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Identification of Sulphur Oxidizing Bacteria on Charcoal Made of Salak Fruit Seeds for Hydrogen Sulfide Removal in Biogas

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Abstract: Alternative energy sources to substitute fossil fuels have been developed, biogas being one of them. However, H_2S needs to be removed in biogas because it promotes corrosion in the equipment using biogas. The H_2S can be removed from biogas by biological processes. H_2S was removed by biofiltration, in which H_2S degrading bacteria immobilized on the packing material inside a column. This study aimed to determine the genera of microorganisms that degrade H_2S in biogas. Moreover, this research aimed to investigate the ability of these microorganisms to degrade H_2S. The novelty of this research is the use of charcoal from salak fruit seed as a packing material for immobilization the microorganisms in the biofilter; therefore, the packing material is more durable and does not rot. The isolated sludge taken from liquid wastewater treatment in the tofu industry was tested for sulfide degradation. Then, the best of bacteria to degrade sulfide was immobilized on the surface of charcoal made of salak fruit seeds and after acclimatization and the bacteria grew well. We tested their capability of forming biofilms on the surface of the charcoal of salak fruit seeds. Further identification showed that the isolate was *Bacillus cereus* with a similarity value of 98%. An experiment to remove H_2S of biogas using a biofilter column with immobilized *Bacillus cereus* bacteria on the surface of the charcoal of salak fruit seeds showed that the *Bacillus cereus* bacteria could degrade H_2S of biogas that flew in the biofilter through the surface of charcoal of salak fruit seeds. The highest removal efficiency was obtained for H_2S (RE) at the packing height of 80 cm; 97.15% of which was achieved at a biogas flow rate of 30 L/hour, the H_2S concentration was 142.48 ppm for 4 hours.

Keywords: *Bacillus cereus*, biogas, biofilm, removal, H_2S.

鉴别萨拉克果实种子上的硫氧化细菌，以消除沼气中的硫化氢

摘要：替代化石燃料的替代能源已经开发出来，其中之一就是沼气。但是，沼气中硫化氢的含量需要去除，因为它会促进利用沼气的设备中的腐蚀。从沼气中消除硫化氢可以使用生物过程来完成。通用作包装材料。从污泥中分离出的生物膜形成细菌是从将豆腐工业废物转化为沼气的液体废水处理中获取的。为了确定莎蓦果实种子作为包装材料的能力，进行了从污泥到莎蓦果实种子表面的生物膜细菌固定化试验。两天后，观察到蝾螈果实种子表面被细菌覆盖。从从污泥中取出的细菌的固定化结果中，获得了3种具有降解硫化物能力的最佳分离物。然后选择3种最佳分离株，即分离株7，11和12，以重新测试它们在蝾螈果实种子表面形成生物膜的能力。据观察，分离物12是最好的。进一步鉴定表明，分离株12为蜡样芽孢杆菌，相似度值为98%。利用生物过滤器柱去除硫化氢沼气的实验表明，蜡样芽孢杆菌能够降解硫化氢的沼气，这些沼气通过蝾螈果实种子表面在生物过滤器中流动。在填料80厘米处获得的硫化氢（回覆）去除效率最高，为97.15%，在沼气流速为30大号/小时时达到，硫化氢浓度进入142.48 ppm持续4小时。

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关键词：蜡样芽孢杆菌, 沼气, 生物膜, 消, 硫化氢.

1. Introduction

Alternative energy sources to replace petroleum have been developed, one of them is biogas. Biogas is the product of the fermentation of livestock, industrial parks, natural decomposition processes, wastewater treatment processes, agricultural practices, and food treatment [1]. The composition of biogas depends on the source of material; however, the main composition of biogas often consists of methane. According to [2], the composition of biogas consists of (CH_4) (40-75%), carbon dioxide (CO_2) (25-60%), nitrogen (N_2), hydrogen sulfide (H_2S), carbon monoxide (CO), and oxygen (O_2). oxide (CO), and oxygen (O_2).

Biogas applied in cooking, lighting, and fuel processes for biogas transportation has been widely applied to generate electricity [3]. Using biogas as alternative energy will reduce greenhouse gas emissions, as CH_4 has 21 times more greenhouses [4]. However, the presence of hydrogen sulfide (H_2S) in this biogas causes metal corrosion [5].

The removal of H_2S from biogas can be conducted in several ways, namely, physically, chemically and biologically. Biological processes are seen as more efficient, more environmentally friendly and less costly. One method to remove sulfide compounds in biogas by a biological process is to apply biochemical techniques by employing the biofiltration method. Biofiltration technology is divided into three fundamental methods, which include biofilter (BF), bio-scrubber, and bio-trickling filter (BTFs) [6]. This biofiltration provides advantages over other methods, such as low cost and high efficiency. It produces no recent waste, environmentally friendly operations, and is stable over an extended period [7]. BF and BTF have been extensively used to remove H_2S. The BTF method possesses more advantages than the BF method, and also can overcome the limitations of traditional BF. For example, BTF has more stable operation, easy monitoring of physicochemical parameters, unnecessary gas pre-humidification, works with extra excessive H_2S concentrations and possesses higher flow rates [8]. Conventionally, major components of BTF systems include microbial communities, biofilms, and packings. The mechanism of H_2S removal using BTF is quite complex, flowing biogas through biofilms there is contact between the gas phase and the liquid phase, further decomposed by microorganisms (especially sulfur oxidizing bacteria) [4].

The operating principle of biofiltration by microbes is due to their capability of capturing along with digesting sulfide compounds. If the gas containing sulfide compounds flows into the biofilter, the microbes will absorb and degrade the sulfide compounds, and the sulfide content on the gas leaving the biofilter will decrease [4]. The advantage of the biofilter process is because the number of microbes can increase during the operation of removing sulfide compounds, it can happen for microbes will grow and develop by consuming sulfide compounds. Biofiltration refers to the process of treating gaseous pollutants in a medium bed (packing) when pollutants are degraded by microorganisms. While in bio-trickling filtration, there is liquid dropping on the medium bed to produce optimum conditions in the biofilm layer [9]. In a biofilter, pollutants are discharged directly to biofilm, while in a bio-trickling filter, the pollutants are transferred through a liquid that is dropped on top of the filter medium.

In biofiltration process, there is a packing for the growth and development on pollutants degrading bacteria. The efficient operation of a biofilter depends on the selection of the packing material [10]. Based on the media employed, the biofiltration system can be divided into systems with a natural, synthetic, or a combination of both. It has high porosity, good water retention capability, capacity to maintain the microbial community [11]. Some of the natural materials frequently used in the biofiltration process are soil, peat soil, compost, wood chips, coconut skin, poultry feathers, lava rock, gravel, chitosan, biochar, and earthworms [12]. Organic support materials provide physical and chemical stability namely the presence of lignin, hemicellulose. The presence of functional groups like (phenolic hydroxyl, methoxyl, carboxylate) non-toxic [13].

Charcoal made of salak fruit seeds is porous, uneasily damaged, firm organic material with high water absorption and is relatively uniform in size. Charcoal of salak fruit seeds contain chemicals like carbon, nitrogen and phosphorus which are needed by pollutant degrading bacteria. Currently, charcoal of salak fruit seeds is in abundance and widely unused; therefore, the price is still relatively low. Efforts to use charcoal of salak fruit seeds as a biofilter medium have been unmade. One cheap technique to solve the removal of hydrogen sulfide in biogas is by using charcoal of salak fruit seeds as a medium for sulfide degrading bacteria in biofilter.

2. Materials and Methods

2.1. Characterization of the Biofilter Packing

The proposed packing was produced by pyrolysis of salak fruit seeds for 1 hour at 350°C. Then, it was

analyzed to determine the characteristics of the charcoal of salak fruit seeds. We analyzed carbon content (TOC test method), nitrogen content (Kjeldahl analysis), sulfide content (colorimetric test method), and conducted physical analysis of density, porosity, humidity and pH. This stage was carried out to determine the carrying capacity of the packing material used as a medium for developing microorganisms.

2.2. Microorganisms and Mineral Medium Composition

The thiosulfate medium with an appropriate amount is sterilized in autoclave at 121°C for 15 minutes [9]. Furthermore, 5 grams of active sludge, which is a culture source of H_2S oxidizing bacteria, was inoculated in a rotary shaker containing 100 mL of thiosulfate medium, and incubated for 9 days at 30°C, 180 rpm. During the incubation process, the pH was constantly monitored, and the thiosulfate medium solution was added every 24 hours. During the propagation process, the number of bacterial cultures was counted so that it could be seen when the logarithmic phase occurred. To count the number of bacterial cells, total plate count (TPC) was used. The composition of media sulfur consisted of KH_2PO_4 8 g/L, K_2HPO_4 8 g/L, $MgSO_4{\cdot}7H_2O$ 0.5 g/L, (NH_4) $2SO_4$ 0.3 g/L, $CaCl_2$ 0.25, $FeCl_3.6H_2O$ 0.02 g/L, Yeast extract 16 g/L.

2.3. Immobilization of Bacterial Culture on Salak Fruit Seeds

Inoculated bacterial cultures on 40 mL of thiosulfate medium were inoculated on 90 grams of salak fruit seeds in Erlenmeyer flask at 30°C [9]. During the immobilization process, pH changes were monitored and controlled, and the thiosulfate medium was replaced with recent ones every 24 hours for 14 days. To determine the formation of biofilms on the surface of charcoal of salak fruit seeds after immobilization, the researchers analyzed the presence of bacteria on the charcoal surface by counting the number of organic cells attached to it. The number of organic cells can be decided by the plate count method by sprinkling it on an agar medium (Pour plate).

2.4. Isolation of Bacteria

Bacteria were put into a 250-mL Erlenmeyer flask containing 50 mL of enriched media for bacteria and then incubated for 24 hours in an incubator at 30°C. After that, the dilution to 10^{-7} was done. Then, 1 mL of diluted solution (10^{-7} to 10^{-4}) was taken, and inoculated on each medium and finally incubated it at 30°C for 24 hours. The growing bacterial colonies were selected morphologically and addressed to a new medium and purified in a new medium by doing a streak plate. The purified separate colony was then transferred to the medium to slant as a stock culture.

2.5. Characterization of Bacteria

2.5.1. Isolation of Bacterial Genomic DNA

Isolation of bacterial genomic DNA was carried out by growing the bacteria on the medium, shaken at 37°C, for 1 night. Furthermore, 15-mL culture was centrifuged at 3,000 rpm for 15 minutes at 40°C. The supernatant was first removed, then, 750 µl of lysis buffer was added to the pellets (100 mM Tris HCl pH 8,100 mM NaCl, 50 mM EDTA, 2% SDS), then pulverized, and added with 10 mg of Proteinase K. Proteinase-K was incubated at 55°C for 30 minutes and centrifuged at 3,000 rpm; for 15 minutes at 40°C. After that, the supernatant was transferred to a 1.5-mL Eppendorf tube. At the next step, 700 µl of phenol was added, lightly shaken, centrifuged at 12,000 rpm for 10 minutes. The uppermost layer was transferred in the 1.5-mL Eppendorf tube was added with cold ethanol with a ratio of 1: 1 (v, v) slowly mixed, resulting in delicate DNA threads emerged. In addition, DNA delicate threads were taken and washed with 70% of ethanol, centrifuged at 12,000 rpm for 10 minutes. The supernatant was then removed, pellets were dried and added with TE up to a considerable volume of 200 µl.

2.5.2. Amplification of the 16S rRNA Gene

A thermal cycler was used for 16S rRNA gene amplification. The isolated genome (1 µl) was taken and added with 1.25 µl 8F Primer (5'-AGAGGTTGATCCTGGCTCAG-3'), 1492R primer (5'- GTTTACCTTGTTACGACTT-3'), 12.5 µl GoTaq Green Master Mix and 9 µl Nuclease Free Water. Thus, the comprehensive volume (25 µl) was available in the PCR (Polymerase Chain Reaction) tube. The PCR procedure begins with the initial denaturation stage at 94°C appropriate to 5 minutes followed by a further process on 30 cycles consisting of a denaturation process at 94°C for 1 second. The following step is sticking the primer at 55°C for 1 minute and lengthening for 72°C for 1 minute. After 30 cycles, the final process of lengthening at 72°C for 10 minutes was continued and the PCR process stopped at 12°C. The PCR results were then viewed by electrophoresis on 0.8% agarose gel.

2.5.3. 16S rRNA Gene Base Sequence Analysis

The results of the 16S rRNA gene coding amplification for the bacterial isolate were then determined for the DNA base sequence. The process of determining the DNA base sequence was carried out by the 1st BASE Sequencing, Malaysia. The results of determining the DNA base sequence were then read with baser DNA. At that time, the obtained DNA base sequence was used to find comparisons about the DNA sequences of various microorganisms experiencing a familiar relationship in the NCBI (National Center for

Biotechnology Information) gen bank through the BLAST (Basic Local Alignment Search Tool) method (http: blast.ncbi.nlm.nih.gov, Blast.cgi). Matching results using the BLAST method were then selected with the closest kinship and a phylogenetic tree was compiled using the Molecular Evolutionary Genetics Analysis 6 (MEGA 6) program.

2.5.4. Analysis of the Kinship of Bacterial Isolates Based on Base Sequence of 16S rRNA Nitrogen Genes

The genetic relationship on bacteria was recognized through analyzing the nitrogen base sequence 16S rRNA gene. The nitrogen base sequence of isolates obtained from sequencing by the 1st BASE Sequencing was analyzed with Baser DNA program to obtain a nitrogen base sequence that could be compared with nitrogen base sequence strain reference at the Genbank National Center for Biotechnology Information (NCBI) by Basic Local Alignment Search Tool (BLAST). The nitrogen base sequence of isolates and nitrogen base sequence of reference or comparison strains were used as analysis materials to determine relationships in the form of phylogenetic trees. Phylogenetic trees in this analysis were represented using the Molecular Evolutionary Genetics Analysis 6 (MEGA 6) program and the neighbor-joining method. The formed phylogenetic tree was evaluated using the bootstrap method (1,000 replications) to determine the level of robustness and accuracy of the analysis. Bootstrap values were indicated by the numbers found on the branches of the phylogenetic tree. According to [14], the higher the bootstrap value, the more reliable or trustworthy it is.

2.5.5. Biofilter Testing

In this analysis, several parameters were observed to be used to obtain results according to the focus of this study is the concentration of sulfide is in the form of H_2S gas. Experiments were carried out with various biogas flow rates. The biogas concentration follows the output of the digester in the field. Observations were performed after the sulfur oxidation bacteria were immobilized on the charcoal of salak fruit seeds for 6 days and the bacteria acclimatized on the surface of charcoal of salak fruit seeds in the biofilter column for 5 days. Furthermore, on the 6th day after acclimatization, biogas sampling was carried out starting at t = 0 hours, at a certain biogas flow rate and at the axial position of the inlet, which was available 0, 20, 40, 60, and 80 cm. the biogas sample taken was poured into a sample bottle containing Zn-Acetate solution as a H_2S capture solution, then the solution containing H_2S gas is analyzed for its concentration by reacting with $FeCl_3$ and a solution of N, N-Dimethyl-1,4-Phenylen Diammonium Dichloride. The turbidity of the solution was also measured using a Spectrophotometer at a wavelength of 670 nm. The absorbance value was then compared with a standard curve. Count the number of microorganisms in the packing after each process using the Total Plate Count method.

3. Results and Discussion

Isolate was identified and characterized to determine the genus of the superior isolate selected to degrade sulfides. Identification was carried out phenotypically and genotypically. Phenotypically, the bacteria were tested for their morphological, physiological and colonic characteristics. Then, genotypically bacteria were tested based on the profile of an organism's genetic material (mainly DNA).

3.1. Phenotypic Identification of Bacteria

The results of the morphological identification concerning the superior isolate obtained are presented in Figure 1. From the results of microscope observations with a magnification of 1,000 times, with gram staining, gram-positive isolates were colored purple (dark), while the bacterial cells of the isolate were rod-shaped.

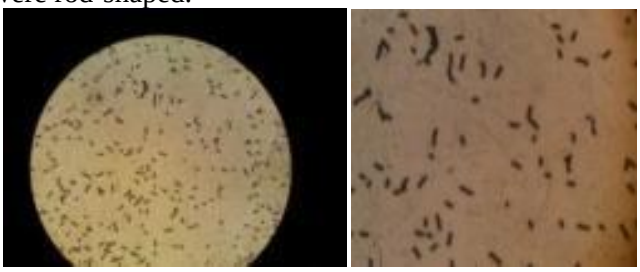

Fig. 1 Morphological identification of isolate

3.2. Genotypic Method of Bacterial Identification

This genotypic method is carried out in two groups, namely, fingerprint or pattern-based techniques also sequences-based techniques or DNA sequences [15]. In this study, rep-PCR technique was used as a proxy for fingerprint analysis along with identification using 16S rRNA and gyrB genes as a representative sequence-based technique.

3.3. Fingerprint Analysis Using rep-PCR

This technique exclusively employs a systematic method of producing a series of fragments from the chromosomal DNA of an organism. These fragments are then separated using size to produce a profile or fingerprint that is unique to the organism and its dearest relatives. Broadly speaking, there are two general approaches with respect to fingerprinting techniques for determining bacterial strains [16]. First is based on RFLP analysis that detects sequence variations by comparing the size and number of restriction fragments produced by cutting DNA by restriction enzymes. Second involves multiple variations on amplicons of unusual sizes which are amplified products with primers. The bands obtained as a result of DNA analyzing isolates 12 are shown in Figure 2.

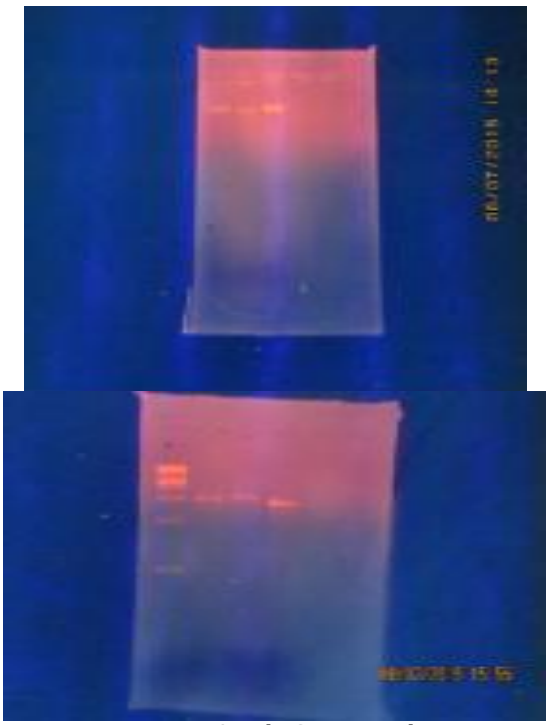

Fig. 2 DNA and PCR test results

3.4. Amplification of 16S rRNA Gene

A thermal cycler was used to perform 16S rRNA gene amplification. The isolated genome was taken in the amount of 1 µl and then it was added with 1.25 µl 8F primer (5'- AGAGGTTGATCCTGGCTCAG-3 '), 1492R primer (5'- GTTTACCTTGTTACGACTT-3'), 12.5 µl GoTaq Green Master Mix and 9 µl nuclease-free water so that the total volume in the PCR (Polymerase Chain Reaction) tube was 25 µl. The PCR procedure began with the initial denaturation stage at 94°C for 5 minutes and was followed by a further process with respect to 30 cycles consisting of a denaturation process at 94°C for 1 second, sticking the primer at 55°C for 1 minute, and lengthening for 72°C for 1 sixty seconds. After 30 cycles, the final process of lengthening at 72°C for 10 minutes was continued and the PCR process was stopped at 12°C. The PCR results were then viewed by electrophoresis on 0.8% agarose gel.

3.5. Analysis of the 6S rRNA Gene Base Sequence

The results of the amplification of the 16S rRNA gene coding to the bacterial isolate were then determined for the DNA base sequence. The process of determining the DNA base sequence was carried out by the 1st BASE Sequencing, Malaysia. The results of the DNA base sequence determination were read with baser DNA. Then, the obtained DNA base sequence was used to provide comparisons of DNA sequences of various microorganisms that were the same or had close kinship in the NCBI (National Center for Biotechnology Information) Genbank through the BLAST (Basic Local Alignment) method. Search Tool (http:blast.ncbi.nlm.nih.gov, Blast.cgi). Matching results operating the BLAST method were then selected with the closest kinship and a phylogenetic tree was compiled using the Molecular Evolutionary Genetics Analysis 6 (MEGA 6) program.

3.6. Analysis of the Kinship of Bacterial Isolates Based on the 16S rRNA Nitrogen Gene Base Sequence

The genetic relationship of bacteria was recognized by analyzing the nitrogen base sequence 16S rRNA gene. The nitrogen base sequence of isolates obtained from sequencing by 1st BASE Sequencing was analyzed applying the Baser DNA program to obtain a nitrogen base sequence that could be compared with the corresponding strain reference at the Genbank National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST). The nitrogen base sequence of isolates and nitrogen base sequence of reference or comparison strains were used as analysis materials to determine the relationship in the form of phylogenetic trees. The phylogenetic tree in this analysis was constructed using the Molecular Evolutionary program.

3.7. Genetics Analysis 6 (MEGA 6) and Neighbor-Joining Method

The formed phylogenetic tree was evaluated using the bootstrap method (1,000 replications) to determine the level of robustness and accuracy of the analysis. Bootstrap values are indicated by the numbers found on the branches of the phylogenetic tree. According to Hall (2013) [14] the higher the bootstrap value, the more reliable or trustworthy it is. The phylogenetic tree results of the analysis are presented in Figure 3, based on the order of the 16S rRNA gene nitrogen base using the MEGA 6 program. Based on the formed phylogenetic tree, isolate 12 had a remarkably familiar relationship with the *Bacillaceae* family, namely *Bacillus cereus*, indicated by a similarity value of 98% and a bootstrap value of 98 who used hydrogen sulfide oxidizing bacteria using *Bacillus cereus*.

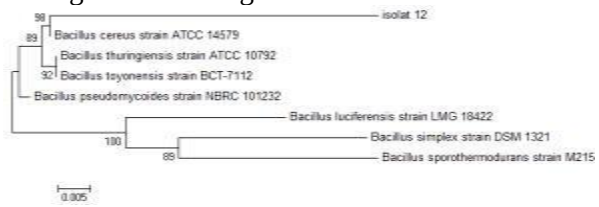

Fig. 3 Isolate phylogenetic tree

Research conducted in [17] obtained consortium bacteria with Sulfurimonas (13.7%) followed by Synechococcus (9.4%), Hydrogenophaga (6.5%), Methanosaeta (2.4%), and Acidithiobacillus (2.1%).

3.8. Sulfide Degradation Test in the Biofilter

According to the experiment on removing H_2S in biogas using a packed bed of salak fruit seeds and

Bacillus cereus bacteria immobilized on the surface of the salak fruit seeds in the biofilter, the experimental data correlating the H_2S concentration and removal efficiency (RE) at various axial positions in the column, flow rates and times used in this research are shown in Figure 4.

Figures 4a-4d show that H_2S concentration of biogas which flowed in the biofilter through the surface of charcoal of salak fruit seeds decreased after leaving the biofilter. It seems that the *Bacillus cereus* microbe are able to degrade H_2S of biogas. Figures 4a-4d also show that all the biogas that flows in the biofilter at various concentrations of H_2S decreases the concentration of H_2S after leaving the biofilter.

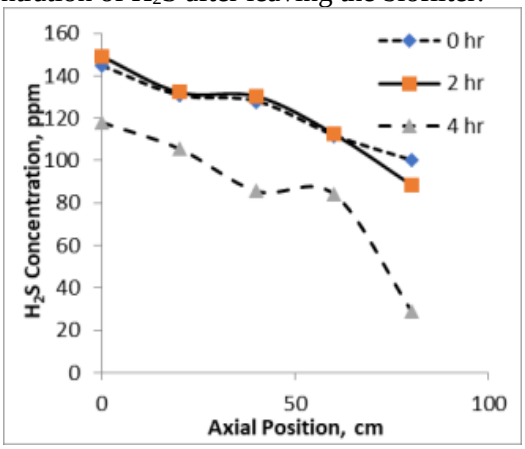

Fig. 4a Decrease in the H_2S concentration of biogas after treating charcoal of salak fruit seeds with *Bacillus cereus* in the biofilter

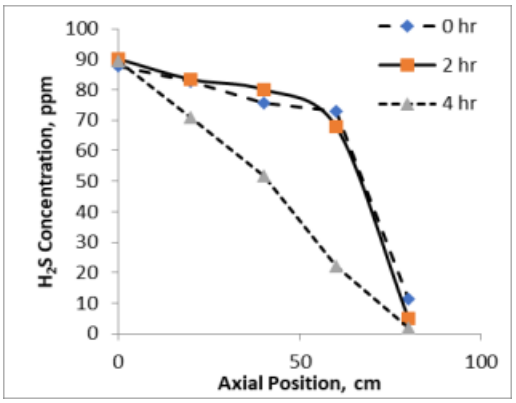

Fig. 4b Decrease in the H_2S concentration of biogas after treating charcoal of salak fruit seeds with *Bacillus cereus* in the biofilter

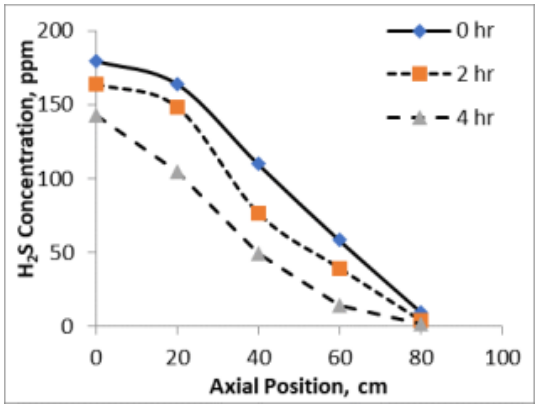

Fig. 4c Decrease in the H_2S concentration of biogas after treating charcoal of salak fruit seeds with *Bacillus cereus* in the biofilter

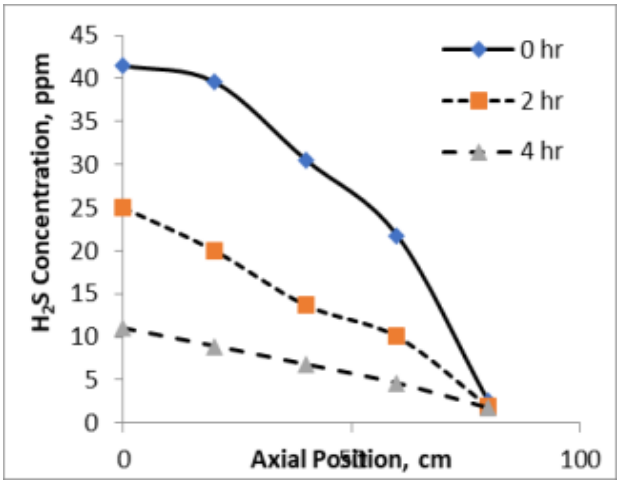

Fig. 4d Decrease in the H_2S concentration of biogas after treating charcoal of salak fruit seeds with *Bacillus cereus* in the biofilter

For the same flow rate and time, H_2S removal efficiency increases with the increasing distance from the column bottom. This happens as a larger distance from the column bottom will provide a longer contact time between the biogas and the biofilm. The farther the axial position from the column inlet, the lower the H_2S concentration at the column outlet and the higher the RE is. This is logical because the farther the axial position, the longer biogas will pass through the biofilm. As a result, contacts between the H_2S and the biofilm are equally longer, which causes the H_2S degradation by the bacteria in the biofilm to also get longer. The increasing length of H_2S degradation process by biofilms causes a larger amount of H_2S degraded by bacteria, in this way the H_2S concentration decreases even more. Figures 4a-4d also show the increasing time effect on the higher the RE. Its causes the biofilm thickness on the surface of charcoal of salak fruit seeds to increase accordingly because of more microbes available. As a result, the H_2S biodegradation process by *Bacillus cereus* bacteria is also better. Therefore, the reduction in H_2S concentration is getting more significant. The longer the H_2S removal time, the greater the efficiency of H_2S removal. This finding is consistent with the results of [18]. That research on H_2S removal using biofilters containing polyethylene and compost showed that good performance eliminating H_2S was obtained in conditions of high inlet concentrations of 43 to 3023 $mg \cdot m^{-3}$ and an EBRT 6 to 13 s at low temperatures from -1 to 10°C in this biofilter. The highest elimination capacity of H_2S obtained was 1214 $g \cdot m^{-3} \cdot h^{-1}$ at the EBRT of 6 s and 556 $g \cdot m^{-3} \cdot h^{-1}$ at the EBRT of 13 seconds, respectively. When studying H_2S removal using compost material with microbial consortiums in biofilter after the addition of biochar to packing materials, under temporary state operations, the biofilter showed a rapid response to shock loads, and restored its performance completely when normal load was restored [19]. However, RE compost and biochar biofilters dropped

from >99 to ~70% when EBRT was changed from 119 to 80s. Biofilters containing sulfide-oxidizing bacteria (SOB) from sludge can reduce the hydrogen sulfide concentration from 0.33% to 0.16% within 5 days, with a removal efficiency of 83% for hydrogen sulfide treatment [20]. *Acidithiobacillus caldus* species can remove H_2S in biogas (33.9%-49.9%) [17]. A conclusion was drawn that charcoal of salak fruit seeds can be used as packing for a biofilter to remove sulfide in the gas phase. It turned out that its operating performance was equivalent to that of the other packing materials based on the literature review results. In the biofilter column with a packing height of 80 cm and a column diameter of 8 cm, the highest RE was acquired at 97.15%.

4. Conclusions

The use of charcoal of salak fruit seeds as packing in biofilters has never been studied. The charcoal of salak fruit seeds as immobilization medium for bacteria in the biofilter has good prospects. The bacteria were able to stick and grow well on the surface of charcoal of salak fruit seeds. The use of salak seed charcoal as packing in the biofilter was an advantage: for a long time, the materials were not rotten, unlike the fresh salak fruit seeds. The charcoal of salak fruit seeds used as packing biofilter yields better results than the use of other packing materials such as polyethylene and compost. The usage of isolate from a consortium of bacteria obtained from sludge taken from tofu industrial waste treatment was a novelty in the process of removing H2S from biogas. Based on the formed phylogenetic tree, isolate had a remarkably familiar relationship with the *Bacillaceae* family, namely *Bacillus cereus*, indicated by a similarity value of 98%. The experiment for removing H_2S contained in biogas through a biofilter column with *Bacillus cereus* bacteria on the surface of the charcoal made of salak fruit seeds demonstrated that its operating performance was equivalent to other packing materials based on the literature review results. In the biofilter column with a packing height of 80 cm and a column diameter of 8 cm, the highest RE was acquired at 97.15%.

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