Process optimization for enhanced biogas production from bagasse

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***Abstract*—With sugarcane being one of the major crops produced globally and with the production hitting 100 million tonnes in Indian context, there needs to be strategies for the effective waste management. Sugar cane bagasse being an excellent raw material for biofuel generation, its proper utilization could be a medium for revenue generation and stabilization. Certain waste products otherwise discarded in poultry industry like feathers are rich source of keratin protein, the beneficial effects of which were investigated in this study along with metallic cofactors for effective generation of biogas. This in addition to generation of biofuel could also help in value addition of otherwise discarded waste products as well as put forth a better option for management of waste generated in the related industries. They also act as cheaper and easily available raw materials for generation of biogas. The study emphasizes on the significance of delignification as an important pretreatment for bio methanation of bagasse which facilitates easier availability of carbon source for the beneficial microbial population essential for the bio methanation process. The study also determined the importance of metallic ions like iron, nickel, cobalt, magnesium and zinc which can act as cofactors and prosthetic groups for functional enzymatic reactions possessing direct as well as indirect roles in enhancing biogas production. Supplementation of poultry feather along with as sources of nitrogen has improved the production of biogas**

***Index Terms*—Anaerobic digestion, bagasse, biogas, poultry feather, urea, metal cofactors, renewable energy.**

I. INTRODUCTION

India comes under tropical region, enriched with plenty of sunlight, climate and terrain that is conducive to large scale production of biomass. We have an agrarian economy which would result in large-scale production of agricultural by-products like rice straw, bagasse, molasses, wheat straw and the like, which have high energy content. Total exploitable amount to such waste exceeds 500 million tonnes which is equivalent to 19500MW of energy. But maximum part of these waste is dumped /remain unutilized rendering it as potential environmental hazard. Energy conservation and Energy production from cheaper resources are the need of time. Biotechnology can make a lot of contribution in this context, by increasing the acceptability of biomass, biogas, and fuel alcohol as feasible alternative. Advantages of bioenergy generation will be ecofriendly, less polluting, cheap, plenty etc. If bioenergy generation is coupled with the tapping of unutilized biomass, wasteland utilization for biomass production or treatment of solid/liquid waste, then pollution abatement and resources utilization will be simultaneously achieved. Development of reactor designs, gene manipulation of microorganisms has made the task easier and bioenergy from waste has become a reality.

With the energy requirement of developing countries like India increasing at a very fast rate the burden on imports also increases. The Indian policy on biofuel is focused exclusively on non-food crops raised on waste land which is unsuitable for cultivation of food crops. The policy allows 100% foreign equity for biofuel project directed at domestic consumption. The initial few steps towards second generation biofuel in India have been conducted on the jatropha plant (*Jatropha curcas*), which is cultivated for biodiesel. However, the prospects soon faded due to issues such as significantly lower yields, low reliability for farmers and lack of capacity across the entire value chain. In cellulosic ethanol, the progress has been largely at the level of pilot projects. Selected research projects have been taken up by the industries through joint ventures and collaborations.

Similarly, waste from marine, poultry and livestock domestication results in waste products like chicken feather, scales of fish, blood, skin and hides of animal.

These are very rich source of protein and their C:N ratio is low making it beneficial for microbial growth. Similarly using bagasse which are generated as by-product of sugar processing industries which are widely distributes around the country for bio methanation comes with benefits like near-zero fuel costs, increased fuel efficiency leading to an increase in the economic viability of sugar mills generating more secure, diverse, reliable and widespread supply of electricity for local consumers, creation of greater employment opportunities for local populations, lower emissions of CO_2 and other gases than from conventional fossil-fuel generation.

The economic development potential of bagasse Bio methanation should not be under-estimated. Most cane producing countries is or extremely poor, with high unemployment and low rate of access to electricity supply. Many cane-producing countries are heavy users of coal in the power generation sectors, including India and China. The application of Clean Development Mechanism (CDM) of Kyoto protocol, giving a monetary value to carbon dioxide (CO_2) emission reduction, could therefore be an important driver for bagasse Bio methanation in cane producing country.

India is also major importer of oil, giving scope for ethanol and compressed natural gas from cane to alleviate a high import burden and reduce emission from oil consumption. The amount of energy that can be extracted from bagasse is largely dependent on two main criteria: moisture content and the technology used for energy production.

Bio methanation from sugarcane waste (bagasse) provides one of the best examples of renewable-based bio methanation yet it remains largely unexploited. The advantages of bagasse as a fuel for bio methanation are numerous, ranging from the environmental to the social and economic. Biofuel like bioethanol, biohydrogen, biodiesel, biogas and so on is a source of energy originated from biomass-recently living organism or their metabolic by products. Example is bio methanation of bagasse using of cow dung. It is renewable energy-unlike natural resources such as petroleum, coal and nuclear fuel.

II. MATERIALS AND METHODS

Materials:

Fresh cow dung, tap water, 750 mL conical flask, measuring cylinder, wooden stand for gas collection, beakers (100/250/500mL) rubber stopper with one and two holes, rubber tube, sieve, sodium hydroxide, urea, poultry feather, hydrochloric acid, ferrous chloride ($FeCl_2$), magnesium chloride ($MgCl_2$), zinc sulfate ($ZnSO_4$), cobalt chloride ($CoCl_2$), copper (II)chloride ($CuCl_2$), nickel chloride ($NiCl_2$), plastic beaker, conical flask, bagasse, grinder, autoclave.

Method:

The fermentation mixture (containing 30g cow dung) was poured into a 750mL conical flask closed with single holed rubber stopper connected with tubing to an inverted conical flask filled with water and closed with double holed rubber stopper. The outlet tubing of the inverted conical flask was placed in a conical flask containing 100mL of water. Fermentation gases produced were collected in the bottle by water displacement method and daily displaced volume was measured using a measuring cylinder which was equal to the daily biogas produced from the particular set up. This set up was used throughout the study to assess the cumulative biogas production over a period of 10 days.

A. Bio-methanation of bagasse under different pre-treatment conditions

The bagasse was collected from different sources, washed twice with tap water, sun dried to remove water. The dried bagasse was cut in to size of 1-2 cm with a sharp scissors, ground to further reduce its size, sieved and collected in separate jars and levelled. A batch of the sample was subjected to alkali treatment where 10g of sieved bagasse was soaked in 100mL NaOH (1%) for 24 hours. After 24h every batch of bagasse was washed thoroughly with tap water to attain near neutral pH (7-7.5). This process also facilitates in the removal the residual lignin and excess alkali. Another batch of bagasse was subjected to heat treatment where it was autoclaved at 15psi at 121°C for 15min then suddenly cooled with help of ice bucket. Another set were subjected to boiling at 100°C for 15min with the temperature calibrated by help of lab thermometer. In another test, 10g raw sieved bagasse powder was mixed with 100mL 0.1 N NaOH solution, stirred and autoclaved at 15psi at 121°C for 15min then suddenly cooled with help of ice bucket. The total volume was 600mL and the mentioned three conditions were tested with control (with no

substrate), test 1 (10g raw sieved bagasse), test 2 (8g delignified bagasse), test 3 (10g sieved and autoclaved bagasse), test 4 (100°C treated bagasse), and test 5 (0.1N NaOH treated and autoclaved bagasse). The whole mixture of different samples was poured in to 750mL conical flask, closed with one-holed rubber stopper and the biogas production analyzed using water displacement as specified above.

B. Effect of urea supplementation on bio methanation of bagasse

Slurry was prepared by mixing 30g of fresh cow dung with substrate (except in control), 1g urea and tap water to make up final volume to 600mL. The cumulative biogas production was estimated using the above-described technique.

C. Effect of different amounts of urea on bio methanation of pre-steamed bagasse

Test samples were prepared by mixing 30g of fresh cow dung with 10g of sieved, autoclaved bagasse and mixed with urea concentrations varying from 1g (test 1), 2g (test 2), 3g (test 3), 4g (test 4) and 5g (test 5) separately. The slurry was poured into 750mL conical flask, and was closed with one-hole rubber stopper. The fermentation gases were collected in an inverted bottle containing water which was closed with two-holed rubber stopper, connected to the reaction mixture with rubber tubing. The space occupied by the fermentation gases in the inverted bottle displaces water of same amount with other tubing. This daily displaced volume of water was measured accurately using measuring cylinder which is equal to the daily biogas production from particular experiment set up.

D. Effect of supplementation of poultry feather nitrogen source on bio methanation of bagasse

Test samples were prepared by mixing 30g of fresh cow dung with different substrates as in section *A* with 1g of poultry feather and poured into a 750mL conical flask connected to conical flask to check for biogas production as in the above sections.

E. Effect of supplementation of hydrolyzed poultry feather nitrogen source on bio methanation of bagasse

Poultry feather collected from chicken slaughter house was washed twice and sun dried. The feather was ground to a fine powder and collected in separate jar and levelled them. 1g of powdered poultry feather was hydrolyzed with 100mL of 6N HCl for 1hour.

Slurry was prepared by mixing 30g of fresh cow dung with different substrates and 1g of hydrolyzed poultry feather and tap water and the total amount was made up to 600mL and poured into a 750mL conical flask to check for biogas production.

F. Effect of $FeCl_2$ supplementation on bio methanation of bagasse

25ppm 100mL solution of $FeCl_2$ was mixed with 500mL of fermentation mixture containing test samples along with 1g urea and checked for the levels of biogas produced as in above sections.

G. Effect of $MgCl_2$ supplementation on bio methanation of bagasse

Slurry was prepared by mixing 30g of fresh cow dung with different substrates mentioned in section *A* along with 100mL 25ppm $MgC1_2$ solution, mixed well and the total volume was made up to 600mL and poured into a 750mL conical flask and biogas production was estimated.

H. Effect of $ZnSO_4$ supplementation on bio methanation of bagasse

Slurry was prepared by mixing 30g of fresh cow dung with different substrates mentioned in section *A* along with 100mL 25ppm $ZnSO_4$ solution, mixed well and the total volume was made up to 600mL and poured into a 750mL conical flask and biogas production was estimated as in previous sections.

I. Effect of $CoCl_2$ supplementation on bio methanation of bagasse

Fermentation mixture was prepared by combining 30g of fresh cow dung with different substrates mentioned in section *A* along with 100mL 25ppm $CoCl_2$ solution, mixed well and the total volume was made up to 600mL and poured into a 750mL conical flask and biogas production was estimated using water displacement method.

J. Effect of $CuCl_2$ supplementation on bio methanation of bagasse

The mixture was prepared by combining 30g of fresh cow dung with different substrates mentioned in section *A* along with 100mL 25ppm $CuC1_2$ solution, mixed well and the total volume was made up to

600mL and poured into a 750mL conical flask and the cumulative biogas production was recorded.

K. Effect of $NiCl_2$ supplementation on bio methanation of bagasse

Slurry was prepared by mixing 30g of fresh cow dung with different substrates mentioned in section *A* along with 100mL 25ppm $NiCl_2$ solution, mixed well and the total volume was made up to 600mL and poured into a 750mL conical flask and biogas production was estimated for a period of 10 days.

L. Synergistic effect of $FeCl_2$ and $ZnS0_4$ supplementation on bio methanation of bagasse

The test samples contained 100mL 25ppm $FeCl_2$ and 100mL 25ppm $ZnSO_4$ solution mixed with 400mL of fermentation slurry as describe in section A and the total volume was made up to 600mL (using tap water) and were then poured separately into a 750mL conical flasks and observed for biogas production as in previous sections.

M. Synergistic effect of $FeCl_2$ and $MgCl_2$ supplementation on bio methanation of bagasse

Slurry was made by mixing 100mL 25ppm $FeC1_2$ and 100mL 25ppm $MgC1_2$ solution with 400mL of fermentation slurry as describe in section A and the total volume was made up to 600mL (using tap water) and were then poured separately into a 750mL conical flasks and observed for biogas production as described in previous sections.

N. Synergistic effect of $MgCl_2$ and $ZnSO_4$ supplementation on bio methanation of bagasse

Slurry was made by mixing 100mL 25ppm $MgC1_2$ and 100mL 25ppm $MgCl_2$ solution with 400mL of fermentation slurry as describe in section A and the total volume was made up to 600mL (using tap water) and were then poured separately into a 750mL conical flasks. Then the fermentation flask was connected with an inverted conical flask filled with water. The daily displaced water was collected and measured.

O. Synergistic effect of urea, $MgCl_2$, $FeCl_2$ and $ZnSO_4$ supplementation on bio methanation of bagasse

Slurry was made by mixing 100mL 25ppm $MgCl_2$, 100mL 25ppm $FeCl_2$ and 100mL 25ppm $ZnSO_4$ solution with 300mL of fermentation slurry and the total volume was made up to 600mL (using tap water) and were then poured separately into a 750mL conical flasks and observed for gas production.

III. RESULTS

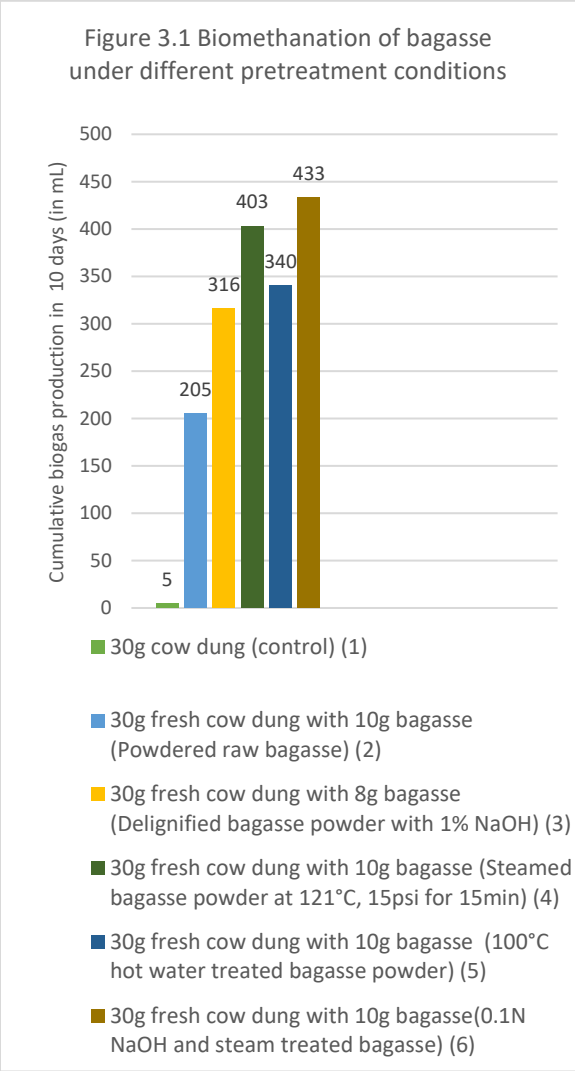

Table 3.1 Bio-methanation of bagasse under different pretreatment conditions

Sample	Condition	Fermentation mixture	Cumulative biogas production in 10 days (in mL)
1	Control	30g cow dung	5
2	Powdered raw bagasse	30g cow dung+10g bagasse	205
3	Delignified bagasse powder treated with 1% NaOH	30g cow dung+8g bagasse	316
4	Steamed bagasse powder (15psi,	30g cow dung+10g bagasse	403

	121°C for15 min)		
5	100°C hot water treated bagasse powder	30g cow dung+10g bagasse	340
6	0.1 N NaOH and steam treated bagasse	30g cow dung+10g bagasse	433

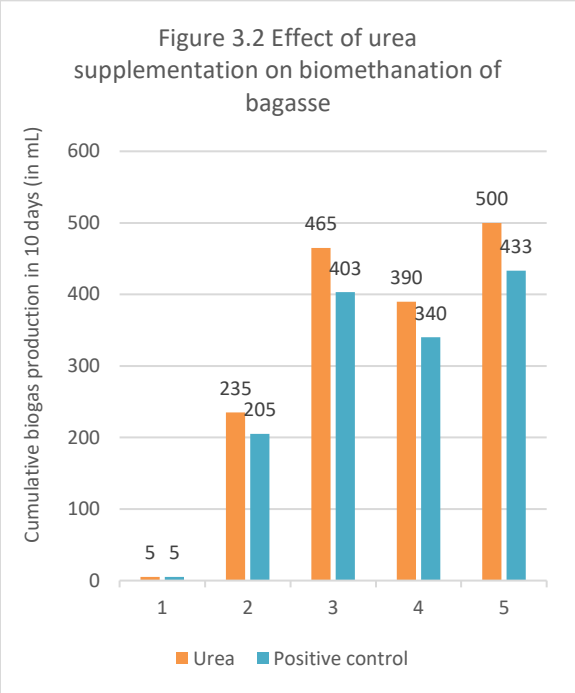

Table 3.2 Effect of urea supplementation on bio-methanation of bagasse

Sample	Condition	Fermentation mixture (FM)	Cumulative biogas production in 10 days (in mL)	Positive control (FM)
1	Control	30g cow dung	5	5
2	Powdered raw bagasse	30g cow dung+10g bagasse+1g urea	235	205
3	Steamed bagasse powder (15psi, 121°C for15 min)	30g cow dung+10g bagasse+1g urea	465	403
4	100°C hot water treated bagasse powder	30g cow dung+10g bagasse+1g urea	390	340
5	0.1 N NaOH and steam treated bagasse	30g cow dung+10g bagasse+1g urea	500	433

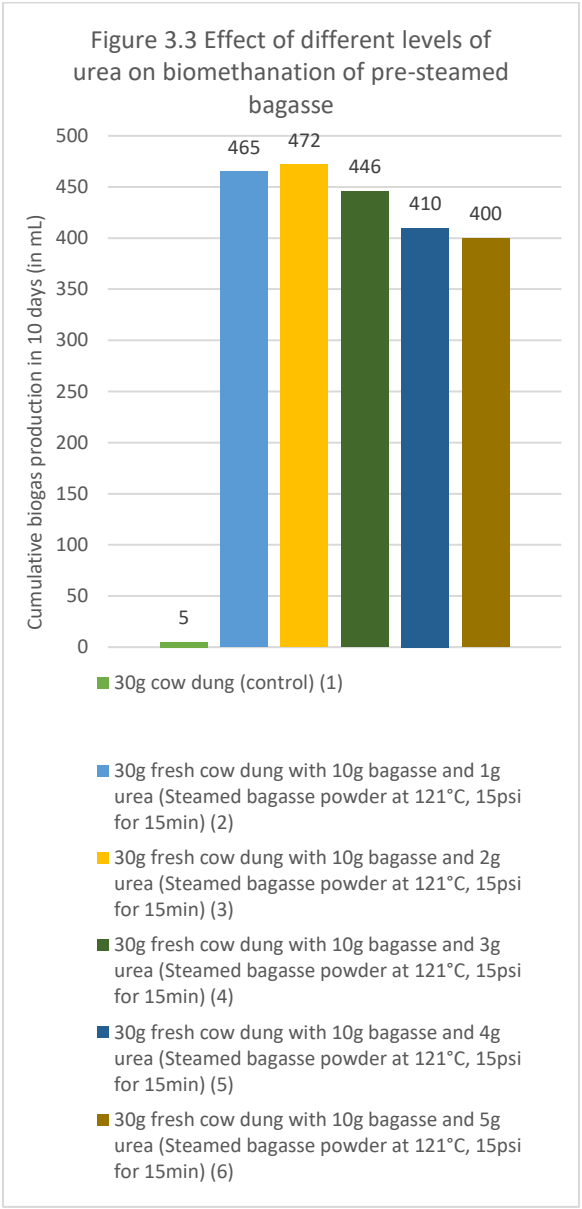

Table 3.3 Effect of different levels of urea on bio-methanation of pre-steamed bagasse

Sample	Condition	Fermentation mixture	Cumulative biogas production in 10 days (in mL)
1	Control	30g cow dung	5
2	Steamed bagasse	30g cow dung+10g bagasse+1g urea	465
3	Steamed bagasse	30g cow dung+5.5g bagasse+2g urea	472

4	Steamed bagasse	30g cow dung+10g bagasse+3g urea	446
5	Steamed bagasse	30g cow dung+10g bagasse+4g urea	410
6	Steamed bagasse	30g cow dung+10g bagasse+5g urea	400

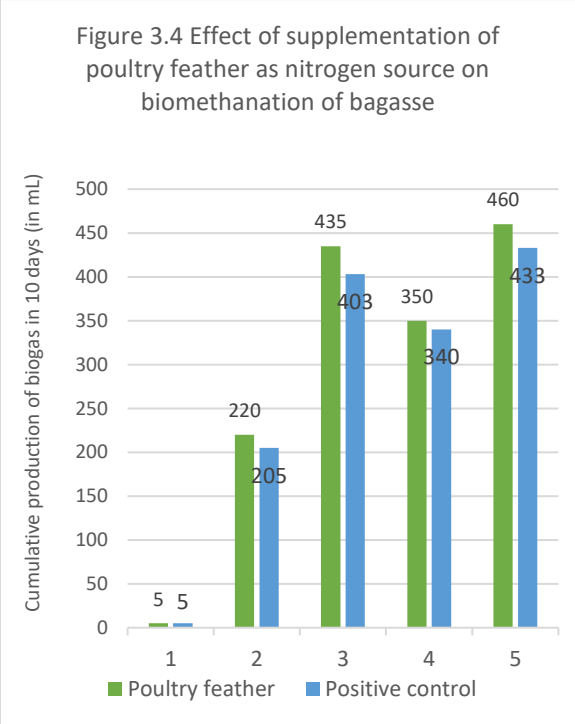

Table 3.4 Effect of supplementation of poultry feather as nitrogen source on bio-methanation of bagasse

Sample	Condition	Fermentation mixture (FM) (30g cow dung+10g bagasse)	Cumulative biogas production in 10 days (in mL)	Positive control (FM)
1	Control	30g cow dung	5	5
2	Powdered raw bagasse powder	FM+1g poultry feather (PF)	220	205
3	Steamed bagasse powder (15psi, 121°C for15 min)	FM+1g poultry feather	435	403
4	100°C hot water treated bagasse powder	FM+1g poultry feather	350	340
5	0.1 N NaOH and steam treated bagasse	FM+1g poultry feather	460	433

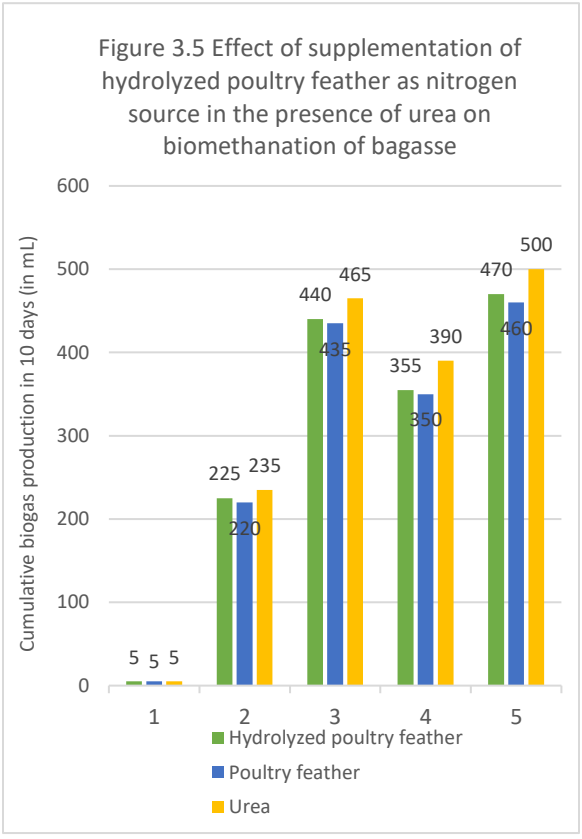

Table 3.5 Effect of supplementation of hydrolyzed poultry feather as nitrogen source in the presence of urea on bio-methanation of bagasse

Sample	Condition	Fermentation mixture (FM) (30g cow dung+10g bagasse)	Cumulative biogas production in 10 days (in mL)	Positive control (FM+1g PF)	Positive control (FM+1g urea)
1	Control	30g cow dung	5	5	5
2	Powdered raw bagasse powder	FM+1g hydrolyzed poultry feather (HPF)	225	220	235
3	Steamed bagasse powder (15psi, 121°C for15 min)	FM+1g hydrolyzed poultry feather	440	435	465

4	100°C hot water treated bagasse powder	FM+1g hydrolyzed poultry feather	355	350	390
5	0.1 N NaOH and steam treated bagasse	FM+1g hydrolyzed poultry feather (HPF)	470	460	500

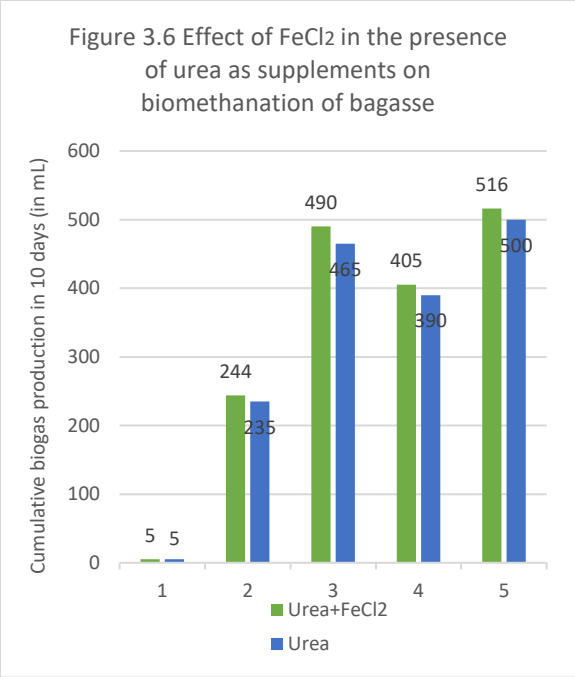

Table 3.6 Effect of $FeCl_2$ in the presence of urea as supplements on bio-methanation of bagasse

Sample	Condition	Fermentation mixture (FM) (30g cow dung+10g bagasse+1g urea)	Cumulative biogas production in 10 days (in mL)	Positive control
1	Control	30g cow dung	5	5
2	Powdered raw bagasse powder	FM+25ppm $FeCl_2$	244	235
3	Steamed bagasse powder (15psi, 121°C for15 min)	FM+25ppm $FeCl_2$	490	465
4	100°C hot water treated bagasse powder	FM+25ppm $FeCl_2$	405	390
5	0.1 N NaOH and steam treated bagasse	FM+25ppm $FeCl_2$	516	500

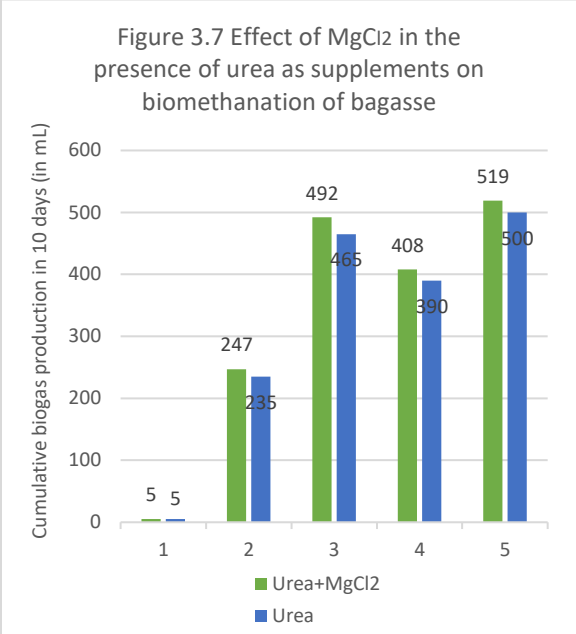

Table 3.7 Effect of $MgCl_2$ in the presence of urea as supplements on bio-methanation of bagasse

Sample	Condition	Fermentation mixture (FM) (30g cow dung+10g bagasse+1g urea)	Cumulative biogas production in 10 days (in mL)	Positive control
1	Control	30g cow dung	5	5
2	Powdered raw bagasse powder	FM+25ppm $MgCl_2$	247	235
3	Steamed bagasse powder (15psi, 121°C for15 min)	FM+25ppm $MgCl_2$	492	465
4	100°C hot water treated bagasse powder	FM+25ppm $MgCl_2$	408	390
5	0.1 N NaOH and steam	FM+25ppm $MgCl_2$	519	500

	treated bagasse

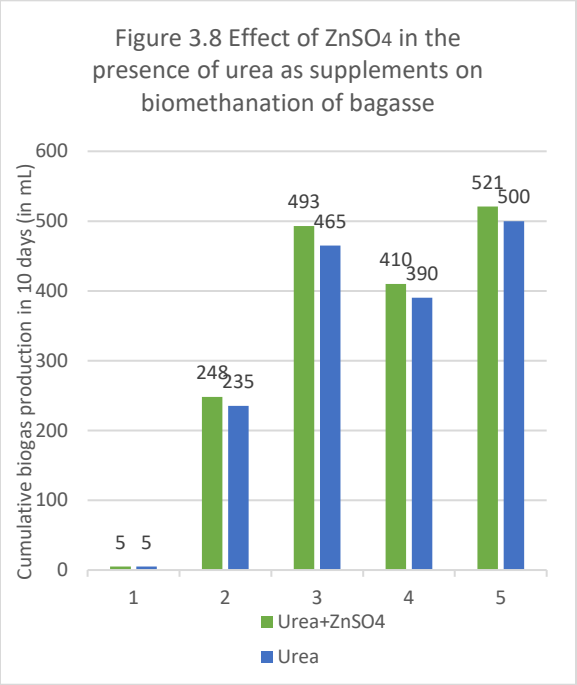

Table 3.8 Effect of $ZnSO_4$ in the presence of urea as supplements on bio-methanation of bagasse

Sample	Condition	Fermentation mixture (FM) (30g cow dung+10g bagasse+1g urea)	Cumulative biogas production in 10 days (in mL)	Positive control
1	Control	30g cow dung	5	5
2	Powdered raw bagasse powder	FM+25ppm $ZnSO_4$	248	235
3	Steamed bagasse powder (15psi, 121°C for15 min)	FM+25ppm $ZnSO_4$	493	465
4	100°C hot water treated bagasse powder	FM+25ppm $ZnSO_4$	410	390
5	0.1 N NaOH and steam treated bagasse	FM+25ppm $ZnSO_4$	521	500

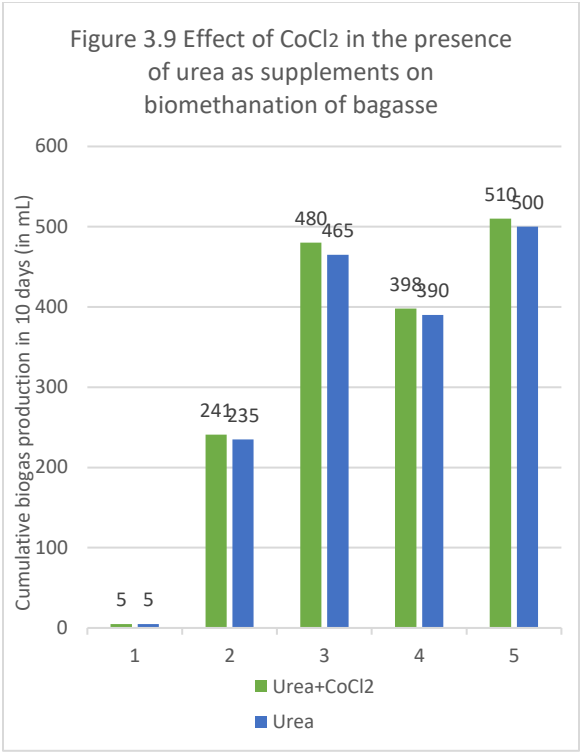

Table 3.9 Effect of $CoCl_2$ in the presence of urea as supplements on bio-methanation of bagasse

Sample	Condition	Fermentation mixture (FM) (30g cow dung+10g bagasse+1g urea)	Cumulative biogas production in 10 days (in mL)	Positive control
1	Control	30g cow dung	5	5
2	Powdered raw bagasse powder	FM+25ppm $CoCl_2$	241	235
3	Steamed bagasse powder (15psi, 121°C for15 min)	FM+25ppm $CoCl_2$	480	465
4	100°C hot water treated bagasse powder	FM+25ppm $CoCl_2$	398	390
5	0.1 N NaOH and steam treated bagasse	FM+25ppm $CoCl_2$	510	500

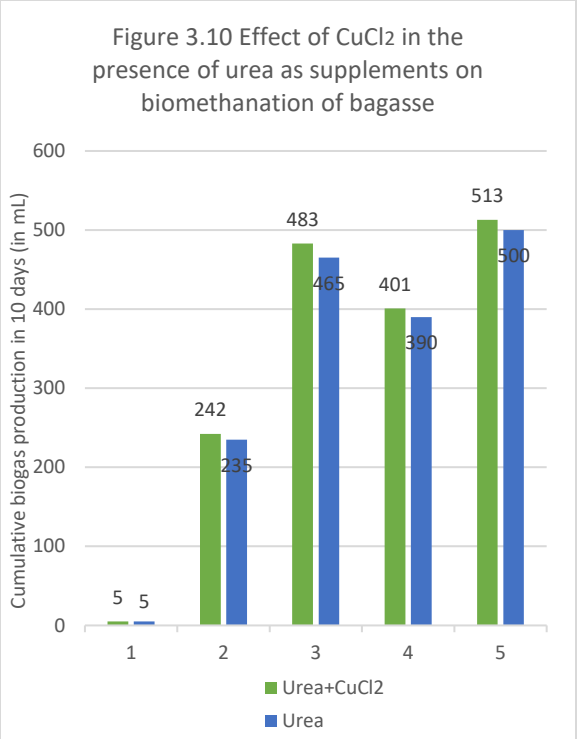

Table 3.10 Effect of $CuCl_2$ in the presence of urea as supplements on bio-methanation of bagasse

Sample	Condition	Fermentation mixture (FM) (30g cow dung+10g bagasse+1g urea)	Cumulative biogas production in 10 days (in mL)	Positive control
1	Control	30g cow dung	5	5
2	Powdered raw bagasse powder	FM+25ppm $CuCl_2$	242	235
3	Steamed bagasse powder (15psi, 121°C for15 min)	FM+25ppm $CuCl_2$	483	465
4	100°C hot water treated bagasse powder	FM+25ppm $CuCl_2$	401	390
5	0.1 N NaOH and steam treated bagasse	FM+25ppm $CuCl_2$	513	500

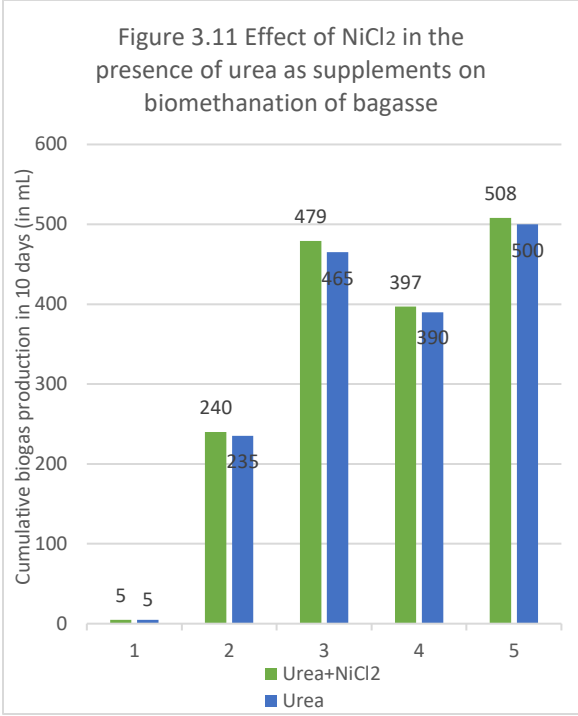

Table 3.11 Effect of $NiCl_2$ in the presence of urea as supplements on bio-methanation of bagasse

Sample	Condition	Fermentation mixture (FM) (30g cow dung+10g bagasse+1g urea)	Cumulative biogas production in 10 days (in mL)	Positive control
1	Control	30g cow dung	5	5
2	Powdered raw bagasse powder	FM+25ppm $NiCl_2$	240	235
3	Steamed bagasse powder (15psi, 121°C for15 min)	FM+25ppm $NiCl_2$	479	465
4	100°C hot water treated bagasse powder	FM+25ppm $NiCl_2$	397	390
5	0.1 N NaOH and steam treated bagasse	FM+25ppm $NiCl_2$	508	500

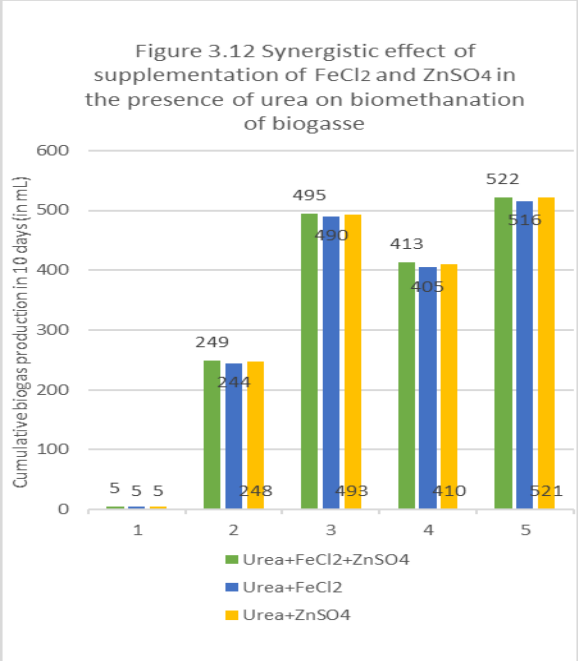

Table 3.12 Synergistic effect of supplementation of $FeCl_2$ and $ZnSO_4$ in the presence of urea on bio-methanation of bagasse

Sample	Condition	Fermentation mixture (FM) (30g cow dung+10 g bagasse+ 1g urea)	Cumulative biogas production in 10 days (in mL)	Positive control (FM+25 ppm $FeCl_2$)	Positive control (FM+25 ppm $ZnSO_4$)
1	Control	30g cow dung	5	5	5
2	Powdered raw bagasse powder	FM+25ppm $FeCl_2$+25 ppm $ZnSO_4$	249	244	248
3	Steamed bagasse powder (15psi, 121°C for15 min)	FM+25ppm $FeCl_2$+25 ppm $ZnSO_4$	495	490	493
4	100°C hot water treated bagasse powder	FM+25ppm $FeCl_2$+25 ppm $ZnSO_4$	413	405	410
5	0.1 N NaOH and steam treated bagasse	FM+25ppm $FeCl_2$+25 ppm $ZnSO_4$	522	516	521

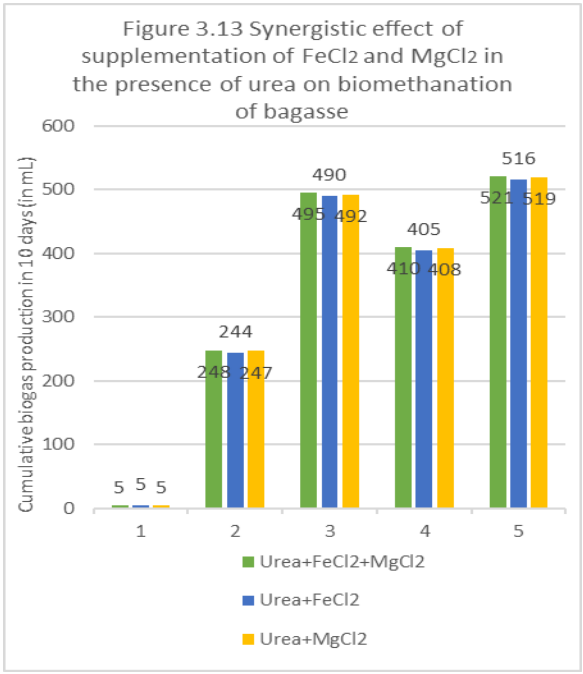

Table 3.13 Synergistic effect of supplementation of $FeCl_2$ and $MgCl_2$ in the presence of urea on bio-methanation of bagasse

Sample	Condition	Fermentation mixture (FM) (30g cow dung+10 g bagasse+ 1g urea)	Cumulative biogas production in 10 days (in mL)	Positive control (FM+25 ppm $FeCl_2$)	Positive control (FM+25 ppm $MgCl_2$)
1	Control	30g cow dung	5	5	5
2	Powdered raw bagasse powder	FM+25ppm $FeCl_2$+25 ppm $MgCl_2$	248	244	247
3	Steamed bagasse powder (15psi, 121°C for15 min)	FM+25ppm $FeCl_2$+25 ppm $MgCl_2$	495	490	492
4	100°C hot water treated	FM+25ppm $FeCl_2$+25 ppm $MgCl_2$	410	405	408

	bagasse powder				
5	0.1 N NaOH and steam treated bagasse	FM+25ppm $FeCl_2$+25 ppm $MgCl_2$	521	516	519

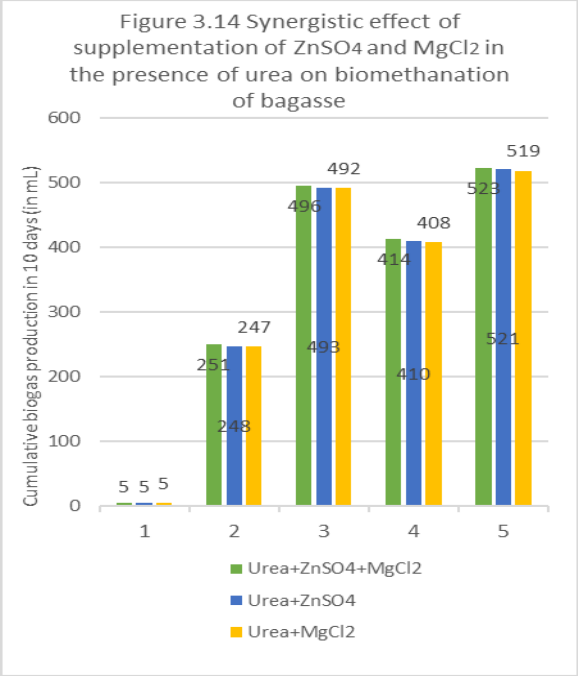

Table 3.14 Synergistic effect of supplementation of $ZnSO_4$ and $MgCl_2$ in the presence of urea on bio-methanation of bagasse

Sample	Condition	Fermentation mixture (FM) (30g cow dung+10g bagasse+ 1g urea)	Cumulative biogas production in 10 days (in mL)	Positive control (FM+25 ppm $ZnSO_4$)	Positive control (FM+25 ppm $MgCl_2$)
1	Control	30g cow dung	5	5	5
2	Powdered raw bagasse powder	FM+25ppm $ZnSO_4$+25ppm $MgCl_2$	251	248	247
3	Steamed bagasse powder (15psi, 121°C for15 min)	FM+25ppm $ZnSO_4$+25ppm $MgCl_2$	496	493	492
4	100°C hot water treated bagasse powder	FM+25ppm $ZnSO_4$+25ppm $MgCl_2$	414	410	408
5	0.1 N NaOH and steam treated bagasse	FM+25ppm $ZnSO_4$+25ppm $MgCl_2$	523	521	519

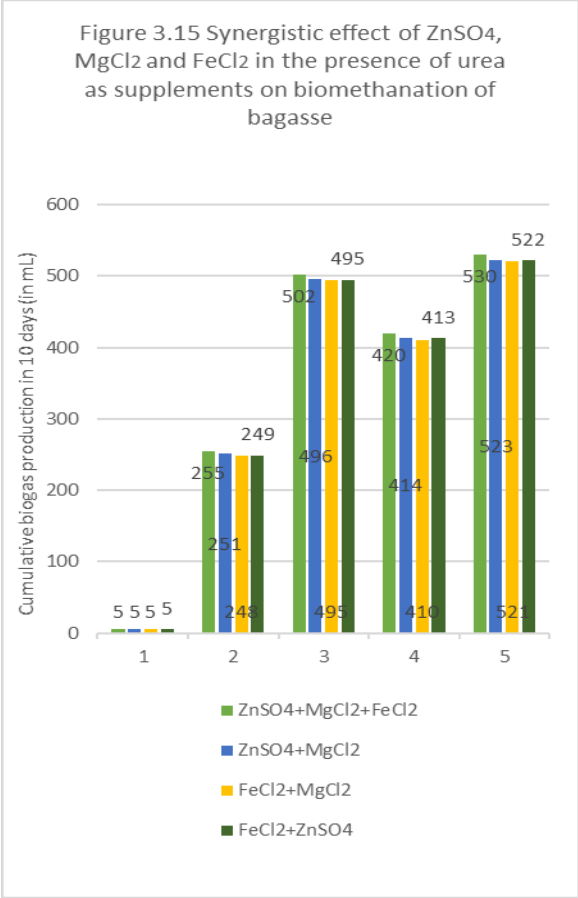

Table 3.15 Synergistic effect of $ZnSO_4$, $MgCl_2$ and $FeCl_2$ in the presence of urea as supplements on bio-methanation of bagasse

Sample	Condition	Fermentation mixture (FM)	Cumulative biogas	Positive control (FM+2	Positive control	Positive control

		(30g cow dung+ 10g bagasse+1g urea)	s production in 10 days (in mL)	5ppm $ZnSO_4$ +25ppm $MgCl_2$)	(FM+ 25ppm $FeCl_2$ +25ppm $MgCl_2$)	(FM+ 25ppm $FeCl_2$ +25ppm $MgCl_2$)
1	Control	30g cow dung	5	5	5	5
2	Powdered raw bagasse powder	FM+25 ppm $ZnSO_4$ +25ppm $MgCl_2$ +25ppm $FeCl_2$	255	251	248	249
3	Steamed bagasse powder (15psi, 121°C for15 min)	FM+25 ppm $ZnSO_4$ +25ppm $MgCl_2$ +25ppm $FeCl_2$	502	496	495	495
4	100°C hot water treated bagasse powder	FM+25 ppm $ZnSO_4$ +25ppm $MgCl_2$	420	414	410	413
5	0.1 N NaOH and steam treated bagasse	FM+25 ppm $ZnSO_4$ +25ppm $MgCl_2$ +25ppm $FeCl_2$	530	523	521	522

IV. DISCUSSION

Fig: 3.1 and Table 3.1 show the levels of bio methanation of bagasse under different condition of physical and chemical treatment. Here, 1% NaOH was used as delignifying agent. As we know lignin and hemicellulose make a protecting covering around cellulose, which is degraded by the alkali pretreatment which also increases the surface area for accessibility for enzyme. Heat treatment reduces the hemicellulose content and increases surface area for enzyme action on cellulose.

In this experiment we found that when we combine physical and chemical method that is, 0.1N NaOH treatment and steaming at 15 psi at 121°C for 15min (Best Combination) gave best results with 433mL biogas production over a period of 10 days, whereas powdered raw bagasse produced only 205mL biogas.

Fig: 3.2 and Table 3.2 show the effect of urea supplementation on bio methanation of bagasse. Bagasse having C:N ratio 150:1, this much higher than required C:N ratio for bio methanation that is, 25:1 to 40:1. In order to increase C:N ratio we added urea as a nitrogen source, we added lg of urea in 600mL solution, which increased the biogas production.

Urea is a cheap organic source of nitrogen, the addition of which greatly enhanced biogas production. In the case of best combination, the biogas production increased from 433mL to 500mL by just adding 1g of urea.

Fig: 3.3 and table 3.3 show the effect of different levels of urea on bio methanation of pre-steamed bagasse condition, in order to optimize the urea concentration, which will maximize biogas production, a series of experiments were conducted with varying concentration of urea, starting from lg to 5g, and it was found out that 2g urea addition was the most beneficial for maximizing biogas production, with maximum gas yield of 472mL in 10 days.

Fig: 3.4 and Table 3.4 show the effect of supplementation of poultry feather as nitrogen source on bio methanation of bagasse. Poultry feather is a waste generated in slaughter houses, the indiscriminate and unscientific disposal could create problems to the environment thereby polluting it. Poultry feather is a rich source of protein keratin, that can supplement urea as source of nitrogen. Keratin is rich in cysteine and methionine. Best combination resulted in 460mL biogas with poultry feather supplementation, whereas non-supplemented samples only yielded 433mL biogas.

Fig: 3.5 and table 3.5 show the effect of supplementation of hydrolyzed poultry feather as nitrogen source in the presence of urea on bio methanation of bagasse. The keratin protein in poultry feather is not easily convertible in to simpler amino acid form so that its microbial utilization becomes difficult. To solve this problem, the poultry feather

was pretreated using 6N HCI t to convert keratin polymer into oligomeric form. The best combination produced 470mL biogas, whereas samples with poultry feather not subjected to acid pretreatment only resulted in 460mL biogas.

Fig: 3.6 and Table 3.6 show the effect of $FeC1_2$ supplementation in the presence of urea on bio methanation of bagasse. Metal ions act as cofactor in metabolic pathway, especially ferric ion acts as part of coenzyme M, which helps in final conversion to CH_4. In the case of best combination, biogas production increased from 500mL to 516mL after adding 25ppm $FeC1_2$.

Fig: 3.7 and Table 3.7 show the effect of $MgC1_2$ supplementation in the presence of urea on bio methanation of bagasse. Mg^{2+} ions help in the conversion of complex polymeric carbon to simpler monosaccharides and are also required for adenosine triphosphate (ATP) synthesis and other metabolic process. Addition of 25ppm $MgC1_2$ resulted in an increase in biogas production from 500mL to 519mL.

Fig: 3.8 and Table 3.8 show the effect of $ZnSO_4$ supplementation in the presence of urea on bio methanation of bagasse. Zn^{2+} ions act as cofactor for many enzymes like carbonic anhydrase. Zinc finger domain is also required for almost all transcription factors. After adding 25ppm $ZnSO_4$ resulted in an increase in biogas production from 500mL to 521mL.

Fig: 3.9 and Table 3.9 show the effect of $CoC1_2$ supplementation in the presence of urea on bio methanation of bagasse. Isomerases are the largest subfamily of B_{12}- dependent enzymes found in bacteria. B_{12} requires Co^{3+}. After adding 25ppm $CoC1_2$, biogas production increased from 500mL to 510mL.

Fig: 3.10 and Table 3.10 Effect $CuC1_2$ supplementation in the presence of urea on bio methanation of bagasse. Cu^{2+} ions act as cofactor for superoxide dismutase and cytochrome oxidase. After the addition of 25ppm $CuC1_2$ to the fermentation mixture, the biogas production improved from 500mL to 513mL.

Fig: 3.11 and Table 3.11 show the effect of $NiC1_2$ supplementation in the presence of urea on bio methanation of bagasse. Ni^{2+} acts as prosthetic group of methyl co-enzyme M reductase, Ni^{2+}-Fe^{2+} hydrogenase, CO dehydrogenase. After adding 25ppm of Ni^{2+} ions, the levels of biogas produced increased from 500mL to 508mL under best combination.

Fig: 3.12 and Table 3.12 show the synergistic effect of $FeC1_2$ and $ZnSO_4$ supplementation on bio methanation of bagasse. In this test, the best suitable combination for metal ions for maximum gas production were checked. Biogas production increased from 521mL to 522mL after the addition of 25ppm each of both $FeC1_2$ and $ZnSO_4$.

Fig: 3.13 and Table 3.13 show the synergistic effect of $FeC1_2$ and $MgC1_2$ supplementation in the presence of urea on bio methanation of bagasse. After adding 25ppm each of both $FeC1_2$ and $ZnSO_4$, biogas production increased from 519mL to 521mL under best condition set up.

Fig: 3.14 and Table 3.14 show the synergistic effect of $ZnSO_4$ and $MgC1_2$ in the presence of urea on bio methanation of bagasse. After adding 25ppm of both ZnS04, and MgC12 biogas production increases from 519mL to 523mL under best condition set up.

Fig: 3.15 and table 3.15 show the synergistic effect of $FeC1_2$, $ZnSO_4$ and $MgC1_2$ in the presence of urea on bio methanation of bagasse. After addition of 25ppm each of $FeC1_2$, $ZnSO_4$, and $MgC1_2$ the level of biogas production recorded was 530mL in 10 days.

V. CONCLUSION

Cutting and grinding of bagasse results in increase in surface area for enzymatic action. These can now easily delignify and the cellulose could be used for metabolic purposes.

Delignification is the one of essential preliminary step for bio-methanation of bagasse. This can be done either by chemical and biological method.

Heat pretreatment is mainly helpful for the removal of hemicellulose component from lignocellulosic material.

When bagasse is treated with NaOH, it breaks the ester bond between and within the complex polymer cellulose, hemicellulose and lignin. This results in the formation of fermentable sugars from cellulose and hemicellulose. Whereas lignin made up of aromatic component is washed out after sieving during removal of excess alkali as sodium salt of aromatic compounds.

When bagasse was pre-treated with steam, it removed hemicellulose component thereby permitting cellulose to be easily available to the microflora for their utilization as carbon source.

When pre-treated bagasse was supplemented with metal ions, the biogas production increased, because these metal ions could act as cofactors and as prosthetic group for functional enzymatic action. Some metals like iron, nickel, and cobalt have a direct role in methanogenesis, while others like magnesium, zinc, and copper have an indirect role.

Bagasse has a C:N ratio of 160:1 whereas the optimum range for bio methanation is 30:1 to 40:1 which required nitrogen supplementation. Better biogas production was observed upon addition of urea and poultry feather. Both urea and poultry feather are cheap sources of nitrogen. Poultry feather being a waste generated from poultry farm, it could be used as a nitrogen source which could also to help control pollution and aid in waste management.

Pre-treatment of lignocellulosic biomass for biogas production through physical, chemical, and thermal methods is an attractive proposal but more research and their optimization to needs to done; it has certain limitations such as pH regulation, void space, delignification etc. which demands more pilot studies.

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