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RESEARCH ARTICLE

Effects of magnesium ions in microbial cells adhesion of attached growth system for the enhancement of biogas production

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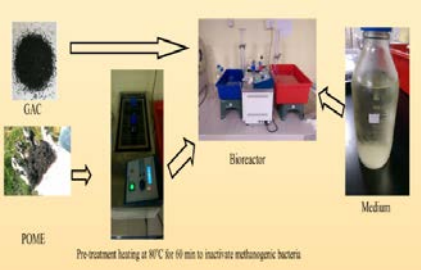
Graphical abstract



Abstract

This research aims to improve the biogas production by employing cell immobilisation technique under thermophilic conditions. The thermophilic fermentative biogas production was carried out by immobilising the anaerobic sludge obtained from palm oil mill treatment plant on granular activated carbon (GAC) in repeated batch mode. Different concentration of magnesium ions (Mg^{2+}) (0.25, 0.5, 0.75, 1.0 and 1.5 g/l) on biogas production was investigated at 60°C with an initial sucrose concentration of 5 g/l as feedstock. The effect of Mg^{2+} supplementation at the initial stage of immobilisation process is important to increase the formation of biofilm in the attached growth system. This study had found that Mg^{2+} could enhance the biogas production capacity with optimum Mg^{2+} concentration of 0.75 g/l.

***Keywords*:** Biogas, biofilm, thermophilic, magnesium, immobilisation

INTRODUCTION

Biogas production via anaerobic dark fermentation has generated a lot of interest among the researchers and has led to a proliferation of studies, which heightened the need to improve the biogas production system performance. The issue of low-yield of biogas is the main bottleneck in current biogas production technologies, which need to be addressed. One of the available solution to improve the biogas yield is by direction of metabolic fluxes that can be accomplished through thermophilic fermentation.

On the other hand, lower cell density was the main challenge faced by several reported studies operated under thermophilic conditions, since most of them were focused on the suspended cell cultures [1]. Therefore, biogas production by thermophilic dark fermentation requires further improvement on cell density, in order to achieve the goal for a superior biogas performance. As an alternative, strategies have been proposed in keeping the culture density and at the same time increasing biogas performance by employing attached growth system under thermophilic condition. There are several approaches to enhance the biogas production through attached growth studies, including optimization of cation ions that could exert their influence during immobilisation process.

Cation such as magnesium ion (Mg^{2+}) is essential for the growth and cell division of cells. However, the need of this cation for growth is depending on the reaction of the organism itself [2]. Simple chemically define media is a good option to explain whether the specific bacteria involve in the process could stimulates the growth with the addition of the cation such as Mg^{2+}. Therefore, this study was employed to determine the effect of Mg^{2+} in microbial cells adhesion of attached growth system for the enhancement of biogas production using define media with sucrose as the sole carbon source.

EXPERIMENTAL

The anaerobic sludge obtained from United Oil Palm Industries Sdn Bhd in Nibong Tebal, Penang was used as the seed sludge in this study. The efficiency of the seed sludge was enhanced by heating at 80°C for 1 hour to inhibit the methanogenic activity [3]. The thermally treated sludge was further used for batch fermentation study using 500 ml Duran bottle with 250 ml of total working volume. The total working volume for each bottle consists of 25 ml sludge that were enriched with 225 ml of synthetic medium, containing sucrose (5 g/l), 1 g/l NH_4Cl, 1.5 g/l $K_2HPO_4.3H_2O$, 0.75 g/l KH_2PO_4, 2 g/l NaCl, 0.05 g/l $CaCl.2H_2O$, 0.5 g/l cystein HCl, 2 g/l yeast extract, 2.6 g/l $NaHCO_3$, and 1 ml/l trace element [4]. The granular activated carbon (GAC) of 25 g was also added to the mixture which functions as host and attached media for cell growth. Study on the effect of Mg^{2+} supplementation was conducted by adding different concentration of magnesium chloride in the ranges of 0.25 g/l to 1.5 g/l in the mixture. Sample without any supplementation of Mg^{2+} was also prepared for fermentation and was assigned as a control sample (blank). The pH of mixture was adjusted to 5.5 using 1 M NaOH or 1 M HCl. Prior to incubation, the bottle was purged with nitrogen to provide an anaerobic condition for dark fermentation process. The samples were incubated at 60°C with 150 rpm of agitation. The batch cultivation was repeated for ten cycles, whereby each of the cycle was conducted for 24 hours to ensure the biofilm formed on the GAC generated a stable biogas production. The biogas composition was measured using

gas analyzer (Model GA5000) and the liquid samples were analysed for total carbohydrate using phenol sulphuric acid method to obtain the sugar consumption during fermentation [5].

RESULTS AND DISCUSSION

Biogas production

Figure 1 shows the volume of biogas produced for different concentration of Mg^{2+}. Based on the observation, the performance of biogas production was enhanced in the presence of Mg^{2+}. The control samples without the addition of Mg^{2+} presented the lowest volume of biogas compared to others. The data had proven that Mg^{2+} could influence the biofilm development, similar to the claim by Pasternak et al. [2] which have reported that Mg^{2+} capable to increase the abundance of attached cells, by reducing the repulsive force between the negatively charged bacterial and substratum surface. Meanwhile, Feng et al. [6] in their study had found that 0.3 g/l of Mg^{2+} have significantly enhanced the biogas production performance to 256 ml H_2/l using hydrogen-producing zymogenous bacteria YUAN-3 (*Ethanoligenens harbinense*). In the case of this study, the adhesion of microbes onto GAC due to the presence Mg^{2+} and the correlation with the increment of biogas performance, indicated that Mg^{2+} plays the role to facilitate cell adhesion in the immobilisation process and improved the biogas production. The results showed that Mg^{2+} with concentration in the range of 0.75 to 1.5 g/l have depicted high but almost similar biogas production. Hence, in terms of economical aspect for medium preparation, Mg^{2+} with concentration of 0.75 g/l was chosen as the optimal value for enhancement of biogas production. In addition, the performance of sugar consumption by microbial cells was the highest with the supplementation of Mg^{2+} at the concentration of 0.75 g/l.

Furthermore, a lighted wooden splinter has been used to confirm the presence of hydrogen gas in the biogas collected. When the lighted wooden splinter was contacted with the gas collected, a pop sound was heard, hence proved the existence of the hydrogen gas in the biogas collected.

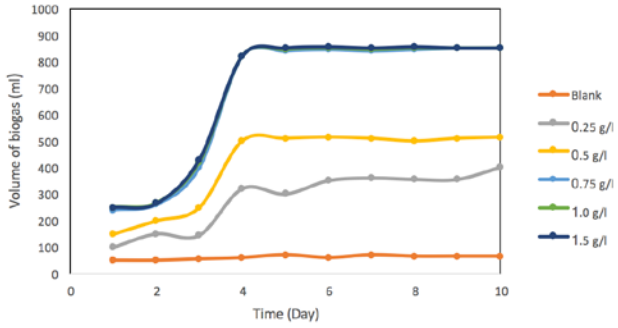


Figure 1 Production of biogas with different range of Mg^{2+} concentration.

Total carbohydrate analysis

Figure 2 shows the percentage of sugar consumption by the microorganisms in different concentration of Mg^{2+}. The control batch fermentation illustrates the lowest percentage of sugar consumption which is 38.25%. The supplementation of Mg^{2+} is crucial as it is essential for the normal cell division of microorganisms [7]. Therefore, the low sugar consumption detected in this study was presumably due to failure of microorganisms to grow in the complete absence of Mg^{2+}. In addition, less biofilm development during the fermentation will cause the population level of microorganisms to decrease, thus causing the low sugar consumption to convert the energy into the biogas production. Moreira et al. [8] has mentioned that higher amount of biofilm will be developed when the consumption of sugar level increased.

Besides that, the sugar consumption by microorganisms could also be influenced by increasing the concentration of cation. For instance, the percentage of sugar consumed by the microorganisms will be increased at higher Mg^{2+}. Figure 2 illustrates the percentage of sugar consumption is higher for the supplementation of 0.50 g/l Mg^{2+} compared to 0.25 g/l Mg^{2+}. According to Zhang et al. [9], the system with higher level of Mg^{2+} offers an advantage to the growth of sulphate reducing bacteria.

The optimum percentage of sugar consumption by microorganisms remain constant at range of 88 % - 89 % for concentration of Mg^{2+} at 0.75 g/l, 1.00 g/l and 1.50 g/l. Ng et al. [10] reported that the growth rate of the species (amount of microorganisms attached as biofilm) were positively correlated to the amount of glucose (amount of sugar consumption). This is because, the addition of Mg^{2+} will reduce the electrostatic repulsion between the negatively charged bacteria and increase the formation of biofilm in the attached growth system. Biofilm formation is one of the most successful strategies for survival in a natural environment, which protect and facilitate the growth of bacteria under unfavourable conditions, such as turbulent flow or limited access to nutrients [11][12].

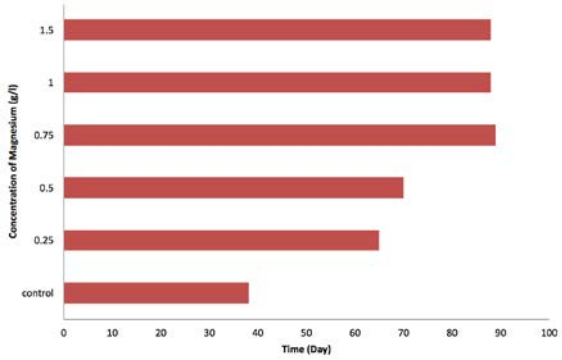


Figure 2 Concentration of magnesium versus the percentage of sugar consumption based on total carbohydrate analysis.

CONCLUSION

In this research, the effect of Mg^{2+} at different concentrations has been studied in attached growth system for the enhancement of biogas production. Supplementation of Mg^{2+} has aid in reducing the electrostatic repulsion between negatively charged bacteria into the seed sludge for a good adhesion towards the granular activated carbon, and also biogas performance. The optimum range of Mg^{2+} obtained in this study was 0.75 g/l by considering the performance of biogas produced and sugar consumption by the immobilised cells.

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