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Biopolishing of Domestic Wastewater Using Polyvinyl Alcohol – Supported Biofilm of Bacterial Strain *Bacillus velezensis* Isolate JB7

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ABSTRACT

Water pollution occurs due to the discharge of domestic waste mixed with residential, industrial, commercial, and agricultural wastewater. Conventional water treatment methods using aerobic/anaerobic methods can cause problems with the production of high green gases and result in the greenhouse effect. Microbial-based domestic sewage treatment technology using polyvinyl alcohol biofilm supporting media was introduced as an alternative measure to overcome this problem. The objective of the study was to determine the performance of polyvinyl alcohol beads in polishing domestic wastewater. In this study, the bacterium *Bacillus velezensis* isolate JB7 was used together with PVA as a raw material to treat domestic sewage wastewater more efficiently and stably. The results of the study show the effectiveness of domestic wastewater treatment in several factors such as pH value, chemical oxygen demand, phosphorus, nitrate, nitrite, ammonia, and total suspended solids. As conclusion, domestic wastewater treatment methods using polyvinyl alcohol beads are seen to be effective, reducing the use of sewage waste plant construction sites and able to avoid the use of non-recyclable materials such as plastics and synthetics.

Keywords: biofilm process; sewage effluent; PVA carrier; water recycling.

INTRODUCTION

Nowadays, human-caused environmental pollution is increasingly a concern. This is because the problem of pollution that occurs will affect the daily lives of all living beings in this world. Due to the increasingly alarming rate of pollution, the government has responded to pressure from nearby communities and environmental organizations by enforcing stricter laws (Abdel-Raouf et al., 2012). Malaysia's increasing urbanization is the result of the country's increasing population day by day. Pollution due to urbanization in Malaysia is a serious problem that needs to be addressed. Environmental pollution has negative effects on water, soil, and air. Solutions to this problem and corrective actions are increasingly being considered. Water pollution is largely due to waste disposal in metropolitan areas. Water that has been contaminated by human daily consumption is known as domestic wastewater. It is a mixture of water that has been used in the residential, industrial, commercial, or agricultural sectors. Organic matter, nitrogen, phosphorus and other uncooled chemicals such as heavy metals and foreign matter are the most common contaminants found in domestic wastewater (Yaakob, 2014).

Domestic wastewater is the most polluting source of natural water bodies in Malaysia. According to reports, COD, BOD, and ammoniacal-nitrogen were among the pollutants that polluted more than 25% of Malaysia's river basin

(Al-Ajalin et al., 2020). Due to the potential eutrophic effects and other dangerous impacts on receiving aquatic bodies, the presence of nutrients (nitrogen and phosphorus) in domestic wastewater has drawn a lot of attention (Zulkifli et al., 2022; Zainuddin et al., 2022). As a result, nutrient removal from wastewater has recently become a critical concern for wastewater treatment facilities. When compared to other approaches, the biological treatment process for nutrient removal is widely used due to its ease of operation, higher efficiency, lower maintenance, cost benefit, and so on (Qu et al., 2015).

Single unit simultaneous nitrification and denitrification (SND) processes, in which nitrification and denitrification occur concurrently in a single unit under identical operating conditions, have been used as promising nitrogen removal technologies in recent years. This single stage nitrogen removal process was introduced only after the pioneering discovery of *Paracoccus denitrificans*' aerobic denitrification ability, that is, the microbe's ability to respire oxygen and nitrate simultaneously (Robertson & Kuenen 1984; Rout et al., 2017). Wehrfritz et al., (1993), using the same microbe, proposed the heterotrophic nitrification and aerobic denitrification (HNAD) model. According to the model, the microbe, *Paracoccus denitrificans*, can perform nitrification using organic carbon sources, which is known as heterotrophic nitrification, and can also perform denitrification under aerobic conditions, which is known as aerobic denitrifying microbe. Until then, varieties of heterotrophic nitrifying and aerobic denitrifying novel microbes have been reported, including *Pseudomonas* sp. (Li et al., 2015), *Rhodococcus* sp. (Chen et al., 2012), *Agrobacterium* sp. (Chen & Ni 2012), *Acinetobacter* sp. (Ren et al., 2014), *Bacillus* strains (Hasan et al., 2012; Alias et al., 2022), *Klebsiella pneumoniae* strain, *Marinobacter* sp. (Zheng et al., 2012) and *Aeromonas* sp. (Chen et al., 2014). These denitrifiers could be used to remove nitrogen in a single reactor under aerobic conditions. However, most of the research on these denitrifiers has been done in shake flasks with pure strains.

Keeping functioning bacteria in wastewater treatment systems is easy using biofilms, in which bacteria are immobilised. Due to its remarkable flexibility, high rate of organic matter and nitrogen removal, ease of operation and maintenance, and low generation of extra sludge, biofilm reactors are increasingly in demand for use in the modernization of wastewater treatment facilities around the world (Hong et al., 2020). Strongly biofilm-forming bacteria co-aggregate with other microbes to create biofilms (Rickard et al., 2004). A highly effective bioaugmentation technique for pollutant removal in wastewater treatment involves inoculating certain contaminant-degrading and biofilm-forming bacteria into biofilm reactors (Li et al., 2013). However, there are certain real-world issues with wastewater treatment. For instance, the contaminant-degrading bacteria may not be efficiently immobilised in biofilms and may therefore be easily washed out of the system, while the bacteria that create biofilms may not successfully remove pollutants. Additionally, simply mixing biofilm forming bacteria with degrading bacteria may result in mutual growth inhibition, slow biofilm development, difficult operation, and other problems (Li et al., 2016). Both the fixation of functional bacteria in the wastewater treatment system and high-efficiency pollutant removal can be accomplished by the presence of a single bacterial species with the capacity to effectively form biofilms and breakdown pollutants simultaneously. It is necessary to discover these microorganisms and test their usefulness in the treatment of wastewater.

Different sizes and shapes of biocarriers, most of which are constructed of polyethylene (PE), high-density polyethylene (HDPE), or polypropylene (PP), are frequently employed in biofilm-based treatment (Mao et al., 2017; Hasan et al. 2020). Gel beads made of polyvinyl alcohol (PVA) are another material that has lately been utilised as biocarriers in biofilm-based treatment. PVA gel beads are an intriguing possibility for use in biofilm-based treatment since they are synthetic biocarriers with a high specific surface area (1,000 – 2,500 m^2/m^3) that allows the culture a of large number of microorganisms (Rajpal et al., 2021). Additionally, PVA gel beads can be easily suspended in water due to their specific gravity being near to that of water (Hao et al., 2006). Additionally, PVA gel is dependable for use in biofilm-based treatment since it is not biodegradable and is not soluble in water. PVA gel beads are a promising media for use in biofilm-based treatment because of these benefits.

Therefore, this study focuses on the bio-purification of domestic wastewater using biofilm process as one of the alternative measures to treat wastewater treatment. The biofilm process is a developed technology, in which PVA as biocarrier

is added to a suspended growth reactor to prepare a surface for the biofilm. This is to increase the concentration of microbes as well as the rate of biofilm decomposition of contaminants so that several removal mechanisms can be carried out including biodegradation, bioaccumulation, biosorption, and biomineralization. In this study, a bacterial strain was previously isolated and identified as *Bacillus velezensis*. Research on *Bacillus velezensis* from the past mostly focuses on the treatment of brewery wastewater (Agunbiade et al., 2022), slaughter wastewater (Deng et al., 2022), and pulp and paper wastewater (Verma et al., 2020). However, research on *Bacillus velezensis* as a bacterial biofilm for treating domestic wastewater is scarce.

MATERIALS AND METHOD

Domestic wastewater sampling

Sewage wastewater sampling was carried out at Keris Mas College sewage area, Universiti Kebangsaan Malaysia coordinate 2°55'48"N 101°47'18"E. Wastewater sampling was done after it goes through the main treatment process. This test is estimated to require 40 liters of domestic sewage in total. Sewage samples will be stored in 20-liter bins and stored at room temperature, which is at an approximate temperature of 27.1 to 27.9 °C.

Growth medium

Mineral salt medium is the medium that is seen as the most suitable and contains a high nutritional composition as the germination medium of *Bacillus velezensis* isolate JB7 bacteria. Mineral salt medium is one of the media often chosen for the preparation of media for bacterial germination performed in the laboratory (Macwilliams & Liao 2006). Mineral salt medium (MSM) according to Zajic and Supplisson (Zajic & Supplisson 1972) was prepared by dissolving 1.8 g K_2HPO_4, 4.0 g NH_4CI, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g NaCl, 0.01 g $FeSO_4 \cdot 7H_2O$ in 1 L of distilled water. Bacteriological agar was added (15 $g \cdot L^{-1}$) into the solution where a solid basal medium was required. Next, the pH value should be adjusted to 6.90 and then the medium is autoclaved at 121 °C for 15 min. 1.0% (v/v) Refined crude oil needs to be added as a single carbon source. Finally, add vitamin solution (1.0 mL L^{-1}) into the medium to enrich the nutrient content (Mukred et al., 2008).

Preparation of inoculum

The stock preparation for *Bacillus velezensis* isolate JB7 bacteria was similar to the inoculum preparation for *Escherichia coli* bacteria. In a conical flask, bacteria (*Bacillus velezensis* isolate JB7) will be cultured into the mineral salt medium that has been produced. The preparation of the inoculum takes a total of 15 liters. As a result, 1.5 L of bacterial stock will be placed in a conical flask, with the remaining 13.5 L of mineral salt medium.

PVA preparation method

According to Sun et al., (2020) and slightly processed through readings and laboratory capacity at Universiti Kebangsaan Malaysia PVA solution was prepared by completely dissolving 11.5 g of polyvinyl alcohol polymer powder in 100 mL of deionized water and stirred for 1 hour under 85 ℃ to form PVA solution homogeneous by using a medium-sized magnetic stirrer. The homogeneous PVA solution was left for a while at room temperature. 1.5 g of NaOH powder was dissolved in 30 mL of deionized air. A 5% NaOH solution was added to a homogeneous PVA solution to produce an alkaline PVA solution. The ultrasonic process is performed on an alkaline PVA solution to remove foam. The alkaline PVA solution was added droplets into a cross-linking agent (saturated boric acid solution containing 1% $CaCl_2$) using several tools with different mouth openings such as burettes, distilled water bottles and syringes. The tools used having 3 different aperture sizes were selected to be the manipulated variables in this study. The spherical beads that have been formed need to be magnetically stirred for 1 hour to ensure that the spherical beads harden perfectly. The gel beads were carefully removed and washed with deionized air until the pH had become neutral (Sun et al., 2020). PVA spherical beads then need to be stored in tap water at room temperature so that the condition does not change.

Preparation of PVA and bacteria

For the preparation of the mixture between PVA and bacteria conical flasks are still used as

a mixing place. A conical flask containing a medium of mineral salts and bacteria will contain PVA beads of 10% of the contents of the water treatment container to be used. The conical flask containing the mineral salt medium, bacteria and PVA produced will be left for 7 days to ensure that the PVA produced has been mixed with bacteria to produce a perfect biofilm.

Bacterial based domestic wastewater treatment

PVA that has been mixed with bacteria or better known as biofilm will be removed from the mineral salt medium and prepared for the process of domestic wastewater treatment. A total of nine 1200 mL containers were used for testing domestic wastewater treatment in this experiment. About 640 mL of domestic wastewater obtained from the sewage treatment plant of Keris Mas College, Universiti Kebangsaan Malaysia will be put into each beaker accurately. For the beginning of the experiment, small-sized PVA beads that have been mixed with bacteria will be placed in the first 3 beakers of 10% of the working volume. Next, the other containers will be filled with PVA mixed with bacteria with medium and large PVA bead sizes. Each size of PVA bead will be put in 3 beakers filled with water because to avoid possible errors. Next, the PVA-filled container was stirred using flocculator for 24 hours a day throughout the experiments. Readings for parameters such as chemical oxygen demand (COD), phosphorus, nitrate, nitrite, total suspended solids (TSS) and pH values will be analyzed in some time such as hours 0, 2, 4, 24, 48, 120, 144 and 216.

Fourier transform infrared spectroscopy (FTIR)

Samples of PVA beads of the three sizes measuring 0.5 cm, 0.4 cm and 0.3 cm were put into sample plastic and labeled before being sent to the Integrated Analysis Laboratory, Department of Chemical and Process Engineering, Universiti Kebangsaan Malaysia to analyze PVA using an FTIR spectrophotometer. A total of 6 samples of PVA beads sent consist of 3 PVA beads of different sizes that have not yet been used and 3 PVA beads of different sizes that have been used in domestic wastewater treatment.

Biomass measurement

To evaluate the development of bacteria (Bacillus *velezensis* isolate JB7) in the beaker under the stirrer, biomass measurements were taken. Measurements were taken every 0, 2, 4, 24, 48, 120, 144 and 216 hours during the bio treatment of sewage wastewater effluent using microorganisms (Bacillus *velezensis* isolate JB7). The biomass calculation method was explained by Khalid et al. (2016). A 15 mL domestic wastewater effluent sample was collected and deposited in a centrifuge test tube. Then, for 10 minutes, spin the sample at 8000 rpm to separate the biomass from the supernatant with a centrifuge. Afterwards, the supernatant from the biomass pallet was separated and tested for pH, COD, and Total Suspended Solids (TSS) values. The supernatant was separated from the biomass, and the biomass pallet was then rinsed with distilled water for 10 minutes before filtering the distilled water and biomass with filter paper. The filter paper should first be dried in an oven at 105 °C for four hours. By weighing the original blank filter paper, weigh the dry reading of the filter paper using the gravimetric method. Dry the biomass overnight with filter paper at 105 °C, then weigh the biomass with the filter paper.

Analysis of wastewater

For the bio-purification treatment of domestic sewage using the bacteria *Bacillus velezensis* isolate JB7, COD and TSS levels were tested every 0, 2, 4, 24, 48, 120, 144 and 216 hours. The concentrations of COD was analysed according to the reactor digestion method (Method: 8000), while measurement was determined using a HACH DR 3900 spectrophotometer (Hach, Loveland, CO, USA). Throughout this study, the pH was monitored using a pH meter (Metrohm 827 pH Lab, USA). TSS was determined according to Standard Methods (APHA 2012).

RESULTS AND DISCUSSION

FTIR analysis of PVA

The first microscopic characteristic performed on polyvinyl alcohol beads (PVA beads) was FTIR analysis. FTIR absorption analysis was performed to determine the functional clusters found in the three types of PVA bead samples

that had not been used during the domestic wastewater treatment. The IR transmission spectra of samples of 3 different size types that have not yet been used are shown in Figure 1.

Figure 1a shows the FTIR analysis of large size PVA beads. To understand the appearance of peaks in the above FTIR, a step -by -step process can be used. The result that can be inferred is about the number of peaks. Based on Figure 1a, there are more than five peaks identified in the FTIR results of large -sized PVA beads. This thus indicates that the chemicals analyzed are not simple chemicals. The peak in Figure 1a contains a single bond area (3000–4000 cm^{-1}). No broad absorption bands were found, informing no hydrogen bonds in the material. There are sharp bonds at about 1400 cm^{-1} indicating the existence of oxygen bonds. No peaks were found between 2000–2500 cm^{-1} indicating no aromatic structure on the bond. Based on the interpretation of the above results, several conclusions can be drawn. Among them is that the material analyzed has no hydrate component. There are no double or triple bonds in the material.

Figure 1b shows the analysis of the FTIR results of medium -sized PVA beads. The results show that many peak numbers are detected, informing the complex structural material. In a single bond area (500–1800 cm^{-1}), several peaks were detected. There is a sharp bond at about 1400 cm^{-1} indicating there is an oxygen bond. Figure 1c shows the FTIR results of small -sized PVA beads. To analyze the results in the above FTIR, a step -by -step process can be used. The results that can be inferred are related to the number of peaks. According to Figure 1c, it can be seen that there are more than five identifiable peaks in the FTIR results of small -sized alcohol PVA beads. This shows that the chemicals analyzed are not easy chemicals. There are sharp bonds at about 1400

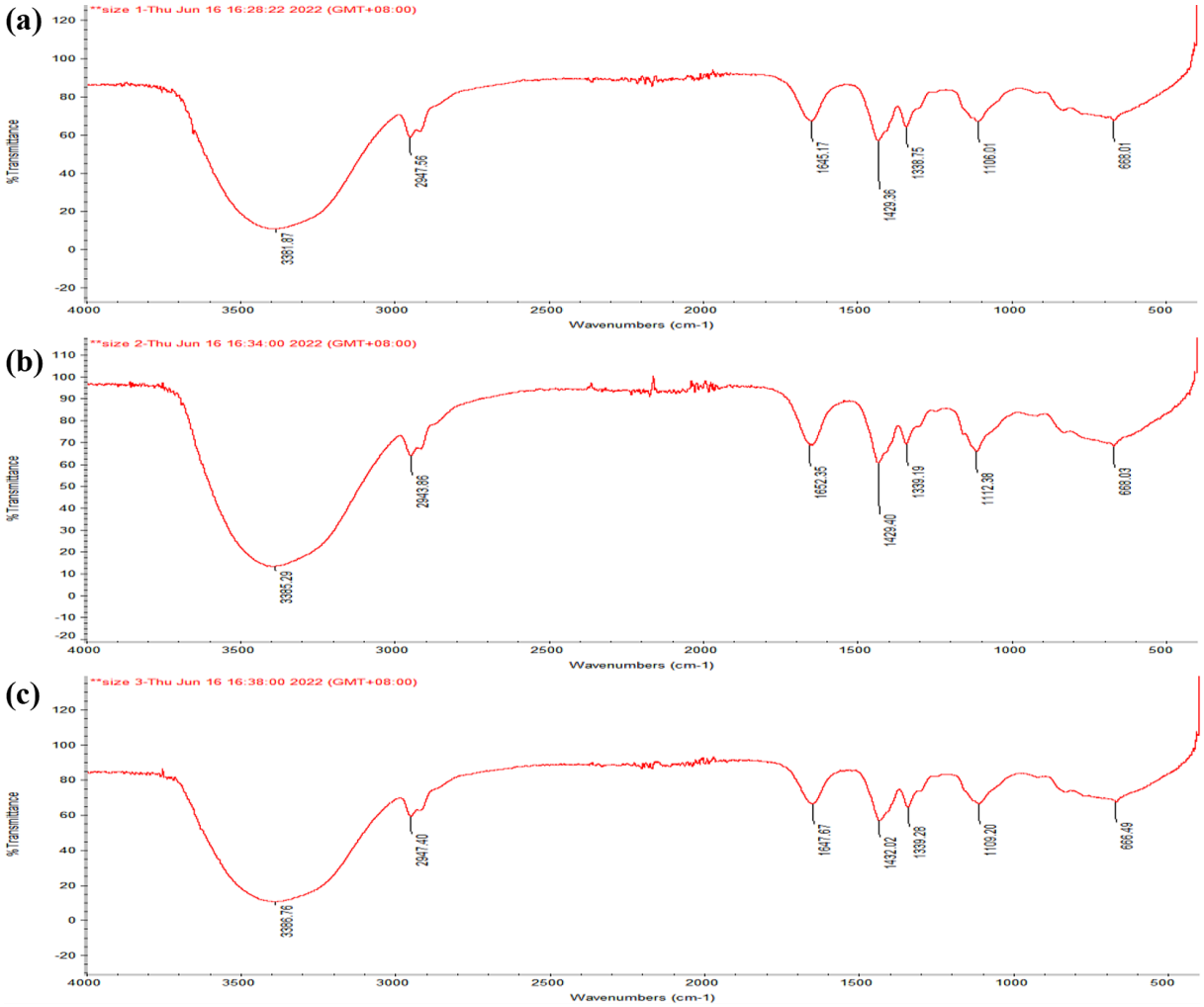

Figure 1. FTIR analysis of (a) large, (b) medium, and (c) small size PVA beads before the treatment process

cm^{-1} indicating the existence of oxygen bonds. No peaks were found between 2000–2500 cm^{-1} indicating no aromatic structure on the bond. Based on the interpretation of the above results, several conclusions can be drawn. Among them is that the material analyzed has no hydrate component. There are no double or triple bonds in the material.

Figure 2 shows the FTIR analysis of PVA beads after the treatment process. The result that can be obtained based on Figure 2a is that there are five peaks that can be identified in the figure. This thus indicates the presence of functional clusters found in large -sized PVA beads. There are wide absorption bands found that indicate the existence of hydrogen bonds in the material. No sharp bonds could be seen indicating no existence of oxygen bonds. No peaks could be identified at approximately 2500–2800 cm^{-1} indicating no aromatic structure on the bond. Based on the results of the study for large -sized PVA beads, no double or triple bonds could be detected while the functional clusters identified for this sample did not show any overlapping.

Figure 2(b) shows the results after FTIR for medium-sized PVA beads. Based on Figure 3b, only three peaks can be identified in the figure. This thus indicates the lack of presence of functional clusters in medium-sized PVA beads. Moreover, no sharp peaks can be seen as a result of the absence of oxygen bonds. No peaks were identified at about 1000 cm^{-1} indicating no aromatic structure on the bond. Figure 2(c) shows the analysis of the results after FTIR for small -sized PVA beads. The finding that can be obtained for Figure 2c is that there are more than five peaks that can be identified in the figure. This proves the presence of functional clusters on small-sized PVA beads. Next, there is a wide absorption band found at about 3400 cm^{-1} indicating the presence of hydrogen bonds in the PVA bead material. No sharp bonds can be identified in the figure while the occurrence of overlapping also does not occur.

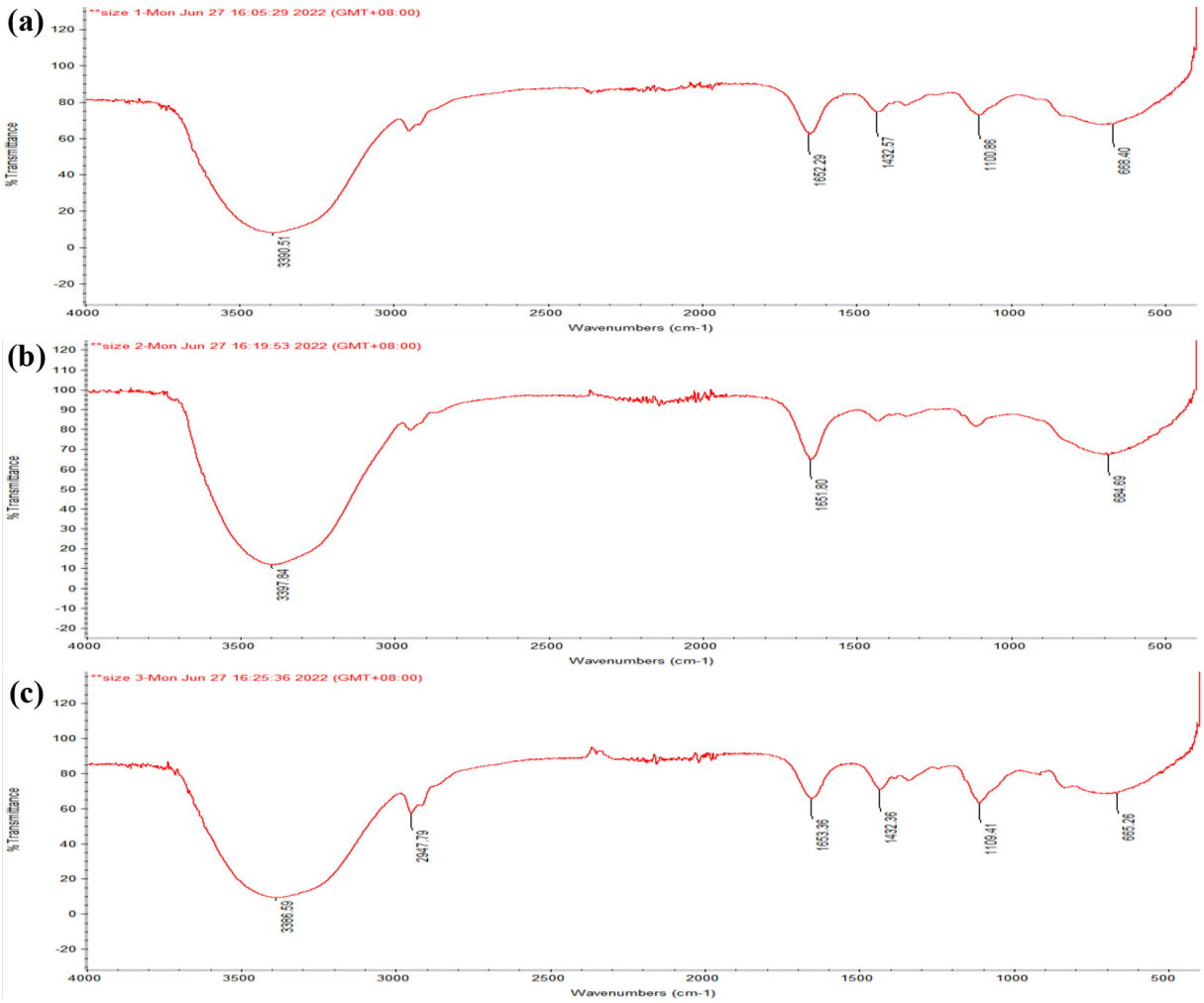

Figure 2. FTIR analysis of (a) large, (b) medium, (c) small size PVA beads after the treatment process

No peaks were found between 2000–2500 cm^{-1} indicating no aromatic structure on the bond. Based on the interpretation of the above results, several conclusions can be drawn. Among them is that the material analyzed has no hydrate component. There are no double or triple bonds in the material.

Domestic wastewater treatment efficiency

COD removal

Figure 3 shows a trend of COD value against time made based on data taken from the analysis of treated water samples based on COD parameters at the corresponding times of 0, 2, 4, 24, 48, 120, 144 and 216 hours. Based on the figure, large-sized PVA beads recorded a COD value of 24 mg/L at hour 0 and recorded the highest COD value within 24 hours of 32 mg/L. After the 216 hours treatment, the COD removal efficiency was 25.9%. Medium-sized PVA beads started at a COD of 25.3 mg/L and ended with 17.5 mg/L after 216 hours with removal efficiency of 30.8%. Small-sized PVA beads recorded a COD of 24 mg/L at 0 hours and recorded a minimum COD value of 16.90 mg/L after 216 hours. The small-sized PVA showed the highest removal efficiency due to the large surface area for biomass attachment and contact area with the pollutants in the wastewater compared to the medium and large-sized PVA beads. The COD indicates the amount of oxidant that essentially reacts with a sample under certain conditions. COD values are used to measure pollutants in wastewater and natural water.

According to Jain & Singh (2003), COD concentrations in ice sources usually range from 20 mg/L or less in unpolluted waters to more than 200 mg/L in waters receiving effluent. Industrial wastewater typically has COD ranging from 100 mg/L to 60 000 mg/L. Based on a study by Herlina et al. (2019), the COD value in domestic wastewater (laundry) is 671 mg/L. In this study, aerobic and anaerobic bacteria were used to treat domestic wastewater. After 10 days of treatment, the COD value was 600 mg/L. The concentration of COD in both studies showed the same trend, that is, in the first hour or day, the COD concentration increased because the bacteria were still exposed and did not result in a shock load. As a result, bacteria have not been able to degrade organic matter. Bacteria also cause concentration of the effluent leading to high COD values in the shock with the addition of food. The trend of decreasing COD value started after 24 hours, and the COD value decreased until 216 hours (Herlina et al. (2019).

Total suspended solid removal

Figure 4 shows a figure of the value of total suspended solids (TSS) against time made based on data taken from the analysis of treated water samples based on cod parameters at time equivalent of 0, 2, 4, 24, 48, 120, 144 and 216 hours. Based on the figure, the large-sized PVA beads started with a maximum value of 7.50 mg/L TSS at hour 0. Next, the amount of suspended solids decreased until after 216 hours, the amount of suspended solids recorded by the large-sized polyvinyl alcohol beads was 6.41 mg/L with removal efficiency of 14.5%. For medium-sized polyvinyl alcohol beads, the total value of suspended solids recorded at the beginning of hour 0 was as much as 7.35 mg/L and experienced a decrease until it reached a minimum value of 6.37 mg/L after 216 hours with removal efficiency of 13.3%. Finally, the small-sized polyvinyl alcohol beads recorded the maximum value at hour 0, which was 7.25 mg/L. Next, the amount of suspended solids decreased over time and finally

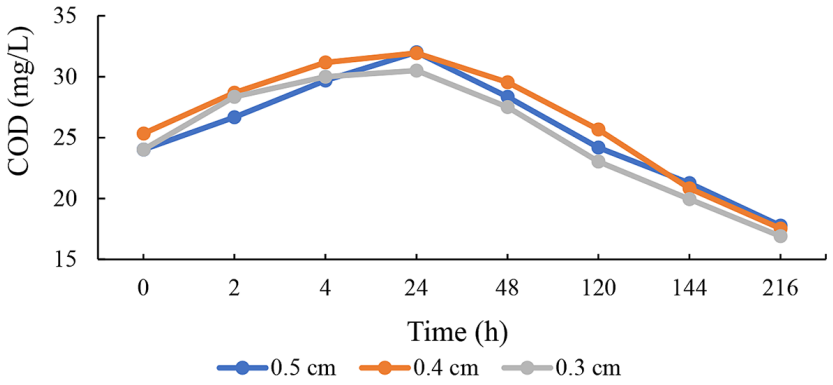

Figure 3. COD reduction trends

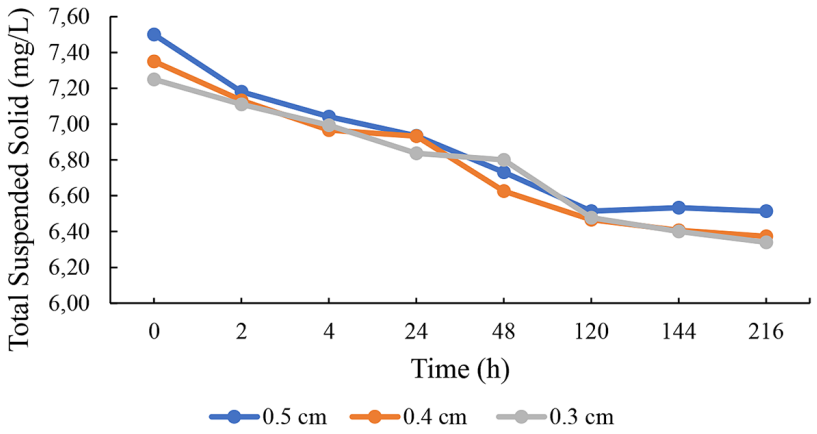

Figure 4. Total suspended solids reduction trends

recorded a value of 6.34 mg/L after 216 hours with removal efficiency of 12.5%. According to He et al., (2019), the ratio of inorganic content of suspended solids in water during rainy days can reach as high as 60% in drainage systems combined with sand in bed sediments originating from the waterbed. Based on the study of He et al. (2019), the amount of suspended solids reached 1400 mg/L at the maximum value and experienced a significant decrease of up to 60% to 500 mg/L. The findings of the study conducted by the researchers also experienced a significant decrease in the amount of suspended solids, from a value of 7.50 mg/L at 0 hours to 6.40 mg/L after 216 hours. This shows the success of the treatment method used (He et al., 2019).

CONCLUSIONS

In this study, the use of 3 sizes of polyvinyl alcohol beads (PVA beads) with a new type of bacteria, *Bacillus velezensis* isolate JB7 was studied to see the effectiveness of the use of polyvinyl alcohol of various sizes and the effectiveness of using this technology in wastewater treatment to curb water pollution problems that often occur. in Malaysia. To produce the best PVA beads, PVA bead manufacturing was repeated several times by changing various types of manipulated variables such as the dosage for each material, the most suitable heat level for a material, the height of the titration process performed, the time taken for the forming process. It was found that PVA powder should be stirred at 85 °C for approximately one hour and during the titration process, boric acid or cross -linking agent should be maintained at 60 °C and stirred continuously throughout the titration process at 550 rpm. For the height of the weaving process, the most ideal height is at a height of 8 cm in order to avoid the formation of bubbles and tails on the surface of the PVA beads. In addition, to produce PVA in different sizes, tools with openings of various sizes should be used such as burettes, distilled water bottles and syringes. To determine the effectiveness and optimization of domestic wastewater treatment using biofilm technology, water samples were taken in a certain time interval (0–216 h) and analyzed using the parameters of total suspended solids (TSS), phosphorus, nitrate, nitrite, chemical oxygen demand (COD) and pH value. Further research about the tools used for the production of PVA beads, the use of several types of bacteria and the wastewater treatment time be increased to a longer period for better treatment performance need to be done.

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