Performance of Paracoccus Solventivorans SP as Sulphur Oxidizing Bacteria to Remove Odour and H$_2$S Has in the Biological Treatment of Malodour

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Abstract. The presence of hydrogen sulphide (H$_2$S) is one of the most problematic contaminants in biogas which emitted from industrial processes causing odour nuisance to the surrounding community. Thus, the ability of sulphur oxidizing bacteria (SOB) could eliminate H$_2$S by changes it into non-odorous elemental sulfur or sulfate was investigated. Basically, most of identified sulphur oxidizing bacteria belongs to the *Thiobacillus*, *Thiothrix*, *Thiomicrospira*, *Achromatium*, *Desulfuromonas* and *Paracoccus* which occurs in heterotrophic bacteria isolated from soil and marine environment. In corresponding of that, about 400 million of palm oil mill effluent (POME) pond found in Malaysia that could be one of the potential supply places for isolation of SOB as it contains elevated concentration of H$_2$S which can support the growth of SOB. To date, many reports utilized SOB can eliminate the high concentration of H$_2$S in environments. Hence, in this study the bacteria of *Paracoccus solventivorans* ATCC 700252 were used as SOB and mixed with media by biofiltration process. Based on final result, these bacteria demonstrated reduction of H$_2$S with removal efficiency 31% to 64.02% with sulphate content production is 30 mg/l compare without SOB, the removal efficiency of H$_2$S about 23% to 46% with sulphate content production is 12 mg/l after 28 days. The suitable condition for the growth of sulphur oxidizing bacteria affected on H$_2$S removal efficiency from palm oil mill biogas by biofilter system. Besides that, the odour reduction for media with SOB is higher (73.5%) compared to media without SOB (66.9%). The reason for this phenomenon is due to the ability of SOB to absorb the odorous ‘rotten egg’ smell and changes into elemental sulfur that is no longer hazardous. In this regard, the utilization of *Paracoccus solventivorans* ATCC 700252 will create a new industry for application in the H$_2$S removal and protect the environment from odour pollution.

1. Introduction

Normally, biogas is derived from anaerobic digestion of sewage sludge, agro industry biowastes and livestock manure. The components that contain in biogas includes methane (CH$_4$), carbon dioxide (CO$_2$), dinitrogen (N$_2$), water (H$_2$O), oxygen (O$_2$), hydrogen sulfide (H$_2$S), ammonia (NH$_3$), hydrocarbon contents and siloxanes [10]. Other than that, the effect due to high concentration of H$_2$S...
in biogas will caused corrosion of concrete and steel, compromises the uses of cogeneration units, become toxic to humans and produces pollution from odors. In addition, the content of sulfide in the liquid stage also will caused corrosion in system of water transport and accumulation of metal sulfides [8].

Conventionally, different processes involving physical (adsorption, absorption, and dilution), chemical (chemical absorption, neutralization, and combustion) and biological (activated sludge and biofilter) methods have been used to remove H2S from waste gas [14]. However, for physical and chemical methods have some disadvantage with high operating costs and production of secondary pollutants when the concentration of H2S is high [2]. In contrast, the biological methods are cost-effective compared to the physical and chemical processes for the removal of H2S in biogas [6]. Otherwise, this technology is a promising alternative because it is energy-savings, eco-friendly and low operating costs. However, biofiltration is a method, which is used especially for odours elimination. Hence, the process of biofiltration efficiency that use immobilized microorganisms technology is one of the potential effective method to improve the efficiency of biogas treatment [9].

In this case, biological removal of sulfides is based on the activities of sulphur oxidizing bacteria (SOB). The SOB is able to oxidize sulphur from H2S to sulphate ion or elemental sulphur and use it as a source of energy. Most of identified sulphur oxidizing bacteria belongs to the *Thiobacillus, Thiothrix, Thiomicrospira, Achromatium, Desulfuromonas* and *Paracoccus* which occurs in heterotropic bacteria isolated from soil and marine environment. Therefore, *Paracoccus* strains can oxidize and reduced sulfur compounds such as thiosulfate, thiocynate, carbon disulfide, carbonyl sulfide and elemental sulfur to gain energy for autotrophic growth [5]. Thus, *Paracoccus solventivorans ATCC 700252* are chosen as SOB as it is one of the identified bacteria in the palm oil itself. They are also suitable for the high rate of sulfide oxidation with modest nutritional requirements and extremely high affinity for sulfides and oxygen. These properties allow them to successfully compete with chemical oxidation of sulfides in both the natural environment and bioreactors with a limited supply of oxygen. Thus, the main objective of this study is to focus on the utilization of *Paracoccus solventivorans ATCC 700252* to eliminate the high concentration of H2S and odor pollution in environments and also to evaluate the influence of immobilization parameter for efficiency of bacterial adhesion on surface media.

2. Experimental

2.1. Carrier Characteristic

Raw material of recycles paper mill sludge (RPMS) in greyish clumps were dried and sieved at 0.75mm. Then, RPMS were formed as small ball by adding 8% w/w of polyvinyl alcohol and again oven dried at 110°C for 24 hrs. Lastly, harden of RPMS balls were formed and tested their characteristics as potential media in biofilter.

2.2. Preparation of Immobilized Carriers

In order to build an initial community of microorganisms in the reactor bed, 10g of RPMS was submerged into the 100ml of phosphate buffer (pH 6.0) containing the bacteria of *Paracoccus sp.* Before the immobilization process, the RPMS carriers must be sterilized. The RPMS were stored in autoclaved at 120°C for 15 minutes. 10% of the concentrated bacteria in the inoculum solution were transferred into flask using a sterilized dropper to avoid contamination (SMWW Part 9050). Then the flask was shaken using orbital shaker in order to determine the immobilization parameters such as contact time and agitation rate with constant 8% w/w of PVA concentration. After obtained their optimum, the RPMS were filled into the biofilter column.

2.2.1 Effect of Contact Time

The effect of time on the immobilization of *Paracoccus sp.* on RPMS was determined by incubating the solution at 10 min, 20 min, 30 min, 1 h and 24 h based on the study conducted by Datta et al. [11].

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2.2.2 Effect of Agitation
The effect of agitation rate on the immobilization of Paracoccus sp. on RPMS was studied by incubating the solution at various speeds of 25, 40, 55 and 70 rpm [3].

2.3 Odour Removal Capability
Odour removal capability can be determined by comparing the amount of odour between gas connected at the inlet and outlet of the biofilter that run for 28 days. Odour strength and concentration of the selected odour compounds are measured using two different devices. In order to detect the odour compounds, Scentroid TR8 Odotracker were used. The concentration value are presented in ppm. Then, odotracker were able to detect the concentration of H₂S using its electrochemical sensors. While, for the odour strength measurement, Scentroid Olfactometer SS400 were used. The odour strength is expressed as dilution factor of individual threshold estimate (Z_iti) and reported in units of OU/m³. For the Scentroid Olfactometer SS400, usually 4 to 6 panelist are required for the testing. The panelist were selected based on their olfactosensory organ sensitivity through n-butanol test. Although the n-butanol possess very distinct odor, but at low concentration it is almost the same as ambient odor. This is why n-butanol are chosen to detect the sensitivity of panelist. However, the H₂S and odor concentration will be measured every 2 days from a gas sampling bags Then, the removal efficiency of odour for both odour strength and compound were calculated.

2.4 Analytical Method
About 0.5g of media will be taken from the different parts of the reactors (at the top, middle and bottom parts), mixed with 10 ml of distilled water and vortexed for 2 min. Then, the solution will be determined for total sulfur and sulfate concentrations. The concentrations of sulfur in biofilter will be measured by the gravimetric method and sulfate concentration will be measured by turbidimetric method which according to APHA standard method.

3. Results and Discussions

3.1 Characterization of RPMS
The organic material tested in this study is recycle paper mill sludge that carefully chosen as a potential carriers for biofilter. For the following reasons, solid organic residue that contain high carbon and proportion of cellulose fibers can use as an excellent additive for biocomposites and adsorbent-absorbent materials, yet its potential as an odour biofiltration medium has not been tested. Further, their characteristic test were done to know the suitability and workability of the RPMS itself to be as a potential biofilter media. Their characteristic tests is done included porosity (%), moisture content (%), bulk density (g/ml), water holding capacity (%) and pH test. Based on the result, the RPMS balls with SOB and without SOB has been compared. All the characteristics in both conditions still within the typical limits. Consequently, the porosity of the RPMS balls will prevent pressure drops, whilst the sufficient water holding capacity will lessen drying for the biofilter bed. Most interestingly is the high density, which given its fibrous nature guarantees a long term physical stability. Hence, the RPMS balls could prevent early compaction of the bed and with proper maintenance of the microbial growth, can ensure a longer lasting odour biofiltration system.
Table 1. Final Characteristics of RPMS for both conditions.

| Characteristics          | With SOB | Without SOB | Acceptable Value | Reference            |
|--------------------------|----------|-------------|------------------|----------------------|
| Water Holding Capacity, %| 78.5     | 71          | > 50             | Chen and Hoff, 2009  |
| Moisture Content, %      | 50       | 45          | 40 - 70          | Ima and Mann, 2007   |
| Bulk Density, g/ml       | 0.48     | 0.35        | 0.3 - 0.5        | Chen et al., 2009    |
| Porosity, %              | 60       | 52          | 50 - 80          | Chen et al., 2009    |

3.2 Immobilization of Bacteria on Organic Carrier

The process of bacteria immobilization by adsorption onto organic carrier is influenced by various factors, such as chemical composition of the carriers and physicochemical interactions. However, it is not yet well understood which factors to determine the efficiency of bacterial adsorption. Therefore, in order to elucidate which parameters govern the adhesion of Paracoccus sp. on RPMS with 8% of PVA concentration, we investigated the immobilization process in function of the contact time, and agitation rate.

Contact time is one of the critical variables that would affect the immobilization of bacteria. In this study, the immobilization process was performed at various contact time ranging from 10 min, 20 min, 30 min, 1 h and 24 h. The influence of the contact time on the immobilization of Paracoccus sp. on carrier is presented in figure 1 (a). From the result, it showed that at 10 to 20 min of contact time, there were only 9.12% and 19.91% of enzyme that were successfully immobilized on the organic carrier, respectively. When the time reached 30 min of contact time, the immobilization yield was significantly increased to 60.45%. The increase of contact time had improved the amount of enzyme interacted to the surface of the support and enhancing the immobilization process. Immobilization process required correct alignment of groups located on the enzyme and the support surface, otherwise the adsorption will not occur. As shown in figure 1(a), as the contact time increased to 24 h, the immobilization yield was significantly decreased to 14.21%. This is due to the detachment of enzyme from the support surface. According to Datta et al. [11] during the physical adsorption, detachment of carrier from the support can be occurred when it was immersed in the immobilization solution for a long period of time.

The immobilization of Paracoccus sp. was carried out at a constant temperature of 25°C with different agitation rate of 25, 40, 55 and 70 rpm to determine the optimal agitation rate for the immobilization process. Results in figure 1(b) illustrated that the low immobilization yield of 37.78% was recorded at 70 rpm compared to 69.55% of immobilization yield at 55 rpm. Low immobilization yield at 70 rpm of agitation rate can be correlated with the non-uniform distribution of the enzyme in the immobilization mixture during the adsorption process [7].
3.3 Hydrogen Sulphides and Odor Removal Efficiency of Biofilter System

The concentration of \( \text{H}_2\text{S} \) in biofilter systems showed that the most efficient \( \text{H}_2\text{S} \) removal occur at carrier that contained immobilized bacteria on day 14. Based on the result until day 14, the removal efficiency of \( \text{H}_2\text{S} \) significantly highest from 31% to 64.02% for carrier that contained SOB compared to the carrier without SOB is lower between 25.52% to 46.84%. However, high removal efficiency for \( \text{H}_2\text{S} \) (64.02%) was decreased to 40.35% within 12 days as shown in figure 2(a). This phenomenon is occur due to the effectiveness of sulfur oxidizing bacteria getting lesser day by day. This factor also effect due to the temperature, moisture content and limited supply of oxygen [1]. Furthermore, it was supported by Tosati et al., [4] stated that use sulfur oxidizing bacteria can eliminate the high concentration of \( \text{H}_2\text{S} \) in environments thus can control odor pollution. Previous study by Nunthaphan et al., [13] had reported \textit{Paracoccus pantotrophus NTV02(PCP)} was able to oxidize elemental sulphur and produced sulphate as an product of the oxidation process. However, to date, no study has reported the capability of \textit{Paracoccus} sp. in the oxidation of \( \text{H}_2\text{S} \) and in this present study, it was the first time \textit{Paracoccus} sp.involved in the biological technology of \( \text{H}_2\text{S} \) in palm oil mill.

Luckily, for odor strength of removal efficiency occur in carrier contains SOB (44.81% - 72.15%) is higher than carrier without SOB (31.84% - 66.89%) within 28 days as shown in figure 2(b). This is due to the high removal of \( \text{H}_2\text{S} \) can change and absorb the odorous ‘rotten egg’ smell and changes into elemental sulfur that is no longer hazardous. Thus, the objective in this study is successfully achieved.

![Figure 1. Effect of contact time (a) and agitation rate (b) by the \textit{Paracoccus sp.} immobilization on RPMS carriers.](image)
4. Conclusions

The present study emphasizes the important to understand the growth requirement of the species *Paracoccus solventivorans* ATCC 700252 to oxidize hydrogen sulfide thus to remove odor pollution by proving the reduction of hydrogen sulfides in environment and produce lower concentration of odor. In this way, hydrogen sulfide removal by the biological process was efficient process under a suitable condition for the growth of SOB. Plus, the efficiency of RPMS media to remove odor also can be utilized for another application.

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