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Anaerobic Bioconversion to Value-Added Byproducts from Two Promising Biomass in the Future, Crude Glycerol and Sewage Sludge

HAYATO TOKUMOTO* and CHIE KIMURA

Department of Chemical Engineering, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531

* E-mail: tokumoto@chemeng.osakafu-u.ac.jp

* TEL & FAX: +81–72–254–9301

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Sewage sludge generates an enormous amount of new biomass in urban cities. As new biomass, crude glycerol is co-produced from biodiesel production and its generation is expected to increase in the future. Sewage sludge containing 0.01% (v/v) crude glycerol showed the highest methane production rate of 10.82 mL/d/g-TS on day 7, which was 1.52 times higher than the control sludge without supplementation of crude glycerol. Addition of crude glycerol at 0.4% (v/v) resulted in production of valuable resources such as hydrogen and 1,3-propanediol (1,3-PDO). A strongly alkaline solution of crude glycerol enhanced the solubilization of organic matter to various organic acids from the solid phase of the sewage sludge, which resulted in a significant decrease in total solid (TS) content in the sludge compared with the control, an estimated 60% decrease from the first day. Anaerobic co-digestion of sewage sludge and crude glycerol as a co-substrate is considered to be a cost effective method for incineration of sewage sludge (with high water content) and should help to reduce landfill capacity. The proposed process promotes the possibility of biodiesel production by anaerobic bioconversion of crude glycerol.

Key words: Sewage sludge, Crude glycerol, Hydrogen, methane, 1,3-Propanediol

1. Introduction

The production of sewage sludge is increasing in accordance with the extension of sewer distribution networks and new installation of wastewater treatment plants. The cost of the treatment and disposal of sewage sludge is estimated to comprise half of the total cost of wastewater treatment[27]. Therefore, minimizing the negative impact of sewage sludge on the environment is a major challenge. Anaerobic digestion is an appropriate technique for reducing the volume and weight of excess sludge before final disposal, and it is employed worldwide as the oldest and most important process for sludge stabilization. Anaerobic sewage sludge digestion can remove pathogens and thereby help to protect humans and the environment[9]. Nordberg et al.[18] reported that highly concentrated methane gas is obtained from sewage sludge during anaerobic digestion which is useful as a way to partly recover the bioenergy of sludge[8,20]. However, conventional anaerobic digestion can be inefficient because of the low organic content in a combined sewer system. Co-digestion in the presence of higher organic content such as food waste could be a reliable option to enhance the activity of anaerobic microorganisms. A proper mixture provides synergistic and complementary advantages that offset the lack of carbon sources in the sewage sludge and dilute harmful or excessive substances which may inhibit the action of anaerobes in food waste[14]. A review of the literature suggests that for co-digestion of sewage sludge in the presence of organic waste, inclusion of crude glycerol with a large amount of organic matter derived from waste food oil makes the ideal substrate mixture for anaerobic digestion of sewage sludge.

Biodiesel is a major biofuel that is used in place of fossil fuels for mitigating greenhouse gas emissions[17]. Biodiesel is mainly produced from the transesterification of vegetable oils with alcohol[21], the main byproduct of which is glycerol. Crude glycerol is a major byproduct of biodiesel production. It is estimated that for every 9 kg of biodiesel produced, about 1 kg of crude glycerol is formed[7]. The significant increase of late in the production of biodiesel has created a glycerol surplus that has resulted in a dramatic 10-fold decrease in crude glycerol prices[30]. In the past, the biodiesel industry considered glycerol a desirable co-product that could contribute to the economic viability of biodiesel production; nowadays, glycerol is often regarded as a waste stream with an associated disposal cost. Tokumoto and Tanaka reported that glycerol was readily decomposed when sewage sludge, acting as a fermentation promoter, was added to the anaerobic reactor[26]. Overall, the fermentation resulted in the generation of hydrogen, 1,3-propanediol (1,3-PDO) and various organic acids.

The main objective of this work was to evaluate the use of crude glycerol as a co-substrate to enhance the productivity of the anaerobic digestion of sewage sludge. The effect of the total solid (TS) content in sewage sludge on the productivity was examined. There was also an attempt to investigate the TS removal efficiency during the anaerobic digestion.

2. Materials and methods

2.1. Seed sludge

The seed sludge used for the anaerobic co-digestion test was sampled from the Senboku sewage disposal plant (Sakai, Japan). The seed sludge was collected on the same day as the anaerobic co-digestion test because otherwise it underwent decay within a few days. The collected sludge contained a large amount of water (about 99.5%), thus, the TS concentration in the sludge was only 4.0 g-dry/L. Therefore, the sludge was centrifuged to condense it and the TS concentration was adjusted to 20.4 g-dry/L and 42.8 g-dry/L as needed.

2.2. Co-substrate

Crude glycerol was derived from biodiesel fuel produced by the transesterification reaction of waste cooking oil from the cafeteria at our university. The crude glycerol contained 554 g/L of glycerol and 482 g/L of total organic carbon.

2.3. Anaerobic digestion test of sewage sludge using crude glycerol as the co-substrate

All anaerobic digestion tests were performed in auto sampler vials (PerkinElmer 20-CV) with butyl rubber and aluminum seals at 310 K without shaking. Glass vials with a capacity of 21.6 mL were filled with 5 mL of a mixture of 4 mL of sewage sludge (TS concentration was set at 4.0 g-dry/L, 20.4 g-dry/L or 42.8 g-dry/L) and 1 mL of crude glycerol (glycerol concentration was adjusted to the desired level). The head space of the vials was replaced with 100% nitrogen under 1.2 atm.

2.4. The production of methane and hydrogen from the anaerobic digestion

During the anaerobic co-digestion, gas in the headspace of the vials was removed in 0.5 ml samples using a 2.5 ml gas-tight syringe (Ito Co., Shizuoka, Japan). The gas composition (H_2, N_2, CH_4, CO_2) was analyzed by gas chromatography (GC-8APT; Shimadzu, Kyoto, Japan) using a stainless-steel 80/100 mesh Porapak Q column (3 m×3.0 mm) and a thermal conductivity detector. Argon was used as the carrier gas at a rate of 20 mL/min. The injector, oven, and detector temperatures were 373 K, 343 K, and 373 K, respectively. A 80% N_2/20% CO_2 gas mixture and CH_4 gas were used as the standard gases. There were three independent samples measured for each condition.

2.5. The concentrations of organic acid, glycerol and 1,3-PDO in the fermentation broth from the anaerobic digestion

The concentrations of several organic acids were determined by high-performance liquid chromatography (HPLC) using a Shimadzu LC-10AD VP pump equipped with two ion-exclusion chromatography columns (Shim-pack SCR-102H; 8×300 mm; Shimadzu) with post column pH-buffered conductivity detection (Shimadzu CDD-6A). The mobile phase consisted of a 5 mM p-toluenesulfonic acid (PTSA) solution at a flow rate of 0.8 mL/min. Mixtures of 5 mM PTSA and 100 mM ethylenediaminetetraacetic acid were used as post-column reagents, both at flow rates of 0.8 mL/min. The column temperature was maintained at 313 K.

The concentrations of glycerol and 1,3-PDO were measured by HPLC using a HSS-1500 system (Jasco International Company, Limited, Tokyo, Japan) equipped with a size-exclusion column (Shodex SUGAR KS-801, 8×300 mm; SHOWA DENKO Company Limited, Tokyo, Japan) and a differential refractometer (RI-2031; Jasco). The mobile phase was ultrapure water at a flow rate of 0.4 mL/min. The column temperature was maintained at 343 K. The retention time of glycerol and 1,3-PDO was confirmed by injecting a sample containing a known amount of glycerol and 1,3-PDO.

3. Result and discussion

3.1. The effect of using crude glycerol as a co-substrate on the productivity of the anaerobic digestion of sewage sludge

Crude glycerol is strongly alkaline with a pH of 12.6. Therefore, a preparatory experiment was conducted to investigate the relationship between the crude glycerol concentration in the reactor and the pH of a mixture of the sewage sludge (4 mL) and the crude glycerol (1 mL). The pH increased moderately in accordance with increasing crude glycerol concentrations. Generally, the sewage sludge has the capability to provide buffering action because it contains a large amount of salt (e.g. potassium, sodium). When the crude glycerol concentration was over 0.4% (v/v), the pH rapidly increased to 8.93. Thus, the minimum concentration of crude glycerol used as a co-substrate was set at 0.01% (v/v) (pH value: 6.59) and the maximum concentration was set at 0.4% (v/v).

Figure 1 shows the biogas production from the anaerobic co-digestion of sewage sludge with different concentrations of crude glycerol. The control sludge without crude glycerol as a co-substrate produced methane over time. Glycerol degraded rapidly on day 1 in the sludge supplemented with 0.01% (v/v) crude glycerol. The highest methane production and rate occurred on day 7 and was estimated at 1.58 mL and 10.82 mLCH_4/d/g-TS respectively (Fig. 1a). Other researchers have come to similar conclusions, but the supplemented crude glycerol concentration varies greatly across the reported studies. Fountoulakis et al.[10] found that digestion of sewage sludge produced 10.4±0.97 mL CH_4/d/g-TS before the addition of crude glycerol and 16.4±1.20 mL CH_4/d/g-TS after the addition of crude glycerol (1% (v/v) in the feed). It would appear that the large amount of organic materials (e.g. methanol, organic acids, amino acids and free fatty acids) in the crude glycerol enhanced the activity of the methanogenic bacteria in the sewage sludge. A significant decrease of methane production in the digester fed with sewage sludge

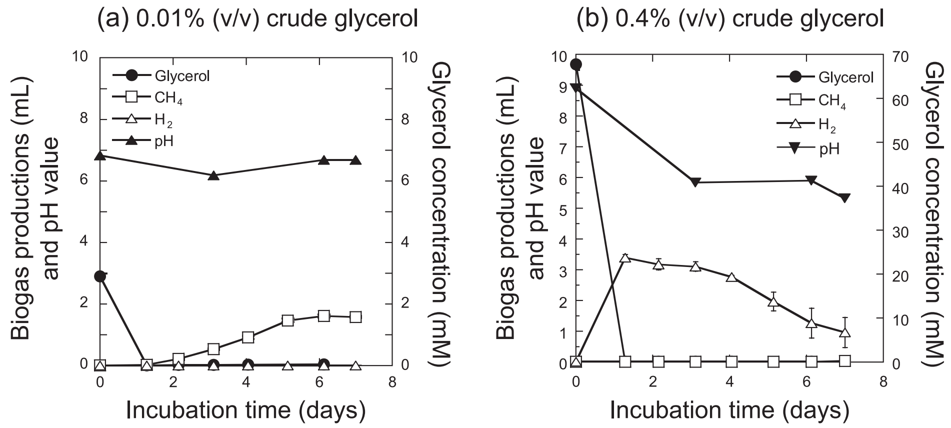

Fig. 1. (a) Biogas production, pH value and glycerol concentration in the anaerobic co-digestion of sewage sludge with 0.01% (v/v) crude glycerol. (b) Biogas production, pH value and glycerol concentration in the anaerobic co-digestion of sewage sludge with 0.4% (v/v) crude glycerol. (●) Glycerol concentration, (□) Methane, (△) Hydrogen. Sewage sludge contained 4.0 g/L TS. The co-digestion was incubated at 310 K for 7 days. Data are means ± standard deviations of results from three independent samples.

supplemented with 0.4% (v/v) crude glycerol was observed. This occurred due to low pH (5.33) and the build-up of volatile fatty acids (VFA). This is strongly supported by the previous work by Holm-Nielsen et al.[13], who found that with low concentrations (<5 g/L) of glycerol, VFA and individual fatty acids showed no signs of organic loading; however, when the glycerol content was increased, organic overloading tended to occur. The optimal crude glycerol concentration for methane production may depend on the properties of the seed sludge. In this study, a supplement of 0.01% (v/v) crude glycerol had a positive effect on methane production from the anaerobic digestion of the sludge.

Figure 1b shows the hydrogen production from the anaerobic co-digestion of sewage sludge with crude glycerol. Hydrogen was only detected from the sludge supplemented with 0.4% (v/v) crude glycerol. The production reached a maximum of 3.38 mL on day 1 when the glycerol was completely decomposed. When the anaerobic digestion of 6.0% (v/v) glycerol occurred in the presence of fermentation promoter, hydrogen generation reached 9.85 mL after 7 days of incubation[26]. Although the hydrogen decomposed over time, the rate of hydrogen production was independent of the methane concentration. Generally hydrogen is rapidly taken up and converted to other products by hydrogen-consuming microorganisms during the methanogenic stage of anaerobic digestion[12,20]. To harvest hydrogen, the anaerobic digestion of glycerol must be blocked at the hydrogen and acetic acid formation stage, namely, the acidogenic stage of anaerobic digestion. In a previous study, it was found that sterilized sludge produced hydrogen from the anaerobic digestion of sewage sludge because sterilization of the sewage sludge screened hydrogen-producing spore-forming microorganisms (against heat shock) from the sludge microflora[28]. However, this process required high temperature, which is unfavorable for maintaining an economical bioprocess. Another study showed that the photosynthetic bacterium *Rhodopseudomonas palustris* is capable of the photofermentative conversion of glycerol, both pure and crude, to hydrogen[23]. However, the usefulness of this process is limited because of difficulties with light penetration and uniform light distribution[15]. The process proposed herein can achieve hydrogen production through the simple addition of 0.4% (v/v) crude glycerol to sewage sludge. Compared with the studies described above, our experimental protocol is more advantageous for hydrogen production from anaerobic digestion.

Figure 2a shows the 1,3-PDO concentration in the fermentation broth from the anaerobic co-digestion of sewage sludge supplemented with different concentrations of crude glycerol. An increase of crude glycerol concentration to 0.4% (v/v) resulted in a significant production of 1,3-PDO, which achieved a high concentration and yield on day 2, estimated at 38.4 mM and 0.57 mol/mol-glycerol respectively. Even after reaching the maximum, the 1,3-PDO was not decomposed. These results suggested that the productivity of the anaerobic digestion of sewage sludge was primarily related to the supplemented crude glycerol concentration. In fact, a supplement of 0.01% (v/v) crude glycerol improved the methane productivity whereas 0.4% (v/v) crude glycerol promoted both hydrogen and 1,3-PDO productivity.

Figure 2b shows the composition of the organic acids in the fermentation broth from the anaerobic co-digestion of sewage sludge with two different concentrations of crude glycerol. Total production of organic acids increased as the crude glycerol concentration increased. The sludge supplemented with 0.4% (v/v) crude glycerol produced a significant amount of total organic acids and attained a maximum concentration of 31.7 mM on day 6. After that, the concentration became almost constant. Propionic acid (26%) and acetic acid (38%) were the major organic acids followed by butyric acid (14%). It appears that a significant amount of organic acid lead to a pH (5.33) decrease in the digester, which likely slowed methanogenic activity. In other words, a significant amount of organic acids may prevent the conversion of intermediates such as hydrogen and 1,3-PDO into methane.

Although there is not a general consensus, many studies have demonstrated that anaerobic fermentation under alka-

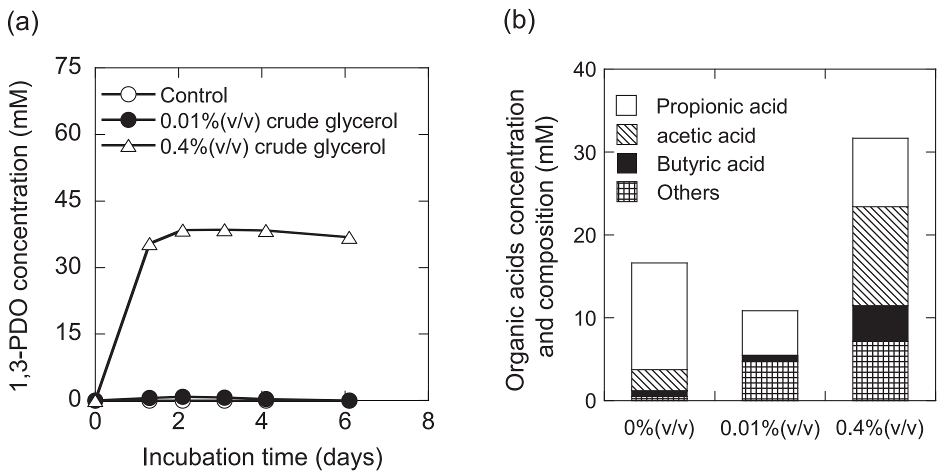

Fig. 2. (a) 1,3-PDO concentration in the fermentation broth from the anaerobic co-digestion of sewage sludge with different concentrations of crude glycerol.
Sewage sludge contained 4.0 g/L TS. Crude glycerol concentration was set at (○) 0 (control), (●) 0.01% (v/v), (△) 0.4% (v/v). The co-digestion was incubated at 310 K for 6 days. Data are means of the results from three independent samples. (b) Total organic acid concentration and composition in the fermentation broth after 6 days incubation. Data are means of the results from three independent samples.

line conditions can significantly improve VFA production from sewage sludge[3,31]. It has been observed that alkali improves the solubilization of organic matter from the solid phase of the sludge and then increases its bioavailability for acidogens in anaerobic reactors. Therefore, it seemed that the strong alkaline nature of the crude glycerol resulted in the solubilization of organic matter from the solid phase of the sludge which in turn, enhanced the activity of the acetogenic bacteria.

3.2. The effect of TS content in sewage sludge on the productivity of the anaerobic digestion

Figure 3 and Table 1 shows the 1,3-PDO concentration and yields in the fermentation broth from the anaerobic co-digestion of different TS contents of sewage sludge in the presence of 0.4% (v/v) crude glycerol. An increase of TS content to 20.4 g/L resulted in a higher production of 1,3-PDO. The concentrated sludge (de-watered by centrifugation) showed a maximum production and yield of 45.7 mM and 0.64 mol/mol-glycerol on day 2, which was 1.13 times higher than from the non-concentrated sludge containing 4.0 g/L TS. However, a further increase of TS content to 42.8 g/L had a negative effect on 1,3-PDO production. The anaerobic digestion of 6.0% (v/v) glycerol in the presence of fermentation promoter yielded a conversion of 0.424 mol/mol-glycerol after 7 days of incubation[27]. In a previous study, the production of 1,3-PDO by a newly isolated *C. butyricum* strain was investigated[19]. The highest 1,3-PDO concentration obtained from a continuous culture was 407–631 mM, with a conversion yield of 0.67 mol/mol-glycerol. Another study of a fed-batch fermentation with sucrose as the co-substrate yielded 1.10 M 1,3-PDO and 0.62 mol/mol-glycerol with a productivity of 1.61 g/L/h in the presence of *Klebsiella oxytoca* M5a1, a mutant strain deficient in lactic acid biosynthesis[29]. The first report of a pilot-scale production

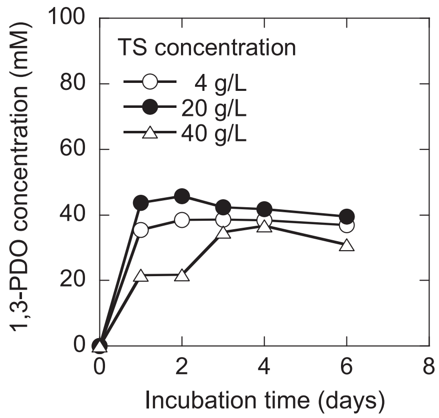

Fig. 3. 1,3-PDO concentration in the fermentation broth from the anaerobic co-digestion of different TS concentrations of sewage sludge with 0.4% (v/v) crude glycerol.
TS concentration in sewage sludge was set at (○) 4.0 g/L, (●) 20.4 g/L, (△) 42.8 g/L. The co-digestion was incubated at 310 K for 6 days. Data are means of the results from three independent samples.

of 1,3-PDO using *Klebsiella pneumoniae* M5a1 obtained 773 mM with a yield of 0.52 mol/mol-glycerol[5]. Although the maximum concentration and yield of 1,3-PDO in this study is not the highest among the above described studies, there are some advantages to this process for producing 1,3-PDO. First, there is no requirement for a single pure bacterial strain that can produce 1,3-PDO from glycerol and second, the instruments necessary to carry out the digestion can be directly installed at the sewage disposal plant so that the high concentration of sewage sludge can be remediated immediately. Additionally, the sludge concentration favors not only 1,3-PDO production but also the sludge treatment, which allows more sludge to be treated at a time than using an anaerobic digestion process to treat the non-concentrated sludge.

Table 1. Comparison of the yields of major digestion products (1,3-PDO, acetic acid, and propionic acid) from the anaerobic co-digestion of sewage sludge with different concentrations of crude glycerol on day 2 when glycerol was decomposed completely. Sewage sludge contained 20.4 g/L TS. Crude glycerol concentration was set at (a) 0 (control), (b) 0.01% (v/v), (c) 0.4% (v/v). The co-digestion was incubated at 310 K for 6 days. Data are means of the results from three independent samples.

	Yield of digestion products (g/g-TS)		
	1,3-PDO*[1]	Acetic acid	Propionic acid
(a) Control	0	0	0
(b) 0.01% (v/v) crude glycerol	0.313	0.0526	0.0348
(c) 0.4%(v/v) crude glycerol	0.529	0.0747	0.0406

*[1] 1,3-PDO yield is the ratio of 1,3-PDO production to digested glycerol.

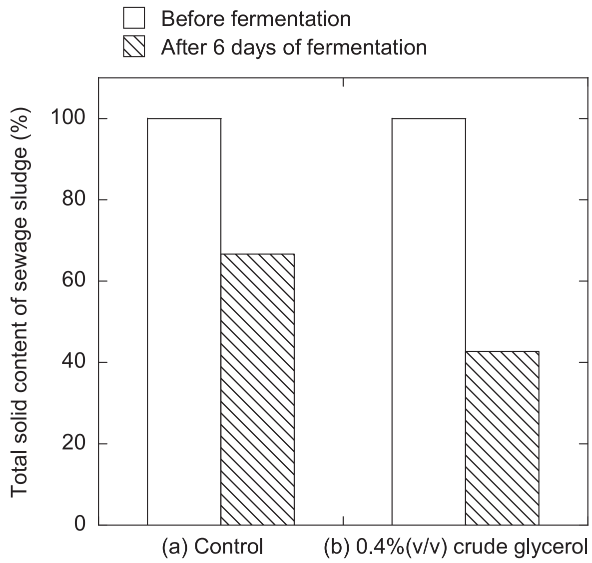

Fig. 4. (a) TS content in sewage sludge from the anaerobic digestion of sewage sludge after 6 days of incubation. Sewage sludge contained 20.4 g/L TS.
The digestion was incubated at 310 K for 6 days. Data are means of the results from three independent samples. (b) TS content in sewage sludge from the anaerobic co-digestion of sewage sludge with 0.4% (v/v) crude glycerol after 6 days of incubation. Except for the addition of crude glycerol, the co-digestion was performed under the same conditions. Data are means of the results from three independent samples.

3.3. The TS removal efficiency during the anaerobic digestion of sewage sludge using crude glycerol as a co-substrate

Recently, the disposal of sewage sludge to sanitary landfills has gradually decreased, whereas the desire to re-use treated sludge as a soil amendment or as a biofuel has increased. The trends in sewage sludge management in many European countries between 1992 and 2005 showed that although landfilling of sludge has not been significantly reduced since 1992, incineration and recycling have increased steadily. Incineration aims to reduce the volume of sludge and render it inert (biologically and chemically) before landfilling. However, this process requires significant amounts of energy and is therefore more costly compared with conventional treatment options. In Europe, one of the most desirable current uses of treated sludge is in agriculture as a soil amendment. The anaerobic digestion process is a well-known technology that improves sewage sludge quality for agricultural use[24], while at the same time reducing the volume and weight of the excess sludge. In this study, the anaerobic digestion of sewage sludge in the absence of crude glycerol as a co-substrate resulted in a slight decrease in the TS content of the sludge in 6 days, which was a 30% decrease from the first day (Figure 4a). Surprisingly, the sludge supplemented with 0.4% (v/v) crude glycerol showed a significant decrease in the TS content of the sludge in 6 days, calculated as a 59% decrease from the first day (Figure 4b). At the same time, the total organic acid concentration reached 8.68 g/L on day 6, which was 3.81 times higher than the yield from the control sludge. The results proved that the use of a strongly alkaline crude glycerol solution resulted in the solubilization of organic material to various organic acids from the solid phase of the sludge. Some researchers have found that depending on the concentration, VFA may have a stimulating or inhibiting effect on bacterial growth. Sometimes stimulation (at low concentration) and inhibition (at high concentration) have been identified for the same bacteria[22,25]. Amon et al.[1] showed that when the VFA concentration significantly exceeded 5.0 g/L, the anaerobic digestion process was no longer stable and organic overloading was likely. Ginkel and Logan[11] found that an increase of VFA, particularly acetic acid, could inhibit further growth of hydrogen-producing microorganisms, resulting in a decrease in the hydrogen production rate. From the research reported herein, supplementation of 0.4% (v/v) crude glycerol enhanced the activity of acetogenic bacteria in the sludge but the resulting metabolic products limited their growth. The growth inhibition of the acetogenic bacteria, which appeared to be a drawback of the anaerobic digestion process, would result in a significant decrease in the excess sludge. Moreover, excessive cell growth could be unfavorable to the 1,3-PDO synthesis because increased carbon flow could contribute to biomass synthesis rather than 1,3-PDO production[2,4,6,16]. In summary, sludge supplemented with 0.4% (v/v) crude glycerol results in both 1,3-PDO formation in good yield from glycerol and a significant decrease in the excess sludge.

4. Conclusion

A supplement of crude glycerol had a positive effect on the productivity of the anaerobic digestion of sewage sludge. The effect depended on the crude glycerol concentration. Crude glycerol supplementation at 0.01% (v/v) increased methane production from the anaerobic digestion. An increase of crude glycerol concentration to 0.4% (v/v) resulted in the production of valuable resources such as hydrogen and 1,3-PDO. The use of concentrated sludge improved the 1,3-PDO yield and the sludge disposal capacity more than the non-concentrated sludge. A strongly alkaline crude glycerol supplement enhanced the solubilization of organic material to various organic acids from the solid phase of the sludge, which resulted in a significant decrease of TS content in the sludge compared with the control sludge without crude glycerol supplementation.

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References

1) Amon, T., B. Amon, V. Kryvoruchko, V. Bodiroza, E. Potsch, and W. Zollitsch. 2006. Optimising methane yield from anaerobic digestion of manure: effects of dairy systems and of glycerine supplementation. Int. Congr. Ser. 1293: 217–220.
2) Biebl, H., K. Menzel, A.P. Zeng, and W.D. Deckwer. 1999. Microbial production of 1,3-propanediol. Appl. Microbiol. Biotechnol. 52: 289–297.
3) Cai, M.L., J.X. Liu, and Y.S. Wei. 2004. Enhanced biohydrogen production from sewage sludge with alkaline pretreatment. Environ Sci. Technol. 38: 3195–3202.
4) Cheng, K.K., H.J. Liu, and D.H. Liu. 2005. Multiple growth inhibition of *Klebsiella pneumoniae* in 1,3-propanediol fermentation. Biotechnol. Lett. 27: 19–22.
5) Cheng, K.K., J.A. Zhang, D.H. Liu, Y. Sun, H.J. Liu, M.D. Yang, and J.M. Xu. 2007. Pilot-scale production of 1,3-propanediol using *Klebsiella pneumoniae*. Process. Biochem. 42: 740–744.
6) Colin, T., A. Bories, C. Lavigne, and G. Moulin. 2001. Effects of acetate and butyrate during glycerol fermentation by *Clostridium butyricum*. Curr. Microbiol. 43: 238–243.
7) Dasari, M.A., P.P. Kiatsimkul, W.R. Sutterlin, and G.J. Suppes. 2005. Low-pressure hydrogenolysis of glycerol to propylene glycol. Appl. Catal. A: General. 281: 225–231.
8) Dimitriou, I. and H. Rosenqvist. 2011. Sewage sludge and wastewater fertilization of Short Rotation Coppice (SRC) for increased bioenergy production–Biological and economic potential. Biom. Bioenerg. 35: 835–842.
9) Forster-Carneiro, T., V. Riau, and M. Pérez. 2010. Mesophilic anaerobic digestion of sewage sludge to obtain class B biosolids: Microbiological methods development. Biom. Bioenerg. 34: 1805–1812.
10) Fountoulakis, M.S., I. Petousi, and T. Manios. 2010. Co-digestion of sewage sludge with glycerol to boost biogas production. Waste. Manage. 30: 1849–1853.
11) Ginkel, S.V. and B.E. Logan. 2005. Inhibition of biohydrogen production by undissociated acetic and butyric acids. Environ. Sci. Technol. 39: 9351–9356.
12) Harper, S.R. and F.G. Pohland. 1986. Recent developments in hydrogen management during anaerobic biological wastewater treatment. Biotechnol. Bioeng. 28: 585–602.
13) Holm-Nielsen, J.B., C.J. Lomborg, P. Oleskowicz-Popiel, and K.H. Esbensen. 2007. Online near infrared monitoring of glycerol-boosted anaerobic digestion processes: evaluation of process analytical technologies. Biotechnol. Bioeng. 99: 302–313.
14) Kim, H.W., H.S. Shin, S.K. Han, and S.E. Oh. 2007. Response surface optimization of substrates for thermophilic anaerobic codigestion of sewage sludge and food waste. J .Air Waste Manage. 57: 309–318.
15) Li, C. and H.H.P. Fang. 2007. Fermentative hydrogen production from wastewater and solid wastes by mixed cultures. Crit. Rev. Environ Sci. Technol. 37: 1–39.
16) Lin, R., H. Liu, J. Hao, K. Cheng, and D. Liu. 2005. Enhancement of 1,3-propanediol production by *Klebsiella pneumoniae* with fumarate addition. Biotechnol. Lett. 27: 1755–1759.
17) Moon, C., J.H. Ahn, S.W. Kim, B.I. Sang, and Y. Um. 2009. Effect of biodiesel-derived raw glycerol on 1,3-propanediol production by different microorganisms. Appl Biochem. Biotechnol. 161: 502–510.
18) Nordberg, Å., M. Edström, M. Uusi-Penttilä, and Å.C. Rasmuson. 2012. Selective desorption of carbon dioxide from sewage sludge for in-situ methane enrichment: Enrichment experiments in pilot scale. Biom. Bioenerg. 37: 196–204.
19) Papanikolaou. S., P. Ruiz-Sanchez, B. Pariset, F. Blanchard, and M. Fick. 2000. High production of 1,3-propanediol from industrial glycerol by a newly isolated *Clostridium butyricum* strain. J. Biotechnol. 77: 191–208.
20) Parkin, B.G.F. and W. Owen. 1986. Fundamentals of anaerobic digestion of wastewater sludges. J. Environ. Eng. 112: 867–920.
21) Rehman, A., R.G.S. Wijesekara, N. Nomura, S. Sato, and M. Matsumura. 2008. Pre-treatment and utilization of raw glycerol from sunflower oil biodiesel for growth and 1,3-propanediol production by *Clostridium butyricum*. J. Chem. Technol. Biotechnol. 83: 1072–1080.
22) Roche, C., H. Albertyn, N.O. Van Gylswyk, and A. Kistner. 1973. The growth response of cellulolytic acetate-utilizing and acetate-producing Butyrivibrios to volatile fatty acids and other nutrients. J. Gen. Microbiol. 78: 253–260.
23) Sabourin-Provost, G. and P.C. Hallenbeck. 2009. High yield conversion of a crude glycerol fraction from biodiesel production to hydrogen by photofermentation. Bioresource Technol. 100: 3513–3517.
24) Shizas, I. and D.M. Bagley. 2004. Experimental determination of energy content of unknown organic in municipal wastewater streams. J. Energ. Eng. 130: 45–53.
25) Stewart, C.S. 1975. Some effects of phosphate and volatile fatty acids salts on the growth of rumen bacteria. J. Gen. Microbiol. 89: 319–326.
26) Tokumoto, H. and Tanaka, M. 2012. Novel anaerobic digestion induced by bacterial components for value-added byproducts from high-loading glycerol. Bioresource Technol. 107: 327–332.
27) Wei, Y.S., R.T. Van Houten, A.R. Borger, D.H. Eikelboom, and Y.B. Fan. 2003. Minimization of excess sludge production for biological wastewater treatment. Water. Res. 37: 4453–4467.
28) Xiao, B.Y. and J.X. Liu. 2009. Biological hydrogen production from sterilized sewage sludge by anaerobic self-fermentation. J. Hazard Mater. 168: 163–167.
29) Yang, G., J. Tian, and J. Li. 2007. Fermentation of 1,3-propanediol by a lactate deficient mutant of *Klebsiella oxytoca* under microaerobic conditions. Appl. Microbiol. Biotechnol. 73: 1017–1024.
30) Yazdani, S.S. and R. Gonzalez. 2007. Anaerobic fermentation of glycerol: a path to economic viability for the biofuels industry. Curr. Opin. Biotechnol. 18: 213–219.
31) Yuan, H.Y., Y.G. Chen, H.X. Zhang, S. Jiang, Q. Zhou, and G.W. Gu. 2006. Improved bioproduction of short-chain fatty acids (SCFAs) from excess sludge under alkaline conditions. Environ Sci. Technol. 40: 2025–2029.