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Impact of Ni(II), Zn(II) and Cd(II) on biogassification of potato waste

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Abstract: *A **s**tudy was conducted on anaerobic digestion of potato waste and cattle manure mixture, inoculated with 12 % inoculum and diluted to 1:1 substrate water ratio at 37±1°C. Initially pH of substrate was found to be 4.5 to 5.0. Lime and sodium bicarbonate solutions were employed to adjust the pH to 7.5. Biogas production continued upto 10 and 7 days, when lime and sodium bicarbonate solutions were used to adjust the pH, respectively. Biogassification potential was studied in response to different ratio of waste and cattle manure. Biogas production rate was higher when potato waste and cattle manure were used in 50:50 ratio. Effect of two different concentrations (2.5 and 5.0 ppm) of three heavy metals viz. (Ni (II), Zn (II) and Cd (II)) on anaerobic digestion of substrate (potato waste –cattle manure, 50:50) was studied. At 2.5 ppm, all the three heavy metals increased biogas production rate over the control value. The percentage increase in biogas production over the control was highest by Cd, followed by Ni and Zn. In all the treatments, methane content of biogas increased with increase in time after feeding. Various physico-chemical parameters viz. total solids, total volatile solids, total organic carbon and chemical oxygen demand considerably declined after 7 days of digestion and decline was greater in presence of heavy metals as compared to control. The physico-chemical parameters revealed maximum decrease in the presence of 2.5-ppm concentrations of heavy metals with the substrate. Among all the three heavy metals employed in the study, Cd^{++} at 2.5 ppm was found to produce maximum biogas production rate. The use of three heavy metals to enhance biogas production from potato and other horticultural waste is discussed.*

Key words: *Biogas, Potato waste, Heavy metals, Anaerobic digestion, Cattle manure.*

Introduction

Solid wastes constitute a major environmental pollution problem in urban and semi-urban areas of not only industrially advanced but also developing countries of the world. Anaerobic digestion of solid waste is being considered as an effective method to serve the purpose of both waste management and energy generation.

India is pioneer in the field of use of anaerobic digestion for biogas production from cattle dung. The installation of over 23.56 lakhs biogas plants has considerably improved energy scenario of rural India (India, 1996), However, due to ever-increasing energy demand, attempts are being made to use alternative feedstock such as agricultural residues, and food processing waste including rotten and spoiled fruits and vegetables for biogas generation. There are about 18,533 food processing units in India, among these, fruits and vegetables sector is the major solid waste sources, generating about 5.6 million tonnes in the form of peel skin, coreseeds, pomace, leaves and other unusable residues (Raju and Ramlinghaih, 1997).

The process of biogas production involves hydrolysis, acidogenic and methane phases. Methanogenic bacteria are very sensitive to fluctuations in process parameters such as pH, temperature, oxygen and organic loading rate. However, the bacteria involved in other two phases of biogas production are not so sensitive to such fluctuations (Abbasi and Ramasami, 1999; Rai, 1995). It has also been established that acetate is the precursor of approximately two third of methane produced in anaerobic digestion, H_2 and CO_2 accounting for the remainder (Murray and Vadanberg, 1987).

Catalytic effects of heavy metals on anaerobic digestion of vegetable waste have not been given due attention. Little is known about the stimulatory or inhibitory effect of trace heavy metals on the conversion of acetate to methane. Since several trace elements are already known to be present in the vegetable waste, the present work was undertaken to study the effect of three heavy metals (viz. Ni^{++}, Zn^{++} and Cd^{++}) on anaerobic digestion of potato waste: cattle manure, methane production rate, pH, total solids (TS), total volatile solids (TVS), total organic carbon (TOC) and chemical oxygen demand (COD) were investigated.

Materials and Methods

The stimulatory effect of Ni^{++}, Zn^{++} and Cd^{++} on biogassification potential was determined using fixed anaerobic batch digester. The substrates used for anaerobic batch digestion were prepared by mixing potato tuber (*Solanum tubericum L.*) waste with fresh cattle manure and with water to obtain substrate water ratio of 1:1. The substrate was inoculated with 12% active inoculum obtained from cattle manure biogas plant. The inoculated substrate was filled in digester and sealed.

Setting up of digester: The sealed digesters were placed in hot air oven (37±1°C). The gas transfer pipe from each digester inside the oven was connected to a gas collection bottle outside the oven. The gas collecting bottles were filled with brine solution upto the brim, and then sealed using rubber cork. Synthetic adhesive was applied on the surface of the rubber cork to make the system leak proof. The outlet from each gas collection bottled was put into a measuring cylinder for the collection of displaced brine solution. The anaerobic digestion of diluted substrate was conducted for a period of 7 days at (37±1°C)

Measurement of biogas: Biogas produced was collected daily using liquid displacement method. Methane content of collected gas was analyzed using Gas Chromatograph (Jain *et al.,* 1980).

pH optimization: In order to optimize the pH of the substrate, 100% potato waste was treated with sodium bicarbonate ($NaHCO_3$) and lime solution ($Ca(OH)_2$), respectively, to obtain the desired pH values.

Optimization of cattle manure: Potato waste ratio: To ascertain the ratio of cattle manure: Potato waste for optimizing gas production and methane concentration, various ratios of cattle manure and potato wastes were used viz. 30:70, 40:60, 50:50 and 100%, respectively. Lime solution was used to maintain at pH 7.5 for all the ratios.

Effect of heavy metals: To study the effect of various concentrations of heavy metals (Ni^{++}, Zn^{++} and Cd^{++}) on biogas production, cattle manure and potato waste (50:50) was used and 2.5 and 5.0-ppm concentration of each of three heavy metals were added, respectively.

Estimation of physico-chemical parameters: COD of the substrate before and after treatments were measured by Open reflux method (APHA, 1989). TOC was determined by Walkley method (Jackson, 1973). Total solids and total volatile solids were determined by the method given by standard methods for examination of water and waste water (APHA, 1989). Nitrogen of the substrate was determined by macro Kjeldhal method (Jackson, 1973).

Tests were made in replicates and the results were compared with those of controls.

Results and Discussion

The paste of potato waste was used as a substrate for biogas production by anaerobic digestion. The pH of substrate before digestion was found to be 4.5-5.0.

Effect of pH: In the present study, pH of substrate (potato waste) was found to be 4.5 – 5.0. Therefore, in order to maintain pH of substrate to about 7.5, sodium bicarbonate ($NaHCO_3$) and lime solution ($Ca(OH)_2$) were employed. It was observed that when initial pH of the substrate was adjusted to 7.5, cumulative biogas production rate was 946 and 2389 ml $l^{-1}d^{-1}$ with lime solution and sodium bicarbonate, respectively. However, biogas production continued for longer period and methane content was higher when lime was used to maintain pH. Therefore, lime solution was employed to adjust pH of substrate in all further experiments.

It is well known that optimum pH requirement for biogas production is between 7.0 – 7.5. Therefore, it was felt necessary to adjust pH of substrate to about 7.5. Acidic and alkaline pH are detrimental to methanogenic bacteria. At the pH of about 6.0 acid condition exhibit acute toxicity to these bacteria. If acid production is not stopped at this pH, acidogenic bacteria may continue to produce acid until pH drop to 4.5 or 5.0. At this pH, digester is said to be stuck or pickled. Thus, to obtain effective biogas production, pH should be controlled using addition of suitable alkali. (Pfeffer, 1980)

Table – 1: Effect of various cattle manure: potato waste slurry ratio (v/v) on biogas production*.

Cattle manure : potato waste ratio (v/v)	Total biogas produced in 13 days (ml)
30:70	2290
40:60	2365
50:50	2625

*pH of substrate was adjusted to 7.5 using lime solution in all cases

Effect of cattle manure and potato waste ratio: Total biogas production was higher when cattle manure: potato waste (v/v) was 50: 50 (2625 ml) than in 30:70 (2290 ml) and 40:60 (2365 ml), respectively (Table 1).

Maintenance of substrate pH (cattle manure and potato waste, 50:50) is relatively better; therefore, mixture of cattle manure and potato waste, 50:50 was employed for further experiments. It is also observed that the pH in both the cases continued to decline.

Ranade *et al.,* (1987) studied anaerobic digestion of vegetable market waste in laboratory scale biogas plant of floating dome design and obtained maximum production rate of 17.5 l/d. At higher loading rate slurry being acidic affected biogas production. Dhala and Rajor (1989) reported that cabbage waste, with 5% cow dung, produce 8.5 l/d gas/kgm dry wt. with CH_4 content of 55%.

Effect of heavy metals: In the present study the effect of three heavy metals viz. Ni^{++}, Zn^{++} and Cd^{++} at two concentrations each (2.5 and 5.0 ppm) on total biogas production during seven days of methanogenasis was studied. In these experiments, the cattle manure: potato waste slurry was kept at 50: 50 and initial pH of the substrate in each case was adjusted to 7.5 using lime solution.

Effect of Ni^{++}: The catalytic effect of different concentration of Ni^{++} on biogassification potential of substrate, methane contents and pH was investigated and results are shown in Table 2. In all Ni^{++} treatments, biogas production started on the very first day of the experiment. The biogas production rate generally declined with time. Total biogas production rate after 7 days of feeding was 1989, 3398 and 2002 ml $l^{-1}d^{-1}$ in control, Tc (no Ni^{++}), T2.5 (2.5 ppm Ni^{++}) and T 5.0 (5.0 ppm Ni^{++}) treatments, respectively. Thus results show that total biogas production rate increased in T 2.5 (170.8% increase) and total biogas production was also highest at 2.5 ppm Ni^{++}. At 5.0 ppm Ni^{++} concentration, the rate as well as total biogas production was almost same as in case of control.

In all the treatments, methane content increased with the increased time after feeding. On the first day of feeding the

Table – 2: Biogas production rate, methane content and pH profile during anaerobic digestion of substrate (potato waste + cattle manure) supplemented with Ni^{++}.

Treatments	Tc			T2.5			T5.0		
Days after feeding	Biogas production rate (ml $l^{-1}d^{-1}$)	Methane content (%)	pH	Biogas production rate (ml $l^{-1}d^{-1}$)	Methane content (%)	pH	Biogas production rate (ml $l^{-1}d^{-1}$)	Methane content (%)	pH
0	0	0.00	7.5	0	0.00	7.5	0	0.00	7.5
1	936	5.52	5.8	1293	7.50	6.0	930	5.80	5.8
2	638	11.50	5.6	1545	13.30	5.8	448	15.40	5.6
3	349	22.70	5.5	369	23.00	5.8	541	26.40	5.5
4	39	27.20	5.5	111	37.20	5.5	53	35.80	5.8
5	4	29.50	5.8	57	40.90	5.3	8	38.90	5.5
6	10	48.80	5.7	16	42.10	5.5	4	44.50	5.5
7	13	56.20	5.5	7	58.10	5.5	18	50.50	5.8
Total biogas production, rate (ml $l^{-1}d^{-1}$)									
	1989			3398			2002		
Total biogas yield (ml)									
	9945			16990			10010		

Note: -ml $l^{-1}d^{-1}$ = ml gas produced per liter digester volume per day
- Tc : Potato waste +Cattle manure (Control)
- T5.0 : Potato waste +Cattle manure +5.0 ppm Ni^{++}.
- Total biogas yield = Biogas production rate x digester volume.
- T2.5 : Potato waste +Cattle manure +2.5 ppm Ni^{++}.
- Each value is the mean of two replication.

Table – 3 : Biogas production rate, methane content and pH profile during anaerobic digestion of substrate (potato waste + cattle manure) supplemented with Zn^{++}.

Treatments	Tc			T2.5			T5.0		
Days after feeding	Biogas production rate (ml $l^{-1}d^{-1}$)	Methane content (%)	pH	Biogas production rate (ml $l^{-1}d^{-1}$)	Methane content (%)	pH	Biogas production rate (ml $l^{-1}d^{-1}$)	Methane content (%)	pH
0	0	0.00	7.5	0	0	7.5	0	0	7.5
1	1099	4.90	5.8	1100	5.2	6.0	865	6.4	6.0
2	393	9.00	5.9	591	6.6	5.8	354	9.4	5.5
3	18	16.90	5.6	215	19.5	5.5	275	21	5.4
4	5	24.60	6.0	121	30.9	5.5	115	28.4	5.5
5	12	26.20	6.2	16	33.3	5.6	9	32.5	5.3
6	6	42.10	5.8	9	44.1	5.5	5	42.2	5.5
7	2	46.30	5.5	2	56.5	5.8	3	58.9	5.8
Total biogas production, rate (ml $l^{-1}d^{-1}$)									
	1535			2054			1626		
Total biogas yield (ml)									
	7675			10270			8130		

Note: - ml $l^{-1}d^{-1}$ = ml gas produced per liter digester volume per day
- Tc : Potato waste +Cattle manure (Control)
- T5.0 : Potato waste +Cattle manure +5.0 ppm Zn^{++}.
- Total biogas yield = Biogas production rate x digester volume.
- T2.5 : Potato waste +Cattle manure + 2.5 ppm Zn^{++}.
- Each value is the mean of two replication.

methane content was 5.52, 7.5 and 5.8 % in Tc, T 2.5 and T 5.0 treatments, respectively. On 7th day of feeding, methane content of biogas increased to 56.2, 58.1 and 50.5 % in the three treatments, respectively. Thus during anaerobic digestion of substrate up to 7 days the methane content was observed to be highest in case T 2.5 treatments. In all the treatments, pH of substrate also declined during anaerobic digestion and it reached to toxic level (5.8 to 6.0) even after one day of feeding and did not recover.

Effect of Zn^{++}: The catalytic effect of different concentrations of Zn^{++}on biogassification potential of substrate, methane contents and pH was investigated and the results are shown in Table 3. In all Zn^{++} treatments, biogas production started on the very first day of experiment. The biogas production rate generally declined with time. Total biogas production rate after 7 days of feeding was 1535, 2054 and 1626 ml $l^{-1}d^{-1}$ in control, Tc (no

Zn^{++}), T2.5 (2.5 ppm Zn^{++}) and T 5.0 (5.0 ppm Zn^{++}) treatments, respectively. These results show that total biogas production rate increased in T 2.5 (133.8% increase) and total biogas production was also highest at 2.5 ppm Zn^{++}. At 5.0 ppm Zn^{++} concentration, the rate and total biogas production was almost same as in case of control.

In all the treatments, methane content increased with the increased time after feeding. On the first day of feeding the methane content was 4.9, 5.2 and 6.4 % in Tc, T 2.5 and T 5.0 treatments, respectively. On 7th day of feeding, methane content of biogas increased to 46.3, 56.5 and 58.9 % in the three treatments, respectively. Thus during anaerobic digestion of substrate upto 7 days the methane content was observed to be highest with T 2.5 treatments. In all the treatments, pH of substrate also declined during anaerobic digestion and it reached to toxic level (5.8 to 6.0) even after one day of feeding and did not recover thereafter.

Effect of Cd^{++}: The catalytic effect of different concentration of Cd^{++} on biogassification potential of substrate, methane contents and pH was investigated and results are shown in Table 4. In all Cd^{++} treatments, biogas production started on the very first day of experiment. The biogas production rate generally declined with time. Total biogas production rate after 7 days of feeding was 472, 1192 and 1008 ml $l^{-1}d^{-1}$ in control, Tc (no Cd^{++}), T 2.5 (2.5 ppm Cd^{++}) and T 5.0 (5.0 ppm Cd^{++}) treatments, respectively. The results showed that total biogas production rate increased in T 2.5 (252.5% increase) and T 5.0 (13.6%) and total biogas production is highest at 2.5 ppm Cd^{++}.

In all the treatments, methane content increased with the increase time after feeding. On the first day of feeding, the methane content was 10.5, 12.3 and 11.9 % at Tc, T 2.5 and T5.0 treatments respectively.

On 7th day of feeding, It was 48.2, 58.3(6th day) and 48.3 (5th day) %. During anaerobic digestion of substrate up to 7 days, the methane content was observed to be highest with T2.5 treatments. In all the treatments, pH of substrate also declined during anaerobic digestion, it reached to toxic level (5.8 to 6.0) even after one day of feeding and did not recover thereafter.

In the present results, 2.5 ppm concentration of all the three heavy metals was found to be as optimum concentration. Hence, the maximum biogas production was found at this concentration. Either total absence of heavy metals or presence of excess amount of heavy metals can affect the activity of methanogenic bacteria and so methanogenic process. Anaerobic reactors are known to be very susceptible to inhibition by heavy metals. It is for this reason that several high COD industrial waste, otherwise suitable for anaerobic treatments, is not subjected to this process. They contain significant concentrations of heavy metals such as Co, Ni, Zn, Cd and Hg. (Hayeb and Thesis, 1978; Speece *et al.,* 1983; Wate *et al.*, 1983).

Total absence of heavy metals can be detrimental to the growth of microorganism involved in anaerobic digestion (Abbasi and Ramasami, 1999). However, the stimulatory effects of Ni and Zn on methane production are attributable to their essentiality for methanogenic bacteria. For example, Ni has been shown to be essential for the growth of methanogenic bacteria such *Methanobacterium thermoautotropicum* and *Methanobacterium bryanti*. (Grat and Thauer, 1981; Hausinger, 1987; Speece *et al*, 1983; Whitman and Wolfe, 1980). Similarly, Zn has also been reported as an essential element for most of

Table – 4: Biogas production rate, methane content and pH profile during anaerobic digestion of substrate (potato waste + cattle manure) supplemented with Cd^{++}.

Treatments	Tc			T2.5			T5.0		
Days after feeding	Biogas production rate (ml $l^{-1}d^{-1}$)	Methane content %	pH	Biogas production rate (ml $l^{-1}d^{-1}$)	Methane content %	pH	Biogas production rate (ml $l^{-1}d^{-1}$)	Methane content %	pH
0	0	0	7.5	0	0	7.5	0	0	7.5
1	344	10.5	5.8	618	12.3	6	806	11.9	6.2
2	0	-	5.7	140	19.8	5.8	0	-	5.7
3	68	23.4	5.5	74	27.6	5.7	130	28.5	5.7
4	45	28.1	5.5	210	40.2	6	60	36.2	6
5	0	-	6	80	50.3	5.8	12	48.3	5.8
6	0	-	5.8	70	58.3	5.5	0	-	5.7
7	15	48.2	5.5	0	-	5.5	0	-	5.8
Total biogas production rate, (ml $l^{-1}d^{-1}$)									
	472			1192			1008		
Total biogas yield (ml)									
	2360			5960			5040		

Note: - ml $l^{-1}d^{-1}$ = ml gas produced per liter digester volume per day
- Tc : Potato waste +Cattle manure (Control)
- T5.0 : Potato waste +Cattle manure +5.0 ppm Cd^{++}.
- Total Biogas Yield = Biogas production rate x digester volume.
- T2.5 : Potato waste +Cattle manure + 2.5 ppm Cd^{++}.
- Each value is the mean of two replication.

Table – 5: Initial parameters of substrates (Potato waste+cattle manure) during anaerobic digestion.

Heavy metals	C/N ratio	Total solid (TS) (gm)	Total volatile solid (TVS) (gm)	Total organic carbon (TOC) %	Chemical oxygen demands (COD) ppm
Ni	28.00	212.40	193.60	42.00	95500.00
Zn	30.00	207.30	195.90	44.25	97000.00
Cd	29.10	211.20	174.00	40.75	90000.00

Table – 6: Percentage reduction in total solid, total volatile solid, total organic carbon and chemical oxygen demands during seven days of anaerobic digestion of substrate (Potato waste+ cattle manure).

Heavy metals	Treatments	Total solid (TS)	Total volatile solid (TVS)	Total organic carbon (TOC)	Chemical oxygen demands (COD)
Ni	Tc	18.99	19.28	4.76	20.41
Ni	T2.5	33.00	36.46	11.43	26.70
Ni	T5.0	26.69	27.40	3.57	23.56
Zn	Tc	27.20	29.10	8.47	17.52
Zn	T2.5	34.58	36.10	13.22	32.98
Zn	T5.0	28.00	31.10	10.17	22.68
Cd	Tc	15.80	17.50	6.75	8.80
Cd	T2.5	27.50	25.06	10.43	22.20
Cd	T5.0	22.00	22.40	9.20	16.70

organisms, including bacteria. (Abbasi and Nipaney, 1992). Cd, Pb and Hg, which are well known for their toxicity also revealed catalytic effect on anaerobic digestion process (Velsen and Lettinga, 1980). The recent report by Abbasi and Krishnakumari (1996) has emphasized the catalytic effect of Ni and Cd at 2.5-ppm level on anaerobic digestion of water hyacinth, which is in confirmity with the result of present study. Further studies on the optimization of biogas production are in progress.

Effect of heavy metals on substrate parameters: In the present study, the effect of three heavy metals on various physico-chemical parameters viz. total solids (TS), total volatile solids (TVS), total organic carbon (TOC) and chemical oxygen demand (COD) of the substrate were studied at the beginning and at the termination of experiments (after seven days). Initial parameters of substrates viz. C/N ratio, total solids (TS), total volatile solids (TVS), total organic carbon (TOC) and chemical oxygen demand (COD) with each heavy metals are given in Table 5. All the parameters declined after 7 days of anaerobic digestion. As mentioned earlier, Ni^{++}, Zn^{++} and Cd^{++} at the concentration of 2.5 ppm showed the maximum catalytic effect on biogas production. Similarly, all the physico-chemical parameters viz.- total solids, total volatile solids, total organic carbon and chemical oxygen demand also showed maximum reduction in this particular treatment (Table 6).

The solid content of a substrate is represented in the form of total solids and total volatile solids. Volatile solids are biodegradable part and it consists of soluble and insoluble organics. The soluble organics are capable of being directly utilized as growth substrates, whereas insoluble organics are converted to soluble organic by enzymatic hydrolysis process before utilization. Volatile solid content of substrate is about 80–85 % of total solids.

Reddy *et al.* (1997) observed that COD reduced during anaerobic digestion when various concentrations of Ni, Cr and Zn were added with the substrate. Reduction in TS, TVS was also reported during process of anaerobic digestion (Hall and Hall *et al.*, 1985; Hill, 1983). The results of present study on potato waste: cattle manure digestion is in confirmity with these reports.

Thus from the present study, it can be concluded that when heavy metals (Ni^{++}, Zn^{++} and Cd^{++}) at a concentration of 2.5 ppm are added into the biogas digester containing potato waste- cattle manure, biogas production enhanced considerably. Also the present investigation showed that biogas production can be accessed only for a very short period because pH of the substrate decreases to a toxic level even after 2-3 days of anaerobic digestion and it does not recover afterwards.

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