

ISOLATION OF BACTERIAL STRAIN FOR BIODEGRADATION OF FATS, OIL AND GREASE

(Pemencilan Strain Bakteria untuk Biodegradasi Lemak, Minyak dan Gris)

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Abstract

Fat, oil and grease (FOG) deposition is one of the major problems that harm the environment and cause dissatisfaction for human. Uncontrolled and un-pre-treated FOG removal from the kitchen could lead to its accumulation in the piping system. Problems include the interference of fat with the aerobic microorganisms that are responsible in treating the wastewater by reducing oxygen transfer rates and for anaerobic microorganisms; their efficiency could also be reduced due to the reduction of the transport of soluble substrates to the bacterial biomass. Biodegradation could be one of the effective means to treat FOG. The main objective of this study is to isolate bacterial strains from the FOG waste and identify the strains that are capable in biodegrading FOG waste. FOG sample was collected from a sewer manhole. Enrichment technique was applied, followed by isolation of bacterial strains to determine which strain is able to degrade the FOG deposition. Some morphology for the bacterial strain was done to determine its characteristics.

Keywords: fat, oil, grease, bacterial strain

Abstrak

Pemendapan lemak, minyak dan gris (FOG) adalah salah satu masalah utama yang membahayakan alam sekitar dan menyebabkan kekusaran dalam masyarakat. Penyingkiran FOG yang tidak terkawal dan tidak terawat daripada dapur boleh membawa kepada pengumpulan dalam sistem saluran paip. Masalah termasuk gangguan lemak dengan mikroorganisma aerobik yang bertanggungjawab dalam merawat air dengan mengurangkan kadar pemindahan oksigen dan anaerobik mikroorganisma; kecekapan mereka dapat juga dikurangkan akibat daripada pengurangan pengangkutan larut substrat untuk biojisim bakteria. Biodegradasi boleh menjadi salah satu cara yang berkesan untuk merawat FOG. Objektif utama kajian ini adalah untuk mengasingkan strain bakteria daripada bahan buangan FOG dan mengenal pasti strain yang mampu melakukan biodegradasi sisa FOG. FOG sampel diambil dari tempat sisa pembentungan. Teknik pengkayaan adalah digunakan, diikuti dengan pengasingan strain bakteria untuk menentukan strain yang dapat mengurai pemendapan FOG. Morfologi beberapa untuk strain bakteria telah dilakukan untuk menentukan ciri-ciri.

Kata kunci: lemak, minyak, gris, strain bakteria

Introduction

FOG is a type of effluent produced by many types of industries which consist of mixtures of various fats, oils, waxes and other constituents that are related to fat. This effluent has been discharged into water bodies and oily sludge discharge into the environment indiscriminately, untreated or in conditions that does not comply with the standard discharge limits [1]. They have also been linked with high toxicity to the aquatic environments and other ecological damages to the water bodies [2]. The normal source of FOG in the sewage system is animal fat, vegetable fat, and oils used to cook and prepare food. While it is the industries that produce the majority of FOG effluent, the amount of FOG effluent added to the sewage system by individual households is also high. In a study conducted by [3] it is reported that kitchen grey water is the highest contributor of oil and grease in domestic grey water, though oil and grease is present in all grey water streams. Wastewaters containing FOG, derived from

vegetable oils, with a significant amount of linoleic acid have been reported by [4], to be difficult to treat due to the presence and concentration of mixtures of linoleic, palmitic and myristic acids inside the effluent.

In countries where food habits result in a large amount of residual FOG in wastewater, it has become increasingly difficult to fulfil the discard requirements [5]. Uncontrolled and un-pretreated FOG removal from the kitchen could lead to its accumulation in the piping system. Based on a study done by [6], the fatty compound inside the municipal sewage system alone contributes around 30 – 40 % of the total COD of the effluent. FOG waste at first is discharged in its liquid form and when it reaches the pipe in the sewer system, its temperature will start to cool down and later it will solidify and cause blockage in the pipe. This will not only create clogging problems for the effluent flow inside it, but also cause unpleasant odour problems to the surrounding [7]. If this problem is left untreated and not maintained properly, the pipe will be constricted and this results in the reduction of the sewage pipe capacity requiring more frequent pipe cleaning and more frequent pipe replacement. And the pipe constriction also causes sewage backups and overflows, which in conclusion will all be lead to a higher cost of operation and maintenance. In terms of biological ecology, as stated by [8], problems include the interference of fat with the aerobic microorganisms that are responsible in treating the wastewater by reducing oxygen transfer rates and for anaerobic microorganisms, their efficiency could also be reduced due to the reduction of the transport of soluble substrates to the bacterial biomass [9].

Biological degradation or biodegradation in short, is a process by which organic substances are broken down by the enzymes produced by living organisms. Biological treatment is used for environmental remediation (known as bioremediation) purposes as an alternative to other type of treatments such as mechanical and chemical treatments. Usually biodegradation process involves the use of enzyme produced by a microorganism on its target compound and transforming it chemically into other compounds which is less or not harmful [10]. Biodegradation of oily effluent using various types of microorganisms such as bacteria, molds, and yeasts, which have demonstrated effective degradability of oil-wastewater, has attracted attention in recent time [11-14].

Biodegradation of FOG begins with the breakdown of the complex molecule by extracellular enzymes produced by microorganisms. Microorganisms produce many different types of lypolytic enzymes such as lipases and esterases (carboxylesterases). Some microorganisms also produce biosurfactant during their degradation process of the FOG and it is secreted outside the cells into the environment. The physiological role that biosurfactant production allows has yet to be clarified, but there are some speculations that it promotes growth of microorganisms, especially on water-immiscible substrates by reducing the interfacial tension and making the substrate more bioavailable and some of them are from *Pseudomonas*, *Rhodococcus*, *Mycobacterium*, *Nocardia*, *Flavobacterium*, *Corynebacterium*, *Clostridium*, *Acinetobacter*, *Thiobacillus*, *Bacillus*, *Serratia*, *Arthrobacter*, and *Alcanivorax* genera [15]. The type of biosurfactant produced is genus and sometimes even species specific.

After the FOG has been exposed to biosurfactant and degraded by enzymes, the fatty acids and glycerol are consumed by the microorganisms that are capable of utilizing them like *E. coli*, *pseudomonas*, *acinetobacter*, and various bacilli [16]. The fatty acids are oxidized to acetyl-CoA via a pathway called β -oxidation. If the fatty acid has an even number of carbon atoms, then the entire chain is degraded to acetyl-CoA. If the fatty acid chain is an odd-chain fatty acid, then the last fragment is propionyl-CoA which is converted to acetyl-CoA through a variety of possible pathways. β -oxidation of fatty acids, in combination with the tricarboxylic acid cycle and respiratory chain, provides more energy per carbon atom than any other energy source [17].

Materials and Methods

Sample collection

Samples of liquid and solid FOG waste were collected from a sewer manhole owned by Indah Water Konsortium (IWK) at Kepong. The exact location was approximately 20-30 meters from a restaurant in Kepong which were suspected to be the biggest contributor of the FOG deposition in pipes. The pipe was made from verified clay (VCP) and its diameter was 225 mm and the samples were collected with the help of IWK engineers and workers. After collection, the samples were put inside a container and brought to IIUM to be stored in a refrigerator at 4 °C for further experimental work.

Isolation of FOG utilizing bacterial strains

Enrichment

In order to isolate the strains that could potentially degrade FOG, first the enrichment process needs to be done. This was done by adding FOG to distilled water along with the nutrient. The enrichment nutrient used is known as Mineral salts medium (MSM) and it was prepared according to the method described by [18] by mixing 2.5g NaCl, 4.74g K_2HPO_4 , 0.56g KH_2PO_4 , 0.5g $MgSO_4 \cdot 7H_2O$, 0.1g $CaCl_2 \cdot 6H_2O$, 0.5g NH_4NO_3 and 20 g agar. The MSM was added to 1 litre of distilled water in a Schott bottle and the pH was adjusted. The media was autoclaved for 15 minutes at 121°C which is the usual conditions used for liquid sterilization. 1 g of FOG was added to three shake flasks containing 100 mL of MSM. The broth was then incubated for 1 week at 150 RPM and at 37°C in the dark.

Selection

For the selection process, Nutrient Agar was prepared by mixing 23 g Nutrient Agar powder with 1 litre of distilled water in a Schott bottle. The media was autoclaved for 15 minutes at 121 °C. After sterilization, nutrient agar plates were prepared in petri dishes and using the inoculation loop, the previously enriched sample from the three flasks was streaked and inoculated in triplicates on the nutrient agar plates. The plates were incubated at 37 °C and were left for a few days until colonies appear on the plate. Pictures were taken in order to see the growth pattern of the colonies [19]. Blank medium (without any inoculation) was also prepared in order to confirm that the growing bacterial strains are really those that were taken from the enrichment medium. Colonies that grew fast were selected to be streaked again around 3 to 5 times more until pure strains were obtained [19]. After the pure strain was obtained, it was then transferred and subcultured into another agar medium called Tween-Peptone agar. The purpose of using this agar was to screen for lipid-degrading microorganism. Tween-Peptone agar was prepared by mixing Tween-Peptone agar containing 10.0 g peptone, 5.0 NaCl, 0.1 g $CaCl_2$, 5 ml Tween 80 and 18 g agar in 1 litre of distilled water in Schott bottle and finally autoclaved for 15 minutes at 121 °C. After sterilization, Tween-Peptone agar plates were prepared in petri dishes and using the inoculation loop, the pure culture from the nutrient agar was streaked and inoculated on the Tween-peptone agar. Strains that grew and showed an opaque halo around the colonies were selected for further experiments.

Characterization and Identification of Bacterial Strain

After the selection of the bacterial strain, the strains were characterized by its morphology and gram stain following standard laboratory methods [20]. Strain morphology identification was done by preparing a smear on a microscope slide and heat fix it. Heat fixing was necessary to make sure the strains were attached firmly onto the slide. The morphology of the strains on the slide was observed under a light microscope. For gram staining test, firstly the smear was prepared just like the strain morphology identification procedure. After that, the smear was immersed in a crystal violet solution and left standing for approximately 10 seconds. The crystal violet stain was then rinsed off the slide with water. Next, Lugol's solution was poured on the bacterial smear for another 10 seconds. The extra Lugol's solution was rinsed off with more water. Then several drops of alcohol were added to the slide that acts as a decolorizer. The alcohol was rinsed off the slide when it was no longer colored by the previous stains as the alcohol runs off the slide. A typical time for this was approximately 5 seconds. And lastly, a counterstain solution, safranin was put on the slide over the bacterial smear. The counterstain was allowed to remain on the smear for approximately 40 seconds and then was rinsed off with water. The slide was allowed to air dry, it was observed under a microscope to examine the slide containing the bacterial smear. Gram-positive (+) bacteria will be colored blue/purple, and Gram-negative (-) bacteria will have a red/pink look.

Results and Discussion

During the enrichment process, the MSM medium which was originally transparent and slightly cloudy in appearance turned very cloudy and yellowish after 7 days of incubation completed, this is an indication of the bacterial growth occurred at enrichment stage. The enrichment was prepared in triplicates and were later used in the selection stage. In the selection stage, enriched sample was streaked on the nutrient agar plates and they were left incubated, until there were appearances in microorganism's colonies on the surface. The growth can be seen clearly as fast as on the first day of incubation. Sub culturing was also done by streaking again and again on new nutrient agar plates until pure cultures were finally obtained. Nevertheless, the colonies inside the nutrient agar plates were then transferred to new Tween-peptone agar plates to observe its lipid-degradation ability, where the Tween-peptone agar is selective for the biodegradable bacterial strains.

The sample from the latest subculture plates (*Bacillus subtilis*) were transferred in three shake flasks (triplicates) which contained 100 mL of MSM broth and 1 mL palm cooking oil as an energy and carbon source. They were incubated in shaker for one week at 37 °C and 250 rpm. After 7 days of growth under this condition, we were able to see that the palm oil in the three flasks was significantly reduced when compared to its first day of incubation. There were no large and visible oil drops floating on the surface anymore. This was because the bacterial strain was able to degrade the oil and utilize it as their carbon and energy source for their growth and proliferation. This confirms the study done by [21] which also used *Bacillus subtilis* in his study. Other studies [15-16] also confirmed that *Bacillus* genus was capable of degrading FOG.

Characterization and identification of bacterial strain

For bacterial morphology, samples from the latest subculture plate were chosen to be smeared onto microscope slides as shown in Fig. 1. Upon observation under the light microscope, the sample taken had a rod-shaped morphology. Other 5 strains found were kept in the lab for further experiment.

The gram staining process was done only to the sample that had undergone the latest microscope observation. The solutions used for the gram staining test were crystal violet solution, Lugol's solution, 95% alcohol (decolorizer) and safranin (counterstain). After the slide for gram staining was ready, it was then observed under the microscope and it can be seen that the strains were coloured blue. This indicated that the sole bacterial strain isolated was a gram-positive bacterial strain. The characteristics obtained on the bacterial strain are summarized in Table 1.



Figure 1. Bacterial strains observed under the microscope

Table 1. Summary of bacterial identification and characterization tests

Test	Characteristic Observed
Strain morphology/shape	Rod-shaped
Gram-stain	Gram positive (+)

According to the Bergey's Manual of Systematic Bacteriology [22], the rod-shaped bacteria and its gram positive traits were highly related to Bacillus genus. Bacteria from Bacillus genus is usually Gram-positive, rod-shaped appearance and a member of the phylum Firmicutes. Bacillus species can be obligate aerobes or facultative anaerobes, and test positive for the enzyme catalase. The species observed under the microscope was highly likely to be a Bacillus subtilis. This was due to the fact that Bacillus subtilis is a strong bacterial species that is able to tolerate harsh and extreme conditions of environment mainly due to its ability to form tough and protective endospores under stressful living conditions [23].

Conclusion

FOG problem is an emerging problem due to current lifestyle, especially in the trend of fast food consumption. Uncontrollable dispose into the sinks will eventually lead to problems in the sewer system which could result in higher cost of repairing. The experiment was mainly aimed to utilize the biological treatment in treating the FOG, which is considered as an alternative treatment to the conventional chemical and mechanical treatment more environmentally friendly and lower in terms of cost. Bacterial strains were successfully isolated from FOG deposit to find the most capable one in degrading the lipid constituents in the FOG. One of the strains was applied with a drop of cooking oil, growth was obtained after a few days and the oil drop was not seen which indicates that it was successfully degraded by the bacteria.

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