METHANOGENIC FERMENTATION OF FAT-CONTAINING WASTEWATER MEDIATED BY IRON

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1. ABSTRACT

Long chain fatty acids (LCFA) are potential inhibitors of bacteria involved in anaerobic digestion because of their surface activity. Precipitation of long-chain fatty acids with iron can improve the anaerobic degradation due to their precipitation and reducing surface properties. Degradation of stearic acid was improved in the presence of iron (II). The methane production was increased 1.6 times as compared to control. Iron-containing soil was applied for degradation of vegetable oil as model case. The methane production was increased 1.5 times as compared to control. Yield of methane production was 0.09 and 0.06 L/ g COD in experiment and control respectively. Optimum COD/Fe ratio was found 20 mg/mg. Iron (II) can be produced in the treatment system from iron (III) hydroxide or iron containing minerals.

2. INTRODUCTION

Fats are hydrolyzed to long-chain fatty acids and glycerol in the anaerobic digestion. Long-chain fatty acids are degraded via β-oxidation cycle in the anaerobic digestion as well as in the aerobic process (Hanaki et al, 1981). LCFA are well-known inhibitors to various microorganisms at millimolar concentrations and cause some serious problems in anaerobic treatment systems (Rinzema, 1994). It is possible that both acetogens and methanogens, involved in the β-oxidation, suffer greatly from LCFA inhibition (Roy et al., 1986; Koster & Cramer, 1987). Other inhibition mechanism can be adsorption of the surface active LCFA onto the cell wall/membrane leading to the damage of transport function.
It is well known that addition of soluble calcium salt reduced the inhibitory effect of LCFA (Hanaki, 1981). However, calcium plays no role in aerobic post treatment. Iron not only diminishes the toxicity of LCFA in anaerobic digestion but form ammonium salt with produced ammonium from anaerobic stage (Ivanov et al, 1997). This ammonium salt of iron can be used as fertilizer. The ferric and ferrous ion form non-dissolved salts with hydrogen sulfide thus diminish sulphide concentration and inhibitory effects on methanogenic bacteria. Another mechanism of positive effect of iron on the anaerobic digestion is based on the competition between sulfate-reducing and methanogenic bacteria for hydrogen. Under the presence of ferric, the sulfate-reducing bacteria are out-competed for hydrogen by bacteria reducing ferric (Achtnich et al. 1995). Positive effect of iron may be related also with the precipitation of LCFA. The formation of dissolved and un-dissolved iron complexes or salts with the fatty acids probably prevents the inhibition of methanogenesis. The aim of this paper was to study the anaerobic degradation of fats mediated by iron. Different parameters were studied in presence of iron including improvement in methane production, COD removal efficiency, methanogenic biomass, and Volatile fatty acid(s) accumulation and results are compared when iron was absent in the system.

3. MATERIAL AND METHODS

The experiments were performed in the bottles with a working volume of 250 and 500 ml. The bottles were completely mixed by magnetic stirrer at 300 rpm. All experimental arrangement was setup in a constant temperature room at 35±1°C. Serum bottles were flushed with nitrogen gas for five minutes before setting up an experiment. Serum bottles then connected with gas measuring system (Challenge AER-100 respirometer: Challenge Environmental Systems, Inc. USA) to monitor gas production. Inoculate was the anaerobic sludge from the anaerobic

digester of municipal wastewater treatment plant at the city of Kwangju, Korea. Propionate-acclimated culture was used for fermentation of propionic acid. Propionate acclimated culture is maintained in Master Culture Reactor (MCR), as described (Young & Tabak, 1993). Chemicals added to the basal medium in different experiments are shown in table 1

Table 1 Experimental condition

Material added	Function
0.5 g COD/L of stearic acid+0.5 g Fe(II)/L	Experiment
0.5 g COD/L of stearic acid	Control
1.0 g COD/L of iron propionate	Experiment
1.0 g COD/L propionic acid	Control
1.0 g COD/L of ethanol+ 2.0 g/L iron (III) hydroxide, 0.5 g/L iron as iron-containing soil, 0.5 g iron-containing ore	Experiment
1.0 g COD/L of ethanol + $HgCl_2$	Abiotic control
11.5 g COD/L of vegetable oil (corn oil)+ 145, 288, and 576 mg/L iron (II) as iron-containing soil	Experiment
11.5 g COD/L of vegetable oil (corn oil)	Control

Analysis The gas chromatographic method was used to determine the concentration of volatile fatty acids (VFA) as described (Hyun et al, 1998). VFAs were analyzed by Gas Chromatograph (Hewlett Packard Series II, 5890, USA) using a flame ionization detector. The analysis of gas was performed by standard method (standard method 2720 C, 1995). CHROMPACK capillary column combined with TCD detector was used. Iron (II) analysis was performed by standard phenanthroline method (standard method 3500-Fe D., 1995). Specific coenzyme F_{420} was monitored by their autofluorescence using Luminescence spectrophotometer (Perkin Elmer, Luminescence Spectrometer, LS 50 B, UK). Coenzyme F_{420} was extracted from biomass according to Dolfing, & Mulder (1985) with little modification. 5 ml of homogenized sludge (vortexed well) in 50-mL tubes was taken. Sludge was boiled for 20 minutes and immediately cooled to room temperature. After centrifugation for 15 min at 10,000 x g. Coenzyme F_{420} was measured using synchronous fluorescence. The emission spectrum range was from 300 to 600 nm with 10 nm optical slit.

4. RESULTS AND DISCUSSION

Fermentation of stearic acid The methane production was increased 1.6 times as compared to control (Fig.1). Yield of methane production was 0.224 L/g COD (as stearic acid) and 0.140 L/ g of COD in control. The ratio between methane and CO_2 production was high in experiment when iron was present, showing that iron has significant positive impact on methanogenesis (Fig. 2.).

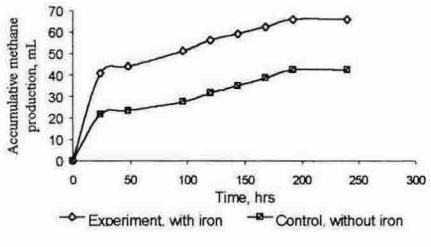

Fig.1. Accumulated methane producing during fermentation of stearic acid.

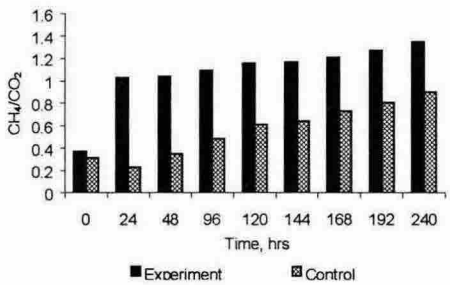

Fig.2. Ratio of CH_4 and CO_2

Fermentation of propionate There was no biogas production in the experiment whereas Propionic acid degraded completely (Fig.3). It shows that there was no degradation of ferrous (II) propionate under anaerobic conditions.

Production of iron (II) in digester. Iron (III) hydroxide, iron-containing soil and iron ore were added both in control and experiment. Control was abiotic process using $HgCl_2$ (0.42 mg/L) to stop the bacterial activity. Adopted sludge with iron reducing bacteria and iron ore was used as iron source then long acclimation period was reduced (Fig.4).

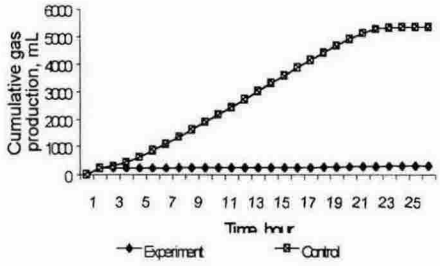

Fig.3. Dynamics of gas production from fermentation of iron (II) propionate

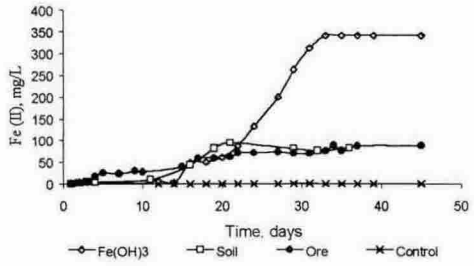

Fig.4. Iron reduction using different iron sources

Fermentation of vegetable oil mediated with iron-containing clay

Four different COD/iron doses were applied. Remaining COD in the system after complete fermentation was lowest when iron dose was 576 mg/L. improvement in COD removal efficiency with increase in iron concentration is shown in Fig. 5. Coenzyme F420 plays a key metabolic role in methanogenic bacteria as an electron carrier in both anabolic and catabolic redox reactions. This coenzyme is only found in methanogens (Dolfing and Mulder, 1985). Therefore, F420 was used as measure of methanogenic activity for comparison. Methanogenic activity didn't improved when applied iron dose was 145 mg/L (Fig. 6). However, when iron dose increase to 288 and 576 metheogenic activity improved. Acetic and propionic acids were accumulated in control, showing inhibitory effect of LCFA on the acetoclasitc methanogenic process. No accumulation of acetic and propionic acids was observed, when applied iron dose was 576 mg/L. (Fig.7). This clearly indicates positive impact of iron on digestion of fats. Methane production was increased 41% compare to control when iron-containing clay was present (Fig. 8). Average rate of methane production was 0.16 L/L hr higher than control (0.011 L/L hr). Yield of methane production was 0.09 L/g COD and 0.06 L/ g of COD in control.

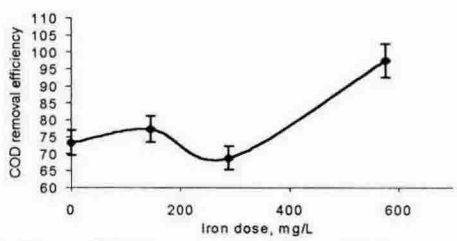

Fig.5. COD removal efficiency improvement with increase in iron dose.

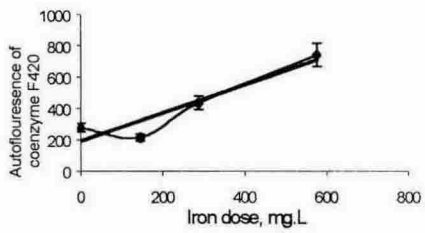

Fig.6. Autoflouresence of Coenzyme F_{420} with increase of iron dose.

5. CONCLUSION

1. Addition of iron showed positive impact on the digestion of LCFA. Methane gas production was increased 1.6 times as compared to control. Yield of methane production was 0.224 L/g COD (as stearic acid) and 0.140 L/ g of COD in control.
2. Iron (II) can be produced inside the treatment reactor. Iron-containing soil among three ferric sources, i.e. Iron (III) hydroxide, iron-containing soil, and iron ore was found best with respect to moderate iron reduction and bioavailability. Long acclimation period can be reduced using adopted sludge.
3. Iron containing soil can be used for the anaerobic treatment of fat-containing wastewaters. Optimum COD/Fe (II) was found 20. COD conversion improved with increase in iron dose. Increases in iron dosage also increase in methanogenic biomass. Acetic and propionic acids were accumulated when iron dose was 0, 145, and 288 mg/L but on VFA accumulation was observed in case of iron dose 576 mg/L showed that iron reduced the toxicity of LCFA methanogens.

6. REFERENCES

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