enrichment of (homo)acetogens was adopted from Chang et al. [7] except for the addition of

0.1 g/l KCl, 0.2 mg/l $Na_2WO_4{\cdot}2H_2O$, and 14 mg/l $Al_2(SO_4)_3{\cdot}12H_2O$ to the trace minerals. The stock salt solution and trace mineral solution were anaerobically prepared, and 2 g/l of yeast extract was added in order to reduce the lag phase and to provide a nutrient source for growth. The pH of the medium was adjusted to 7.2 using 1 N NaOH prior to the boiling. The medium was then cooled under N_2/CO_2 (4:1) (Sinilgas Co., Gwangju, Korea) purging to ensure the anaerobic conditions and then stored in serum vials (Wheaton Scientific Co., Millville, NJ, USA) sealed with butyl rubber stoppers (Bellco Glass, Inc., Vineland, NJ, USA). Medium was autoclaved (Vision Scientific Co., Bucheon, Korea) at 121 °C for 15 min. Vitamin solution [9], $Na_2S{\cdot}9H_2O$ (2.5 %), and cysteine-$HCl{\cdot}H_2O$ (0.3 g/l) were prepared separately. The same media compositions were used for the isolation experiments.

Bioreactor setup

A gas-lift reactor with a working volume of 0.5 l was used in this experiment (Fig. 1). The reactor was sealed with butyl rubber stoppers to prevent atmospheric oxygen penetrating into the reactor. All reactor parts and tubing (Masterflex PharMed BPT tubing, Cole-Parmer, Vernon Hills, IL, USA) were then autoclaved before the reactor setup. The reactor temperature was maintained at 30 °C by circulating water through the water jacket surrounding the reactor. Next, a 10-l gas bag (FlexFoil Grab Bags, SKC Gulf Coast Inc., Houston, TX, USA) was filled with $CO/H_2/CO_2$ (4.5:4.5:1) (Sinilgas Co., Gwangju, Korea), and a peristaltic pump (Watson Marlow 505S, Watson-Marlow Inc., Falmouth, Cornwall, UK) was set between the gas bag and reactor. The mixture gas was passed through a Ti-NTA solution [25 mM Ti(III), 100 mM nitrilotriacetic acid], which is an efficient, sulfur-free reducing reagent used in the preparation of anaerobic media to retain a low redox potential [22], before entering into the reactor via a sintered glass filter. Pure N_2 gas (Sinilgas Co., Gwangju, Korea) was purged through the reactor, and medium was injected simultaneously through the sample ports using 50-ml gas-tight hypodermic syringes (Korea Vaccine Co., Ltd., Ansan, Korea) fitted with a 0.20-μm disposable syringe filter unit (Advantec MFS Inc., Dublin, CA, USA). N_2 purging was then stopped, and the peristaltic pump was operated at a gas flow rate of 175 ml/min. The substrate was continuously recovered and recycled to the gas bag using the method described in [9].

k_La measurement

The gaseous substrate must be dissolved in a liquid medium in order to be taken up by microbes. The transfer of gases between gas and liquid phase in a bioreactor then relies on diffusion through a liquid film at the interphase between a gas bubble and the liquid phase of a cell [18]. Considering the laminar fluid flow, CO absorption can be assumed to be controlled by the liquid phase [10], and the gas concentration at the gas–liquid interface will be in equilibrium with the CO concentration in gas phase. The overall volumetric mass transfer rate ($R = M/h$) for a liquid during the gas transfer process can be described by

$$R = k_La(C^* - C_L), \quad (1)$$

where k_La is the mass transfer coefficient (h^{-1}) based on the liquid-phase resistance to the mass transfer, a is the bubble surface area per unit volume, $C^*(M)$ the saturated dissolved gas concentration, and $C_L(M)$ the real dissolved gas concentration in the liquid. In the absence of microorganisms, Eq. 1 can be solved to obtain

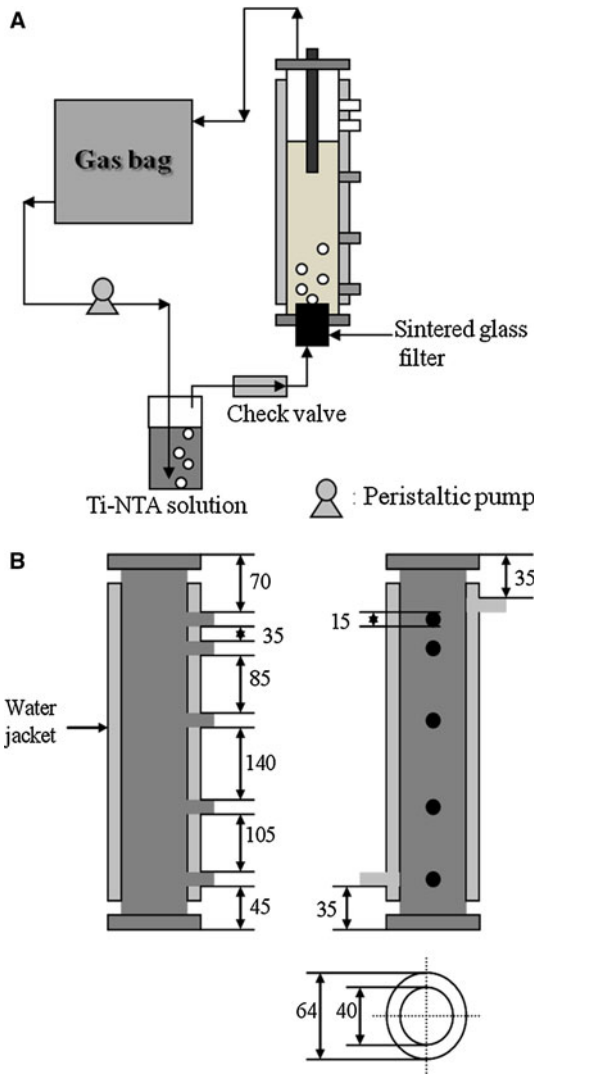

Fig. 1 Schematic (**a**) and dimensions (**b**) of the reactor (dimensions not to scale)

$$\ln\left(1-\frac{C_L}{C^*}\right) = -(k_L a)t. \quad (2)$$

Equation 2 is in linear form and can be used to directly obtain $k_L a$ by finding the slope of the line. To determine $k_L a$, CO was pumped into the reactor through a sintered gas filter installed at the bottom of the reactor (Fig. 1); the function of the sintered glass filter is to achieve a small bubble size by splitting the larger gas bubbles. The desired flow rate of the substrate was then achieved using a peristaltic pump, and a bubble meter (DryCal DC-2, Bios International Corp., Butler, NJ, USA) was used to measure the gas flow rate. After ensuring anaerobic conditions in the reactor, the continuous flow of CO was maintained by the peristaltic pump (Watson Marlow 505S, Watson-Marlow Inc., Falmouth, Cornwall, UK). During operation, liquid medium samples containing the dissolved gas were taken from the reactor every 20 s using gas-tight syringes. The liquid samples were immediately transferred to Pyrex glass tubes and heated in the heating block (Multi-Block Heater, Barnstead International, Dubuque, IA, USA) for 20 min at 95 °C. Then, 0.2 ml of gas from the head space (C_L) was measured using a gas chromatograph thermal conductivity detector (GC-TCD) (GC: ACME 6100, Young Lin Instrument Co., Anyang, Korea). Triplicate values of dissolved CO (C_L) were plotted against the time, and the resultant data were fitted using a hyperbolic curve to obtain the saturated concentration of CO (C^*). Fitted values of C_L and C^* were then used to determine $k_L a$ using Eq. 2; Chang et al. [6] used a similar method to measure C_L.

Hungate roll tube preparation and isolation

The Hungate roll tube technique [21] was used to count microorganisms in the enriched culture and to isolate (homo)acetogens. In order to maintain anaerobic conditions, the medium (CBBM) in Hungate roll tubes was purged with O_2-free N_2 and 2 % agar was added to the medium before sealing the tubes using butyl rubber bung followed by crimping with aluminum seals. The Hungate roll tubes were then pressurized at 101.3 kPa with the CO and H_2 in 1:1. After autoclaving, the medium bottles were cooled to 55 °C, and Bromocresol green (BCG; 0.4 %, w/v) was then added to the medium to detect acid-producing microbes. Serially diluted samples were then inoculated to the tubes. These Hungate roll tubes (with inoculum and all nutrients) were immediately rolled under cooled water and placed into the incubator at 37 °C. Total colony numbers [colony forming units (CFU/ml)] were counted after 8 days of incubation.

The individual colonies were picked up by using an autoclaved metal rod and transferred to the serum vials, which were pressurized to 101.3 kPa with H_2 and CO (1:1). This step was performed in the anaerobic glove box (Coy Laboratory Products, Inc., Grass Lake, MI, USA). The microbes were then grown in a shaking incubator (SI-600R, Jeio Tech, Daejeon, Korea) at 37 °C and 180 rpm for the cultivation of isolates. Enough cell mass was achieved in the first liquid culture after 3 days, and the liquid cultures were sent to a sequencing company (Macrogen, Seoul, Korea) to sequence the 16S rRNA gene.

16S rRNA gene sequence analysis

Genomic DNA of isolates was extracted using the InstaGene™ Matrix (BIO-RAD). The PCR reaction was performed with 20 ng of genomic DNA as the template in a 30-µl reaction mixture by using a EF-Taq (SolGent, Korea) as follows: activation of Taq polymerase at 95 °C for 5 min, 35 cycles of 95 °C for 45 s, 55 °C, and 72 °C for 1 min each were performed, finishing with a 10-min step at 72 °C. Universal primers 27F 5′ (AGA GTT TGA TCM TGG CTC AG) 3′ and 1492R 5′ (TAC GGY TAC CTT GTT ACG ACT T) 3′ were used. The 16S rDNA sequences were assembled by using MEGA version 4.0. The taxonomic classification of the isolates was achieved by searching for the closest 16S rRNA gene sequence deposited in the GenBank database using BLAST software (http://www.ncbi.nlm.nih.gov). Note that the 16S rRNA gene sequences determined in this study have been deposited in the GenBank under the following accession numbers: JN688156 (Cow-5, 6, 8 and 22), JN688157 (Cow-16), JN688158 (Cow-7), and JX041515 (Chicken-3, 8, 10, and 11).

Analyses

The optical density (OD) was measured at a wavelength of 600 nm using a UV–VIS spectrophotometer (Lambda12, PerkinElmer, Waltham, MA, USA) to determine the cell mass. The pH was measured using a pH meter (780 pH meter, Metrohm, Herisau, Switzerland). Carbon dioxide and carbon monoxide were measured using a gas chromatograph (GC; ACME 6100, Young Lin Instrument Co., Anyang, Korea) equipped with a thermal conductivity detector (TCD) and Carboxen-1000 column (Supelco, Bellefonte, PA, USA). During GC operation, the oven temperature was initially maintained at 60 °C for 5 min and was then increased to 215 °C at a rate of 30 °C/min. The injector and detector temperatures were 150 and 200 °C, respectively, and the flow rate of the carrier gas (He) was 30 ml/min.

Hydrogen was measured using a GC-TCD (GC-2010, Shimazu, Japan) equipped with a capillary column (CP-Pora Plot Q, Shimazu, Japan). For this measurement, the oven temperature was controlled at 33 °C, and the injector

and detector temperatures were maintained at 110 °C; nitrogen was the carrier gas at a rate of 20 ml/min.

Volatile fatty acids and solvents were measured using a GC (ACME 6000, Young Lin Instrument Co., Anyang, Korea) equipped with a flame ionization detector (FID) and capillary column (AquaWax, Alltech, Deerfield, IL, USA). The oven temperature was increased from 50 to 200 °C at a rate of 12 °C/min. Both the injector and detector temperatures were 250 °C and nitrogen was the carrier gas (1 ml/min). An aliquot (0.25 ml) of the centrifuged sample was acidified by adding 0.5 M phosphoric acid, and *n*-propanol was used as the internal standard.

Results and discussion

Determination of k_La

Chang et al. [9] reported that the CO mass transfer rate was critical for cultivating *E. limosum* KIST612 using a gas-lift reactor; the k_La of CO was found to be 72 h^{-1}. They suggested that the CO mass transfer limitation could be avoided by maintaining the dissolved CO concentration at a high k_La condition even at high cell density and low CO partial pressure. In an earlier study (Chang IS, Ph.D. thesis), it was also determined that a serum vial has a k_La value of around 13 h^{-1} during a shaking incubation of 150 rpm [8]. Thus, we decided to find the maximum performance of our system in terms of k_La before the microbial enrichment process. By adjusting the gaseous substrate pump speed (rpm), the k_La was measured at various flow rates before a maximum measurement of 80.28 h^{-1} at 175 ml/min was attained. Since this value was higher than our previously reported [9] values, the reactor was then operated at 93 rpm (175 ml/min) using a peristaltic pump. Note that although we subsequently achieved a higher k_La of 94.32 h^{-1} at 110 rpm (207 ml/min), we decided to maintain the reactor operation at a lower gas flow rate (175 ml/min) because of secure system operation and maintenance. The results of dissolved CO concentration (C_L) as a function of time and plot of Eq. 2 for k_La at 175 ml/min are depicted in Fig. 2, and also the measured values for k_La are shown in Fig. 3.

Enrichment of (homo)acetogens from animal feces

The enrichment of (homo)acetogens were successfully done in 8 days from the initial time of inoculation in the gas lift reactor. Throughout these experiments, the OD and pH were monitored daily; a biomass (OD) increase came with a pH drop due to the production of acetate and butyrate. The cow, rabbit, and chicken fecal sample inoculated reactors showed a greater biomass increase and pH

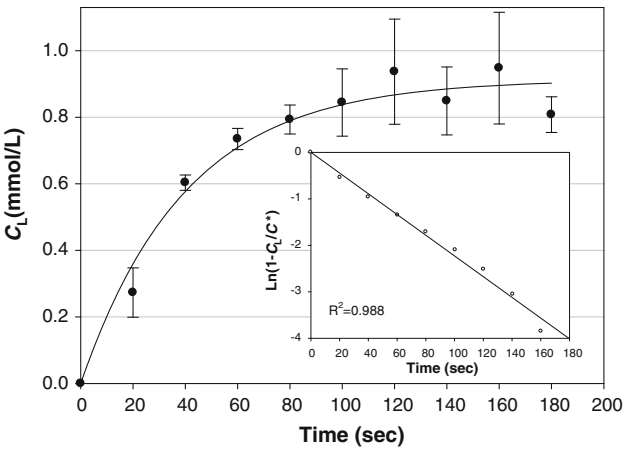

Fig. 2 Measurement of volumetric mass transfer coefficient (k_La) in a gas lift reactor at a CO flow rate of 175 ml/min. Each point for the dissolved CO concentration (C_L) is obtained from the row mean of triplicate values using simple error graph on SigmaPlot 10. The curve is fitted using hyperbolic function to get the saturated CO concentration (C^*). The fitted values of C_L and C^* are used to find k_La using Eq. 2

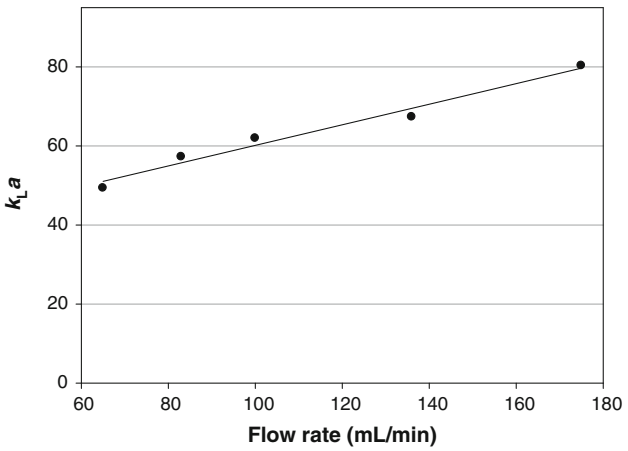

Fig. 3 Measured k_La at various flow rates (65, 85, 105, 145, 175 ml/min). Each value was obtained from the triplicate measurements of C_L and C^*

drop than the goat fecal sample, in addition to a lowering of the CO partial pressure. The aim here was to see the significant decrease of CO from the gas phase. The results presented in Table 1 showed that the final CO ratio in these three reactors decreased from 45.0 to 30.23, 31.02, and 23.29 %, respectively, whereas the goat fecal sample inoculated reactor only displayed a minimal decrease. The decrease of CO partial pressure is justified in that (homo)acetogens can utilize CO as sole carbon and electron source via the acetyl-CoA pathway [13]. Therefore, the decrease in CO partial pressure indicated that CO was used as the substrate, though the H_2 consumption was relatively lower than the CO consumption; this finding can likely be explained by the fact that CO is a hydrogenase

Table 1 Comparison of substrate consumption ratio and organic chemicals produced from different animal fecal samples using a gas-lift reactor

Animal fecal sample	Culture Time (h)	Max OD_{600}	Final pH	CFU/ml[a]	Final gas ratio in mixed gas (%)			Final products concentration[b] (mg/l)			
					CO	H_2	CO_2	Acetate	Ethanol	Butyrate	Butanol
Cow	192	0.94	5.61	3.83×10^9	30.23	46.59	23.18	531	87	165	10
Rabbit	192	1.16	5.50	1.03×10^9	31.02	45.04	24.67	478	105	198	ND
Chicken	192	1.49	5.60	8.30×10^8	23.29	43.48	33.23	711	498	389	251
Goat	192	0.53	6.61	3.25×10^8	44.98	39.64	15.38	328	44	83	ND

ND not detected

[a] CFU: colony-forming units. Results are the average of triplicate counting

[b] Product: based on the GC chromatogram, the presented values are the difference between final and initial concentration of the products in the gas-lift reactor

inhibitor [17]. However, due to the possibility of CO loss during the reactor operation, it was decided to confirm the CO-utilizing ability of the individual colonies. As such, it was clear that methanogenesis did not occur, since no methane was detected during operation. This could be due to the fact that CO has high affinity for the metals ions; it binds with them resulting in their reduced activity due to uptake hydrogenases inhibition [5]. Also methanogens are very sensitive to acidic pH; thus pH drop during the enrichment due to the accumulation of fatty acids could be another reason attributable to inhibit the methane production.

The formation of products further confirmed the different strengths of enrichment. All reactors had acetate formation as the main product, which indicates that (homo)acetogens were successfully enriched; butyrate and ethanol were also significantly detected as products. The chicken fecal sample inoculated reactor displayed relatively high ethanol production; we posit here that the presence of ethanol-producing microorganisms may be dominant in the chicken feces-enriched culture. A number of ethanogenic acetogens isolated from ruminant animal waste, agricultural lagoon, duck pond sediment, and sewage digesters have been previously reported. These acetogens, *C. autoethanogenum*, *C. ljungdahlii*, *C. carboxidivorans* P7, *C. ragsdalei* P11, and *B. methylotrophicum* [1, 2, 20, 27, 30], have been studied with respect to syngas fermentation for ethanol production. Among them, *C. ljungdahlii* was also isolated from chicken yard waste [27].

Isolation of (homo)acetogens from the enriched culture

Serially diluted enriched cultures were inoculated into Hungate roll tubes in order to isolate the individual colonies. CO disappearance from the Hungate tubes was monitored, and color change around colonies due to acid formation was observed. The exterior view of acid-producing colonies changed the surrounding color of the medium from an initial blue to light green within 48 h from the time of inoculation. Since this acid accumulation could be from the yeast extract, the whole CO disappearance from the tubes was delayed for 8 days. Thus, the presence of CO/H_2-utilizing and acid-producing (homo)acetogens was confirmed. The results of the colony count shown in Table 1 indicated that four fecal sample-enriched cultures had cell numbers of over 10^8 CFU/ml. Since cow feces had the highest cell numbers while chicken feces had the highest concentrations of acetate and ethanol, they were selected to confirm the microbial acetogenic activity. In the cow feces-enriched culture, 24 colonies were selected, and 18 colonies were selected from the chicken feces-enriched culture.

Confirmation of isolates as (homo)acetogens

The results of individual colony cultivation in the liquid culture are depicted in Table 2. The overall substrate consumption and product profile is provided along with the 16S rRNA gene sequence analysis. Among 42 colonies cultivated in the liquid cultures, 10 isolates showed evidence of CO/H_2 consumption, coupled with product formation. The remaining 32 colonies showed fatty acid production, but they did not consume the CO/H_2. Hence, CO/H_2 consumption was only shown by the colonies that have acetyl CoA pathways. Six isolates from cow feces (samples 5, 6, 7, 8, 16, and 22) produced acetate and butyrate, while Cow-7 produced acetate but not butyrate. Ethanol was produced by Cow-5, 6, 7, and 8 only. Three isolates from chicken feces (samples 3, 8, and 10) produced acetate and a small amount of ethanol related to type strains, but no butyrate was produced. On the other hand, Chicken-11 had the greatest acetate concentration. This difference in the final product concentration between the isolates gives support to the hypothesis that each isolate may possess a unique metabolic pathway or that differences may be due to food differences and individual

Table 2 Confirmation of microbial acetogenic activity and taxonomy results of isolated microbes from enriched culture of cow and chicken fecal sample

Isolate	CO and H_2 consumption (mmol)		Concentration of products[a] (mg/l)			Closest related bacterium (accession no., % identity)	Accession no.
	CO	H_2	Acetate	Ethanol	Butyrate		
Cow-5	0.21 ± 0.01[b]	0.74 ± 0.01	523 ± 14	42 ± 7	348 ± 7	*Oscillibacter* sp. GH1 (JF750939.1, 99 %)	JN688156
						Oscillospiraceae bacterium NML 061048 (EU149939.1, 99 %)	
Cow-6	0.21 ± 0.01	0.75 ± 0.02	543 ± 74	42 ± 3	359 ± 44	*Oscillibacter* sp. GH1 (JF750939.1, 100 %)	JN688156
						Oscillospiraceae bacterium NML 061048 (EU149939.1, 100 %)	
Cow-7	0.20 ± 0.01	0.73 ± 0.01	554 ± 28	7 ± 1	ND	*Acetobacterium* sp. Mic1c04 (AB546243.1, 99 %)	JN688158
						Uncultured bacterium gene (AB509206.1, 99 %)	
Cow-8	0.21 ± 0.02	0.72 ± 0.02	250 ± 132	25 ± 7	188 ± 88	*Oscillibacter* sp. GH1 (JF750939.1, 100 %)	JN688156
						Oscillospiraceae bacterium NML 061048 (EU149939.1, 100 %)	
Cow-16	0.22 ± 0.01	0.74 ± 0.03	140 ± 119	ND	150 ± 8	*Oscillibacter* sp. GH1 (JF750939.1, 99 %)	JN688157
						Oscillospiraceae bacterium NML 061048 (EU149939.1, 99 %)	
Cow-22	0.20 ± 0.01	0.71 ± 0.01	81 ± 8	ND	106 ± 9	*Oscillibacter* sp. GH1 (JF750939.1, 100 %)	JN688156
						Oscillospiraceae bacterium NML 061048 (EU149939.1, 100 %)	
Chicken-3[c]	0.07 ± 0.01	0.25 ± 0.07	517 ± 31	13 ± 4	ND	Mixed culture (*Escherichia coli* + *Clostridium* sp.)	JX041515
Chicken-8[c]	0.07 ± 0.02	0.29 ± 0.09	533 ± 22	10 ± 14	ND	Mixed culture (*Escherichia coli* + *Clostridium* sp.)	JX041515
Chicken-10[c]	0.05 ± 0.01	0.23 ± 0.04	545 ± 51	10 ± 18	ND	Mixed culture (*Escherichia coli* + *Clostridium* sp.)	JX041515
Chicken-11[c]	0.20 ± 0.01	1.42 ± 0.10	822 ± 60	35 ± 4	ND	Mixed culture (*Escherichia coli* + *Clostridium* sp.)	JX041515
Clostridium ljungdahlii	0.19 ± 0.01	0.94 ± 0.04	1,062 ± 48	139 ± 59	ND	–	
Clostridium autoethanogenum	0.20 ± 0.01	0.89 ± 0.04	986 ± 309	96 ± 7	ND	–	

ND not detected

[a] Product: based on the GC chromatogram, the presented values are the difference between the final and initial concentration of the products in the gas-lift reactor

[b] All values are the average of triplicate data. Data were obtained from 72 h of cultivation

[c] Chicken-3, 8, 10, and 11 were isolated under the same condition during the second enrichment in the gas-lift reactor since we did not get single isolates during the first enrichment

digestive systems; further study is clearly needed in this area. In any case, it is clear that (homo)acetogenic consortia successfully occurred.

Table 2 presents the similarity between isolates and their closest related bacteria as a 16S rRNA gene sequence, as identified during a comparison with a GenBank database via a BLAST analysis. (Cow-4 was deleted.) Five isolates (Cow-5, 6, 8, 16, and 22) were identified as *Oscillibacter valericigenes* (with 98 % similarity), and one (Cow-16) showed a slightly different sequence (<5 bp) compared to the others. These had not yet been reported as (homo)acetogens. In a previous report, *O. valericigenes* was found to be a mesophilic, strictly anaerobic, non-sporulating, gram-negative bacterium that ferments pentose

(arabinose, ribose, xylose) and hexose to fatty acids, though no (homo)acetogenic activity was reported [15]. Cow-7 was identified as *Acetobacterium* sp. (with 99 % similarity), which is a known acetogens. Meanwhile, from the GenBank database via a BLAST analysis, Chicken-3, 8, 10, and 11 were found to be *Escherichia* sp. although the culture showed CO consumption. Thus, it is anticipated that *Escherichia* sp. was the main strain in the first liquid culture. However, after several transfers, *Clostridium* sp. becomes dominant. The same has been proven by the genome analysis of chicken-11 strain (data not shown).

Conclusion

A high mass-transfer gas-lift reactor was used to successfully enrich (homo)acetogens from animal feces. This enrichment was rapidly performed within 8 days, and subsequent enumeration of the cell number and culture profiles of isolates supported the successful enrichment of the (homo)acetogens. Ten strains that were isolated from the cow feces- and chicken feces-enriched cultures demonstrated notable acetogenic properties. Shortening the enrichment time will certainly reduce the overall time to get isolates. Thus, it is expected that such a high mass-transfer gas-lift system can be used as a rapid enrichment apparatus for obtaining (homo)acetogens from syngas or H_2/CO.

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