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Experimental analysis on the effects of trace metals as micronutrients in enhancing biomethane production

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Abstract

Anaerobic digestion (AD) of microbial biomass has proven to be a significant breakthrough technique in producing biogas rich in methane. The quantity of biogas obtained by anaerobic digestion processes varies significantly based on the nature and characteristics of the substrates used. This research work focuses on the use of trace metals such as Fe, Cu, Zn, Mn, Mg, Ni in proper proportions to enhance the microbial consortium thus aiding in the production of biogas of desired quality. The substrate used for this study is Food Waste and Cow dung. Food waste from the college canteen was used as the substrate and cow dung was used as an inoculum for providing a catalytic effect in the anaerobic reactor. Food waste and cow dung in the ratio 75:25 was fed into the anaerobic digesters with varying concentrations of micronutrients supplemented to the reactors operating at a pH range maintained between 6.8 and 7.2 under room temperatures (22–27 ºC). The effect of these micronutrients on the anaerobic digestion process was observed by analysing the biogas yield, pH, alkalinity, total solids, and volatile solids of the samples. Sulphates of Fe, Cu, Mn, Ni and Chlorides of Zn and Mg was used in this study. Fe, Cu, Zn, Mn, Mg, Ni were fed to the anaerobic reactor at varying concentrations to arrive at the optimum dosage for the chosen substrates. The optimum dosage for the chosen substrate concentration was taken as that concentration which yielded maximum biogas yield with less retention time. Fe at concentrations varying from 1 mg/l–5 mg/l was fed to the anaerobic reactor and the optimum dosage for the chosen substrate concentration was noted at 1 mg/l. The reactor with an Fe concentration of 1 mg/l showed an increase in biogas production rate of about 68% compared to the sample without Fe supplementation as well as the ones with other dosages greater than 1 mg/l and less than 1 mg/l of Fe dosage. Each nutrient is subjected to an individual dosage analysis before arriving at the optimum dosage and then a mixture of the arrived optimum dosages will be analysed for further study. The process set-up will be conducted for a minimum retention period of 20 days and terminated when the results show a deep fall in the biogas production for consecutive days. Biogas produced for the nutrient supplementation of 1 mg/l of Fe, 0.5 mg/l of Cu, 1 mg/l of Zn, 0.5 mg/l of Mn, 1 mg/l of Mg and 0.5 mg/l of Ni yielded a biogas of 850 ml/g VS in 10 day retention time. Triplicate samples study were conducted and biogas yield measured daily to arrive at concordant results. The results showed an increase in the biomethane yields of the substrate by about 60% compared to the reactors which had no micronutrient supplementations. Furthermore, the study summarized that not all micronutrients are essential for a successful microbial metabolism to take place in an anaerobic digester as the micronutrient Manganese at varying dosages of 0.5 mg/l, 1 mg/l and 1.5 mg/l showed an antagonistic effect on the microbial activity in the reactor. The results obtained from this study showed a significant improvement in the quantity of biogas produced from the substrates

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supplemented with micronutrients at optimum dosages thus arriving at an efficient and effective method for treating waste in a sustainable way.

Keywords Anaerobic digestion, Biogas, Micronutrients

Introduction

Due to rapidly increasing costs associated with various waste management practices and due to the increase in public awareness relating to waste management, conversion of food waste (FW) to energy is becoming a more sustainable practice recommended globally (Hamed et al. 2007). Over-exploitation of fossil fuels and the increase in energy demand resulted in a decrease in fuel abundance in earth's natural reserves (CasperD'Silva et al. 2021). This is one reason that is attributed for the major nations targeting to cut off fossil fuels usage and GHG emissions supporting the Kyoto Protocol (De Filippis et al. 2010). Biogas produced during AD depends on a wide range of factors mostly pertained to the substrates used, microbial pathways and the changing environmental conditions (Kadam et al. 2017). Martinez-Sosa et al. (2009) confirmed the high concentrations of fatty acids as one reason behind the production of a high amount of biogas in an AD process. Biomass is an abundant source of renewable energy capable of producing energy throughout the year (Kapoor et al. 2020) which is yet not fully explored globally in terms of its wide applicability. Moreover, the applicability of the derived digestate as biofertilizer and the carbon neutrality establishes the significance of the process (Adya et al. 2021).

Nutrients play a significant role in the functioning of microorganisms during anaerobic digestion. Nutrients are essential requirements for any microbial species for monitoring their growth, metabolic and enzymatic functions (Fermoso et al. 2009). Wei et al. (2015) and Zheng et al. (2014) suggested the impacts of pretreatment methods and effects of co-digestion in biogas production from AD and reported that less attention has been made to nutrient supplementation for AD stimulation. Choong et al. (2016 and Sibiya et al. (2015) suggested the significance of nutrients in enhancing the metabolism of microorganisms by promoting effective digestion process. Bougrier et al. (2018) and Moestedt et al. (2016) reported that methanogens are highly dependent on micronutrients. Pobeheim et al. (2010) and Takashima et al. (2011) studied the effects of macro nutrients in the growth of microorganisms and how it improved the metabolism of anaerobic microorganisms by acting as buffering agents. Even though the effect of micronutrient supplementation has been shown to improve the AD process, it is not always advisable to do so as there has been reports which shows that all nutrients does not necessarily stimulate the bacterial growth (Sibiya et al. 2015).

Although various substrates have been studied to produce biogas rich in biomethane by various methods, studies using micronutrients for enhancing biomethane production are less explored. Choong et al. (2016) and Ortner et al. (2015) suggested that the nutrient requirements are not well-understood and varies depending on feedstock characteristics and the microbial species involved.

The objective of this study involves the supplementation of Iron (Fe), Copper (Cu), Nickel (Ni), Zinc (Zn), Magnesium (Mg), Manganese (Mn), at various dosages into a sample containing food waste seeded with cow dung to function as an inoculum to enhance the methanogenic pathways. Angelidaki et al. (2009) reported the advantage of using fresh active inoculum or digestates from existing biogas plant as seed for accelerating AD process. Isha et al. (2021) has kept the reactor set up idle for about 1–2 months for the methanogenesis to initiate the AD process. This study hypothesizes the role of micronutrients in improving the performance yield of the reactor in producing methane that reduces the retention time of the reaction and hence aiding towards a sustainable approach in AD process. Parameters such as pH, total solids, volatile solids, volatile fatty acids (VFA), alkalinity, nitrate nitrogen, and variation in C/N ratio were studied for exploring the future opportunities and for scaling up of the biogas reactor to larger scale to be used by the college community.

Materials and methods

Substrate collection

Substrates used for the study were collected from the nearby residential areas and the college canteen. Cow dung (CD) to be used as inoculum was collected manually from the dairy farm near the college campus. The collected samples were cleared off from any type of inert particles and stored in closed airtight containers for future use. The waste collected from the college canteen and nearby residential houses were deprived of any acidic substrates fruit extracts and peels for the smooth functioning of the reactor. Left over brown rice was separated from the collected waste and used as the substrate for this study. The substrates were subjected to an initial

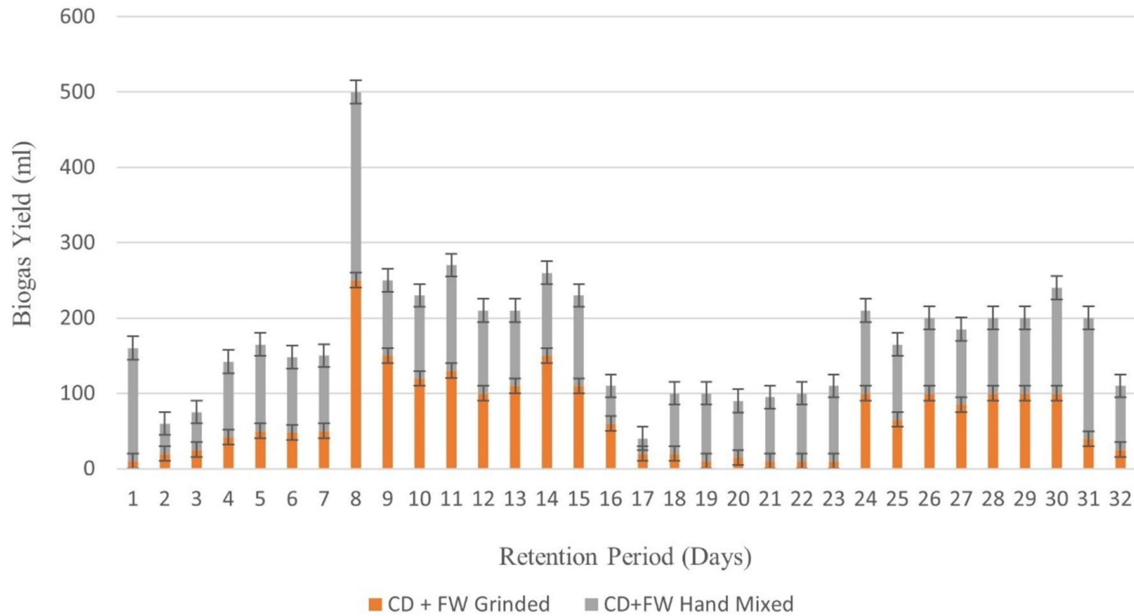

Fig. 1 Comparison of pretreatment methods

pretreatment by grinding of the collected waste and cow dung before initializing the AD process. This method of pretreatment aids in the initiation of the hydrolysis stage of the microorganisms in the production of biomethane. Moreover, it was noteworthy that pretreatment by hand mixed samples yielded concordant results compared to grinded/pulverized mixes. Micronutrients used for the study was prepared from salts of sulphates and chlorides. As shown in Fig. 1, it was observed that hand mixed samples produced a remarkably higher biogas compared to grinded samples.

Experimental analysis by batch test method

Batch tests were run to analyse the amount of biomethane produced from the substrates. The reactor set up used to run the AD process is shown in Fig. 2. Each set up was loaded with food waste, cow dung and micronutrients in the appropriate proportion and dosage. Various proportions of food waste to cow dung in the order 50:50, 60:40, 40:60, 80:20, 20:80, 70:30, 30:70, 75:25, and 25:75 was analysed to arrive at the most appropriate mix which yielded maximum biogas yield with less retention time. Food waste to cow dung ratio was proportioned at 75:25 and micronutrients were supplied at varying dosages starting from 0.25 mg/l to 1.5 mg/l for each micronutrient supplied to the reactor. The dosage which gave the maximum biogas in shortest retention time was taken as the optimum dosage for the study. The total volume of the reactor was 500 ml, and the working volume was 400 ml. All the tests were run in triplicates to arrive at concordant results. Biogas production was studied by water displacement method. Once the reactor was fed with the required volume of substrate in diluted form initial precautions were noted to maintain the reactor volume in required temperature and pressure. Care was taken to maintain the system in anaerobic condition throughout the study. Nitrogen gas was fluxed before initiating the study to maintain the reactor set up in anaerobic condition. The initial pH of the system was maintained at 7.5 to aid in the AD process. Digestate from a running biogas plant was provided as a seed to accelerate the biomethane production process. Proper mixing of the reactor manually ensured that the reactor volume was kept in suspended condition to aid the microbes in bio methanation process.

Once the reactor was loaded with the required substrates and corresponding micronutrients in the required proportion proximate analysis for the samples were conducted. The sample pH was monitored daily to monitor the progress of microbial reaction taking place in the reactor. VFA analysis was done to have a control on the reactor set up. Further studies were done to analyse the Total Solids, Volatile Solids, Moisture Content, Alkalinity of the sample chosen for the study. Analysis of nitrate

Fig. 2 Batch test set up in lab

nitrogen of the sample was conducted to study the C/N ratio of the substrate composition. The initial parameter study to identify the characteristics of the blank sample was performed and was used as the reference to study the variation in parameters of the sample's dosage with Fe, Cu, Zn, Mn, Mg, Ni at varying dosages of 0.25 mg/l, 0.5 mg/l, 0.75 mg/l, 1 mg/l, 1.5 mg/l, respectively. The entire process study was done in triplicates for a retention period of a minimum of 20 days until the gas production from the reactor terminates.

The biogas produced from the samples were analysed in the Standard Biogas analyser in the Lab (Gasboard-3200Plus Handheld Biogas Analyzer). The analyser displays the composition in percentage of various constituents of the gases present, such as methane (CH_4), carbon dioxide (CO_2), hydrogen sulphide (H_2S) and trace amounts of residual gases. The amount of biogas produced was analysed every 2 days for one cycle of the process and care was taken to provide the necessary mixing to reduce the number of residual gases in the reactor. The pH of the reactor was maintained between a range of 6.2 to 7.5 by adding 0.02 N sodium bicarbonate when desired to aid the microbes in bringing out the biological decomposition significantly.

Forms of micronutrients used for the study

The micronutrients used for this study were Iron ($FeSO_4$), Copper ($CuSO_4$), Zinc ($ZnCl_2$), Manganese ($MnSO_4$), Magnesium ($MgCl_2$), Nickel ($NiSO_4$).

Analytical methods

The substrates were studied to analyse the initial characteristics before initiating the process and subjected to final parameters study to analyse the significant biological process in the reactor at the end of retention period or until the gas production in the reactor ceases. Proximate analysis for the determination of Total Solids (TS), Volatile Solids (VS), Moisture Content (MC), Total Alkalinity (TA) of the samples were conducted by the standard APHA 1992, method. Ultimate Analysis for the samples were conducted at the end of the study to analyse the process stability in the anaerobic digestion process. Table 1 summarises the results of proximate and ultimate analysis of the samples used in the study. The micronutrients supplemented to the reactors were strictly dosage specific as any increase or decrease in the dosages of the trace metals supplemented would alter the reaction process. Hence, standard measuring tubes and pipettes were used to provide the optimum dosage of micronutrients to the samples.

pH of the sample was maintained between the range of 6.8 to 7.2. Tinku et al. (2022) for the successful functioning of the biomass in the reactor throughout the test set up. If a considerable drop in pH was noted beyond the required pH range of 6.8–7.2 then 2–3 drops of sodium bicarbonate with 0.02 normality was used to bring the pH to desired level. Proper mixing of the reactors at room temperatures were ensured to avoid settling of the biomass and to aid the microbial biomass in bringing out the biochemical reactions in a profound manner. The biogas produced was measured every day in ml of water displaced from the sample by water displacement method. Volatile fatty acids and total alkalinity were measured by standard titration methods (Anderson and Yang 1992). pH was monitored daily with the help of a pH meter (Systronics Lcd Laboratory Digital pH Meter,1.25 kg, Model Number 802).

Results and discussion

Substrate characteristics

Chandra et al. (2012) reported that methane formation in any biogas digester is highly dependent on the nature of the feedstocks used for the study. The characteristics of the substrates chosen for the study were analysed separately before co-digestion to study the nature of the substrates and the constituents present in them. Both the proximate analyses study and ultimate analyses tests conducted in triplicate samples showed high Total

Table 1 Micronutrients present in the substrates

Micronutrients	Unit	Cow dung alone	Food waste alone	Sample blank: food waste: cow dung
P	%	0.64	0.35	0.10
K	%	0.17	0.13	0.01
Ca	mg/100 g	183.5	200	153
Mg	mg/100 g	143	78.8	67.5
Zn	mg/100 g	5.72	1.4	0.70
Cu	mg/100 g	7.24	0.2	5.68
Fe	mg/100 g	134.24	1.1	71.2

Solids and Volatile Solids with low moisture content values. Lee et al. (2014) reported the significance of the hydrolysis and acetogenesis phases in bringing about an inhibition on the methanogen's metabolism due to lack of nutrients in the anaerobic digester which led to the failure of many successfully running anaerobic reactors. Kurt Moller et al. (2012) suggested that even the lowest supplementation of any micronutrient reduced the accumulation of VFA in the reactor to a significant level.

Micronutrients

Supplementation of micronutrients in required dosages is an efficient method for optimizing anaerobic digestion due to the accumulation of necessary enzymes and for balancing the coenzymes (Moestedt et al. 2016). Zhang et al. (2015) reported micronutrients supplementation to a biogas digester is dosage dependent. Micronutrient supplementations at low dosages produced high gas production with less retention time. Higher dosages led to the failure of the digester and alterations of pH values to an alkaline range which led to the deterioration of the microorganisms in the anaerobic digester. Marcin Zielinski et al. (2019) reported that supplementations enabled biogas production by eliminating the volatile fatty acids production and increases the pH of the microbial biomass.

Iron (Fe) as micronutrient

Fe as a micronutrient can bring about significant changes in the reactor relating to accelerating the biological process in the reactor along with other added micronutrients (Kurt Moller et al. 2012). Fe in the form of green vitriol ($FeSo_4.7H_2O$) was taken in required proportions and diluted to 1000 ml before being fed to the anaerobic digester for the microorganisms to easily absorb the micronutrient in bioavailable form. Fe in varying proportions was fed to the sample of food waste and cow dung mixture in the proportion of 75:25 and compared with the blank sample to study the variations in the process parameters listed in Table 2. Varying dosages of Fe in the range varying from 1 mg/l–5 mg/l were supplied to the anaerobic reactor to study the effect of various dosages.

Initial and final parameter studies conducted on samples taken from the reactor after supplying Fe in required dosages showed that the reactor with an Fe concentration of 1 mg/l showed an increase in biogas production rate of about 68% compared to the sample without Fe supplementation as well as the ones with other dosages greater than 1 mg/l and less than 1 mg/l of Fe dosage. The variation of parameters is shown in Table 2. Initially study was conducted by supplying Fe at higher dosages than the optimal dosage and arrived at the most accurate feedstock with high biogas production, then the same was used to arrive at the optimum dosage. pH of the sample was noted as 6.8 at the start of the experiment. It is noteworthy that the addition of Fe at higher dosages of 2 mg/l–5 mg/l has remarkably decreased biogas production due to an increase in the VFA production which resulted in lowering the pH of the reactors. The pH of the sample dropped significantly in reactors with Fe dosages greater than 1 mg/l owing to VFA accumulation and reduction in alkalinity of the sample. Further change was noted for an Fe dosage of 5 mg/l due to an increase in VFA concentration and an increase in alkalinity, but the pH of the sample dropped.

Three conditions were noted in the anaerobic reactor, first when the pH is stable alkalinity is more than VFA and the amount of biogas produced was significantly higher compared to the control reactor without Fe supplementation. Initially, the digester has good buffering capacity, since the VFA production was less compared to alkalinity but later as the reaction proceeds with an Fe concentration of 2 mg/l the gas production started reducing due to accumulation of VFA. When VFA of the reactor and alkalinity becomes almost equal the natural buffer will become insufficient for the reactor which leads

Table 2 Variation of parameters with varying dosages of Fe

SL No	Sample	Biogas (ml)	VFA (mg HAc/L)	Alkalinity (CaCO3/L)	Total Solids (g/L)	Volatile Solids (g/L)	% C	% N	C/N Ratio
Blank	CD + FD (Hand Mixed)	1609	3245	3250	44.5	42	52.43	1.57	33
1	CD + FD (Grinded)	1517	3079	3500	49.5	34	38.16	1.54	25
2	CD + FD + 1 mg/l Fe	2046	3411	3600	48	45.5	52.66	1.56	34
3	CD + FD + 2 mg/l Fe	836	4905	775	69	49	39.45	1.13	35
4	CD + FD + 3 mg/l Fe	1328	11,545	2500	48.5	30.5	34.94	1.56	22
5	CD + FD + 4 mg/l Fe	1923	11,545	7500	29	26	49.81	1.52	33
6	CD + FD + 5 mg/l Fe	1007	35,615	10,000	73	62.5	47.56	1.30	37

to a drop in pH of the reactor. Alkalinity started reducing but as pH remained stable methanogenic activity was not hindered, and biogas was produced, but as the dosages of the nutrients increased beyond the optimum concentration the methane production will be completely dropped due to reduction in pH which resulted in $C0_2$ increase in the reactor.

Furthermore, the total solids and volatile solids of the samples increased to a considerable extent for optimum dosage of 1 mg/l of Fe and then showed a decreasing trend due to a decrease in methanogenic activity. In this study it was observed that the cumulative biogas yield increased about 68% by addition of 1 mg/l of Fe supplementation. Figure 3 shows the variation of various Fe concentrations with the retention rate in days. The increase in biogas production rate was maximum for a retention period of 20 day incubation duration and then reduction in biogas production was observed due to accumulation of intermediate acids in the reactor. Figure 4 shows the cumulative biogas yield with varying dosages of Fe.

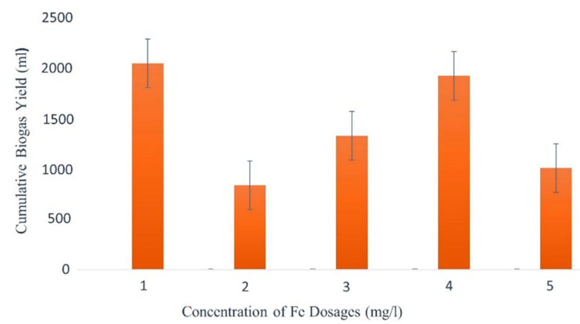

Fig. 3 Cumulative biogas yield with varying dosages of Fe

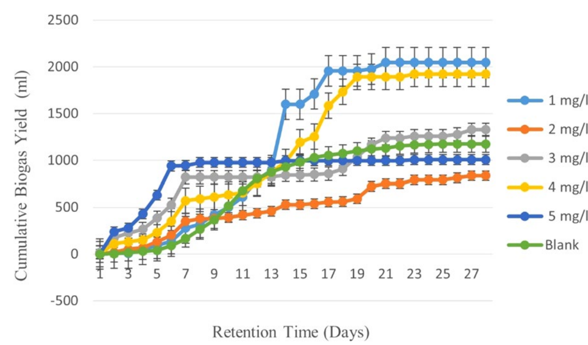

Fig. 4 Cumulative biogas yield with varying dosages of Fe

Copper (Cu) as micronutrient

In this study Cu has been used as a micronutrient to enhance the enzymatic activities in the anaerobic digester and to maintain a good nutrient balance in the digester. Moreover, the supplementation of Cu in varying dosages helped the microbial mass to absorb the Fe more easily in a consortium consisting of Fe and Cu. Varying dosages of Cu ranging from 0.5 mg/l, 1 mg/l, 1.5 mg/l and 2 mg/l, respectively, was fed to the reactor containing Food waste and Cow dung in the ratio 75:25.

It was observed that the biogas produced for 0.5 mg/l of Cu supplementation yielded a higher quantity of biogas compared to all other dosages and hence was taken as the optimum dosage for further analysis. Dosages less than 0.5 mg/l was supplemented to the reactor but proved to be inhibitory in very less retention period of 7 days, similarly dosages greater than 0.5 mg/l of Cu supplementation to the feed stock yielded less amount of biogas than the blank sample and hence was not advisable for the study. However, the C/N ratios obtained for all the samples with Cu supplementations was within the concordant limits of 20:1 to 30:1 (Punal et al. 2000). Accordingly, the C/N ratio obtained for 0.5 mg/l of Cu dosage was 34 which showed successive anaerobic digestion in the sample and process stability. Table 3 shows the variation in parameters with varying dosages of Cu.

Based on the above findings attempt was made to study the combined effects of Fe and Cu supplementations in a feedstock containing food waste and cow dung in the required proportions. Initially one of the digesters was supplied with an optimum concentration of both 0.5 mg/l Fe and 0.5 mg/l Cu, a second digester was supplemented with 1 mg/l Fe and 1 mg/l Cu and a third digester with 1 mg/l of Fe and 0.5 mg/l of Cu. It was observed that the digester with 1 mg/l of Fe and 0.5 mg/l of Cu showed a higher production rate compared to the other two digesters, since the methanogenic activity must have been suppressed due to higher rates of hydrolysis and acidification than methanogenesis (Qiongli Bao et al. 2016) due to imbalance in nutrient absorption by the microbial biomass.

Figure 5 shows the synergistic effects of 1 mg/l of Cu and 1 mg/l of Fe in the biogas yield. It is clearly visible from the graph that 1 mg/l of Fe promoted the biogas production with less retention time, whereas the same dosage of Cu when supplemented to the digester was inhibitory to the biogas yield. Hence, it was decided to study the effects of various elements individually and then make a consortium of the same and provide a supplementation separately to analyse the behavioural pattern of the microbes to nutrient supplementations.

Zinc (Zn) as micronutrient

In this study Zn from Zinc Chloride ($ZnCl_2$) was supplied at various dosages ranging from 0.5 mg/l, 1 mg/l and 1.5 mg/l of Zn, respectively. The test set up was run for a 31 days of incubation duration as compared to Fe

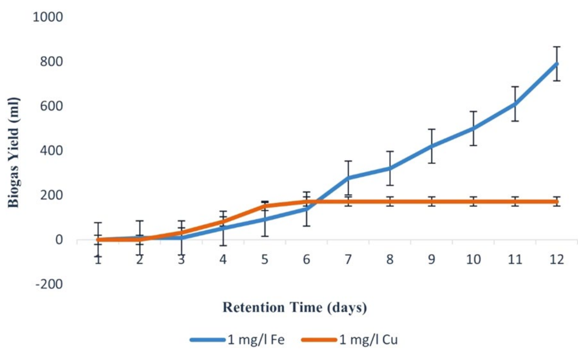

Fig. 5 Synergistic effects of 1 mg/l of Cu and 1 mg/l of Fe in biogas production

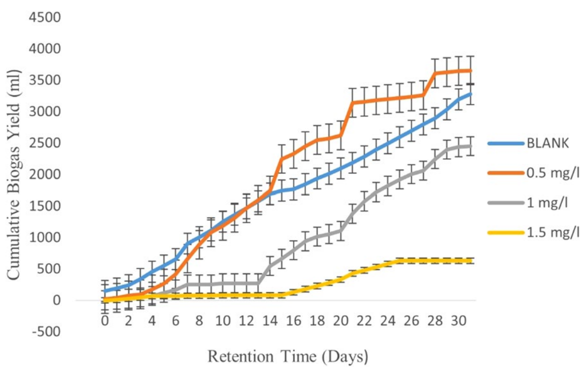

Fig. 6 Cumulative biogas yield for varying dosages of Zn

and Cu nutrient supplementation. An increase in incubation duration was observed in all the reactors, where Zn was added as it accelerated the biomethane production to a considerable extent by decreasing the formation of short chain fatty acids and by decreasing the acetate concentrations in the reactors (Hojae Shim et al. 2019). The digester with 0.5 mg/l of Zinc dosage increased the biogas yield by about 40% with a retention period of 13 days than the blank sample and other reactors. It was observed that the VFA production decreased significantly at 7 days of incubation duration and the biomethane production was significantly increased due to sufficient methanogenic activity in the reactor with a pH of about 7.2. Figure 6 shows the variation in cumulative biogas yield for varying dosages of Zinc. It is visible from the graphs that 0.5 mg/l of Zinc yielded maximum biogas and hence was chosen as the optimum dosage for the designed AD.

Another remarkable pattern of behaviour was observed in the reactor supplemented with 1.5 mg/l of Zinc (Fig. 7). The microbial species showed a uniform behavioural pattern initially and then suddenly showed a decreasing trend in the biogas production after 16 days of incubation duration and then without any active inoculum showed an increasing trend for a few days and then slowly the microbial activity ceased very early after 25 days of incubation duration compared with other digesters which yielded more biogas at 20 days of incubation duration. This behavioural pattern exhibited an inverse growth rate pattern owing to Zn supplementation, also the COD removal efficiency was around 10% which affected the mass balance of the system and decreased the AD process stability.

Magnesium (Mg) as micronutrient

Mg ions from salts of magnesium chloride were used for analyses in this study. Varying dosages ranging from 0.5 mg/l, 1 mg/l and 1.5 mg/l of Mg were supplied to the reactor (Fig. 8) with food waste and cow dung in the required proportion. Kurt Moller et al. (2012) reported that there are only very few studies addressing the significance of Mg in an anaerobic reactor. The reactor with nutrient supplementation of 1 mg/l of Mg showed an increase in biogas production by about 30% than the other reactors and the control. Hence, the optimum dosage of Mg from this study was taken as 1 mg/l. Astals et al. (2016) reported that the addition of Mg to an anaerobic reactor can be inhibitory as well as stimulatory based on the dosage supplemented. The observed result from this study proves 1 mg/l of Mg to be stimulatory for methanogenic activity. The COD removal efficiency was around 60% in the reactor supplied by 1 mg/l of Mg micronutrient.

Manganese (Mn) as micronutrient

There was no considerable increase in biogas production from any of the reactors with Mn supplementations (Fig. 9) compared to the control reactor. This result highlighted the fact that addition of Manganese alone as micronutrient in an anaerobic digester with food waste to cow dung in the ratio of 75:25 had no stimulatory effect in the methanogenic activity. Synergistic effects with other micronutrients were yet to resolve.

Nickel (Ni) as micronutrient

Nickel being a trace metal has significant effects on the anaerobic digestion process and on the COD removal rate of an AD process (Sohail Khan et al. 2021). Svensson et al. (2020) reported the effects of Nickel in enhancing the acetogenic and methanogenic pathways in an AD process. Figure 10 shows the effect of varying dosages of Nickel supplemented to an anaerobic digester compared with a control. It was observed that the reactor with a dosage of 0.5 mg/l of Ni yielded maximum biogas with less retention time. The alkalinity of the sample was observed to be 2000 ($CaCO_3$/L) which prevented the accumulation of long chain fatty acids in the reactor

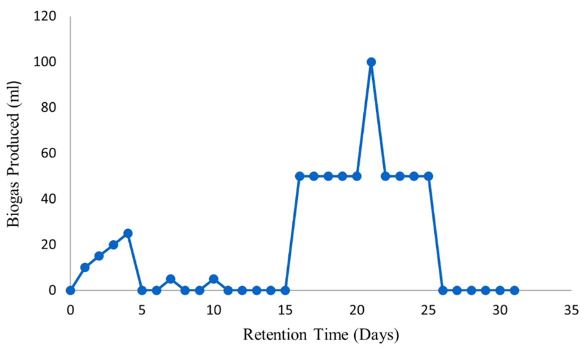

Fig. 7 Daily biogas yield for 1.5 mg/l dosage of Zn

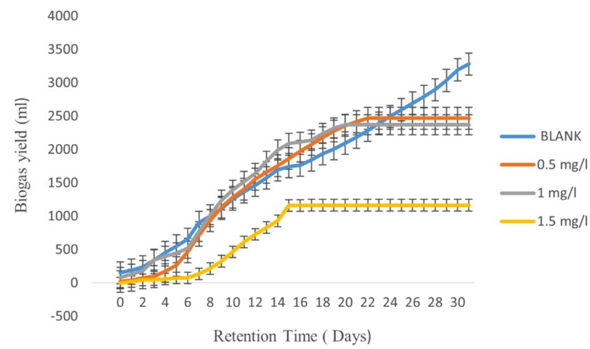

Fig. 9 Cumulative biogas yield for varying Mn dosages

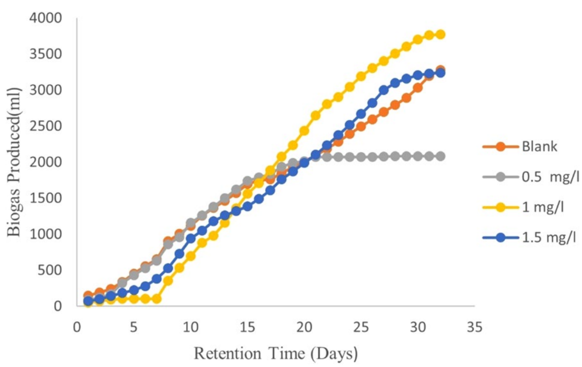

Fig. 8 Cumulative biogas yield for varying Mg dosages

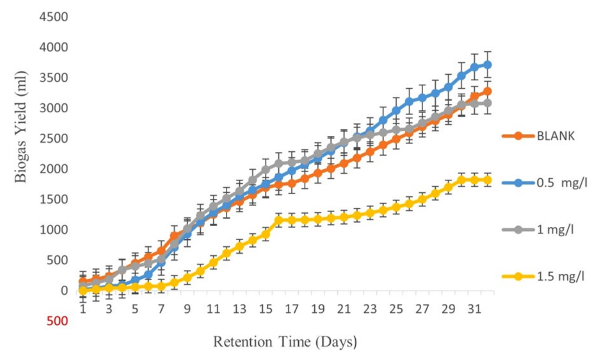

Fig. 10 Cumulative biogas yield for varying dosages of Ni

which enhanced the methanogenic activity. The total solids and volatile solids in the reactor were obtained as 40 g/l and 29 g/l, respectively, which shows an improved process performance of the reactor. The highlighting factor of the addition of nickel was the fact that the biogas production never ceased even at the end of the retention period of 32 days. This is because the addition of Ni enhances the production of methyl co-enzyme M reductase which accelerates the methanogenic phase (Sohail Khan et al. 2021).

Tian et al. (2017) reported that biogas yields with Ni supplemented reactors will occur after 20 day incubation duration, but in this study Ni supplementations at 0.5 mg/l yielded biogas with high biomethane content at 6 day incubation period.

However, the reactors with other concentrations of Ni also yielded methane with high quality but the retention period taken for the yield were comparatively more than the one with 0.5 mg/l of Ni and hence was chosen as the optimum yield for this study, which was then compared with lower variant dosages to arrive at the accurate conclusion.

Variation of VFA with optimum dosages of micronutrients

Table 4 summarizes the VFA variations for the optimum dosages arrived at the end of this study. It was observed that the volatile fatty acids production was remarkably controlled in reactors supplied with optimum dosages of the micronutrients. Manganese supplied to the reactor did not have any significant effect in the digester as the substrates did not require this trace element for the microbial metabolism, whereas nickel added had a synergistic effect and aided in the degradation of the organic matter in the digester.

Conclusions

The study focussed on the use of Iron, Copper, Zinc, Magnesium, Manganese and Nickel as micronutrient supplements in enhancing biogas production in an Anaerobic Digestion process. The batch study was used for determining the effects of micronutrients on food waste and cow dung in low reactors volumes with controlled conditions of pH and temperature. The optimum concentrations of these trace metals were arrived at based on the study. The optimum dosages obtained for Fe was 1 mg/l, 0.5 mg/l for Cu, 1 mg/l for Zn, 1 mg/l for Mg and 0.5 mg/l for Ni, respectively. Manganese as a micronutrient could not contribute much to improving

Table 3 Variation of parameters with varying dosages of Cu

SL No	Sample	Biogas (ml)	VFA (mg HAc/L)	Alkalinity (CaCO3/L)	Total Solids (g/L)	Volatile Solids (g/L)	% C	% N	C/N Ratio
Blank	CD + FD	609	4905	325	44.5	42	52.43	1.57	33
1	CD + FD + 0.5 mg/l Cu	1710	5320	750	48	45.5	52.66	1.56	34
2	CD + FD + 1 mg/l Cu	461	4905	775	69	49	39.45	1.13	35
3	CD + FD + 1.5 mg/l Cu	821	13,205	2750	48.5	30.5	34.94	1.56	22
4	CD + FD + 2 mg/l Cu	167	37,275	10,750	73	62.5	47.56	1.30	37

Table 4 Variation of VFA for optimum dosages of micronutrients

SL No	Micronutrient	Optimum Dosage (ml/g)	VFA (mg HAc/L)
1	Fe	1	3411
2	Cu	0.5	5320
3	Zn	0.5	2415
4	Mg	1	5320
5	Mn	Antagonistic effect	–
6	Ni	0.5	1585

the biogas yield in any of the reactors supplemented with manganese compared to the control reactor. Hence, it was concluded that Mn as a micronutrient depicted antagonistic properties in anaerobic digestion with food waste as substrate. The C/N ratio obtained in all the digesters are between the prescribed range which shows that an optimum C/N ratio alone does not contribute to a successful AD process, but an optimum dosage of micronutrients has significant impact in determining successive anaerobic digestion and process stability. Higher dosages yielded biogas with higher retention period due to the inhibitory action of the micronutrient on the sample. Hence, higher concentrations must have hindered the methanogenic metabolism which further resulted in accumulation of Volatile Fatty Acids in the reactor.

The synergistic effects of the above micronutrients with varying substrates can be explored to enhance the research in this area. In addition, a pilot scale plant can be set and designed for a larger volume which can then be used to convert large amount of waste to energy if explored. The study summarizes that more micronutrients can be explored in future for arriving at a best empirical formula for improving the quality of biomethane produced in the reactor and by completely eradicating CO_2 from the final reactor phase.

Abbreviations

AD Anaerobic digestion
VFA Volatile fatty acids
FW Food waste
CD Cow dung
TS Total solids
VS Volatile solids
MC Moisture content
TA Total alkalinity

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Author contributions

TMC has performed all the lab works and conducted the study on this research work. She has written the manuscript. KSW has contributed ideas in selection of micronutrients required for this study. SJH has contributed to analysing the micronutrients present in the substrates. All authors read and approved the final manuscript.

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Declarations

Competing interests

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References

Adya, I., Tinku, C. D., Paruchuri, M. V. S., Ram, C., & Virendra, K. V. (2021). Stabilization of anaerobic digestion of kitchen wastes using protein-rich additives: Study of process performance, kinetic modelling, and energy balance. *Bioresource Technology, 337*, 125331.

Anderson, G. K., & Yang, G. (1992). Determination of bicarbonate and total volatile acid concentration in anaerobic digesters using a simple titration. *Water Environment Research, 64*(1), 53–59.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, L., Guwy, A., Kalyuzhnyi, J., Jenicek, S., Van Lier, P., & Euverink, J. B. (2009). Defining the BMP of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Science Technology, 59*(5), 927–934.

Astals, S., Romero, M. S., Vila, J., Mata, A. J., & Chimenos, J. M. (2016). The role of additives on anaerobic digestion: A review. *Renewable and Sustainable Energy Reviews, 58*, 1486–1499.

Bougrier, C., Dognin, D., Laroche, C., Gonzalez, V., Benali-Raclot, D., & Cacho Rivero, J. A. (2018). Anaerobic digestion of brewery spent grains: Trace elements addition requirement. *Bioresource Technology, 247*, 1193–1196.

CasperD'Silva, T., Isha, A., Chandra, R., Vijay, V. K., Subbarao, P. M. V., Kumar, R., Chaudhary, V. P., Khan, A. A., Tyagi, V. K., & Kovács, K. L. (2021). Enhancing methane production in anaerobic digestion through hydrogen assisted pathways- A state of the art review. *Renewable and Sustainable Energy Reviews, 151*, 111536.

Chandra, R., Takeuchi, H., & Hasegawa, T. (2012). Improving biodegradability and biogas production of wheat straw substrates using sodium hydroxide and hydrothermal pretreatments. *Energy, 43*, 273–282.

Choong, Y. Y., Norli, I., Addullah, A. Z., & Yhaya, M. F. (2016). Impacts of trace element supplementation on the performance of anaerobic digestion process: A critical review. *Bioresource Technology, 209*, 369–379.

De Filippis, F., & Scarano, F. G. (2010). The Kyoto protocol and European energy policy. *European View, 9*, 39–46.

Fermoso, F. G., Bartacek, J., Jansen, S., & Lens, P. N. L. (2009). Metal supplementation to UASB bioreactors: From cell-metal interactions to full-scale application. *Science of the Total Environment, 407*(12), 3652–3667.

Hamed, M. M., Ruihong, Z., Karl, H., & Fengyu, W. (2007). Characterization of food waste as feedstock for anaerobic digestion. *Bioresource Technology, 98*(4), 929–935.

Hojae, S., Dong, G., Renata, A. T., Qihong, Lu., & Pak, C. C. (2019). Improved anaerobic co-digestion of food waste and domestic wastewater by copper supplementation—Microbial community change and enhanced effluent quality. *Science of the Total Environment, 670*, 337–344.

Isha, A., Chandra, R., Kumar, S., Ahmed, S., Subbarao, P. M. V., Vijay, V. K., Chandel, A. K., & Chaudhary, V. P. (2021). *Potentials and challenges of biogas upgradation as liquid biomethane Biogas production*. Chem: Springer International Publishing.

Kadam, R., & Panwar, N. L. (2017). Recent advancement in biogas enrichment and its applications. *Renewable and Sustainable Energy Reviews, 73*, 892–903.

Kapoor, R., Pooja, G., Bhawna, T., Virendra, K. V., Vandit, V., Indu, S. T., Hesam, K., Dinh, D. N., & Ashok, K. (2020). Advances in biogas valorization and utilization systems a comprehensive review. *Journal of Cleaner Production, 273*, 123052.

Kurt, M., & Torsten, M. (2012). Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Engineering in life sciences., 12*(3), 242–257.

Lee, W. S., Chua, A. S. M., Yeoh, H. K., & Ngoh, G. C. (2014). A review of the production and applications of waste derived volatile fatty acids. *Chemical Engineering Journal, 1235*, 83–99.

Marcin, Z., Marta, K., Marcin, D., & Katarzyna, E. (2019). Effects of nutrients supplementation on enhanced biogas production from maize silage and cattle slurry mixture. *Water Air Soil Pollution, 230*, 117.

Martinez-Sosa, D., Torrijos, M., Buitron, G., Sousbie, P., Devillers, P. H., & Delgenes, J. P. (2009). Treatment of fatty solid waste from the meat industry in an anaerobic sequencing batch reactor: Start up period and establishment of the design criteria. *Water Science Technology, 60*(9), 2245–2251.

Moestedt, J., Nordell, E., Shakeri Yekta, S., Lundgren, J., Marti, M., Sundberg, C., Ejlertsson, J., Svensson, B. H., & Bjorn, A. (2016). Effects of trace element addition on process stability during anaerobic co-digestion of MSW and slaughterhouse waste. *Waste Management., 47*, 11–20.

Ortner, M., Rameder, M., Rachbauer, L., Bochmann, G., & Fuchs, W. (2015). Bioavailability of essential trace elements and their impact on anaerobic digestion of slaughterhouse waste. *Biochemical Engineering Journal, 99*, 107–113.

Pobeheim, H., Munk, B., Johansson, J., & Guebitz, G. M. (2010). Influence of trace elements on methane formation from a synthetic model substrate for maize silage. *Bioresource Technology, 101*, 836–839.

Punal, A., Trevisan, M., Rozzi, A., & Lema, M. (2000). Influence of C: N ratio on the startup of up flow anaerobic filter reactors. *Water Research, 34*, 2614–2619.

Qiongli, B., Yizong, H., Fenghua, W., Sanan, N., Graeme, W. N., Huaiying, L., & Longjun, D. (2016). Effect of nitrogen fertilizer and/or rice straw amendment on methanogenic archaeal communities and methane production from a rice paddy soil. *Applied Microbiology and Biotechnology, 100*, 5989–5998.

Sibiya, N.T., Tesfagiorgis, H.B., Muzenda, E. (2015). Influence of nutrients addition for enhanced biogas production from energy crops: a review. 7th International Conference on Latest Trends in Engineering & Technology (ICLTET'2015) Nov. 26–27, 2015, Irene, Pretoria (South Africa). http://iieng.org/images/proceedings_pdf/4554E1115038.pdf

Sohail, K., Fuzhi, L., Muhammed, K., & Peihong, S. (2021). Multiple effects of different Nickel concentrations on the stability of anaerobic digestion of molasses. *Sustainability, 13*, 2–11.

Svensson, H. B., Anna, S., David, B., Annika, B., Luka, S., & Sepehr, S. Y. (2020). Effect of cobalt, nickel, and selenium/tungsten deficiency on mesophilic anaerobic digestion of chemically defined soluble organic compounds. *Microorganisms, 8*(4), 598.

Takashima, M., Shimada, K., & Speece, R. E. (2011). Minimum requirements for trace metals in thermophilic and mesophilic methane fermentation from glucose. *Water environment research., 83*(4), 339–346.

Tian, T., Sen, Q., Cong, Y., Yihui, T., Yue, Y., & Jiti, Z. (2017). Distinct and diverse anaerobic respiration of methanogenic community in response to MnO2 nanoparticles in anaerobic digester sludge. *Water Research, 123*, 206–215.

Tinku, C. D., Adya, I., Srishti, V., & Ganesh, S. (2022). Anaerobic co-digestion of dry fallen leaves, fruit/vegetable wastes and cow dung without an active inoculum-A biomethane potential study. *Bioresource Technology Reports, 19*, 101189.

Wei, Y., Li, X., Yu, L., Zou, D., & Yuan, H. (2015). Mesophilic anaerobic codigestion of cattle manure and corn Stover with biological and chemical pretreatment. *Bioresource Technology, 198*, 431–436.

Zhang, W., Zhang, L., & Li, A. (2015). Enhanced anaerobic digestion of food waste by trace metal elements supplementation and reduced metals dosage by green chelating agent. *Water Research, 84*, 266–277.

Zheng, Y., Zhao, J., Xu, F., & Li, Y. (2014). Pretreatment of lignocellulosic biomass for enhanced biogas production. *Progress in Energy and Combustion Science, 42*, 35–53.

Publisher's Note

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