Enhancement of Biogas Production from Mixed Organic Substrates Containing Cow Manure and Delignified Spent Coffee Grounds (SCG) by Addition of Effective Microorganism - 4

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Research

Keywords: EM-4, spent coffee ground (SCG), biogas, anaerobic digestion, Gompertz equation

Posted Date: February 3rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-159437/v1>

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Abstract

There have been continuous efforts to find environmentally benign energy to cope steady increase of global energy needs. The spent coffee ground (SCG) is one of agricultural wastes that can be utilized as a raw material to produce biogas. This study aims to produce biogas from mixture of organic substrate containing cow manure and spent coffee ground (SCG) with a C/N ratio of 25:1 with the addition of effective microorganisms-4 (EM-4) to enhance biogas production. The biodegradations were performed in an anaerobic batch digestion (AD) at ambient environment. The variables used of this research were pH (5, 7, and 9) and the loading of EM-4 (6, 9, and 12%). The digestions were run for 60 days and the volumes of biogas produced were measured every two days. The results showed that the addition of EM-4 was able to initiate biogas production earlier, enhance biogas volume, and extend biogas production end time. The best digestion condition for substrate containing cow manure and SCG with a C/N ratio of 25:1 was found to be at pH 9 and EM-4 loading of 9% to obtain 11.28 mL biogas/g COD with enhancement of biogas production of about 100.2 %. The experimental data was fitted really well by the modified *Gompertz* equation as shown by a high determination coefficient ($R^2 > 0.95$).

Introduction

The formation of biogas in the anaerobic process occurs through the accomplishment of four steps organic substrates degradation, namely the hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Many previous studies revealed that the anaerobic digestion (AD) is suitable for the treatment of wastewater with high content of organic substances. As compared to aerobic digestion, the AD process offers some superiorities, such as the larger production of biomethane, inorganic nutrients and humus as fertilizer, from which additional benefits from economic and environmental aspects can be achieved. The AD process is a popular method for treating of industrial and agricultural waste [1] and converting the organic substances to produce biomethane. Indeed, AD is also well suited with the tropical climate [2]. In addition to Carbon-Nitrogen ratio, other important factors also play important roles in AD process, such as pH, temperature, solid particle size, volatile fatty acid, organic loading rate, solid retention time, and nutrient concentration [3].

The growth of the microbes is highly affected by the presence of nitrogen as the major nutrient. However, it is also usually depending on the carbon substance as reflected by carbon-nitrogen ratio. For an anaerobic digestion, the carbon-nitrogen ratio usually ranges from 25 to 30. Although the statement suggests that nitrogen is the limiting nutrient to the AD process. A low supply of nitrogen will cause limited amount of microbial population, while an excess of nitrogen supply can induce the formation of ammonia and cause a longer duration required for carbon degradation. Therefore, the carbon-nitrogen ratio can be used as an indicator of the performance of AD process. The available carbon is an energy source for the microorganisms, while the nitrogen plays its role to control the population of the microorganisms [4, 5].

Cow manure is one of the important livestock wastes generally used in biogas production through anaerobic digestion. The production of biogas as environmentally benign alternative energy from animal manure, especially cows, possesses excellent potential because the residue of the biogas production in the form of slurry can still be utilized as a high-quality organic fertilizer [6]. Cow manure comprises hemicellulose, cellulose and lignin by 18.6%, 25.2% and 20.2%, respectively. Cow manure bears a nitrogen content of 0.3%, phosphate 0.2%, potassium oxide 0.15% and calcium oxide by 0.2%, which provides an approximate C/N ratio of 20–25% [7]. Therefore, cow manure requires an appropriate supplementation with other materials that have a good C/N ratio. The best supplements can be organic materials such as, agricultural waste, kitchen waste and other organic waste [8].

Along with Brazil and Vietnam, Indonesia is regarded as one of the world's largest coffee (*Coffea sp*) producers. Approximately 78% of the total world's coffee beans are produced from Indonesia. Of the total production of 644,000 tons, about 59.2% is exported and the rest 40.8% is used to fulfill the domestic demand [9]. At present, coffee is served to consumers in various forms. Its enjoyment has now become part of the lifestyle, which can be linked to informal, formal and business communication, characterized by the emerging of cafés in numerous regions in Indonesia and many other countries. In accordance with the increasing coffee productivity and consumption in Indonesia, vast amount of coffee waste generation is inevitable. Coffee amps contain 12.4% cellulose, 39.1% hemicellulose, and 23.9% lignocellulose, nitrogen: 2.1–2.79, C/N ratio: 16.91, fat: 2.29, protein: 17.44. [10, 11, 12].

Effective Microorganism-4 (EM-4) is a brownish-colored and sweetly sour (fresh) liquid that is a cocktail of several living microorganisms useful for the anaerobic digestive process for biogas production. Herawati & Wibawa [13] observed that the addition of EM 4 accelerates the degradation of cellulose, hemicellulose, and lignin into compounds required by biogas-producing microorganisms. Generally, those microorganisms consist of photosynthetic bacteria, lactic acid bacteria, yeast, actinomycetes, and yeast. Inoculant microorganism should consist of 90% *Lactobacillus Sp*, which produce lactic acid which can speed up the digestion of lignin and cellulose [14]. The EM-4's inoculate capabilities that can accelerate cellulose, hemicellulose, and lignin degradation can be an excellent choice for use as a mixture of biogas-making raw materials.

This research aimed to observe the utilization of cow manure and SCG mixture as a substrate and the addition of effective microorganisms-4 to enhance the biomethane production. Although animal manure has been reported as the most popular substrate for full-scale biogas production, it resulted in low biogas yields, therefore an effort to enhance biogas production through co-substrate is proposed [15]. The study was conducted in a batch process at ambient temperature and pressure for 60 days observation with biogas yield was recorded at every 2 days interval. The initial COD of the system and carbon-nitrogen ratio were 37,000 mg/L and 25. The research variables were pH solution (5, 7, and 9) and addition of EM-4 (6, 9, 12%). The dependent variables of the research were biogas production and reduction of COD.

Materials

Cow manure.

The cow manure used for the biodigestion experiment was not the fresh manure, but that after naturally fermented into compost and locally known as “compost fertilizer”. The manure was purchased from an agricultural shop in Semarang, Central Java, Indonesia. Prior to biodigestion experiment, the compost fertilizer was analyzed for its total carbon and nitrogen content. The result of the analysis indicated that the content of the total carbon and nitrogen content were 9.3 % and 0.34 %, confirming a carbon-nitrogen ratio of 27.34 [16].

Spent Coffee Grounds

The spent coffee grounds (SCG) were also collected from some café shops in Semarang, Central Java, Indonesia. The SCG waste was dried in an electric oven at 70°C to dryness before further subjected to alkali delignification using 1.5 N sodium hydroxide solution at 120°C for 60 minutes. The delignified SCG was decanted from the solution and washed several times using hot water to remove the residual alkali and further dried in the oven at 120°C. The delignified SCG contained 49.9 % and 2.70% total carbon and nitrogen giving carbon-nitrogen ratio of 17.87 [16].

Biodigestion Experiment

The experiments were run in a batch biodigester (Fig. 1) with the carbon-nitrogen ratio of 25. Based on the carbon and nitrogen content, a carefully weighed substrate mixture (500 g) could be obtained by mixing of 480.63 g cow manure and 19.37 g coffee ground waste.

The substrate feed and EM-4 bacteria mixtures were prepared according to Table 1, and predetermined amount of water with total volume of mixture of 1000 ml. Furthermore, the initial pH of the mixture was set according to Table 1 by addition of a buffer solution. The readily used substrate feed was introduced into the digester and followed by hermetically closed the digester to create an anaerobic condition. As the biodigestion went by, the volume of the produced biogas was recorded. The volume of biogas was quantified using the water displacement method. In addition, the COD measurements using COD reactors and absorbance spectrophotometry were performed at the first day and the 60th day.

Table 1
Variation of the EM-4 and mixture of cow manure and SCG

No		Addition of EM-4 (%)	Cow manure and SCG		Volume EM-4 (mL)	Volume of water (mL)
			Volume (mL)	COD (mg/L)		
1	Control	-	748	37,000	-	252.0
2	pH = 5	6	748	37,000	44.9	207.4
		9	748	37,000	67.3	184.7
		12	748	37,000	89.8	162.2
3	pH = 7	6	748	37,000	44.9	207.4
		9	748	37,000	67.3	184.7
		12	748	37,000	89.8	162.2
4	pH = 9	6	748	37,000	44.9	207.4
		9	748	37,000	67.3	184.7
		12	748	37,000	89.8	162.2

Kinetic Model

Kinetics of the biogas production data obtained from 60 days digestion was evaluated using the modified Gompertz and first-order reaction kinetic models [17]. Both models were developed based on the completion of the anaerobic digestion process, which corresponds to the rate of specific growth of methanogenic bacteria in the digester. The modified *Gompertz* equation can be written as:

$$y(t) = A \cdot \exp \left\{ - \exp \left[\frac{\mu e}{A} (\lambda - t) + 1 \right] \right\} \quad (1)$$

where:

$y(t)$ = cumulative of specific biogas production (mL/g COD).

A = biogas production potential (mL/g COD)

μ = maximum biogas production rate (mL/g COD.day),

λ = lag phase period or minimum time to produce biogas (days),

t = cumulative time for biogas production (days) and

e = a constant (2.718282).

The kinetics constants of A , μ , and λ can be determined through a non-linear regression.

Results And Discussion

Rate of biogas formation

Table 2
Effect of pH and EM-4 loading on biogas production enhancement and COD removal.

No	Variable	Addition of EM-4 (%)	Cumulative biogas volume (mL)	Enhancement of biogas (%)	COD influent (mg/L)	COD effluent (mg/L)	COD removal	
							kg	%
1	Control	-	53.4	-	37000	24666	12334	33.34
2	pH = 5	6	57.6	7.9	37000	23666	13334	36.04
		9	69.5	30.1	37000	22400	14600	39.46
		12	67.5	26.4	37000	21333	15667	42.34
3	pH = 7	6	83.4	56.2	37000	16666	20334	54.96
		9	106.9	100.2	37000	15533	21467	58.02
		12	98.7	84.8	37000	17333	19667	53.15
4	pH = 9	6	71.5	33.9	37000	18200	18800	50.81
		9	86.8	62.5	37000	19400	17600	47.57
		12	84.2	57.7	37000	20200	16800	45.41

Table 2 presents the cumulative and enhancement of biogas production at various pH and EM-4 loading. The control experiment was performed without addition of EM-4 as an anaerobic digestion enhancer. It is clear that the addition of EM-4 to the substrate mixture in the digestion chamber significantly increased the biogas production. The increasing biogas production for pH of 5 was 7.9 to 26.4 %, pH of 7 was 56.2 to 100.2%, and for pH of 9 was 33.9 to 62.5%. Three pH values were applied in the biodigestion experiments, which all resulted in significant enhancement of the biogas production compared to the control experiment. However, biodigestion conducted at pH of 7 (neutral condition) demonstrated the highest enhancement of biogas production than at pH 5 (acidic) and 9 (basic) conditions. Table 2 also clearly shows that the addition of EM-4 at 9% resulted in the highest enhancement of biogas production than that at 6 and 12%. Overall, the EM-4 provided a positive effect to the enhancement of biogas production because EM-4 is basically a cocktail mixture of living microorganisms that may actively improve the completion of anaerobic digestion. Substrate from food waste generally has higher methane potential production, lignocellulosic biomass, animal manure, and sewage sludge [18, 19]. The living microorganisms of EM-4 are believed to demonstrate strong capability to degrade the substrate for SCG and cow manure. Previous researchers [20] examined the biogas production utilized of soluble fraction of SCG and cow manure in a batch digester and the result indicated that there was 10% an increase of biogas production compared to only cow manure as a substrate.

As tabulated in Table 2, the pH of the system also plays important roles in the anaerobic digestion (AD) process. Previous reports also revealed that the performance of the AD is strongly dependent on pH because pH of the system determines the activity of the bacteria groups involve for each stage of AD [19, 3]. Under weak acidic condition (pH 5.5 to 6.5), the biodigestion took place through hydrolysis and acidogenesis. Some intermediate products of AD, such as volatile fatty acids and acetate are produced during acidogenesis stage, which caused the pH to decline in the biodigester and subsequently interfere the formation of biogas. In fact, methanogenesis process during AD is the most sensitive to the pH, which is usually optimum at neutral condition ($\text{pH} = 7.0 \pm 0.2$) [19, 3]. Unfortunately, under basic condition ($\text{pH} = 9$) the AD because suffer from a decline performance due to reduction of the population of methanogenesis group of bacteria leading to the reduction of biogas formation. As expected, such increment of COD removal rate was achieved from anaerobic biodigestion of mixed organic substrate containing cow manure and SCG via the addition of EM-4 [21]. The trend and performance of COD removal were closely similar with the enhancement of biogas production. The anaerobic biodigestion with the EM-4 addition of 9% at neutral condition ($\text{pH} = 7$) resulted in the highest enhancement of biogas production and COD removal.

Table 3
Effect of pH and EM-4 loading on the biogas production

No	Variable	Addition of EM-4 (%)	Estimation first of biogas production	Highest biogas production		Decrease of biogas production	
			(th day)	(th day)	Vol (mL/g COD)	(th day)	Vol (mL/g COD)
1	Control	-	6th	34th	3.72	36th	1.96
2	pH = 5	6	2nd	32nd	5.27	46th	0.41
		9	2nd	30th	7.23	46th	0.61
		12	2nd	26th	7.43	38th	1.96
3	pH = 7	6	2nd	30th	9.46	44th	1.28
		9	2nd	28th	11.28	40th	1.08
		12	2nd	22nd	11.69	38th	0.68
4	pH = 9	6	4th	30th	7.77	42nd	2.03
		9	2nd	28th	9.72	40th	0.68
		12	4th	24th	9.32	38th	1.08

Table 3 and Fig. 2 (a, b, c) present the profile of biogas production as a function of pH of the digestion medium and EM-4 loading. Indeed, all of the studied conditions underwent the same typical biodigestion process, which includes adaptation phase, log phase, stationary phase, and death phase. The biodigestion of mixed substrate under control condition (without addition of EM-4) began to produce biogas at 6th day digestion and reached the highest biogas production at 34th day (3.72 mL/g COD). The biogas production levelled off at 36th day (1.96 mL/g COD). In the contrary, addition of EM-4 resulted in an earlier biogas production from the first day with higher total volume of biogas than that of control condition.

For biodigestion at weak acidic and neutral conditions (pH 5 and 7) with addition of 6 to 12% EM-4, the first biogas production occurred at the 2nd day of digestion. On the other hand, biodigestion at basic condition (pH = 9), the biogas production began at 2-4th days and being faster than control condition. The SCG as the co-substrate of the digestion, which mainly consists of cellulose, hemicellulose and lignocellulose was proven to assist the improvement of digestion performance. Previous investigators explained that the SCG has function to improve the pH value in the biodigester, so that the decline of pH was less than without SCG [20, 15]. The cellulose, hemicellulose and lignin contained in the SCG can be easily utilized by EM-4 to accelerate the biogas production.

The control experiment achieved highest biogas production (3.72 mL/g COD) at the 34th days of digestion. As expected, the digestion system with EM-4 loadings of 6 to 12% with pH of 5 to 9 required a shorter time to obtain highest biogas production. In addition, EM-4 loading also demonstrated longer time to end the biogas production and resulted in larger cumulative biogas volume. Such increase of EM-4 loadings from 6 to 12% were able to accelerate the initiation of biogas production compared with the control experiment. As shown in Fig. 2.b, the digestion at neutral condition (pH = 7) demonstrated the best digestion performance for the time to produce highest biogas and accumulative biogas volume than those of digestion under acidic (pH = 5) and basic (pH = 9) conditions. It is clear that the time to end the biogas production is affected by EM-4 loading, by which a higher EM-4 loading will consume more substrate in the biodigester and resulted in a shorter time to end of the digestion. Unfortunately, the rate of biogas production obtained in this study was lower compared with the biodigestion using domestic and restaurant food waste as substrates, which are classified as high-methane yield substrates for their high lipids and other important nutrient contents [22, 23].

During AD process, the pH of the biodigestion system plays essential roles because pH affects the solubilization process [24], sets up desirable environment for microbes [25] and influences the simultaneously occurring enzymatic reactions [26]. The first process of digestion is in acidic pH and usually maintained at pH of 5.5–6.5, through a short period (hydraulic retention time of about 2–3 days) acid fermentation [27]. This indicated that at pH of 5, the dominant products were acid compounds and a longer time is still required for further biodigestion steps to produce biogas. At higher pH values (neutral and basic conditions), higher biogas production and shorter biodigestion time were observed. However, the digestion at neutral condition (pH = 7) exhibited the best digestion performance for the time to produce highest biogas and accumulative biogas volume than those of other digestion conditions.

Cumulative biogas production

As seen in Fig. 3, all of the EM-4 loadings provided the similar trends for the cumulative of biogas production at any given time. At pH 5, loading of EM-4 at 6 % only resulted in slightly higher cumulative of biogas volume than that of the control experiment. Surprisingly, loading of EM-4 at 9 and 12% produced significantly higher cumulative of biogas volume than that of the control experiment, which were 67.5 and 69.5 mL/g COD, respectively. It can also be seen that addition of EM-4 was able to shorten the initial biogas production, and hence, reduced the lag time (λ) of the *Gompertz's* kinetic model. Addition of EM-4 increased the total microorganisms in the biodigestion system and assisted to produce biogas earlier. A higher value of EM-4 loading resulted in the shorter period of biogas formation due to more microorganisms present in the anaerobic digester. Figure 3.b. presents the wide difference of cumulative biogas production volume between the control experiment and those with EM-4 loadings under neutral condition (pH = 7). Increasing of EM-4 loading resulted in the earlier time to produce biogas (EM-4 loading of 12%). However, the highest cumulative biogas volume was obtained at biodigestion using EM-4 loading of 9%.

This phenomenon occurs because the more EM-4 loading means that larger number of microorganisms present in the biodigestion system and effectively degrade the substrate so that the stationer phase is achieved earlier [28]. The death phase of EM-4 loaded at 12% is achieved earlier than that of 6% and 9% loading. Indeed, this situation is likely due to the excellent performance of EM-4 bacteria that help the process of decaying organic matter, which leads to a faster biogas formation [7]. The speedy substrate degradation process caused a faster the substrate depletion in system, so that the death phase will come very early. Biodigestion experiment with EM-4 loading of 9% produced the highest total biogas volume compared to that of 6% and 12%, which could be due to balance between the substrate availability and that is required for the growth of microorganisms.

Kinetic model of biogas production

The cumulative biogas production data were evaluated using modified *Gompertz* equation. Results of the kinetic model obtained of modified *Gompertz* for all experiments are tabulated in Table 4. The kinetic constant parameters of A , μ , λ , and coefficient of determination were obtained from non-linear regression of *Gompertz* equation. Figure 4 illustrates the comparison of the cumulative biogas production obtained from experiments and simulation using modified *Gompertz* equation.

The value of A that indicated an ability of the experimental condition to generate the cumulative biogas production. The A value of the control experiment was the lowest compared to the other experimental conditions, which was 54 mL/g COD (Table 4). While, the experiment at neutral condition (pH = 7) with EM-4 loading of 9% resulted in the highest the cumulative biogas production (108 mL/g COD). Therefore, pH = 7 can be concluded as the optimum digestion condition and the addition of EM-4 of 9% was proven to enhance the performance of AD to produce biogas.

The value of μ in the modified *Gompertz* equation showed the rate of maximum biogas production. The control experiment suffered from the lowest μ value (2.28 mL/g COD.day) compared to the other experimental conditions, which indicated the slowest biogas production rate. As seen in Table 4 and Fig. 4, the highest μ value was achieved by the AD experiment at neutral condition (pH = 7) and EM-4 loading of 12%, which suggested that the faster biogas production from AD by addition of EM-4. Unfortunately, the values of μ obtained in our experiments were 2.5 to 4.2 mL/g COD.day and being lower compared with those obtained by other previous studies (Table 4) which used cattle manure, municipal solid waste, water hyacinth, and poultry litter as substrates [29, 30, 31, 32].

The other important constant of *Gompertz* equation is λ , which indicates the minimum time to produce biogas. The control experiment and AD experiment at pH = 5 with EM-4 loading of 6% both possessed 14 days of λ . The larger λ value is an indication of the longer the time to produce biogas. Hence, the λ value can be used to indicate the organic soluble materials of the substrate in the liquid phase which consumed faster by microorganisms in the anaerobic process [33]. In practice, the AD is expected to produce biogas as early as possible. The addition of EM-4 has been proven to reduce the λ value (Table 4), and being advantageous for biogas formation. The AD experiment at neutral condition (pH = 7) with EM-4 loading of 12% exhibited the lowest λ value (6 days). As expected, the increase in EM-4 loading

is beneficial to the AD because more microorganisms available in the system to assist the degradation of organic material to form biogas. However, the lag time (λ) values our experiments were higher than those reported by previous studies (Table 4) indicating that degradation of the mixed substrate of cow manure, SCG, and EM-4 was slower. High lignin content in the cow manure could be the cause of the longer degradation time [29].

The comparison between the cumulative biogas production data obtained from experiments and those calculated using the modified *Gompertz* equation are depicted in Fig. 4. The values of coefficient of determination were high enough ranged from 0.978 to 0.993 (Table 4). These values indicate that modified *Gompertz* model is a suitable equation to describe the AD of mixed substrate containing cow manure and delignified spent coffee ground (SCG).

Table 4
Kinetic constants of *Gompertz* equation of biogas production.

Variable	Addition of EM-4	A (mL/g COD)	μ (mL/g COD.day)	λ (days)	R ²	Author
Control	-	54	2.28	14	0.983	This study
pH = 5	6%	58	2.75	14	0.987	
	9%	70	2.50	9	0.978	
	12%	68	2.80	8	0.983	
pH = 7	6%	86	2.94	10	0.983	
	9%	108	4.07	8	0.979	
	12%	100	4.20	6	0.986	
pH = 9	6%	73	2.65	10	0.981	
	9%	88	3.80	10	0.984	
	12%	85	4.01	8	0.993	
	Cattle manure	418.260	9.490	4.460	-	[29]
	Municipal solid waste	522	97	1.2	0.983	[30]
	Water Hyacinth	449.4	27.9	6.625	0.981	[31]
	Poultry litter	390.4	16.5	8.749	0.999	[32]

Conclusion

The addition of EM-4 to an anaerobic digestion of mixed substrates containing cow manure and SCG in provided positive effect on the biogas production, COD removal and *Gompertz* kinetic parameters. Compared with the control experiment without addition of EM-4, the AD experiment with the addition of EM-4 loading increase the biogas production by 44.9 to 89.8%, removal COD of 6.12 to 24.68%, and increased the kinetic parameter of A , μ , and λ . An AD process at neutral condition and EM-4 loading of 9% with substrate containing C/N ratio of 25 resulted the best condition of biogas production, COD removal and biodegradation kinetic parameters. The result of the experiments promised that utilization of SCG with addition of EM-4 can be used to both treatment of solid waste of coffee and generation of biogas as green energy.

Declarations

Conflict of interest

The authors declare no competing financial interest.

Acknowledgements

The authors gratefully thank to the laboratory facilities in Universitas Diponegoro and Wastewater Technology Laboratory of Department of Chemical Engineering, Universitas Diponegoro during the research.

Authors'contributions

PK and IGRH carried out data collection through laboratory experimental studies and drafted the article. IS and ACK designed the conception or design of the work, data analysis and interpretation and critical revision of the article. All the authors read and final approval of the version to be published.

Funding

No any Research Funds or Grants.

Availability of data and materials

All data generated or analysed during this study are included in this published article. The raw data obtained available from the corresponding author upon reasonable request.

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Figures

Figure 1

Equipment of biogas production

Figure 2

Daily Biogas production at various EM-4 loading (a). pH = 5, (b). pH = 7, (c). pH = 9

Figure 3

Cumulative of biogas production of (a) pH of 5, (b). pH of 7, and (c). pH of 9.

Figure 4

Cumulative biogas production: (a). Control, (b). pH of 5, (c). pH of 7, (d). pH of 9