Biodegradation of Grease Trap Waste by Lipolytic Fungi under Local Ambient Conditions

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Abstract: Relatively higher concentrations of fat, oil and grease (FOG) are contained in hotel and restaurant wastewater which makes it difficult to be treated through cost-effective conventional biological treatment systems. The present study is aimed at finding a suitable solution to the persistent problem of proper disposal of FOG. Different lipolytic fungi were isolated from the grease trap waste. Lipolytic activity and the rate of FOG degradation were employed to identify for the most efficient degraders of FOG. Biodegradation of grease trap waste was tested by inoculation of a particular fungal isolate containing approximately $1 \times 10^7$ spores per ml to FOG in a tray type reactor at 30 °C and using coir fibre (size <1.7 mm) as the bulking agent. FOG removal efficiency was observed as 48-88% in 72 hours for isolate F1-F5. The developed protocol for biodegradation of FOG was found to be effective for practical applications to treat grease trap waste.

Keywords: fatty wastewater, FOG, food service industry, lipase

1. INTRODUCTION

With the change in lifestyle of the urban population, where home cooking has been largely replaced by take away food and restaurant meals, large numbers of small hotels and restaurants have opened up in many cities. Use of fat and oil in food preparation has also increased, in order to enhance the flavour and taste of food. As a result, the amount of fat and oil to be discharged with kitchen wastewater has also increased. Elevated FOG levels in wastewater cause blockages in drainage systems and grease accumulation on interior pipe surfaces, as well as causing problems in subsequent treatment stages, due to alteration of settling characteristics of flocs and detrimental impact on oxygen transfer in aerobic treatment process. Long-chain fatty acids, the primary hydrolysis products of fats and oils, have been demonstrated to lead severe inhibition of the biomass in anaerobic treatment processes (Dumore and Mukhopadhyay 2012). Therefore, removal of FOG becomes an essential first step in biological treatment of wastewater streams.

In food service industry, main technique for separating FOG from the wastewater stream is accomplished through gravity separation by installation of grease traps/interceptors. Due to elevated FOG levels in wastewater, numbers of baffles in the grease trap devise needs to increase in order to retain the FOG. However, this method recurrently becomes aesthetically unappealing and causes nuisance in the vicinity of the treatment facility. In addition, physical treatment methods only help to separate FOG from the main wastewater stream, but the separated FOG has to be treated further or disposed of properly. There are chemical treatment methods which use alkaline solutions to enhance the breakdown of fat in the feeding tank, neutralize acidic wastewaters and maintain the required alkalinity level. Lin et al. (2009) reported that chemical hydrolysis with NaOH increases the ratio of soluble COD to total COD and reduces the volatile solid content during anaerobic digestion. However, these chemical methods remove the floating scum and hardened grease adhering to wastewater treatment plant equipment but transfer the pollutant from solid to liquid phase, without actually treating the FOG.

Biotechnological applications such as biodiesel and biogas production have received great attention due to their potential to be used as an alternative energy sources. However, most of them require substantial capital outlay and demand for higher technical input with closer supervision, which may discourage the industry personnel from implementation of advanced biotechnological methods, if it is not a part of their core business. Therefore, there is a growing demand for cost-effective and environmentally friendly FOG treatment methods. The present study is aimed at finding a sustainable solution to the persistent problem of FOG in wastewater. The objectives of the present study are:

1. To screening and isolation of fungi capable of degrading FOG efficiently.
2. To demonstrate at laboratory scale optimum fat, oil and grease degradation with isolated fungi.
3. To study the fat, oil and grease degradation of actual grease trap waste under local ambient conditions.

2. LITERATURE REVIEW

2.1. Sources of FOG Rich Wastewater

Food service industries are considered to be the major consumers of animal fats and vegetable oils. The spent FOG comes out from the commercial kitchen in two basic forms viz yellow grease and brown grease (Gaur et al. 2010). In general, yellow grease can be defined as the inedible, unadulterated FOG that remains in the containers after kitchen operations such as bulk deep-frying (Makkar and Cammerota 2002). This yellow grease usually does not contribute for elevated FOG levels in kitchen effluent. The brown grease is mainly generated as a result of cleaning of dishes, pots, pans, equipment and utensils. The brown grease can be found as recovered materials from grease traps and majority of brown grease had been adulterated and in contact with food particles, detergents/cleaning solutions etc. (Kiepper 2001). In the following section FOG treatment options for brown grease are discussed.

2.2. FOG Treatment Options for Brown Grease

Direct cultivation of lipolytic bacteria in bioreactors in a single or mixed culture in the form of attached or suspended growth was proposed as a biological treatment method to treat FOG rich wastewater. Wakelin & Forster (1997) treated two fast food restaurant wastewaters using a consortium of gram negative bacteria. In this study a novel bioreactor, the weir tank reactor was employed in suspended growth system and a high removal rate (84±96%) of FOG has been achieved. Keenan & Sabelnikov (2000) employed an attached growth system by immobilizing bacteria in porous material which recorded an enhancement of FOG degradation over suspended culturing. However, this method had limitations due to inability to treat shock loads, flush out of biomass and requirement of close supervision.

With the advances in industrial biotechnology, lipase and other hydrolytic enzymes and/or enzymes pools, patented catalytic formulations and bio-additives are produced which have the ability to break down the FOG rapidly into usable forms for microbes such as glycerol and long chain fatty acids (LCFA) (Santis-Navarro et al. 2011; Kumar and Kanwar 2012). However, LCFA thus generated impose negative impacts on the downstream of the process. In literature such unfavorable effects on methanogenic bacteria are well-documented, notably the high mortality due to toxicity (Long et al. 2012). The possible recovery processes to overcome this toxicity, such as precipitation with soluble calcium (Koster 1987), use of bentonite (Mouneimne et al. 2004), adsorption into zeolite (Nordell et al. 2013), use of high dilution ratio with water: active inocula (Palatsi et al. 2009; Wu et al. 2015), have been studied in detail.

However, to date, the exact level and nature of the inhibitory effect of LCFA on methanogenic bacteria is not well understood (Valladão et al. 2011; Long et al. 2012; Wu et al. 2015). Moreover, grease trap waste is a mixture of lipids, fatty acids, food particles and detergents which are discharged from food service kitchens. Therefore, it is difficult to pre-decide the levels of inhibition caused by LCFA. These inconsistencies prevent adoption of hydrolysis of FOG as a pre-treatment option in conventional wastewater treatment systems.

As a consequence, in most food service industries, though they have wastewater treatment facilities, grease trap waste is disposed of with municipal solid waste (without proper treatment) which may cause negative impacts on the environment (Nanayakkara and Witharana 2015). Therefore, it is necessary to find alternative methods to treat FOG collected in the grease trap before disposal.

The present research is to contribute for the development of a sustainable and economical technology to treat/degrade FOG. To this end, lipolytic fungi were isolated from different sources such as grease
trap waste and FOG degradation efficiency was determined using FOG as the sole carbon source for fungi. The feasibility of using the developed bioprocess application for degradation of FOG was also tested using raw grease trap waste under ambient conditions.

3. METHODOLOGY

3.1. Collection, Transport and Storage of Samples

Oil contaminated soil samples were collected from an industrial coconut oil mill, at a depth of 3-5 cm, from three locations of the site. Grease trap waste samples were collected at 3 points of the grease trap chamber: at the surface, interface (between oil and water layer) and chamber wall (scraped off the wall). All the samples were collected into sterilized sealable polythene bags and brought into Microbiology Laboratory, Environmental Engineering Division, Department of Civil Engineering, University of Moratuwa, Sri Lanka within 4 h and stored in a refrigerator at -18°C until use.

3.2. Isolation of Lipolytic Fungi

The minimum salt broth (MSB) was used as the enrichment medium for fungi. A volume of 25.0 ml of MSB was autoclaved at 121°C for 20 min and it was allowed to cool and 2.0 ml of Ciprobid (10 mg/l) was added as an antibacterial antibiotic. A 5g wet weight each from soil samples and grease trap waste samples were added to Erlenmeyer flasks and kept under constant agitation of 100 rpm under room temperature for one hour. Thereafter, samples were incubated at 30°C for 3-5 days and visible mycelia growths were inoculated onto fresh solid media to obtain pure cultures. The remaining portion was used to prepare spread plates on Tributyrin Agar in a tenfold dilution series up to 10⁻⁶ and incubated in triplicates at 30°C for 7 days. The detection of lipase active fungi was based on formation of clear zones/halo zones around the colonies and pure colonies of fungi were obtained by repeated sub culturing on Tributyrin Agar. The colonies having most conspicuous clear zones were selected for further studies. Each isolated pure fungal colony was stored in MSA slants and stored at 4°C and sub-cultured at every six weeks.

3.3. Lipase Activity

3.3.1. Preparation of fungal inocula

A fungal spore suspension was obtained by growing a pure culture of a particular isolate on minimum salt agar and incubating at 30°C for seven days. A total volume of 10 ml sterile distilled water was spread in aliquots on a culture plate and the fungal colony surface was gently scraped using an inoculation needle. The cultures were filtered through Whatman® No. 42 filter paper into a sterile container. An aqueous spore suspension having approximately 1 x 10⁷ spores per 1 ml was obtained using a haemocytometer.

Preparation of lipase enzyme extracts

A volume of 25 ml minimum salt liquid medium containing 1% (w/v) olive oil was prepared and inoculated with 1 ml of spore suspension of a particular fungal strain. A volume of 25.0 ml of sterile distilled water was inoculated in similar manner and treated as the control. Three replicates were tested for each fungus. All the samples were kept at 30°C for 72 hours. Thereafter, media were filtered through Whatman® No. 42 filter paper and centrifuged at 4000 rpm for 10 minutes to remove any suspended solids and mycelial fragments. Supernatants i.e. enzyme extracts were collected and
10 ml from each were taken for the measurement of pH values and remaining suspension (15.0 ml) was used to measure lipase activity.

3.3.2. Determination of lipase unit activity

Lipase unit activity was determined in an emulsified based system having arabic gum as a emulsifier and olive oil as a substrate. A 18 ml volume of an emulsion of arabic gum (5% w:v) and olive oil (5% w:v) were prepared in 50 mM phosphate buffer solution (pH 7.0). Then 2 ml enzyme extract was added and vortex mixed. It was allowed to react for 60 min at 37 °C and enzyme reaction was stopped through the addition of 20 ml of a 1:1 acetone:ethanol mixture. After further agitation for 10 min for total extraction of fatty acids, titration was performed with 0.05 N NaOH until end-point 11.0. The control assay was run by adding the acetone:ethanol mixture prior to the enzyme sample.

3.3.3. Lipase Unit Activity (U/min):

One unit of lipase activity was defined as the amount of enzyme, which produced 1 mmol of fatty acids equivalent per minute under the assay conditions (Eq 1). Where, N is the normality of NaOH.

\[
\text{Lipase Unit Activity} = \frac{N \times \text{Vol of NaOH titrated} \times 1000}{\text{Time of incubation}}
\]

(1)

3.4. Biodegradation of FOG under Optimum Conditions

FOG biodegradation efficiency was determined using lipid portion (i.e., FOG) of grease trap waste on minimum salt agar based medium. The composition of 100.0 mL of the minimum salt agar medium is FOG: 100 μL (1% v/w), K₂HPO₄: 0.7 g, KH₂PO₄: 0.2 g, Trisodium Citrate (Na₃C₆H₅O₇): 0.05 g, MgSO₄: 0.01 g, (NH₄)₂SO₄: 0.15 g and Agar: 1.5 g. Distilled water was added up to 100.0 mL and the pH of the medium was adjusted to 7 ± 0.1. The growth medium was autoclaved at 120 °C, 1 bar pressure for 20 minutes. Thereafter, this growth medium was kept in a bio-safety cabinet and left to cool down to about 80 °C. After that, about 12 - 15 mL of growth medium was poured into a Petri dish and allowed to solidify completely. Then the Petri dish was inoculated with isolated lipolytic fungal isolates as described above, by taking a fraction (approximately 0.5 cm²) from a pure culture of the respective mycelium under aseptic conditions and placing it at the center of the Petri dish. Each inoculation was run in triplicate and control sample (without inoculation) was also tested. Then all the Petri dishes were incubated at 30 °C for 72 hours. Thereafter, the remaining FOG was extracted according to the method developed by Bligh and Dyer (1959) and the weights were recorded.

3.5. Biodegradation of Grease Trap Waste under Ambient Conditions

Biodegradation of grease trap waste was determined under local ambient condition in a tray type reactor (120 mm × 100 mm × 25 mm) after mixing grease trap waste with coir fiber (bulking agent). The inoculation was made with a spore suspension of fungi having approximately \(1 \times 10^7\) spores/ml for each 10 mg/g dry weight of substrate (Table 1). The medium was incubated at room temperature (28 °C) without adjusting the pH of grease trap waste. The initial moisture content of the grease trap waste was kept at a low level (25-35% of moisture). Thereafter, the moisture content in the tray type reactor was increased up to 65% by adding 1.9 ml of distilled water per gram dry weight of solid substrate (Falony et al. 2006).
**Table 1** Environmental Conditions maintained for biodegradation of grease trap waste

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fat, oil and grease</strong></td>
<td>Adjusted to 7± 0.1</td>
</tr>
<tr>
<td><strong>Raw grease trap waste</strong></td>
<td>4.5-6.5</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>4.5-6.5</td>
</tr>
<tr>
<td><strong>Moisture content of the substrate</strong></td>
<td>&lt; 30%</td>
</tr>
<tr>
<td><strong>FOG content in the substrate</strong></td>
<td>100 µL (1% v/w)</td>
</tr>
<tr>
<td><strong>Inoculation</strong></td>
<td>A 0.5 cm² fraction from a pure culture</td>
</tr>
<tr>
<td><strong>Bulking agent</strong></td>
<td>None</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>30 °C ± 0.1</td>
</tr>
</tbody>
</table>

4. RESULTS AND DISCUSSION

A total of five fungi were selected for the study and coded for convenience (Table 2). During screening process of Lipolytic fungi, lipase activity of each fungus was determined and five isolates were selected for further studies (Table 2).

**Table 2** Lipase activity of the isolated fungi

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Enzyme activity (U) /g dry weight ± SD</th>
<th>Final pH of the medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>15.42 ± 2.7</td>
<td>3.8 ± 0.5</td>
</tr>
<tr>
<td>F2</td>
<td>11.62 ± 2.1</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>F3</td>
<td>9.47 ± 1.4</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>F4</td>
<td>18.29 ± 3.2</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>F5</td>
<td>21.75 ± 3.1</td>
<td>3.1 ± 0.5</td>
</tr>
</tbody>
</table>

Biodegradation of FOG (lipid portion of grease trap waste) was determined under optimum conditions using selected isolates of lipolytic fungi, (Table 1). The FOG was provided as the sole carbon source and solid substrate at low moisture levels in a tray type reactor. The degradation efficiencies by lipolytic fungi were determined by taking the initial and final weight of FOG after 72 h (3 days) of incubation time. Fungal isolates F1, F4 and F5 showed significantly high degradation efficiencies (P < 0.05) within 72 h (Figure 1).

The raw grease trap waste was used as the solid substrate (after drying to have relatively low moisture condition between 25-30% for lipolytic fungi and degradation efficiencies were determined under local ambient conditions (Table 1). About 50% degradation efficiencies were achieved under ambient condition for raw grease trap waste (Figure 2).

During the degradation process of grease trap waste fungi are allowed to degrade the FOG and other organic components present in the waste. Fatty acids and other hydrolyzed products that are produced such as glucose and amino acids, may be used to fulfill their nutrient requirement, metabolism and growth. Fungi may reach the end of their life cycle in the reactor by forming spores due to the limitation of food and space. This degraded residue containing fungal spores can be used as an inoculum for the next round of treatment of grease trap waste. Therefore, once the degradation cycle is started, putting them into practice would be easier because no continuous inoculation is needed.
In the present study, the recorded optimum pH for FOG degradation was 7 at 30 °C for selected fungal isolates. However, in literature lipolytic fungi have shown optimum FOG degradation for different pH values: at pH 2.5 and at 45 °C (Mahadik et al. 2002); at pH 5 and at 37-40 °C (Kirsh 1935); at pH 5 and at 30 °C for synthetic substrate (triacylglycerols) and for natural substrate (oil) at pH 7 and at 30 °C (Hee-Yeon et al. 2007); at pH 8.5 and at 40 °C (Ulker et al. 2011); at pH 7-9 and at 30 - 45 °C (Mahmoud et al. 2015). Although, the recorded pH of grease trap waste varied from 4.5 - 6.5, it has been in a compatible range with other reported pH values for FOG degradation. Therefore, the pH of the grease trap waste was not adjusted to the optimum of 7. Also, adjustment of pH of the grease trap waste would entail additional expenditure, which will make the overall cost of wastewater treatment higher.
Maintaining the initial moisture content of the substrate at the required level is vital for germination of spores. It should be less than 30% for effective and efficient degradation of FOG (Table 1). However, evidence for the optimum initial moisture content of the substrate for germination was hardly reported. Nevertheless, optimum moisture content in the reactor for Lipolytic activity was well documented in literature, which was about 65% (Falony et al. 2006; Kotogan et al. 2014); 50-55% (Raimbault and Alazard 1980). In the present study, observed optimum moisture content for degradation of grease trap waste in the reactor was 65%.

5. CONCLUSIONS

The study showed that there is a significant potential for implementation of this developed methodology as a biotechnological application to degrade FOG in the food service industry. The following conclusions can be drawn from the study:

- Grease trap waste can be degraded using lipolytic fungi under local ambient conditions.
- FOG degradation efficiencies of around 50% can be achieved within 72 h with raw grease trap waste.

6. ACKNOWLEDGEMENT

The authors wish to acknowledge the funding received from the Senate Research Council (Grant No: SRC/LT/2012/3), University of Moratuwa, Sri Lanka. Also, this study was supported by the National Research Council (NRC) of Sri Lanka (Grant No: 12-086) to purchase Gas Chromatography/Mass Spectrometer (GC/MS).

7. REFERENCES


