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Effect of Metals on Anaerobic Digestion of Water Hyacinth–Cattle Dung

VIKRAM B. PATEL, ANAMI R. PATEL, MANISHA C. PATEL, AND DATTA B. MADAMWAR*

Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar-388 120 Gujarat, India

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ABSTRACT

The effect of several salts, $FeCl_3$, $NiCl_2$, $CoCl_2$, $CuCl_2$, and $ZnCl_2$, on anaerobic digestion of water hyacinth-cattle dung was examined. Among the salts studied, $FeCl_3$ caused a more than 60% increase in gas production with a high methane content.

Index Entries: Anaerobic digestion; biogas; metal ions; methane; water hyacinth; cattle dung.

INTRODUCTION

In India, a large number of water bodies have been damaged because of excessive growth of aquatic weeds, particularly water hyacinth (*Eichhornia crassipes*), to the extent that they are of no further use for any purpose. Water hyacinth (WH) has grown at an alarming rate to such explosive proportions that all efforts of humans to bring it under control have failed.

Today, utilization is considered an important part of weed management. Therefore, anaerobic digestion of water hyacinth resulting in production of biogas, a valuable source of energy, has attracted universal attention recently (*1–4*). There is also a growing interest in maximizing extraction of methane for energy recovery from water hyacinth. Unfortunately, operating experiences with anaerobic digesters and cost effectiveness has not been consistently good.

*Author to whom all correspondence and reprint requests should be addressed.

Recent evidence indicates that many methanogenic bacteria may require nickel (metal ion) for growth and/or methane formation (*5,6*). Also the conversion of fatty acid to methane and carbon dioxide by methanogens from an anaerobic fixed film reactor treating food processing waste was shown to be stimulated by the addition of nickel (*7*).

No detailed study, however, seems to have been made so far on the effect of various metal ions, such as Co^{2+}, Cu^{2+}, Ni^{2+}, Fe^{3+}, and Zn^{2+}, on anaerobic digestion of water hyacinth. This has prompted us to study this aspect with the ultimate aim of improving the digestion process. The impact of various metal ions on volatile acids, pH, and process stability has also been examined.

MATERIALS AND METHODS

Resources

WH was obtained always from the same pond, which did not get city or industrial effluent. It was dried at 60°C and powdered to 50 mesh. Fresh cattle dung (CD) was obtained locally. All the chemicals used were of analytical grade.

Anaerobic Digesters

Several bench-scale anaerobic digesters were used. Each vessel consisted of a 10-L glass reaction bottle, having a working volume of 6 L and containing 7% (w/v) total solids (TS) (mixture of WH:CD, 7:3, w/w on dry wt basis). All digesters were maintained at 37 ± 1°C. The digesters were fed on a semicontinuous basis: once per day with a mixture of WH (dried at 60°C and powdered to 50 mesh) and CD in the ratio of 7:3 (w/w), containing 7% TS (w/v), with a retention time (RT) of 8 d (loading rate of 8.75 g TS/L of digester/d), which was found to be most suitable for our study (*8*). Prior to feeding, an equal quantity of sludge was withdrawn from the bottom of the digester. Metals were incorporated with feeding material.

A fresh digester was always started by preparing a mixture of powdered water hyacinth and cattle dung in the ratio of 7:3 (w/w) to give a final total solid concentration of 7% (w/v) and using 10% (v/v) inoculum from the running biogas digester of the same type. Steady-state condition was judged by constant mean gas production rate and also from effluent BOD, COD, and volatile acid values remaining constant. Each digester was run for 40 d, i.e., for 5 RTs after reaching steady-state condition. Experiments were carried out in quadruplicate, and average data are presented.

Analysis

Gas was collected and measured by displacement of acidified saturated salt solution (9), making due correction for atmospheric pressure and temperature. Gas composition was analyzed by CIC-make gas liquid chromatography with a stainless-steel Chromosorb 2 column and thermal conductivity detector. Helium served as the carrier gas for methane and carbon dioxide. Identification and percentage of methane and carbon dioxide were based on comparison of retention time and peak area of unknowns with standard amounts of each gas (10). Feed slurry and effluent slurry were routinely analyzed for pH, BOD, COD, TS, and volatile acids as per standard procedures (9).

RESULTS AND DISCUSSIONS

In the present work, we investigated the effect of various metal ions, Co^{2+}, Ni^{2+}, Cu^{2+}, Fe^{3+}, and Zn^{2+}, on anaerobic digestion of a water hyacinth–cattle dung mixture. A trend of enhanced gas production with increased amount of ferric chloride was observed (Table 1). Maximum enhancement (over 61%) was achieved with the addition of 70 μM ferric chloride and gas production declined thereafter. In addition to enhanced total gas production, ferric chloride was also responsible for higher methane content (68%) in the digester gas.

Process stability, as evidenced by lower volatile acids (10–12), consistently increased with increased amount of ferric chloride, indicating that methane-producing bacteria utilize acids at faster rates. It appears that ferric chloride did enhance the methane-forming step of the digestion process. It is seen that the rate-limiting step in the methane fermentation often involves the degradation of fatty acids, which is related to the efficiency of hydrogen utilization by methanogenic bacteria (13–15) because the partial pressure of hydrogen has to be maintained at a very low level in this ecosystem. This in turn favors the degradation of butyrate and propionate. Addition of ferric chloride helps the digester, which otherwise is stressed by the accumulation of fatty acids.

Ferric chloride gives lower values of BOD and COD, indicating greater biodegradation with high process performance. Values of BOD and COD were 13.3 (g/L) and 20.2 (g/L), respectively, in the ferric chloride dosed (70 μM) digester in comparison to values of BOD (17.2 g/L) and COD (27.3 g/L) in control without metal addition. This also indicates that microbial degradation of organic matter was faster in the first stage (acidogenic stage), and in the second stage (methanogenic stage), the methanogens

Table 1
Summary of Effluent Data During Steady-State Periods of Digesters Maintained at 37±1°C in Presence of Metal Salts

Metal concentration µmol	Gas, L/L of dig./d	CH_4, %	BOD, g/L	Volatile acids, g/L	COD removal, %
Control	1.01 ± 0.001200	64	17.2	1.35	62.01
$FeCl_3$ (Anhydrous)					
10	1.04 ± 0.000100	64	20.1	1.21	62.57
30	1.08 ± 0.000081	65	17.8	0.97	64.95
50	1.33 ± 0.000225	66	15.1	0.78	68.86
70	1.61 ± 0.012100	68	13.3	0.54	71.79
90	1.19 ± 0.000081	66	17.8	0.61	66.75
100	1.14 ± 0.001225	65	18.7	0.83	65.92
$ZnCl_2$					
10	1.12 ± 0.001000	63	19.1	1.10	63.27
30	1.15 ± 0.001300	63	18.5	1.00	63.94
50	1.17 ± 0.000300	64	17.6	0.88	64.25
70	1.24 ± 0.000300	64	16.6	0.78	64.95
90	1.38 ± 0.003700	65	17.1	0.70	67.04
100	1.29 ± 0.010800	63	18.3	0.83	66.07
$NiCl_2$ $6H_2O$					
10	1.10 ± 0.000100	63	19.2	1.29	63.13
30	1.23 ± 0.006400	64	18.2	0.97	64.53
50	1.32 ± 0.001200	64	17.0	0.81	67.87
70	1.54 ± 0.005279	65	14.9	0.62	69.70
90	1.15 ± 0.003700	64	18.2	1.03	64.11
100	1.11 ± 0.000300	64	18.9	1.18	63.27
$CoCl_2$ $6H_2O$					
1	1.09 ± 0.001200	63	18.9	0.96	62.84
5	1.32 ± 0.000700	64	16.6	0.85	68.85
10	1.46 ± 0.004300	65	15.3	0.71	71.22
30	1.24 ± 0.015100	64	16.8	0.83	66.34
50	1.24 ± 0.001200	63	17.5	0.91	65.92
100	1.22 ± 0.002800	62	18.0	1.10	63.97
$CuCl_2$ $2H_2O$					
10	1.09 ± 0.003900	63	18.8	1.30	62.43
30	1.13 ± 0.004800	64	17.8	1.12	63.97
50	1.18 ± 0.000700	64	16.9	0.98	65.23
70	1.26 ± 0.002700	65	16.1	0.91	69.13
90	1.40 ± 0.008100	66	15.3	0.83	70.39
100	1.29 ± 0.010300	64	16.9	0.97	65.65

use acetate, hydrogen, and carbon dioxide as precursors for methane production at a faster rate by using metal ions. Table 1 also gives the information on COD removal. This parameter is important because the values suggest the bacterial efficiency (*16*). This shows that presence of ferric chloride improves the bacterial efficiency, thereby increasing biodegradation.

Effects of various other metals, like Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}, were also seen. They also showed increased gas production with enriched methane content. This may be because the presence of Zn^{2+} and Cu^{2+} is required for the activity of hydrogenase and superoxide dismutase in facultative anaerobes. It has been known that many heavy metals are parts of the essential enzymes that drive numerous anaerobic reactions (*17*). Analysis of ten methanogenic strains by Takashima and Speece (*18*) showed the following order of heavy metal composition in the cell: Fe>>Zn>>Ni>>Co=Mo>>Cu. The intracellular concentration in unstressed condition is regarded as indicative of the essential requirement, under optimal nutrient and process conditions. Metals considered the most important in digestion are from the iron family: Fe, Ni, Co. Nickel plays a key role in the factor F430 and is essential for the growth of acetoclastic organisms. The addition of Fe, Ni, and Co results in positive effects on growth of anaerobes, thereby proving the requirement of Fe^{3+}, Ni^{2+}, and Co^{2+} in anaerobic digestion of WH–CD. The role of different metals in this fermentation process is not well understood. However, several studies have found that several metals are required for growth of methanogens (*5,6,19*). Both autotrophic and organotrophic methanogens incorporate nickel during growth. During the process of methane production, coenzyme M is a C_1 carrier, and methyl coenzyme M undergoes a reduction reaction in which methane is liberated. The biological role of nickel is thought to be in the methyl coenzyme M reductase. The prosthetic group of this enzyme contains a nickel-prophinoid designated factor F430 (*20,21*). This is likely to be a common reaction to all methanogens independent of their specific nutritional requirements.

From our study, it seems that anaerobic digestion of water hyacinth–cattle dung has an unusual requirement for some essential metals, such as Fe^{3+}, Zn^{2+}, Ni^{2+}, Co^{2+}, and Cu^{2+}. In all cases, addition of heavy metals showed improvement in digestion performance. Probably these metals are:

1. Essential for various enzymatic reactions;
2. Inhibitors of sulfide toxicity;
3. Agents binding nutrients, such as phosphates, and
4. Biomass stimulants.

It is surprising that even though water hyacinth has an ability to absorb and concentrate large amounts of chemicals, such as lead, cadmium, mercury, nickel, and so forth, its anaerobic digestion still requires external supply of heavy metals. This may be because the water hyacinth used for our study was collected from local ponds where the metal content of the water is low compared to industrial effluent and waste water. Our studies clearly indicate that metal deficiencies can limit anaerobic digester performance in terms of gas production, its methane content, BOD, COD, and volatile acids together, and metal supplementation does substantially improve the digester performance.

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REFERENCES

1. Obeid, M. (1975), In *Aquatic Weeds in the Sudan*, Obeid, M. ed., Natl. Coun. Res., Sudan, and U.S. Natl. Acad. Sci., Washington, D.C., pp. 101–103.
2. Gupta, O. P. and Lamba, P. S. (1976), In *Aquatic Weeds in S. E. Asia*, Junk W., ed., The Hague, pp. 361–367.
3. Wolverton, D. C. and McDonald, R. C. (1976), *New Scientist (USA)* **71 (1913)**, 318–329.
4. Saraswat, N. and Khanna, P. (1986), *Biotechnol. Bioeng.* **28**, 240–246.
5. Schonheit, P., Moll, J., and Thauer, R. K. (1979), *Arch. Microbiol.* **123**, 105–107.
6. Diekert, G., Konheiser, U., Piechulla, K., and Thauer, R. K. (1981), *J. Bacteriol.* **148(2)**, 459–464.
7. Murray, W. D. and Van Den Berg, L. (1981), *Appl. Environ. Microbiol.* **42(3)**, 502–505.
8. Madamwar, D. B., Patel, A. R., and Patel, V. B. (1990), *J. Ferment. Bioeng.* **70(5)**, 340–342.
9. APHA (1976), *Standard Methods for the Examination of Water and Waste Water*, 14th ed., American Public Health Association, Washington, D.C.
10. Varel, V. H., Hashimoto, A. G., and Chen, Y. R. (1980), *Appl. Environ. Microbiol.* **40**, 217–222.
11. Fraser, M. D. (1977), In *Proc. Symposium Fuels from Biogas and Wastes.* Institute of Gas Technology, Chicago.
12. Madamwar, D. B. and Mithal, B. M. (1986), *Biotechnol. Bioeng.* **28**, 624–626.
13. McInerney, M. J., Bryant, M. P., and Pfenning, N. (1979), *Arch. Microbiol.* **122**, 129–135.
14. Boone, D. R. and Bryant, M. P. (1980), *Appl. Environ. Microbiol.* **40**, 626–632.
15. Mountfort, D. O. and Bryant, M. P. (1982), *Arch. Microbiol.* **133**, 249–256.
16. Hills, D. J. and Roberts, W. D. (1981), *Agric. Wastes* **3**, 179–189.
17. Oleskiewicz, J. A. and Sharma, V. K. (1990), *Biol. Wastes* **31**, 45–67.
18. Takashima, M. and Speece, R. E. (1988), In *Proc. Ann. Water Pollut. Control Fed. Confer.*, Dallas, Texas, October 2–6, Water Pollution Control Federation, Washington, D.C.
19. Scherer, P. and Sham, H. (1981), *Acta. Biotechnol.* **1**, 57–65.
20. Diecker, G., Weber, B., and Thauer, R. K. (1980), *Arch. Microbiol.* **127**, 273–277.
21. Whitman, W. B. and Wolfe, R. S. (1980), *Biochem. Biophy. Res. Commun.* **92**, 1196–1201.