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Effects of the Redox Potential of the Acidogenic Reactor on the Performance of a Two-Stage Methanogenic Reactor

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Distillery wastewater was used in a thermophilic laboratory-scale two stage anaerobic digester to test the effects of the redox potential of the first acidogenic reactor on the performance of the system. The digester consisted of first a acidogenic reactor and the an upflow anaerobic sludge blanket (UASB) reactor. The digestor was operated at a hydraulic retention time (HRT) of 48 h. Under these conditions, about 90% of the chemical oxygen demand as measured by the chromate method (COD_{cr}) was removed with a gas production yield of 0.4 l/g-COD removed. The redox potential of the acidogenic reactor was increased when the reactor was purged with nitrogen gas or agitation speed was increased. The increase in reduction potential was accompanied by an increase in acetate production and a decrease in butyrate formation. A similar trend was observed when a small amount of air was introduced into the acidogenic reactor. It is believed that the hydrogen partial pressure in the acidogenic reactor was decreased by the above mentioned treatments. The possible failure of anaerobic digestion processes due to over-loading could be avoided by the above mentioned treatments.

In most cases the strength and the composition of wastewater treated by anaerobic digesters fluctuate. This is one of the limitations in the application of an anaerobic digestion process to treat wastewater, especially high strength wastewater such as distillery wastewater (9).

Conversion of organic materials into methane is a complex process involving at least three classes of bacteria; hydrolytic fermentative bacteria (HFB), syntrophic acetogenic bacteria (SAB) and methanogenic bacteria. The HFB are the major hydrogen producers, and the methanogens are the hydrogen consumers. The SAB metabolize fatty acids to acetate with the production of hydrogen in association with methanogenic bacteria.

Butyrate and propionate producers are the major HFB in an anaerobic digester. They produce more acetate in the place of butyrate and propionate at lower hydrogen partial pressure. When an anaerobic digester is over-loaded, organic materials are actively fermented by the HFB, which grow much faster than the methanogens. Consequently hydrogen partial pressure builds up, inhibiting the metabolism of SAB, and diverting the metabolism of HFB to produce more propionate and butyrate. These lead to the accumulation of fatty acids and to a pH drop (2).

Processes have been developed to avoid the failures due to the over-loading. A two-stage digester can be operated to separate the major H_2 producing HFB from SAB, which are sensitive to H_2. H_2 can be stripped off from a digestor using inert gases (13), and excess electrons are consumed by sulfate-reducing bacteria or by photosynthetic bacteria (3). The rationale for the use of sulfate in an anaerobic digester is that sulfate is a better electron accepter than HCO_3^- (10).

Though O_2 is toxic to the strictly anaerobic bacteria including methanogens in pure cultures (11), methanogens can tolerate low level of dissolved oxygen in a co-cultures with an aerobe (5, 8). Some HFB, known as strictly anaerobic bacteria, consume oxygen. *Clostridium acetobutylium* and *C. butyricum* reduce O_2 to H_2O by the action of NADH oxidase (11, 12). *Propionibacterium shermanii* (14) and *Desulfovibrio desulfuricans* (1) use O_2 as an electron accepter. This paper describes the effects of various treatments, including aeration (to remove excess electrons from the acidogenic reactor) on the per-

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formance of a two-stage anaerobic digester.

MATERIALS AND METHODS

Wastewater

Wastewater collected from Baekwha Distillery Co. (Kunsan, Korea) was used throughout the study. Mixtures of tapioca and rye (50 : 50 or 33 : 67) were raw materials used to produce ethanol. Settleable solids were removed from the wastewater before it was fed to the reactor. The characteristics of the wastewater varied according to the raw material used: pH 3.8-4.2, COD_{Cr} 28-40 g/l, suspended solid 1.6-1.9 g/l.

Experimental Apparatus and Its Operation

A two-stage methanogenic reactor was installed, consisting of a continuously stirred tank reactor (CSTR) type acidogenic reactor and two USAB type methanogenic reactors connected to the acidogenic reactor in parallel (Fig. 1). A three liter fermentor (Model SY-500, Korea Fermentor Co., Inchon, Korea) was used as the acidogenic reactor with a working volume of 1.5 liter. The fermentor was equipped with a pH electrode connected to a pH controller (Model MDL-4CR, Marubishi Co., Tokyo, Japan), and a redox electrode connected to a recorder through a variable resister. The recorder was calibrated using a standard redox solution (250 mV, Metrohm CH-9100, Herisau, Switzerland). The reactor was run at 55°C.

Glass. jars (diameter; 5.5 cm, height; 35 cm, working volume; 650 ml) were used as USAB reactors with a water jacket for temperature control, and with a solid-liquid separator and a feed distributor. One of the methanogenic reactors were operated at 35°C and the other at 55°C.

Effluent from the acidogenic reactor was fed to the methanogenic reactors through a settler (500 ml) where settleable solid were removed. Hydrogen and carbon dioxide gases produced in the first acidogenic reactor were released from the settler. Both effluent and gas from the methanogenic reactors were collected in the effluent receivers which were connected to gas measuring devices. Settleable solids from the methanogenic reactors were separated in the settlers, and returned to the reactors at the rate of 188 ml/h.

The HRT of each reactor was one day. The pH of the acidogenic reactor was maintained around 5.5 by using 5 N NaOH. The pH of the methanogenic reactors was maintained at around 7.2 without control.

Sludge collected from the anaerobic digester of Baekwha Distillery Co. was used to inoculate reactors operated at 55°C, the USAB reactor operated at 35°C was inoculated by the sludge collected from a mesophilic anaerobic digester. The main system had been operated for 2 years before the experiment was started except the UASB reactor operated at 35°C, which was about 6 months old. During the start-up period the headspace of the acidogenic reactor was gassed by oxygen-free nitrogen gas at the rate of 0.3 l/min to maintain the reactor anaerobic. The acidogenic reactor was operated at varying conditions of agitation speed, types of gases, gassing rate and gassing mode as shown in Table 1. The reactor was operated for at least 4 days under each condition before samples were collected for analyses. Just before the start of the experiment the acidogenic reactor experienced a failure in temperature control for 36 h.

Analyses

COD_{Cr} was determined by a standard method (7). Volatile fatty acids and methane were analyzed by gas chro-

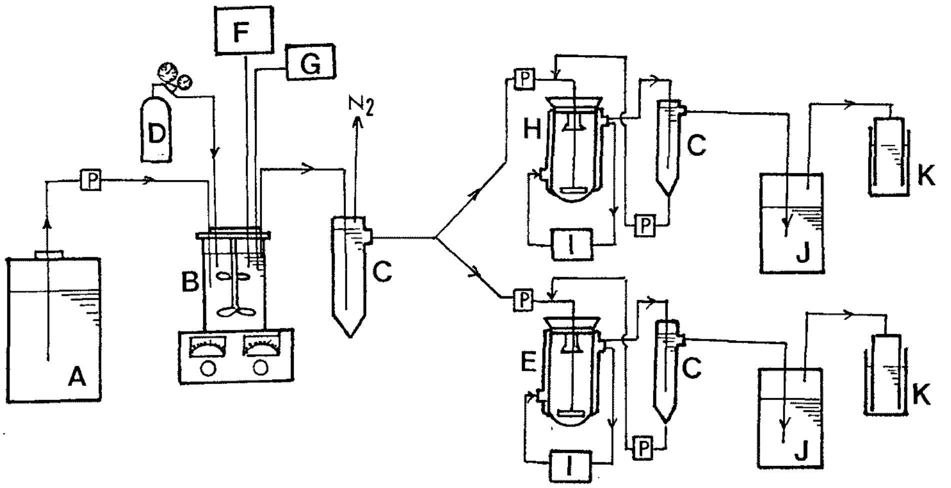

Fig. 1. Schematic diagram of the experimental two-stage anaerobic digester.
A, Raw wastewater Reservoir; B, Acidogenic reactor; C, Settler; D, N_2 gas; E, Methanogenic reactor (55°C); F, Redox electrode; G, pH controller; H, Methanogenic reactor (35°C); I, Temperature controller; J, Reservior of outlet wastewater; K, Gas collector; P, Pump.

Table 1. Operation conditions and the redoc potential changes in the acidogenic reactor.

Run number	Agitation (rpm)	Gassing conditions			ORP (mV)	Running time (day)
		Gas	Rate (l/min)	Mode		
	100	Nitrogen	0.3	Headspace		0- 28
1	100	Air	0.3	Submerged	-115	29- 33
	100	Nitrogen	0.3	Headspace		34- 38
2	300	Nitrogen	0.3	Headspace	-200	39- 52
	100	Nitrogen	0.3	Headspace		53- 83
	300	Nitrogen	0.3	Submerged		84- 98
	100	Nitrogen	0.3	Submerged		99-117
3	300	Nitrogen	0.3	Submerged	-115	118-139
	100	Nitrogen	0.3	Submerged		140-191
	200	Nitrogen	0.3	Submerged		192-210
4	100	Nitrogen	0.3	Submerged	-250	211-216
	100	Nitrogen	0.3	Headspace		217-220
5	100	Air	0.3	Headspace	-400	221-234
6	100	Nitrogen	0.3	Headspace	-480	235-241
7	100	Non	0.3	-	-530	242-250

matographic methods using a Varian 3400 equipped with a flame ionization detector and glass column (2 mm by 2 m) packed with Chromosorb WAW coated with GP-10%, SP-1200/l H_3PO_4. Lactic acid was determined by gas chromatography after methylation.

RESULTS

Redox Potential (ORP) Change

ORP values of the acidogenic reactor varied according to the mode of operation of the acidogenic reactor as shown in Table 1. The lowest value was recorded when the reactor was agitated at low speed without gassing (run 7). Gassing by nitrogen through the headspace increased the ORP value slightly to -480 mV (run 6). When the reactor was gassed by air through the headspace at similar conditions (run 5), the ORP value increased only by about 80 mV.

When agitation speed was increased to 300 rpm under similar conditions (run 2), the redox potential increased remarkably to -200 mV. These results show that hydrogen is super saturated in the reactor agitated at low speed, and removed from the reactor by increasing the agitation speed and by gassing with nitrogen. This effect was more significant, when the reactor was gassed through the culture liquid instead of into the headspace (run 3). Run 4 showed an ORP of about -250 mV when the reactor was operated at a low agitation speed of 100 rpm with the gassing through the culture. A high ORP value of -115 mV was obtained in the run gassed by air through the culture at low agitation speed (run 1). From this result it can be said that oxygen is consumed in the reactor.

Fermentation Products of the Acidogenic Reactor

Fermentation products of each run were determined.

Table 2. Volatile fatty acids (VFAs) production in the acidogenic reactor under different operational conditions.

RUN Number	VFA (mM)		
	Acetate	Propionate	Butyrate
1	23.7	2.7	27.7
2	15.1	4.9	48.1
3	16.0	3.5	39.9
4	17.1	3.8	51.6

Table 2 shows that acetate and butyrate were the major volatile fatty acids formed in the reactor. Propionate was a minor fermentation product under the conditions investigated. This seems to be due to the low pH of 5.5 in the reactor since the growth of propionate producing bacteria such as *Propionibacterium* is poor at low pH. Generally more acetate was produced under higher redox potential. The increase in acetate production was accompanied by a decrease in butyrate production. These results show that more electrons were removed as hydrogen during the acidogenic phases with higher reduction potential values.

Calculation of the oxidation/reduction balance is one way of showing diversions of the electron metabolism in a fermentation process (6). Since the gaseous products were difficult to determine due to the gassing, hydrogen/carbon (H/C) ratios of the soluble products were calculated based on the soluble fermentation products formed including lactate (Table 3). The ratio was calculated using the following equation:

$$H/C \text{ ratio} = (H - 2 * O)/C$$

where H, O and C represent the total numbers of hydrogen, oxygen and carbon in the soluble fermentation

Table 3. Hydrogen/carbon (H/C) ratios of soluble fermentation products during the acidogenesis under different conditions.

Run Number	Fermentation product (mM)				H/C Ratio*
	Acetate	Pro-pionate	Butyrate	Lactate	
5	15.8	4.1	45.9	80	0.40
6	12.9	3.1	52.8	50	0.54
7	14.4	4.0	57.9	50	0.56

*The ratio was calculated from the total numbers of C, H and O in the soluble fermntation products. The number of H was divided by numer of C after subtracting the numbers of H as nH_2O:

$$H/C \text{ ratio} = (H - 2 * O)/C$$

Table 4. COD reduction from the acidogenic reactor at different runs.

Run number	Reduction (%)
1	9.6
2	12.4
3	13.5
4	12.9
5	17.9
6	9.7
7	9.2

products.

As shown in Table 3 the highest ratio was obtained in run 7 which showed the lowest reduction potential, and the lowest ratio in run 5, where air was introduced in the headspace. It is noteworthy that lactate was one of the major products, and that more lactate was produced in run 5 when air was introduced to the reactor. It is believed that the low pH and limited aeration provided selection pressures for the acidophilic aerotolerant lactic-acid bacteria over the strict anaerobes.

COD Removal from the Acidogenic Reactor

COD removed from the acidogenic reactor was determined by measuring the COD values in the raw wastewater and the effluent from the acidogenic reactor as Table 4. At the beginning of the experiment, less than 10% of COD was removed from the acidogenic reactor. This result shows that a steady state was not achieved in the acidogenic reactor up to run 2 after the failure of the temperature controller mentioned in the materials and methods section. Another possibility is that strict anaerobes were severely damaged during run 1 by the aeration, but this is less likely because the ORP reading was -115 mV, the same as run 3.

From run 2 the value fluctuated between 9.2% and 17.9%. Generally more COD was removed during the runs which were operated at a higher ORP. The lowest value of 9.2% was recorded in run 7 which was agitated at low speed without gassing, and when the headspace was gassed by air as in run 5, 17.9% of COD was removed. These results show that about 12% of COD was removed as gaseous products, mainly hydrogen in the runs with nitrogen stripping or when high speed agitation was used, and that in run 5 some of the electrons derived from the oxidation of organic materials were used to reduce oxygen.

Table 5. Fermentability of wastewater in the acidogenic reactor.

Run number	Fermentability (%)
5	31.7
6	30.8
7	26.4

Fermentability was calculated by dividing the sum of COD removed in the acidogenic reactor and the COD content of the fermentation products by the intial COD value.

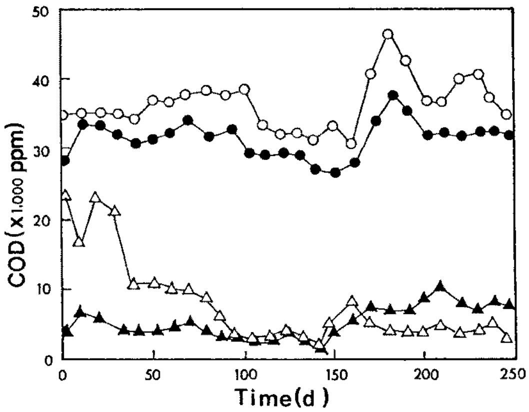

Fig. 2. Influent and effluent COD values of the two-stage methanogenic reactor fed by distillery wastewater.

-○-, Raw wastewater; -●-, Acidogenic reactor; -△-, 35°C UASB reactor; -▲-, 55°C UASB reactor.

Materials Metabolized in the Acidogenic Reactor

HFB oxidize organic materials to various metabolites including, carbon dioxide, hydrogen and soluble products to generate the energy required to synthesize cell materials. The total amount of organic materials metabolized in the acidogenic reactor is the sum of COD removed, and COD in the soluble products and cell mass. Since the cell mass synthesized in an anaerobic system is very small, the total amount of organic materials metabolized in the acidogenic reactor was calculated by adding COD removed to the COD value of the soluble fermentation products (Table 5). About 30% of the organic materials present in the wastewater were metabolized in the acidogenic reactor. The actual value should be slightly higher than the calculated value because the major fermentation products were used for the calculation.

Methanogenesis

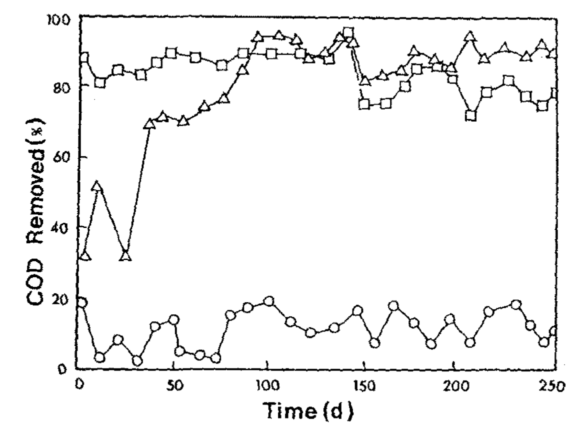

Fig. 3. COD removed in the two-stage methanogenic reactor fed by distillery wastewater.
-○-, Acidogenic reactor; -△-, 35°C UASB reactor; -□-, 55°C UASB reactor.

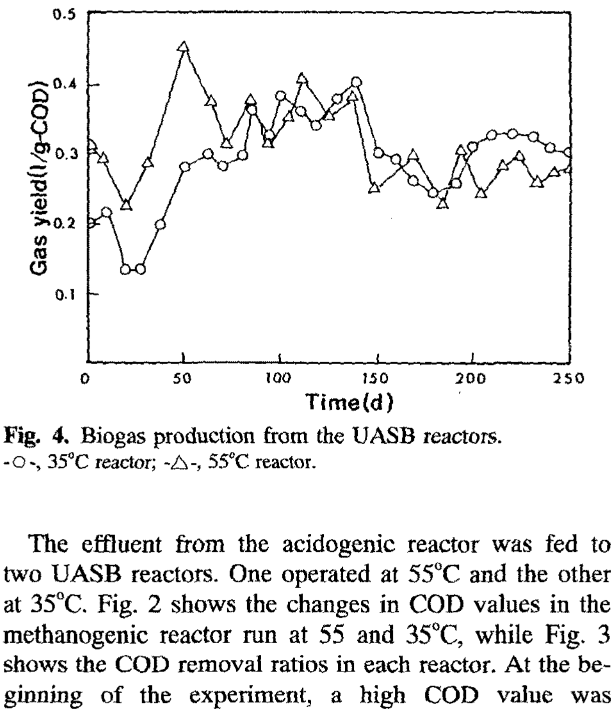

Fig. 4. Biogas production from the UASB reactors.
-○-, 35°C reactor; -△-, 55°C reactor.

The effluent from the acidogenic reactor was fed to two UASB reactors. One operated at 55°C and the other at 35°C. Fig. 2 shows the changes in COD values in the methanogenic reactor run at 55 and 35°C, while Fig. 3 shows the COD removal ratios in each reactor. At the beginning of the experiment, a high COD value was measured in the effluent from the reactor operated at 35°C. This shows that the start-up time for the reactor was about 100 h. After the start-up period, the effluents showed COD values of around 5000 ppm with a COD removal ratio of over 80% by methanogensis alone. From these values it was calculated that a two-stage anaerobic digestion system can remove over 85-95% of COD from the wastewater. These results show that the operational conditions of the acidogenic reactor (which can remove excess reducing equivalent) do not have any negative effects on methanogenesis. This conclusion was further substantiated by the gas yields in the methanogenic reactors. The gas yield (liter of gas produced per gram of COD removed) was about 0.4 as shown in Fig. 4.

DISCUSSION

The availability of electron donors and electron acceptors are the most important environmental factors in an anaerobic ecosystem. In an anaerobic digestion process organic materials are fermented to simple products by the hydrolytic fementative bacteria before being metabolized into methane and carbon dioxide by the action of syntrophic acetogenic bacteria and methanogenic bacteria.

During the fermentative process, the metabolic intermediates are used as the electron acceptors. Under high hydrogen partial pressure conditions the metabolic intermediates are converted to more reduced products such as butyrate and propionate (6).

Acetate is the major product under low hydrogen partial pressure where proton is used as the major electron acceptor to produce hydrogen (6). More reduced products than acetate are oxidized to acetate and hydrogen by syntrophic acetogens in association with the methanogenic bacteria which consume hydrogen during the methanogenic process.

One of the most frequent problems encountered in the anaerobic digestion of wastewater is system failure due to over-loading. In this condition the hydrogen partial pressure is built up, which in turn inhibits the growth of the syntrophic acetogenic bacteria. The consequence is an increase in fatty acid concentrations, especially propionate and a decrease in pH (2).

This problem can be avoided to some extent by employing a two-stage digestion process where the hydrogen producing acidogenic phase is physically separated from the methanogenic phase, which is affected more seriously by the high hydrogen partial pressure (13).

More COD was removed from the acidogenic reactor when the reactor was operated under a high reduction potential (Table 4). These are high speed agitation, stripping the culture with an inert gas, and air supply into the acidogenic reactor. Since hydrogen is the major form of COD removed from the acidogenic reactor, it can be said that those treatments are effective in keeping the hydrogen partial pressure of the acidogenic reactor low. This was verified by the reduction potential readings and by the hydrogen/carbon ratios of the soluble fermentation products.

It is well documented that hydrogen is supersaturated under actively fermenting conditions (4). In this condition hydrogen production is thermodynamically unfavourable. High speed agitation and stripping with an inert gas were effective in removing hydrogen from the acidogenic reactor to keep the hydrogenase activity high (13).

Introduction of air into the acidogenic reactor was another effective method of removing more COD from the reactor without any adverse effects on the overall performance of the anaerobic digestion process. Though molecular oxygen is known to be toxic to strict anaerobes, some strict anaerobes are resistant to O_2, and others can utilize it as an electron acceptor as shown in the introduction section. The aerated acidogenic reactor showed negative redox potential values. These results show that the aeration methods employed in the experiment didn't supply enough oxygen for the complete oxidation of the auto-oxidizeable metabolites such as H_2S. It is believed that O_2 supplied to the acidogenic reactor was completely consumed before the effluent was fed to the secondary UASB reactors, which were reported to be inhibited by O_2 (8).

About 30% of the organic matter in the raw waste water was metabolized in the acidogenic reactor to produce over 120 mM of fatty acids (Table 3). The low degree of fermentability seems to be due to the toxicity of the fatty acids at low pH (16). When air was introduced through the headspace, the acidogenic reactor removed about 18% of the COD of the wastewater, which is about 6.3 g/l. This method might help avoid system instability in an anaerobic digester treating wastewater of varying strengths, especially medium strength wastewater containing organic matter content which can be metabolized completely during acidogenesis.

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