



## Enhancement of Biogas Production from Sewage Sludge by Biofilm Pretreatment Method

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### ABSTRACT

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Since the last two decades, different pre-treatment methods have been proposed to enhance the yield of biogas production from sewage sludge. In order to improve the biogas yield and to find an alternative and economically suitable pre-treatment method, a biological pre-treatment method using a biofilm was studied. The biofilm constituted of four strains of bacteria producing hydrolases was used as an immobilized biocatalyst for the pre-treatment of the sludge. Based on the data obtained during this study, the optimum biofilm was formed within 2 days. The best amount of granular activated carbon (GAC) that has given an optimum biofilm was 4g. An improvement of 16.9% on the ratio of soluble chemical oxygen demand (SCOD) / total chemical oxygen demand (TCOD) and 28.3% in the volatile solids (VS) was achieved after 12 hours of pre-treatment at room temperature ( $30 \pm 2^\circ\text{C}$ ). Comparing to the no pre-treated sludge (control), an increase of 15% in the cumulative biogas production was observed after 14 days of digestion with 30% v/v of inoculum (anaerobic sludge) at  $37^\circ\text{C}$  and 25 days of HRT. It can be concluded that the developed pre-treatment method can be used for the enhancement of the biogas production from sewage sludge at mesophilic temperature range.

#### Keywords:

Biogas; Sewage Sludge; Pretreatment; Biofilm;  
Granular Activated Carbon (GAC)

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## 1. Introduction

With the population growth and the treatment of the wastewater has consequently increased the production of sewage sludge. The current annual production of sewage sludge in Malaysia was estimated to be around 4.9 million tons and it is expected to increase about 7 million cubic meters in 2020 [1, 2]. The biological treatment process of the wastewater leads the concentration of the organic matter in the sludge. It is estimated that about 60% of the plant operation cost is due to sludge treatment only [3, 4]. However, this encourages the reuse and recycling of the sludge to a value-added product. Anaerobic digestion is one of the most applied treatments for the reduction of

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pollutant, solids removal and the recovery of renewable energy (biogas) which is generally used for heat and/or electricity production [5-10]. Currently, this microbiologically driven technology is the most promising and it is widely applied in large and medium-sized wastewater treatment plant. On the other hand, the overall efficiency of this multistage process is limited by the hydrolysis step [11]. Thus, these last two decades, researchers have focused and carried out different pre-treatment methods to improve the solubilization of organic flocs in the sludge. These include thermal, physical, chemical and biological pretreatment methods [3, 12, 13]. Improvement on the biodegradability of organic matters in the sludge as well as enhancement in biogas production rate has been observed after application of these pretreatment methods. Besides the enhancement of biodegradability, biogas yield and dewaterability of sludge, it was noted that biological pretreatment methods were more advantageous than chemical, thermal and physical methods in term of environmental impact and economic feasibility [14]. The application of biological pretreatment methods is eco-friendly, does not require special equipment, extra heat and neither the addition of chemicals.

The high preparation cost of the commercial enzymes has limited their direct application as pretreatment methods and therefore researchers have alternatively studied the addition of crude enzymatic extract [11] or microbial strain producing extracellular enzymes [15, 16]. While, the effect of additional hydrolases extract, suspended endogenous microbial strain producing excess extracellular hydrolases, and the addition of other microbes have been widely studied, but the application of immobilized microbes producing extracellular hydrolases remained unstudied. Thus, this study aimed to improve the solubilization of organic flocs in the sludge by using biological pretreatment method and thus enhance the yield of biogas production. The biological pretreatment method consists the application of biofilm that was constituted of mixed-bacterial culture producing hydrolases (amylase, cellulase and lipase). The sludge was pretreated, and then digested at a mesophilic temperature range in order to see the effect of the pretreatment on the biogas production. Meanwhile, the changes in chemical oxygen demand and volatile solids were analyzed for the assessment of the pretreatment efficiency.

## 2. Methodology

### 2.1 Sample Collection

Sewage sludge was collected from Bunus sewage treatment plant owned by Indah Water Konsortium (IWK), and then it was stored in the chiller at 4°C. The collected sample was containing 3.75 g/L of TS, 2.74 g/l of TVS, 3,5 g/l of TSS, 2.12 g/l of VSS, 2929 mg/l of total COD and 580 mg/l of soluble COD. The bacterial strain used in this study were taken from the environmental-lab stock at Department of Biotechnology Engineering. The isolation study of these bacteria was carried out by Fazil *et al.*, [17]. Granular activated carbon (GAC) was used as the biofilm carrier.

### 2.2 Media Preparation

Nutrient broth was prepared by adding (3 g of yeast extract, 5 g of bacterial peptone and 5 g of sodium chloride) in 1L of distilled water. The GAC was washed with clean water in order to remove ash, and then it was dried in an oven at 50°C for 24 hours.

An overnight culture of each bacteria was prepared by 1 ml of inoculum and 49 ml of nutrient broth, which were incubated at 37°C and 150 rpm shaking speed. The optical density (OD) of each overnight bacterial culture was fixed to (OD=0.1) at 620 nm wavelength, then the subcultures of the same volume were mixed.

### 2.3 Immobilization Process

The attachment of the mixed culture on the surface of the granular activated carbon (GAC) was done in batch mode by using 250 ml conical flask containing an appropriate mass of GAC, 100 mL of nutrient broth, at 37°C and 150 rpm. The determination of immobilization time was done by one-factor-at-time (OFAT) method and the optimization of inoculum volume and mass of carrier was designed by face-centered central composite (FCCCD) option under response surface method (RSM) by using the Design-Expert® software v. 10. After the attachment of microbes on the GAC, the broth was discharged and the GAC was collected with small pore sized (1 mm) tea strainer, then the GAC was washed with distilled water and dried in an oven at 105°C for 2-3 hours. The weight was taken with an analytical balance that is able to measure up to four decimals.

### 2.4 Pretreatment and Anaerobic Digestion Process (ADP)

The pretreatment of liquid sludge was carried out in batch mode by using the same flask used for immobilization. The broth was discarded and the GAC was washed with sterilized distilled water, the 100 mL of sludge was poured into the flasks. The pre-treatment was done at room temperature (30 ±2°C) and 150 in a shaker flask.

The anaerobic digestion process (ADP) was performed in a semi-continuous lab-scale bioreactor by using 500 mL Duran® bottles with two orifices steel cover. The bioreactors were kept in a water bath at 37°C. One orifice was designed for feeding and sampling while the other one was for the collection of biogas. The biogas was measured daily basis by using water displacement methods with acidic water and reversed graduated cylinder. The inoculum to feed ratio was optimized by using One factor-at-time (OFAT) method, while the hydraulic retention time (HRT) was fixed to 25 days.

### 2.5 Other Analytical Methods

The analysis of the different concentration of total solids (TS), total volatile solids (TVS), total chemical oxygen demand (TCOD), and soluble chemical oxygen demand (SCOD) was done following the standard methods [18]. Syringe filter of 0.45µm pore size was used for the preparation of the sample for the soluble COD (SCOD) [19].

## 3. Results and discussion

### 3.1 Immobilization

Biomass dry weight method was used to determine the attached biomass (biofilm) and the additional mass on the initial mass of carrier was due to attached biofilm. During the determination of suitable time for the attachment of the microorganisms on the GAC, 48 hours of retention time have shown higher biomass dry weight (22.65 mg dry biomass /g of GAC) than 24 and 78 hours. Thus, 48 hours was kept as immobilization time for further application. For the optimization of inoculum volume (1, 3 and 5 ml of mixed culture) and mass of carrier (2, 5 and 8 g of GAC) by face-centered central composite (FCCCD), ANOVA was used to analyse the result. The model was significant and the model terms A (mass of GAC) and A<sup>2</sup> were significant, but B (inoculum volume), B<sup>2</sup> and AB were not significant. R square of this model was 0.8185. After the analysis of the 3D and 2D counter plot graph (Figure 1), only the variation in the mass of GAC had an impact on the biomass dry weight, whereas the variation in inoculum volume did not influenced the immobilization of biofilm. Therefore, the

optimum mass of carrier was found to be 4 g and 1 ml of inoculum was economically desired to be used for all immobilization process of the biofilm.

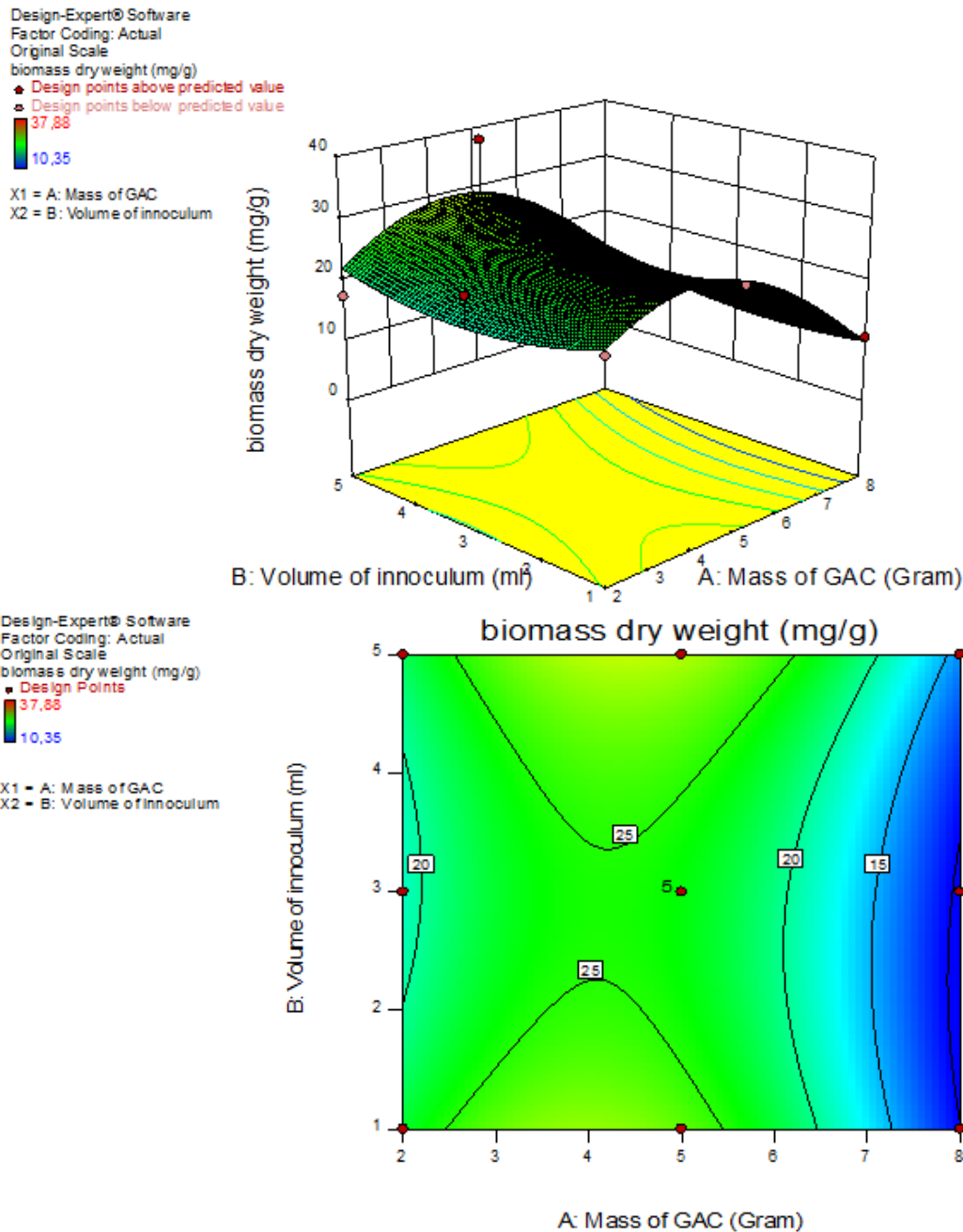


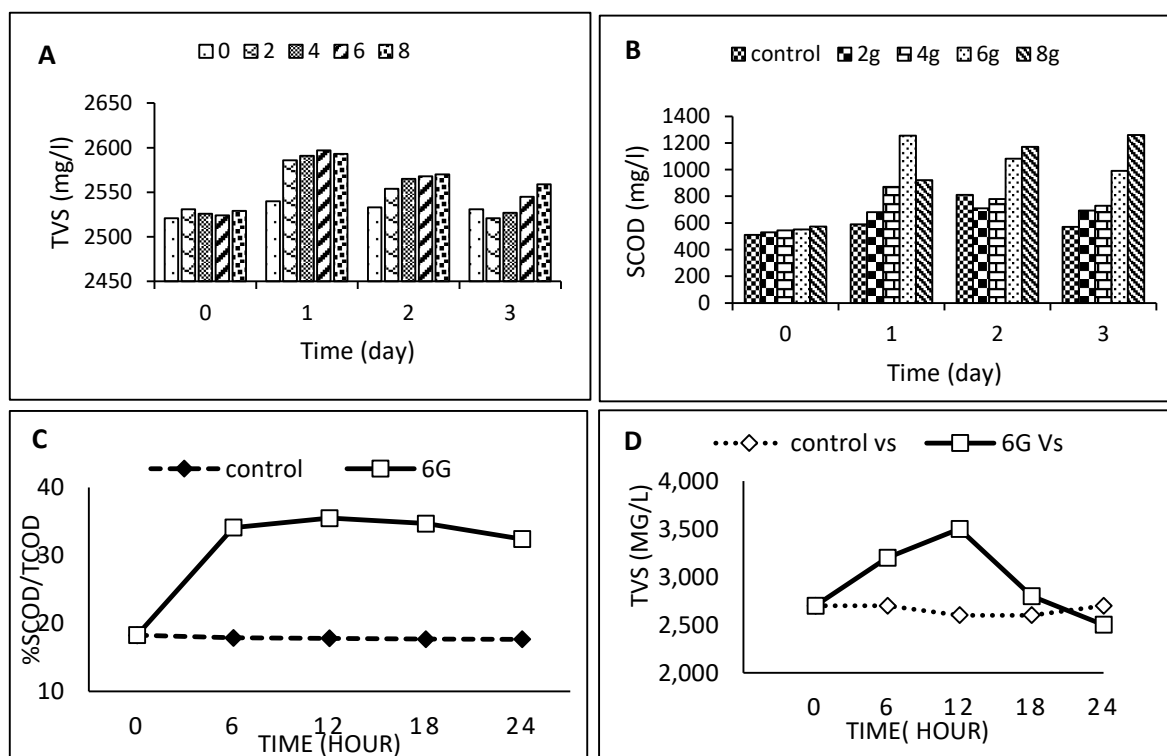
Fig. 1. 3D and 2D counter plot graph for optimization of biofilm immobilization

### 3.2 Pre-treatment Process

Sewage sludge was pre-treated with active biofilm and the best amount of biofilm (among 2, 4, 6 and 8 g of GAC) was determined by OFAT method. The pre-treatment efficiency was studied in term of TVS, TCOD and SCOD changes. This OFAT process was carried out for 3 days, and 6 g of GAC (with attached biofilm) has given an optimum solubilization of organic matters after 24 hours of pre-treatment comparing to the others amounts. It is shown in Figure 2 (A and B), that the process using 6 g of biofilm carrier, the TVS and SCOD have increased from 2520 to 2597 mg/l, and 570 to 1254 mg/l, respectively. Thus, the optimum biofilm was proportional to 6g, but the optimum time

remained to be determined. Therefore, pre-treatment process was subjected to another OFAT study using 6g of biofilm carrier in order to find the suitable time and with the optimum improvement. This last OFAT was carried out for 24 hours and the analysis was done for every 6 hours. Based on the result of the analysis, the effective time of the pre-treatment was 12 hours in which the ratio SCOD/TCOD and total VS increased 16.9% and 28.3%, respectively (Figure 2C and 2D).

After the second OFAT of pre-treatment process, the enhancement observed in term of COD solubilization had the same trend than the one observed in the changes of TVS, and the net improvement in term of TVS was closer to the one obtained in term of SCOD/TCOD. In addition, both results agreed the optimum time of hydrolysis, but the curves of COD changes have slowly decreased whereas the decrease was dramatic in term of VS. This biological hydrolysis was analysed in general, in term of chemical oxygen demand but not specifically for each component such as carbohydrate, protein, and lipids. In a research work using a mixture of protease and amylase liquid extract [11] found a short optimum time than this work (7 hours), however, their COD solubilization ratio (SCOD/TCOD = 11.07%) was lower than the one found in this study. Although, the overall enhancement of organic floc solubilization of this study was obviously lower than the studies carried out by [15] on solubilization of primary sewage sludge by freeze-dried *Lactobacillus brevis* (with 36.9% and 20.7%), and those using other pre-treatment method such as ozone assisted - Ultrasonication pre-treatment by [20] combined Alkaline-Ultrasonic pre-treatment [21].



**Fig. 2.** OFAT 1 of the pretreatment: A) variation of TVS, and B) variation SCOD. OFAT 2 of the pretreatment: C) Variation of SCOD/TCOD ratio OFAT 2, and D) variation of TVS

### 3.3 Anaerobic Digestion Process

The biogas is the product of the product of anaerobic digestion process is biogas which is mainly methane and carbon dioxide as large fraction (60% CH<sub>4</sub> and 40% CO<sub>2</sub>), while traces of other gases such as H<sub>2</sub>, H<sub>2</sub>S, N<sub>2</sub>, O<sub>2</sub>, CO may present [21]. The production of biogas has begun from the first day

and it has gradually increased as depicted on Figure 3 of the cumulative biogas production. The reactor using 30% of inoculum has shown higher biogas production than the control, whereas lower biogas production was observed from the reactors using 10% and 50%. At the end of the process, the total biogas production was calculated and it was observed that the digester inoculated with 30% (v/v) of inoculum has produced the highest biogas volume 172.8 ml, followed by 150.2 ml for the control and the others were lesser than the control (141.4 and 142.4) for the 10% and 50% digesters, respectively.

Yu *et al.*, [4] have reported that 17.8% of enhancement in accumulated biogas production was achieved on 22<sup>nd</sup> day when sludge was pre-treated with amylase and mixture of (amylase protease), in this study 15% of improvement was achieved for only 14 days of digestion.

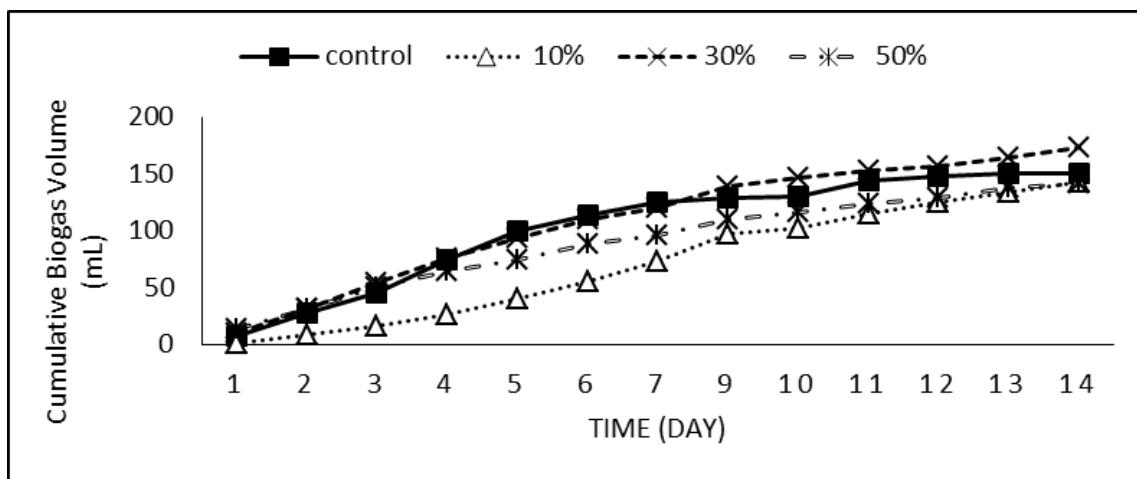


Fig. 3. Cumulative biogas production

#### 4. Conclusions

Biofilm was successfully attached on GAC with a minimum amount of carrier. The pre-treatment method has moderately worked and improved 28.3% in volatile solids concentration and 16.9% in SCOD/TCOD ratio. During the anaerobic digestion process, an enhancement of 15% was achieved in cumulative biogas production within 14 days of digestion. Further and depth study is needed to be done in order to improve the effectiveness of this pre-treatment method.

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