Hydrogen Production by Anaerobic Digestion of Crude Glycerol from Biodiesel Fuel Manufacturing Process – Part I. Inoculum Characteristics and the Effect of Crude Glycerol

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Abstract

In order to utilize crude glycerol, a byproduct from biodiesel fuel production, its conversion through anaerobic fermentation was examined. It was found that crude glycerol enhances hydrogen production while suppressing methane fermentation. Characteristics of the anaerobic fermentation with crude glycerol were examined. There existed an optimum crude glycerol concentration when it was digested with an inoculum of cattle excrement origin.

Keywords: Biodiesel fuel, Crude glycerol, Anaerobic digestion, Hydrogen fermentation

1 Introduction

Production of biodiesel fuel by transesterification of vegetable oil necessarily produces byproduct glycerol, which amounts to ca. 10 wt% of the biodiesel produced. Byproduct crude glycerol contains unreacted methanol, catalyst such as KOH, fatty acids, soap and/or oil cake. These impurities make the use of the byproduct crude glycerol for any purpose difficult. Here, the possibility of converting it to gaseous fuel by anaerobic fermentation is explored.

Anaerobic fermentation (or digestion) is a collection of biotic processes composed of the following steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. In the first step, biomass macromolecules are hydrolyzed to simpler and water-soluble molecules such as sugars, amino acids and fatty acids. In the second step products from the first step are further converted to volatile fatty acids, ammonia, CO_2 and H_2S. The main products of the third step are acetic acid, CO_2 and H_2. In the last step methanogen produce a mixture of CH_4 and CO_2 from the products of previous steps. The collection of microorganic species responsible for anaerobic digestion may differ depending on the operating temperatures and substrates, and acclimation may be necessary for the starting flora to adapt to specific operation conditions. Furthermore, if any of these four steps is disrupted for any reason, the fermentation will stop at that step, leaving the process with the products of the previous step. For instance, if the last methanogenesis step is disrupted somehow, the fermentation products in gas phase will be predominantly CO_2 and H_2.

There are ca. 10 papers known to the authors that deal with anaerobic fermentation of crude glycerol [1-10]. Some of them utilize specific bacterium such as *Enterobacter aerogenes* HU-101 [1] and *Escherichis* coli MG1655 [10] as inoculum, or heat treatment of mixed culture [2, 9] or photofermentation [3, 8] to enhance H_2 production. Only one paper [6] report the use of commonly available inoculum from activated sludge. With the use of proper inoculum and optimized fermentation conditions, some show high biohydrogen production [11]. In the present two-part series paper, we attempted the processing of byproduct crude glycerol from biodiesel production by anaerobic fermentation, using an easily obtainable inoculum from cattle excrement. The effect of crude glycerol as substrate of anaerobic fermentation by this inoculum will be examined.

2 Experimental

2.1 Raw materials

Cattle excrement obtained from Yamanashi Prefectural Dairy Experimental Station, Hokuto City, Japan, was used as starting inoculum for the present experiments. Crude glycerol was obtained from the biodiesel production equipment (Nanko Co. Ltd., Japan, model ME-100, 100 L/day) installed at the Faculty of Engineering, University of Yamanashi. The characteristic of this biodiesel plant is that it does not wash biodiesel to separate glycerol: instead it uses separation-by-sedimentation technique, and settled crude glycerol is withdrawn from the bottom of the reactor tank. Catalyst used for transesterification was KOH, and as a result crude glycerol produced was highly alkaline. Its characteristics and compositions are listed in Table 1.

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Table 1 Characteristics and Compositions of Byproduct Crude Glycerol Employed in the Present Study

pH	12.7
glycerol / wt%	43
methanol / wt%	13
water / wt%	2.5
palmitate / wt%	4.1
oleate / wt%	20
steroids / wt%	0.3
ash / wt%	8.6
potassium / wt%	4.2
sodium / wt ppm	72
phosphorous / wt ppm %	10
Other impurities / wt%	8.5

gas production had levelled off. This procedure was meant to enrich the bacteria consortium present in the cattle excrement to the maximum extent through digestion of the organic matter in the excrement.

2.3 Procedures

Each inoculum prepared was divided into smaller batches, typically 50 mL in a 100 mL flask, and crude glycerol was added up to 14 wt % (see Fig. 2). Again, the air in the head space of each reactor (flask) was replaced with nitrogen, a Tedlar bag was connected and shaking of the entire assembly was started with temperature-controlled water bath shaker. Throughout the present experiment, mesophilic digestion temperature of 35 °C or 37 °C was employed. The amount of gas produced was measured with appropriate interval by water displacement method. Gas composition analysis was done by using a Shimadzu GC-8A gas chromatograph equipped with a thermal conductivity detector. An activated carbon column operated at 70 °C was used for gas separation with Ar carrier gas.

Table 2 Two Types of Inoculums Prepared in the Present Study

	Preparation method	Total solids / wt%	Volatile solids / wt%	pH
Inoculum A	Cattle excrement was sieved (2 kg after sieving) and mixed with 2 L water	5.99	5.07	7.60
Inoculum B	Inoculum A was methane fermented under anaerobic conditions at 37 °C for ca. 150 h until biogas production had levelled off	5.33	4.54	5.59

2.2 Inoculum preparation

The cattle excrement was first sieved with a screen with 1.7 mm opening in order to remove straw mulching and other debris. The sieved cattle excrement was mixed with water of the same weight, agitated, and its pH was measured. This inoculum is designated Inoculum A, as shown in Table 2. Total solids shown in Table 2 is measured as the residue after heating at 105 °C for 24 h, and volatile solids after heating at 550 °C for 24 h.

For Inoculum B preparation, 4 kg of Inoculum A was placed in a 10 L polyethylene tank. The air in the head space was replaced with nitrogen, a Tedlar bag was connected and shaking of the entire assembly was started with temperature-controlled water bath shaker at 37 °C. The amount of gas produced was measured once a day by water displacement method, until when the substrate in the inoculum was almost exhausted, i.e.,

3 Results and discussion

3.1 Organic load of cattle excrement for Inoculum A enrichment

Firstly, fermentation characteristics of Inoculum A with varying organic load were examined. Figure 1 shows the time course of biogas accumulation, with incremental Inoculum A addition at various time intervals. Run #1 (navy diamonds) was run only with the starting 100 g of Inoculum A. Its biogas production leveled off at about 86 h, with the final gas amount being 69 mL at 338 h (biogas production per g substrate is 0.69 mL). Run # 2 (blue crosses) was fed with Inoculum A twice at 72 h interval totaling 300 g of Inoculum A by 144 h, and its biogas production levelled off at ca. 324 h and it produced a total of 451 mL biogas (biogas production per g substrate is 1.50 mL). Run #3 (yellow triangles) was fed with inoculum A 4 times with

48 h interval totaling 500 g of Inoculum A by 192 h, and 620 mL of biogas was produced (biogas production per g substrate is 1.24 mL). Run #4 (purple squares) was fed with the same 6 times with 24 h interval totaling 700 g of Inoculum A by 144 h, and its biogas production levelled off at ca. 270 h producing total of 519 mL biogas (biogas production per g substrate is 0.74 mL).

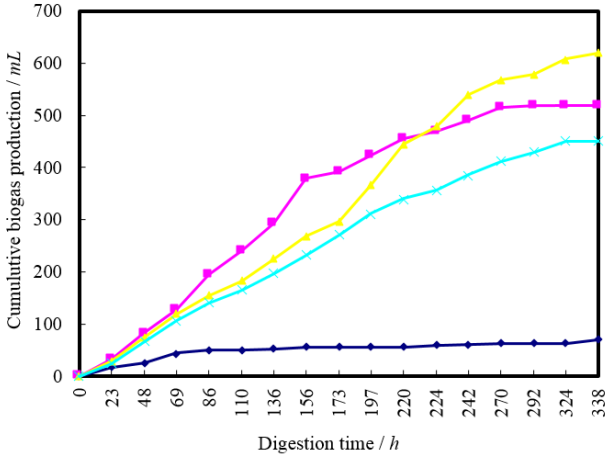

Figure 1 Time course of biogas accumulation with incremental substrate (Inoculum A) addition at different intervals. Navy diamonds: Run #1 with no substrate addition, blue crosses: Run #2 with addition of substrate twice with 72 h interval, yellow triangles: Run #3 with addition of the same 4 times with 48 h interval, purple squares: Run #4 with addition of the same 6 times with 24 h interval. Fermentation temperature was 35 °C.

From these data, one would notice that the amount of produced biogas is almost proportional to the amount of substrate (Inoculum A) added to each fermentation reactor between two runs, Run #1 and Run #4 (the amounts of biogas produced per g substrate are almost the same (see Table 3, column 5). On the other hand, Run #2 and #3 are more efficient, producing almost two times or more biogas per g substrate. It may be possible to consider the amount of produced biogas as a measure of inoculum enrichment. Apparently, there appears to be an optimum interval for substrate addition for the purpose of enriching inoculum through incremental addition of fresh substrate. With the specific conditions of the present study and with Inoculum A as a substrate, 72 h interval appears to give the best biogas production characteristics, hence the best inoculum enrichment.

This also implies that Runs #1 and #4 in which biogas production levelled off at ca. 86 h and ca. 270 h, respectively, substrates are not depleted completely, and there may be some other reason(s) for the biogas production leveling off. This is also apparent from Table 2 that Inoculum B is still hold almost 90 % of volatile solid compared to Inoculum A, even after biogas production stopped in the fermentation of Inoculum A.

Table 3 lists the overall gas compositions obtained for the runs shown in Figure 1 (measured on the accumulated gas). It is noteworthy to mention here that although its proportion is very small, we did observe hydrogen in the produced biogas.

Table 3 Overall Biogas Composition for Runs Shown in Figure 1

Run #	Total substrate fed / g	Substrate feeding interval / h	Total biogas produced / mL	Biogas production per g substrate / mL	Biogas composition / %		
					CH_4	CO_2	H_2
1	100	-	69	0.69	19.5	80.1	0.35
2	300	72	451	1.50	34.2	65.8	0.05
3	500	48	620	1.24	30.1	69.9	0.04
4	700	24	519	0.74	33.5	66.4	0.10

3.2 Biogas production characteristics of crude glycerol as substrate with Inoculum B

Then, we examined the characteristics of crude glycerol as a substrate. For this purpose, Inoculum B was used as a starting inoculum, in order to extract and highlight the characteristics of crude glycerol as substrate, since Inoculum B has stopped its initial fermentation stage already, and any activity observed with crude glycerol addition would be the direct consequence of crude glycerol. Various amount of crude glycerol were added to the inoculum (Run A(0) through A(14)) with the numbers in parenthesis indicating the weight percentage of added crude glycerol. Initial pH of each reactor varied slightly due to the alkalinity of the crude glycerol, thus the initial pH

was adjusted to the intrinsic value of Inoculum B, 5.59, by adding the appropriate amount of 2.0 M HCl. Fermentation was performed at 37 °C for 216 h in a constant-temperature water bath shaker. At the end of the fermentation, pH of all the reactors were in between 4.92–5.52. Two runs for each condition were performed, and the results were shown as an average of the two.

Figure 2 shows the gas compositions obtained for each glycerol concentrations (average of two runs each). Due to the limited number of experiments (two runs each), data fluctuations are not removed completely. For instance, the standard deviation for H_2 production at A(6) is 10.1 mL (±26.4 %). Nevertheless, a few observations may be made in the figure:

From these observations, the following conclusions may be drawn:

1) Crude glycerol as a substrate enhances hydrogen fermentation and suppresses methane production.

2) There exists an optimal concentration of crude glycerol, ca. 7 wt%, for hydrogen production.

3) Crude glycerol also enhances CO_2 production. The activity is also concentration dependent as in the case of hydrogen production, but the active crude glycerol concentration for CO_2 production is shifted to 4–7 wt% compared to that of hydrogen production, which is 6–7.5 wt%.

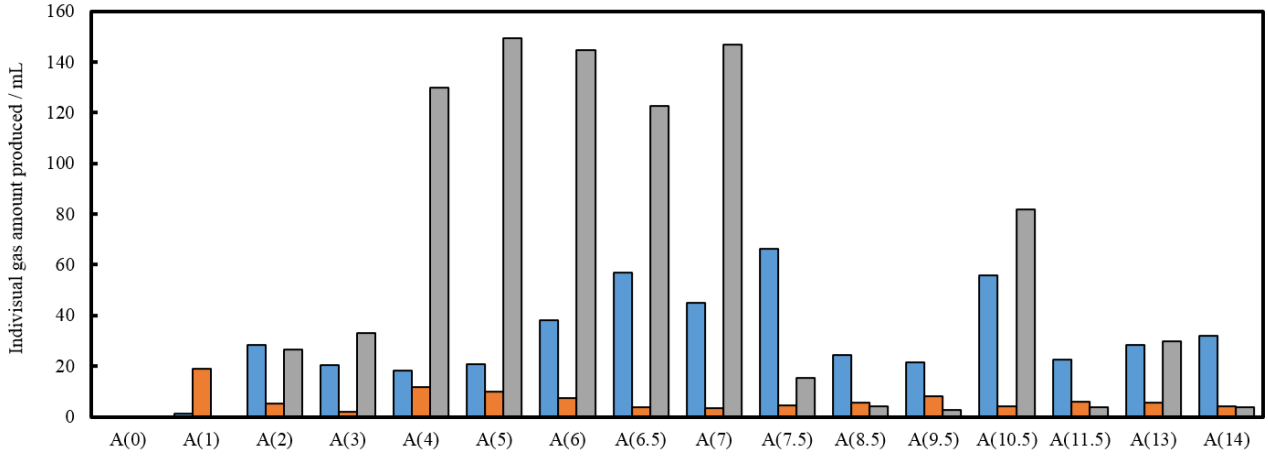

Figure 2 Biogas compositions produced with Inoculum B and various amount of crude glycerol as substrate. Numbers in parenthesis indicate the weight percentage of crude glycerol. Total amount of inoculum and crude glycerol: 100 g. Blue bar: H_2, orange bar: CH_4 and gray bar: CO_2. The fermentation temperature was 37 °C. The standard deviation for H_2 production at A(6) is 10.1 mL (±26.4 %).

1) Run A(0), which had no added glycerol substrate, naturally shows no biogas production, since Inoculum B has already stopped its initial fermentation stage (see Table 2).

2) With 1 wt% of crude glycerol (Run A(1)), hydrogen production is barely noticeable (the produced amount was 1.2 mL), while above 2 wt% (Run A(2) and on), sizable amount of hydrogen production is observed.

3) With a few inexplicable fluctuations (like abrupt H_2 increase at Run A(10.5)), hydrogen production seems to peak at ca. 7 wt% crude glycerol concentration.

4) CO_2 production is also active in the 4–7 wt% crude glycerol concentration region, but not necessarily related to hydrogen production.

4) The hydrogen production efficiency is in between 0.26–0.37 mmol H_2/g-crude glycerol, or 0.32 mmol H_2/g-crude glycerol on an average, in the range of 6–7.5 wt% crude glycerol.

4. Conclusions

In an attempt to process byproduct crude glycerol from biodiesel production, its anaerobic fermentation characteristics were examined using easily obtainable inoculum from cattle excrement. Crude glycerol as a substrate enhanced hydrogen fermentation and suppressed methane production. The phenomenon was dependent on crude glycerol concentration, with the highest hydrogen production in the range of 6–7.5 wt% crude glycerol, producing ca. 0.32 mmol H_2/g-crude glycerol on an average.

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