OPTIMIZATION OF FAT, OIL, AND GREASE (FOG) BIODEGRADATION USING CONSORTIUM OF BACTERIA

BY

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A dissertation submitted in fulfilment of the requirement for the degree of Master of Science (Biotechnology Engineering)

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ABSTRACT

Fats, oils and grease (FOG) is a by-product produced originally by various food preparation activities. Removing FOG is critical because it is implicated in causing sewerage blockages leading to sewage system overflows (SSOs) and it can also deplete of oxygen levels in waterways. Recent studies favoured biological methods over chemical and physical methods for it is more efficient, safe and eco-friendly approach in treating FOG in sewer system. This work aims to study the ability to degrade FOG by bacterial consortium isolated from palm oil mill effluent (POME). The consortium was prepared from bacteria strains which were capable of degrading FOG. Screening test on Tween 20 agar media was applied to identify the consortia capable of degrading FOG and determine best consortium. The results showed that the E_3YZ consortium is best performing since it has largest growth and highest degradation of FOG. Optimization experiments were performed by using different levels of parameters to achieve the maximum removal efficiency of palm oil using the selected consortium. The parameters of pH (6, 7, 8), oil concentration (1, 3, 5% v/v), and bacterial inoculum concentration (2, 6, 10% v/v) were studied. The influence of these three parameters on the amount of oil degraded were investigated. Based on the results of 2 level factorial design, the most impact on the degraded oil was found in the oil concentration followed by the bacterial inoculum concentration then pH level. Oil concentration of 1% , 10% of bacterial inoculum concentration with pH 6 were obtained as the optimum conditions for oil degradation that led to the best degradation of FOG. At the optimum conditions, Consortium E_3YZ, which comprises two strains, *Micrococcus lylae strain DSM 20315* and *Corynebacterium aurimucosum* has shown the best result in the desired degradation of FOG. This consortium showed degradation up to 82.7% of FOG after 20 days of incubation. In conclusion, the study thus indicates that this bacterial consortium can be efficiently used for the biodegradation of FOG waste.

خلاصة البحث

الدهون والزيوت والشحوم (FOG) هي منتج ثانوي ينتج أصلاً عن طريق أنشطة إعداد الطعام المختلفة. إن إزالة FOG أمر بالغ الأهمية لأنه مسبب رئيسي في انسداد المجاري مما يؤدي إلى فيضان مياه الصرف الصحي (SSOs) ويمكن أن يستنفد أيضًا مستويات الأكسجين في المجاري المائية. الدراسات الحديثة فضلت الطرق البيولوجية على الطرق الكيميائية والفيزيائية لأنها نهج فعال وآمن وصديق للبيئة في علاج FOG. يهدف هذا العمل إلى دراسة القدرة على التحليل العضوي لـ FOG الضار عن طريق خليط بكتيري من بكتريا معزولة من مخلفات زيت النخيل المتدفقة (POME).تم تحضير الخليط البكتيري من سلالات البكتيريا القادرة على تحليل FOG. تم تطبيق اختبار الكشف على وسائط أجار (Tween 20). لتحديد المخاليط القادرة على تحليل FOG ثم تحديد أفضل خليط . أظهرت نتائج هذه الدراسة أن الخليط البكتيري E_3YZ هو الأفضل بين المخاليط الأخرى لاحتوائه على أكبر نمو وأكبر تحليل عضوي لـ FOG. في هذه الدراسة، أجريت تجارب التحسين باستخدام مستويات مختلفة من العوامل لتحقيق أقصى كفاءة إزالة لزيت النخيل. تمت دراسة العوامل pH (6، 7، 8)، وتركيز الزيت (1، 3، 5)، وتركيز العزلة البكتيرية (2، 6، 10) تمت دراسة تأثير هذه العوامل الثلاثة على كمية الزيت المتحللة عضويا. استنادًا إلى نتائج LFD 2، تم تحقيق أعلى تحلل للزيت لمجموعة 1% من تركيز الزيت، و10% من تركيز اللقاح البكتيري مع الرقم الهيدروجيني 6 كظروف مثالية للتحلل العضوي. في الظروف المثلى، أظهر الخليط البكتيري E_3YZ ، والذي يتكون من سلالتي ***Micrococcus lylae strain DSM 20315*** و ***Corynebacterium aurimucosum*** أفضل نتيجة في تحلل FOG. أظهر هذا الخليط البكتيري تحللا عضويا يصل إلى 82.7٪ من FOG بعد 20 يومًا من الحضانة. في الختام، تشير الدراسة إلى أن هذا الخليط البكتيري يمكن استخدامه بكفاءة للتحلل الحيوي لمخلفات FOG.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master of Science (Biotechnology Engineering).

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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LIST OF ABBREVIATIONS

BOD	Biochemical Oxygen Demand.
COD	Chemical Oxygen Demand.
CO	Cooking Oil.
CPO	Crude Palm Oil.
DAFs	Dissolved Air Flotation Systems.
DAG	Di Acyl Glyceride.
FFA	free fatty acids.1
FOG	Fats, Oils, and Grease.
FSE	Food Service Establishments.
LB Broth	Luria-Bertani Broth.
LCFA	long Chain Fatty Acids.
LFD	Level Factorial Design.
MAG	Mono Acyl Glyceride.
OD	Optical Density.
O&G	Oil and Grease.
POME	Palm Oil Mill Effluent.
SSO	Sanitary Sewage Overflows.
TAG	Tri Acyl Glyceride.
TPH	Total Petroleum Hydrocarbon.
TSS	Total Suspended Solids.
UCO	Used Cooking Oil.
WWTP	Wastewater Treatment Plant.

LIST OF SYMBOLS

-	Minus Sign
%	Percentage
+	Plus Sign
°C	Degree Celsius
π	Pi
μ	Mu

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Fats, oils and grease (FOG) commonly refers to the fats, oils and grease found in wastewater. It is derived from vegetable oil and animal fats which are found in food or used to prepare food. FOG originates either from food processing such as slaughterhouses, kitchens and restaurants (Long et al., 2012) or from food industry such as vegetable fats, nuts, cereals, butter, meats and lard. Significant sources of FOG are food service establishments (FSEs) and residential homes, where residual food grease and cooking oil is improperly discharged of in drains.

The emergence and prevalence of FOG deposition in sewer system has become a real impendence to global public health and harm the environment that requires effective action (Ma'an, Alam, & Shabana, 2015). The real concern is the accumulation of FOG on internal walls of pipeline and ultimately causing blockage of entire sewer line (Fan, 2014) and sanitary sewage overflows (SSO). This will lead to rise in the maintenance cost that include repair and clean of sewer pipes as well as nuisance to the public because of foul smell.

FOG is one of major lipid compounds refer especially to triglycerides which are formed by association between three fatty acids and glycerol (Simoneit, 1978; Williams et al., 2012). One of physical methods used to remove FOG in municipal wastewater is grease trap. It is a unit of special geometrical design in order to overcome clogging of FOG in pipelines. Grease trap is often made out of concrete, steel or plastic (Weber, 1990).

This device depends on separation and prevention of discharged FOG into a wastewater treatment plant by intercepting FOG compounds before they enter a sewer system. Wastewater which consists of FOG, when enters sewer tanks forms a floating FOG layer on the surface of water because FOG will not mix with water due to different physical properties. Therefore, the FOG layer will be easily and manually removed. Although this method is effective to reduce FOG from wastewater, it requires to be cleaned continuously as well as foul smelling and unpleasant mess (Tzirita et al., 2018).

Another physical method that has been used to remove FOG, biochemical oxygen demand (BOD_5) and suspended solids (TSS) from wastewater stream is a dissolved air flotation systems (DAFs). The removal is achieved by injecting dissolved air in wastewater under pressure where the dissolved air comes out in a form of microbubbles. The microbubbles will attach with contaminants particles to form bulk material, float at the surface of wastewater then removed by a skimming device. However, the DAF requires high energy to remove the collected FOG by skimming device (Lambert & Houtz, 2005). Thus, this makes it very important to find an alternative treatment method which is more efficient and environmentally friendly to remove FOG. The previous studies generally stated that biological treatment method has the ability to remove lipid and oil from both industrial wastewater and domestic wastewater with less harm on environment (Alkhatib et al., 2011).

Biodegradation is a method that has been verified to be an effective method for biodegradation of various oils. This method has many advantages, as it is highly efficient, environmentally friendly and reduces the required time for degradation without releasing any foul odour (Basumatary et al., 2012). The principle of this method is to use the microorganism, which plays a crucial and efficient role, to break down organic compounds in wastewater system and convert them into safe or less harmful products under controlled conditions (Santisi et al., 2015). Bacteria are often used in biodegradation, in addition to algae, fungi and plants. The main factor affecting the efficiency of biodegradation process is selecting suitable microorganism. For instance (Nzila et al., 2017) used a single and mixed consortium of bacteria strains as *Sphingobacterium sp., Pseudomonas poae, Pseudomonas aeruginosa, Pseudomonas libanensis and Stenotrophomonasrhizophila* for treatment of oil and grease (O&G) in municipal wastewater. Both sets successfully degraded cooking oil. However, the bacterial consortium was more efficient than single bacterial strains.

The success of biodegradation technique depends on biodegrading capabilities of the microbial population. Therefore, the mixed cultures that contain a diverse variety of microbes may be used for on eff ective technique in a complex cleaning of FOG where they have abilities to endure more changeable environments and do even more complicated tasks than monocultures (Kumari et al., 2017). Thus, the use of mixed cultures in bioremediation of FOG waste in wastewater is a development in the right direction.

In the current research, the bacteria strains isolated from palm oil mill effluent (POME), is used to degrade the FOG. POME is chosen as source of microorganisms because of its rich constituent of microorganisms which are acclimatized with oil-based waste. Consequently, POME is considered preferable place for various microorganisms (Agualimpia et al., 2016). The main objective of this work is to create a consortium of bacteria that can efficiently biodegrade FOG.

1.2 PROBLEM STATEMENT

FOG release to sewer pipes leads to complications in the wastewater treatment plants, where lipids interfere with aerobic microorganisms which are responsible for the biodegradation in the sewage system. Also, FOG deposition in sewer pipes is causing major issues of clogging in sewage systems. Nevertheless, the needed attention addressing this problem is still very little. The current mechanical and physical methods used for FOG removal from wastewater have an issue of low effectiveness and weak ability to control the amount of FOG in sewer system. Thus, more research is needed to improve the ability on removing the FOG in wastewater.

Therefore, single strains isolated from POME were used in biodegradation of FOG as an alternative for physical methods. The results showed positive outcome (Qedra, 2018). Nevertheless, shorter time and high rate of biodegradation is sought. The use of consortia of bacteria in biodegradation of curd oil showed better results compared to single strains (ADD reference). Therefore applying consortium of bacteria is expected to improve the efficiency of FOG biodegradation.

1.3 SIGNIFICANCE OF THE STUDY

This study was conducted to determine which bacterial consortium is capable to degrade FOG. This may lead to the development and innovation of new types of FOG degrading bacterial consortium and the detection of various bacteria which can be used to remove fat, oil and grease (FOG) from wastewater and solution to the FOG deposition problem in the piping system.

1.4 RESEARCH OBJECTIVES

The general objective of this research is to use bacterial consortium for biodegradation of FOG waste in the sewage system. The specific objectives are:

1- To screen bacterial consortia formed from bacteria isolated from POME for biodegradation of FOG.

2- To study the growth kinetics of the bacterial consortium

3- To optimize FOG biodegradation process parameters using the most effective bacterial consortium.

1.5 RESEARCH SCOPE

This study concentrates on identifying the bacterial consortia that can be used to biodegrade FOG. Used cooking oil will be utilized to resemble FOG. Bacterial consortium was composed of two different kinds of bacteria which were previously isolated from palm oil mill effluents (POME). Various consortia will be formed and compared for best FOG biodegradation. The best consortium was further used in the optimization study using three variables (pH, oil concentration, bacterial concentration) to investigate the optimum parameters. A kinetic study on the growth of the selected consortium was conducted.

1.6 RESEARCH GAP

After reviewing different studies in biodegradation of FOG, it is very clear that most of such researches have paid a little attention to the use of mix culture of bacterial strains in biodegradation of FOG. Consequently, this study intends to improve the biodegradation of FOG using bacterial consortium in order to bridge the gap left behind by previous researchers. Where this study used to screen which bacterial consortium has ability to degrade the FOG because selecting suitable bacterial population is an important step in biodegradation process of FOG and then using the selected bacterial consortium in optimization process to determine the optimum condition by using different levels of parameters including the oil concentration, bacteria inoculum concentration and pH.

1.7 THESIS ORGANIZATION

This research contains five chapters, where the background, problem statement, objectives and scope of the study are discussed in chapter one. The scope of research and significance of research are also discussed in this chapter.

In chapter two, literature review on the origin of FOG and the previous works on their biodegradation are reviewed. While chapter three presents the material, experimental design and analysis techniques used to achieve the objectives. The results and discussion are described in chapter four. Finally, in chapter five the conclusions of the study are outlined with some recommendations taken into consideration for future works.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

This chapter provides an overview of previous works on biodegradation of fat, oil and grease (FOG) using consortium of bacteria. It introduced more details and facts about FOG and various important aspects related to removal process of FOG deposition in pipeline system. In addition, in this chapter information about biodegradation such as types of microorganisms, mechanism of biodegradation and factors influencing biodegradation are discussed. Finally, the bacterial consortium used in bioremediation technique are highlighted.

2.2 LIPIDS

FOG is one type of lipid especially that refers to triglycerides, where they are composed from three fatty acids and one glycerol molecule only, so that called simple lipids. During dehydration process, three water molecules are removed, the glycerol molecule and fatty acids are bonded by ester bond lead to formation of triglyceride molecule as shown in Figure 2.1 (Berg et al., 2002).

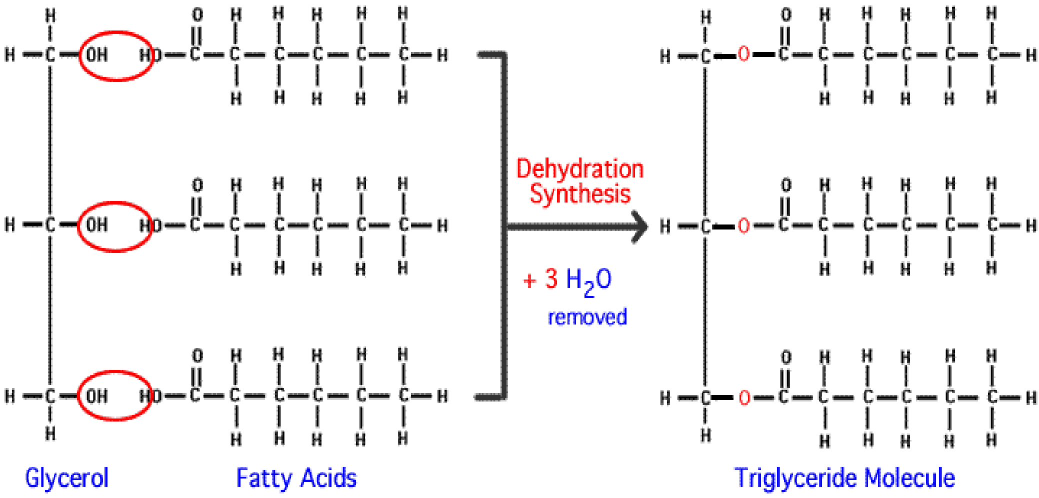

Figure 2.1 Dehydration process (Berg et al., 2002).

Generally, the lipid chain length is different based on number of carbon atoms which often in a range of 2 to 22. Moreover, the lipid is classified depending on the type of bonds into saturated or unsaturated lipid (Wakelin & Forster, 1997; Berg et al., 2002).

The saturated lipids have no double bonds in their chemical structure, they have single bonds only and the term “saturated” refer to hydrogen atoms. Meaning that the last carbon contains 3 hydrogen atoms (-CH_3) (Figure 2.2). Saturated lipids are typically solid at room temperature and have a higher melting point. They mostly come from either animal sources like cheese, butter, whole-fat milk, beef and poultry, or come from plant sources such as coconut and palm oils. Normally, the saturated fatty acids contain between 12-14 carbon atoms (Table 2.1) (Chakraborty et al., 2011).

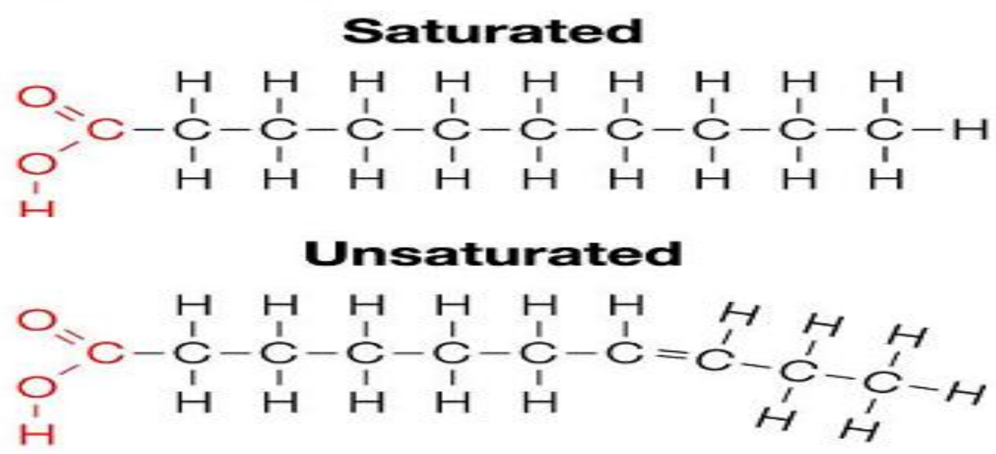

Figure 2.2 Structure of saturated and unsaturated fatty acids (Berg et al., 2002).

Contrarily, the unsaturated lipids have one or more double bonds in their chemical structure, where one alkenyl group (-CH=CH-) replacing (-CH_2-CH_2-) (Figure 2.2). They are classified to monounsaturated lipids or polyunsaturated lipids based on a number of double bonds which are present in their chemical structure. The unsaturated fatty acids usually with 18 carbon atoms. They are normally liquid at room temperature and have a relatively lower melting point. Examples include vegetable oils such as soybean, olive, corn, peanut, safflower oil and sunflower (Berg et al., 2002).

Table 2.1 Some of most common free fatty acids (Berg et al., 2002).

C-Atoms: Double Bonds	Common Name	Systematic Name	Abbrev	Structural Formula
4:0	Butyric	Butanoic	C4:0	CH3(CH2)2COOH
5:0	Valeric	Pentanoic	C5:0	CH3(CH2)3COOH
6:0	Caproic	Hexanoic	C6:0	CH3(CH2)4COOH
7:0	Enanthic	Heptanoic	C7:0	CH3(CH2)5COOH
8:0	Caprylic	Octanoic	C8:0	CH3(CH2)6COOH
9:0	Pelargonic	Nonanoic	C9:0	CH3(CH2)7COOH
10:0	Capric	Decanoic	C10:0	CH3(CH2)8COOH
12:0	Lauric	Dodecanoic	C12:0	CH3(CH2)10COOH
14:0	Myristic	Tetradecanoic	C14:0	CH3(CH2)12COOH
15:0	Valerenic	Pentadecanoic	C15:0	CH3(CH2)13COOH
16:0	Palmitic	Hexadecanoic	C16:0	CH3(CH2)14COOH
17:0	Margaric	Heptadecanoic	C17:0	CH3(CH2)15COOH
18:0	Stearic	Octadecenoic	C18:0	CH3(CH2)16COOH

2.2.1 Definitions of FOG

FOG is a combination of fat, oil and grease. The fat is a chain of fatty acids found in food that may usually be obtained in solid shape, but oil means fats that are liquid at a room temperature, while grease refers to a combination of fats and oils (Table 2.2). Some of facilities that generate FOG involve restaurants, kitchen, mechanic shops, fossil fuel, malls, hotels, car washes (Klaucans & Sams, 2018).