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Rapid degradation of FOG discharged from food industry wastewater by lipolytic fungi as a bioaugmentation application

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ABSTRACT

Fats, oils and grease (FOG) congregate in grease traps and are a slowly biodegradable particulate organic matter, which may require enzymatic or hydrolytic conversion to form readily biodegradable soluble organic matter. The existing treatment methods employ water-based hydrolysis of FOG to form long-chain fatty acids (LCFAs). The LCFAs discharged into wastewater treatment system create functional difficulties, especially the inhibitory effect caused by accumulation of LCFAs. This study aims to find an effective treatment method for this persistent problem encountered in conventional wastewater treatment system. Solid-state degradation by lipolytic fungi was performed in a tray-type reactor as a novel approach of bioaugmentation. Grease trap waste samples were dried to have moisture content of 25–35% and mixed with coir fiber (1% w/v) for proper aeration. Each 10 mg/g dry weight of substrate was inoculated with 1 mL of spore suspension (1×10^7 spores/mL) of lipolytic fungi. Thereafter, moisture content in the reactor was increased to 65%, and incubated at 30°C. Within 72 h of post incubation, degradation efficiency of about 50% was recorded by fungal isolates. The feasibility of using developed protocol for FOG degradation was tested with a laboratory-scale prototype reactor.

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Fatty acid methyl esters; grease trap waste; lipase; long-chain fatty acid; solid-state degradation

Introduction

Oils and fats are mainly used for culinary applications in food service industries, such as restaurants, in order to enhance the taste, flavor and texture of the food product. The fats, oils and grease (FOG) concentration of kitchen wastewater could be as high as 312–836 mg/L [1]; 462–2441 mg/L (authors' unpubished data); 500–4700 [2]; 21,500 mg/L [3]. The FOG removal from food industry wastewater generally accomplish by installing grease traps and/or grease interceptors. FOG in wastewater creates numerous problems in sewer systems as it solidifies inside pipes, consequently reducing the effective pipe diameter and/or completely blocking the pipelines [4]. In aerobic treatment processes, the presence of FOG in high levels may (i) have a detrimental impact on oxygen transfer [5] and (ii) lead to the occurrence of filamentous actinomycetes that are involved in the formation of scum and stable foams [6]. In anaerobic treatment processes, the presence of long-chain fatty acids (LCFAs) has been found to cause severe inhibition of methanogenic microbial community [7,8]. Moreover, in conventional wastewater treatment systems, FOG is considered to be a slowly biodegradable particulate organic matter (commonly referred to as 'sbpCOD') [9] and may require enzymatic or hydrolytic conversion to form readily biodegradable soluble organic matter (rbsCOD) such as glycerol and fatty acids.

As a hydrolysis/treatment option for FOG, direct cultivation of lipolytic bacteria in FOG-rich wastewater has been proposed previously [10,11]. However, this method has limitations due to the relatively long acclimatization period and high energy requirement to maintain aerobic conditions during the treatment process. These limitations were overcome to a certain extent by growing lipolytic microorganisms separately and introducing them as an attached growth system into wastewater, which has a high-FOG content around 20,000 mg/L [3,12]. It is especially noteworthy that these attempts to hydrolyze/treat high-FOG-containing wastewater were focused on the use of lipolytic bacteria rather than fungi.

However, with the advances in industrial biotechnology, lipase and other hydrolytic enzymes are produced by solid-state fermentation (SSF) using fungi. Researchers have shown promising results in the production of enzymes with higher lipase activity [13–17]. Although, these enzymes have been used to catalyze the initial hydrolysis step of FOG to form LCFAs [18,19],

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accumulation of LCFA leads to inhibition of the activity of methanogense [7,20–23]. The possible recovery processes to overcome this inhibitory effect, such as precipitation with soluble calcium [24,25], use of bentonite [26], adsorption with iron-containing clay [27] or zeolite [28], use of high dilution ratio with water: active inocula [29,30], have been studied in detail.

However, to date, the exact level and nature of the inhibitory effect of LCFAs on methanogenic bacteria is not well understood [23,30,31]. 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 [32]. Therefore, it is necessary to find an alternative method to treat FOG collected in the grease trap before disposal. The objective of the present research is to contribute for development of a feasible technology to treat/degrade FOG. To this end, from different sources such as grease trap waste, fungi with higher lipolytic activity were isolated and inocula were prepared (for future bioaugmentation of this study). The FOG degradation efficiencies were determined at different pH and moisture levels using oils, fats and lipid portion of the grease trap waste. The feasibility of the developed methodology for degradation of raw grease trap waste was tested in a tray-type reactor.

Materials and methods

In the present study, solid-state degradation (SSD) was performed in two stages: (i) using extracts of the lipid portion of grease trap waste (i.e. FOG) (ii) using raw grease trap waste (without extraction of lipid portion). Stage 2 was carried out to investigate the degradation under raw field situation. All studies were carried out in triplicate, in tray-type reactors by inoculating the prepared samples with previously isolated lipolytic fungi. The methodology was developed to study the degradation characteristics of FOG in order to comprehend whether lipolytic fungi can be used as an effective treatment option for grease trap waste.

Sample collection

Fifteen grease trap waste samples were collected from hotels and restaurants located in Colombo and its suburbs. The upper layer of the grease trap contents was skimmed off manually from at least three points and combined to yield a total volume of about 1 L. These waste samples were collected into polyethylene containers and chilled during transportation. The initial water content, pH, total FOG content and fatty acid profiles of the grease trap waste samples were determined before storing at −18°C. The samples were thawed (in a water bath at ~40°C), and homogenized before use.

Extraction of FOG

The extraction of the lipid portion from grease trap waste is essential in the determination of the profile and subsequent quantitative analysis. The extraction was carried out according to the method developed by Bligh and Dyer [33]: the grease trap waste samples were homogenized with a mixture of chloroform and methanol in a proportion such that a miscible system is formed with the water in the grease trap waste (see below section). Then, dilution is made with chloroform and water, which separates the homogenate into two layers, the chloroform layer containing all the lipids and the methanolic layer containing non-lipids. The lipid extract is obtained by isolating the chloroform layer.

Following the above method, a known weight of grease trap waste was taken and mixed with water, methanol and chloroform at a ratio of 1:2:0.8, respectively. Subsequently, water and chloroform were added to shift the ratio to 2:2:1.8. The lower chloroform layer was transferred (using a Pasteur pipette) into a clean glass vial and the volume was recorded. Then chloroform was evaporated and the residue was subsequently dried with a stream of N_2 and the final weight of the above glass vial was recorded. Then, the extracted sample was stored in 5 mL of chloroform:methanol (10:1) mixture at −18°C until use.

Preparation of fatty acid methyl esters (FAMEs)

The fatty acid composition of the samples needs to be determined as FAMEs, which are moderately polar and sufficiently volatile to be detectable by Gas Chromatography/Mass Spectrometer (GC/MS). The ester bonds of the fatty acids are hydrolyzed and the free fatty acids that are formed in the process are converted to the corresponding FAMEs [34].

In order to derive FAMEs, acid-catalyzed esterification was performed to produce FAME according to the method developed by Christie [35]. The extracted lipid sample up to 5 mg was dissolved in toluene (1 mL) in a screw capped test tube and 1% sulfuric acid in methanol (2 mL) was added. The mixture was left overnight in a

water bath at 50°C. Water (5 mL) containing NaCl (5% w/v) was added and the esters produced were extracted with hexane (2 × 5 mL) using Pasteur pipettes. The hexane layer was washed with water (4 mL) containing $KHCO_3$ (2% w/v) and was dried over anhydrous sodium sulfate. The solution was filtered to remove the drying agent, and the solvent was removed under a stream of nitrogen to complete dryness. Then the residue was reconstituted in hexane and topped-up to a volume of 10.0 mL in a volumetric flask. A volume of 1.0 mL was taken into a vial for identification and quantification of FAMEs using the GC/MS.

In addition, the FAME profile of unused cooking oils (olive oil, coconut oil, palm oil, sunflower oil, soya oil) and animal fats (butter, chicken and lard) were derived in order to compare it with the fatty acid profile of the waste. The same extraction and derivatization procedure was followed for cooking oils and animal fats in order to maintain consistency of experiments.

GC/MS analysis

The GC/MS was used to identify and quantify the complete fatty acid profile present in a lipid sample. The quantification of fatty acids present in a sample was made by the development of a calibration graph by using the FAME Mix, C_8–C_{24} (CRM18918 SUPELCO 100 mg, Sigma-Aldrich). Agilent 7890 B GC equipped with 5977 A Mass Selective Detector, split-less injector with HP-88 capillary column (length 60 m, internal diameter 0.250 mm, film thickness 0.20 µm). The carrier gas was Helium at a constant flow-rate of 1 mL/min. The MS source and MS quadrupole temperatures were set at 230°C and 150°C, respectively. The oven settings consisted a 5 min isothermal period at 140°C, followed by a temperature ramp to 240°C at 4°C/min and 10 min of hold time period. The total analysis time was 40 min.

SSD of FOG by bioaugmentation

Naturally, fungi are in favor of growing on relatively low-moisture conditions with sufficient aeration. SSD nearly resembles natural growth conditions for fungi. In the first stage, the lipid degradation efficiency of each fungus was determined by extracting FOG from grease trap waste and performing SSD under controlled conditions (Table 1), while providing FOG as the main carbon source. The optimum pH for FOG degradation was determined by adjusting pH in the medium (Figure 1). However, the composition of raw grease trap waste is highly variable and thus bringing up its composition to its optimum level (to be degraded by fungi) is not a practical option under field condition. The recorded average pH of the grease trap waste ranged between 4.5 and 6.5. Therefore, in the second stage, SSD was performed with raw grease trap waste (without extracting FOG) and without adjusting the pH (Table 1). It was also noted that the initial moisture/water content of the grease trap waste (substrate) was the main factor influencing the FOG degradation rate of raw waste. The raw grease trap waste (substrate) was a slurry mixture of having moisture/water between 52 and 93 wt % (Table 2). The presence of high moisture/water contents in grease waste samples decrease the porosity and made the mixing difficult with bulking agent and prevented the uniform distribution of spores within the reactor and resulted in poor FOG degradation efficiencies. Therefore, in order to facilitate the aerobic condition (by mixing the waste with bulking agent) and uniform distribution of spores for optimum FOG degradation, experiments were carried out at different initial moisture conditions (Figure 2). However, in the second stage, during SSD, a considerable amount of heat is generated, and as a result, the substrate was subjected to further drying, which may affected the spore germination and growth of the mycelium. Therefore, once the reactor setup is ready to be used, the moisture level within the reacor need to be increased (to around 65%) during the incubation.

With extracted FOG from grease trap waste

SSD was performed on a minimal salt-agar-based medium with extracted FOG. In general, glucose is

Table 1. Composition of SSD medium.

	Conditions	
Parameter	(1) With extracted FOG	(2) Raw grease trap waste
pH	Adjusted to 7 ± 0.1	4.5–6.5
Moisture content of the substrate	<10%	25–35%
FOG content in the substrate	100 µL (1% w/v)	10 mg/g dry weight
Inoculation	A 0.5 cm^2 fraction from a pure culture	1×10^7 spores/mL
Bulking agent	None	1% w/v coir fiber
Temperature	30°C	30°C

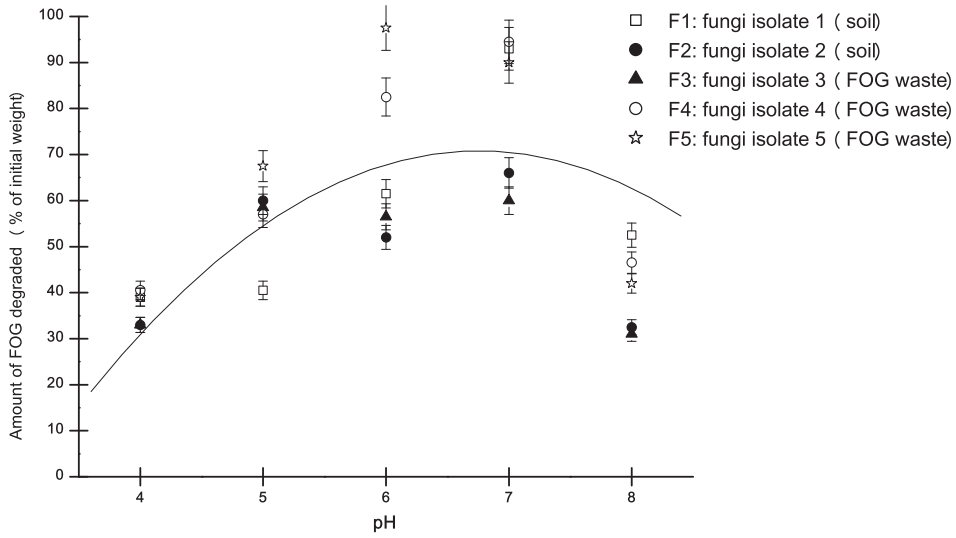

Figure 1. FOG degradation efficiency at different pH levels (Moisture content: ~ 30%).

used as the carbon source in minimal salt medium and the proportion is about 0.2% v/v. In the present study, FOG was used instead of glucose and 100 µL (~1% v/v) was added for each run in order to obtain a better extraction yield of FOG after degradation. The composition of 100.0 mL of the minimal salt-agar medium is K_2HPO_4: 0.7 g, KH_2PO_4: 0.2 g, trisodium citrate ($Na_3C_6H_5O_7$): 0.05 g, $MgSO_4$: 0.01 g, $(NH_4)_2SO_4$: 0.15 g and agar: 1.5 g. Distilled water was added up to the 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 min. 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 previously isolated lipolytic fungal isolates, by taking a fraction (approximately 0.5 cm^2) 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 h. Thereafter, the remaining FOG was extracted according to the method developed by Bligh and Dyer [33] and the weights were recorded. Then derivatization was done to form FAMEs as described above, in order to feed the samples for GC/MS analysis.

Raw grease trap waste (without extracting FOG)

Inoculum preparation for fungi. A fungal spore suspension was obtained by growing a pure culture of particular isolate on minimal 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

Table 2. Physicochemical characteristics of grease trap waste from different locations.

Grease trap waste from different locations		FOG Characteristics (mean ± SD; $n = 3$)		
		pH	Moisture content (wt %)	Total FOG content (wt %)
Small- and medium-sized hotels	W-1	6.1 ± 0.2	78.5 ± 3.5	34.2 ± 2.1
	W-2	5.9 ± 0.2	93.2 ± 1.5	26.5 ± 1.9
	W-3	4.8 ± 0.2	86.5 ± 6.6	29.4 ± 1.5
	W-4	6.5 ± 0.1	90.8 ± 2.1	16.5 ± 1.4
	W-5	4.7 ± 0.2	88.4 ± 4.1	35.4 ± 2.7
Large-sized hotels	W-6	5.6 ± 0.1	85.2 ± 2.6	34.1 ± 2.0
	W-7	5.4 ± 0.1	80.9 ± 5.1	16.3 ± 1.2
	W-8	5.2 ± 0.2	68.9 ± 4.7	37.8 ± 1.8
	W-9	5.3 ± 0.1	74.5 ± 3.7	26.2 ± 2.2
	W-10	5.2 ± 0.2	52.2 ± 1.2	37.1 ± 3.1
	W-11	6.1 ± 0.1	88.6 ± 4.1	18.7 ± 1.3
	W-12	4.5 ± 0.2	90.8 ± 2.1	21.6 ± 1.4
	W-13	5.3 ± 0.2	84.6 ± 3.7	28.4 ± 2.2
	W-14	6.1 ± 0.2	81.7 ± 3.1	19.8 ± 1.6
	W-15	5.5 ± 0.1	79.4 ± 2.8	31.8 ± 3.1

Note: W: grease trap waste; 1–15: location number.

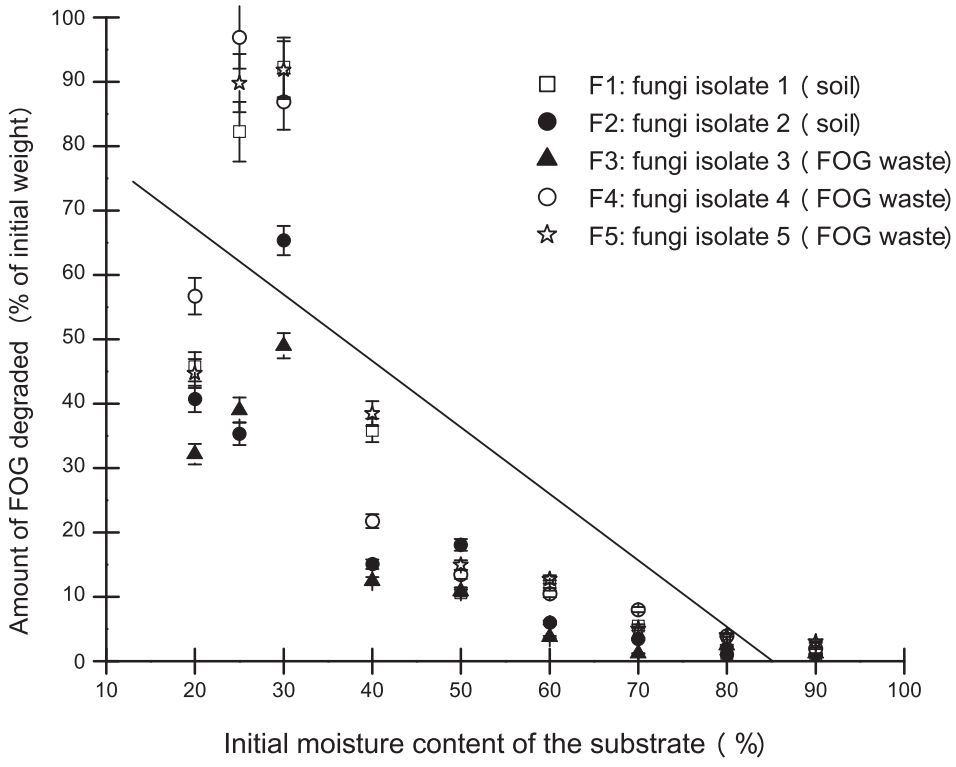

Figure 2. FOG degradation efficiency at different initial moisture levels (at pH 7).

inoculation needle. The cultures were filtered through Whatman® No. 42 filter paper (2.5 µm) into a sterile container. An aqueous spore suspension having approximately 1×10^7 spores per 1 mL was obtained using a hemocytometer. Microscopic and macromorphological characteristics of isolates have been used to identify fungi isolates upto the genus level.

Solid substrate. The grease waste samples dried at 70°C to have different initial moisture levels (10–50%). The optimum initial moisture content was determined (Figure 2) by observing the highest amount of degradation.

Substrate:inoculumn ratio. In most fermentation applications (enzyme production), generally 10^7–10^8 spores/mL is used for 1 g of substrate. In this study, grease trap waste (substrate) had different proportions of FOG (Table 2). Therefore, in order to maintain consistancy of experiments, a quantity of 10 mg of FOG for 1 g dry weight of substrate was used for inoculation by spore suspension of fungi having approximately 1×10^7 spores/mL (Table 1).

Degradation of grease trap waste. SSD was performed with grease trap waste (previously dried to have different moisture levels) by mixing with coir fiber and spore suspension in a tray-type reactor (Figure 3). The incubation was carried out for 72 h by increasing moisture level in the rector by 5% up to 90% by adding 1.9 mL of distilled water per gram dry weight of solid substrate [12] and optimum moisture that is required to be maintained in the reactor was determined (Figure 4). FOG degradation rates of each trial were determined by substracting final FOG content from the initial FOG content of the waste sample according to the method proposed by Bligh and Dyer [33] for total lipid extraction. Finally, FOG degradation rates were determined for all grease trap waste samples (without pH adjustment) with substrate initial moisture condition of 25–30 wt % (before mixing), and incubation was carried out at 30°C for 72 h at the observed optimum moisture content (65%) for reactor operation (Figure 4).

Statistical analysis

The one-way analysis of variance (ANOVA) was performed at 95% confidence interval in order to determine whether there are any statistically significant differences

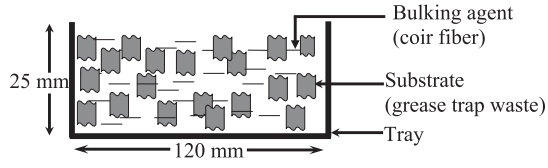

Figure 3. Schematic diagram of the tray-type reactor.

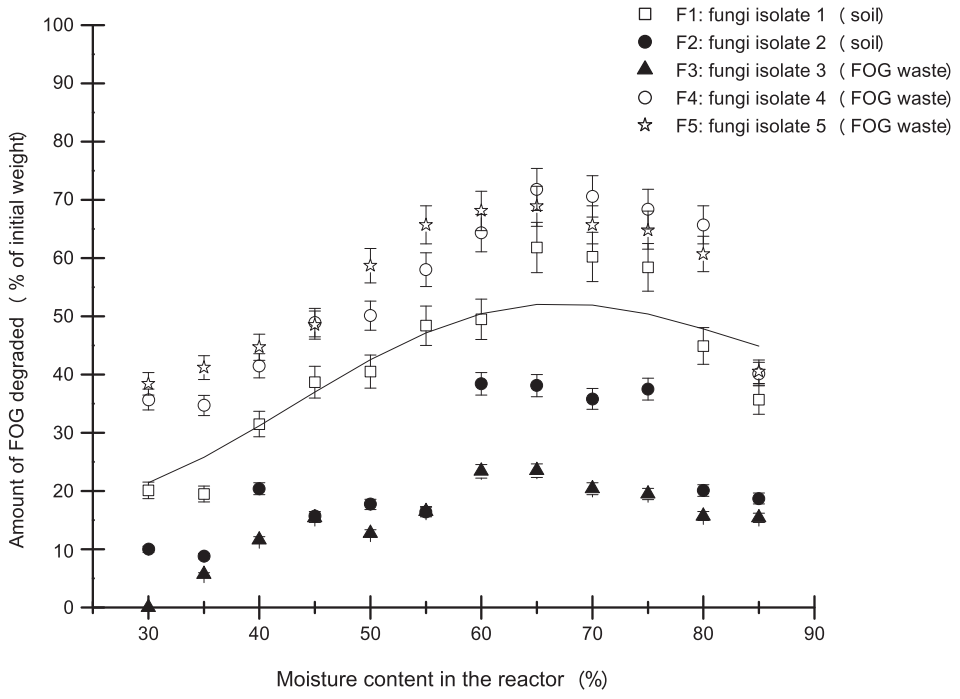

Figure 4. Grease trap degradation efficiency at different moisture levels in the reactor.

between the means of degraded amount of FOG by fungal isolates.

Results

In this section, the physicochemical properties of grease trap waste samples and optimum pH and moisture level for FOG degradation are presented. A comparison was made between the fatty acid profiles of commercial-grade cooking oils and fats, and grease trap waste. The lipid degradation efficiency of grease trap waste, commercial-grade oils and fats by each fungal isolate are also presented.

Physicochemical properties

pH and moisture

The moisture content of grease trap waste samples varied between 52% and 93% (w/w), and the FOG content was varied between 16% and 37% (w/w) (Table 2). The pH of the waste samples was varied between 4.5 and 6.5 (Table 2). The optimum pH was varied between 6 and 7 (Figure 1), and optimum initial moisture level was varied between 25% and 30% (Figure 2) for FOG degradation.

Fatty acid composition of grease trap waste, commercial-grade oils and fats

The main components of cooking oils and animal fats are esters of fatty acids attached to a glycerol molecule. Normally, triglycerides of cooking oils and animal fats consist of different fatty acids and thereby have different physical and chemical properties.

In the present study, on average, palmitic acid 49.5% (w/w) and oleic acid 33% (w/w) were observed to be the most abundant fatty acids present in grease trap waste. Myristic, stearic and linoleic acids had contributed only less than 10% (w/w) to the grease trap waste composition (Table 3, Figure 5). Analysis of commercial-grade oils showed that palmitic acid content was more than 40% (w/w) in palm oil, whereas in olive oil, coconut oil, soya oil and sunflower oil, the palmitic acid content was less than 12% (w/w). Analysis of animal fats showed that palmitic acid content in butter was nearly 40% (w/w), while chicken fats contained 29.5% (w/w) and lard contained 27.5% (w/w) (Table 3). Oleic acid is present at a higher percentage in commercial-grade oils, with more than 70% in olive oil, and about 40% (w/w) in palm oil. In animal fats (chicken and lard), the oleic content was more than 35% (w/w) (Table 3).

SSD of extracted FOG from grease trap waste

The extracted FOG was provided as solid substrate at low-moisture levels in a tray-type reactor to be degraded by lipolytic fungi. The FOG degradation efficiency was determined by taking the initial and final weight of FOG after 72 h (3 days) of incubation time. Detailed studies conducted using the five isolates have shown that the degradation of animal fats by fungi was less

Table 3. Fatty acid profile of commercial-grade cooking oils and animal fats.

	Fatty acid profile as percentage of weight (w/w) (mean ± SD)								
Fatty acid	Olive oil ($n = 3$)	Coconut oil ($n = 3$)	Palm oil ($n = 3$)	Soya oil ($n = 3$)	Sunflower oil ($n = 3$)	Butter fat ($n = 3$)	Chicken fat ($n = 3$)	Lard ($n = 3$)	Oil/grease trap waste ($n = 15$)
Caprylic	0.0*	2.5 ± 1.0	0.0*	0.0*	0.0*	0.3 ± 0.0	0.0*	0.4 ± 0.2	0.0 ± 0.0
Capric	0.0*	4.6 ± 0.7	0.1 ± 0.0	0.0*	0.0*	1.1 ± 0.1	0.0*	0.5 ± 0.3	0.1 ± 0.0
Lauric	0.0*	47.1 ± 2.4	1.0 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	1.9 ± 0.2	1.5 ± 0.1	5.8 ± 0.1	4.1 ± 3.1
Myristic	0.0*	20.2 ± 1.8	1.4 ± 0.1	0.1 ± 0.0	0.5 ± 0.0	11.8 ± 0.5	1.7 ± 0.1	6.1 ± 0.2	3.6 ± 1.4
Palmitic	12.0 ± 0.5	12.2 ± 1.4	42.2 ± 2.1	11.3 ± 0.6	7.5 ± 0.3	39.3 ± 2.0	29.5 ± 1.4	27.5 ± 1.7	49.5 ± 12.0
Palmitoleic	1.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.6 ± 0.0	4.0 ± 0.1	2.6 ± 0.4	0.6 ± 0.1
Stearic	3.7 ± 0.2	3.5 ± 0.2	5.0 ± 0.2	3.1 ± 0.1	3.5 ± 0.7	18.2 ± 0.9	6.9 ± 0.3	9.8 ± 0.6	4.8 ± 1.7
Oleic	78.4 ± 3.9	7.1 ± 0.2	40.0 ± 1.9	24.9 ± 1.1	21.5 ± 1.0	25.8 ± 1.2	35.6 ± 1.7	36.5 ± 1.6	33.0 ± 9.2
Linoleic	4.7 ± 0.2	2.7 ± 0.1	10.1 ± 0.5	60.0 ± 2.8	66.2 ± 3.2	0.9 ± 0.1	20.9 ± 1.0	10.9 ± 0.4	7.0 ± 2.5
Arachidic	0.1 ± 0.0	0.0*	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0*	0.0*	0.0*
Linolenic	0.0*	0.0*	0.0*	4.8 ± 1.2	0.1 ± 0.0	0.8 ± 0.0	1.5 ± 0.1	0.4 ± 0.0	0.0*
Behenic	0.0*	0.0*	0.0*	0.3 ± 0.2	0.5 ± 0.0	0.0*	0.0*	0.0*	0.0*
Erucic	0.0*	0.0*	0.0*	0.0*	0.0*	0.0*	0.0*	0.0*	0.0*
Lignoceric	0.0*	0.0*	0.0*	0.1 ± 0.1	0.1 ± 0.0	0.0*	0.0*	0.0*	0.0*
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

*Below the limit of detection (the average values observed for grease trap waste samples are shown for comparison purposes).

efficient (Table 4) than degradation of cooking oils. Nevertheless, three fungal isolates (F4 and F5) have showed significantly high degradation efficiencies (at the $P < .05$) for cooking oils and animal fats. For each waste type, more than 80% of degradation efficiencies were recorded at least by three fungi isolates within 72 h (Figure 6).

SSD of raw grease trap waste (without extracting FOG)

The raw grease trap waste was provided as solid substrate (after drying to have relatively low-moisture condition between 25% and 30%). About 50% degradation efficiencies were achieved for SSD of raw grease trap waste (Figure 7).

Discussion

Oils and fats are subjected to physicochemical changes during the cooking processes. Also physicochemical properties of grease trap waste are different from one location to another. Therefore, it is important to understand certain properties such as pH, moisture content and fatty acid composition of grease trap waste for development of a feasible technology.

Physicochemical properties of FOG

Oil heated during the frying process may reach temperatures between 150°C and 190°C [36] for a relatively long period of time. Also, the use of same oil repeatedly for economical reasons may cause various physical and

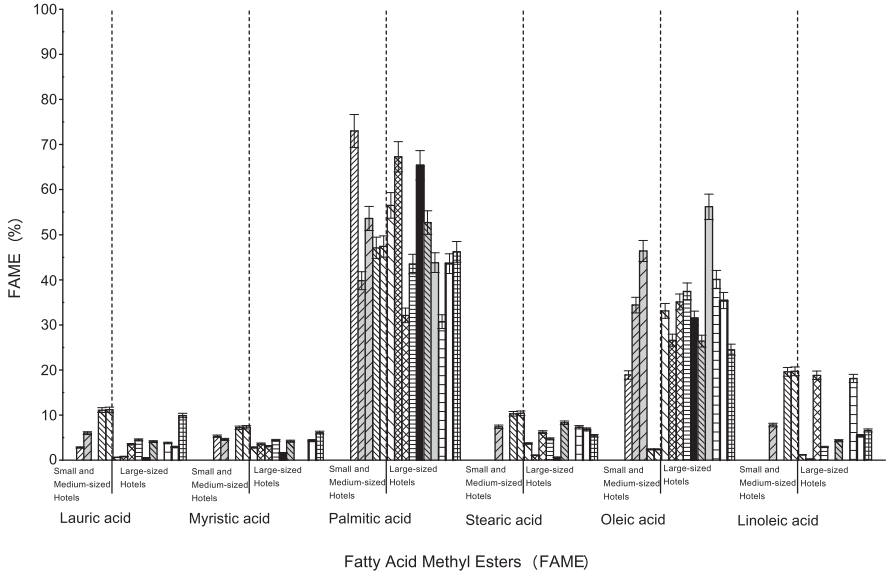

Figure 5. Relative fatty acid percentage (w/w) of grease trap waste (mean ± SD; $n = 3$).

Note: Large-sized hotels: number of rooms >50; small and medium-sized hotels: number of rooms <50.

Table 4. FOG degradation efficiency as a percentage of weight reduction by fungi isolates within 72 h.

	Degradation efficiency by fungi isolates (mean ± SD; $n = 3$)				
Oil/fat type	F1	F2	F3	F4	F5
Olive oil	49.5 ± 3.2	31.4 ± 2.5	31.4 ± 3.8	43.3 ± 5.4	58.1 ± 2.4
Coconut oil	5.4 ± 0.8	4.7 ± 1.2	7.5 ± 1.6	92.0 ± 1.2	64.5 ± 1.7
Palm oil	48.5 ± 1.7	31.4 ± 1.5	19.5 ± 2.5	54.5 ± 2.8	78.1 ± 1.5
Soya oil	5.3 ± 1.2	15.1 ± 1.4	<1	33.2 ± 2.1	35.47 ± 2.4
Sunflower oil	13.4 ± 1.5	31.4 ± 1.2	<1	93.1 ± 6.4	78.9 ± 3.8
Butter fat	<1	<1	<1	10.1 ± 4.1	9.0 ± 2.1
Chicken fat	<1	16.4 ± 1.2	<1	98.0 ± 1.4	96.16 ± 2.4
Lard	<1	9.8 ± 1.2	<1	92.4 ± 2.1	89.12 ± 5.1

Note: F1: fungi isolate 1; F2: fungi isolate 2; F3: fungi isolate 3; F4: fungi isolate 4; F5: fungi isolate 5.

chemical changes in the oil. Some physical changes include increase in viscosity and specific heat and change in surface tension and color [37]. Besides, oils are subjected to thermolytic, oxidative and hydrolytic reactions and result in the formation of many undesired harmful compounds [36] and changes in fatty profile have been cited [38,39].

The physicochemical properties of grease trap waste depend on the type of restaurant, grease trap configuration such as size, inlet/outlet piping, number of baffles [23]. Some physicochemical properties of grease trap waste were investigated in the present study (Table 2). As expected, the average pH of the grease trap waste is slightly acidic (pH 5.5) [4,32] and fatty acid composition was dominated by palmitic acid (~50%) and oleic acid (~33%). However, in reported literature, oleic acid is known to be the abundant fatty acid in food industry wastewater [40]. This is perhaps due to a growing interest in palm oil in Southern Asia while in Mediterranean countries olive oil production is high [41]. Nevertheless, during field visits and discussions with food service industry personnel, it was noted that palm oil (refined, bleached and deodorized) is used as the main oil source for cooking applications. Minor amounts of olive oil are used in the preparation of salads, but the possibility of its presence in wastewater is low. Animal fats, especially chicken fats and lard, may contribute to elevate the levels of palmitic and oleic acid content and may enter the wastewater stream during food preparation (roasting, grilling and frying) and cleaning of ovens, pots, pans and other utensils (Table 3, Figure 5).

Degradation of FOG

Bioaugmentation has emerged as a potential biotechnological application of waste treatment aspects. In this

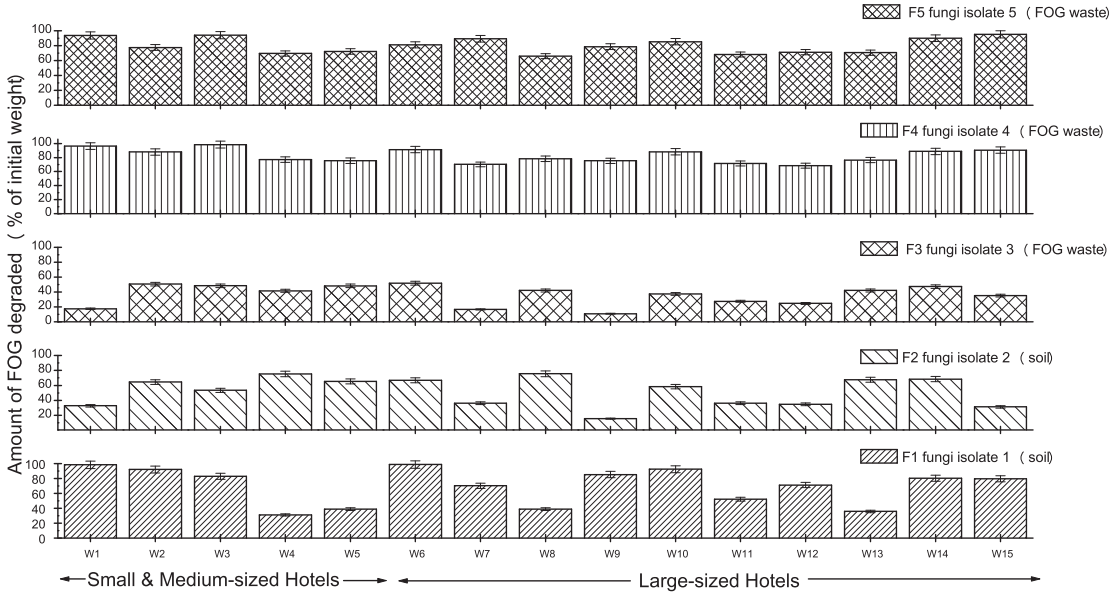

Figure 6. FOG degradation efficiency of selected fungi isolates on extracted grease trap waste (initial oil/fat content: weight of 100 µL volume of oil/fat, incubation time: 72 h, temperature: 30°C.)

Note: W: grease trap waste; 1–15: location number; large-sized hotels: number of rooms >50; small and medium-sized hotels: number of rooms <50.

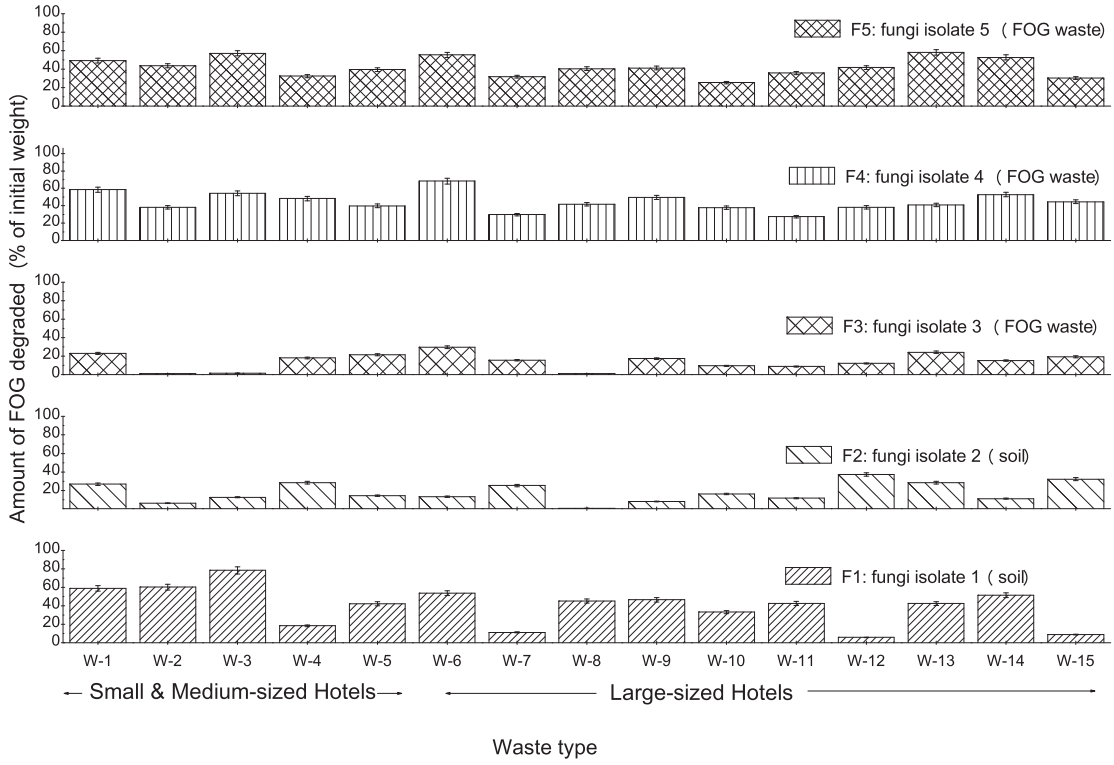

Figure 7. FOG degradation efficiency of selected fungi isolates on raw grease trap waste (initial FOG content: 10 mg/g dry weight, incubation time: 72 h, temperature: 30°C).

Note: W: grease trap waste; 1–15: location number; large-sized hotels: number of rooms >50; small and medium-sized hotels: number of rooms <50.

study, SSD by lipolytic fungi is proposed as novel approach to bioaugmentation. Grease trap waste was used as a substrate for lipolytic microorganisms. As described in the methodology, fungi are grown on solid surfaces (in a tray-type reactor), which were 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 can be used as an inoculum for the second set 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 6–7 at 30°C for selected fungi isolates. However, in literature lipolytic fungi have shown optimum FOG degradation for different pH values: at pH 2.5 and at 45°C [42]; at pH 5 and at 37–40°C [43]; at pH 5 and at 30°C for synthetic substrate (triacylglycerols) and for natural substrate (oil) at pH 7 and at 30°C [44]; at pH 8.5 and at 40°C [45]; at pH 7–9 and at 30–45°C [46]. Although, the recorded pH of grease trap waste varied from 4.5 to 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 6–7. 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 moisture content at the required level in the reactor has become a critical factor for better degradation of grease trap waste. The observed optimum moisture content for degradation of grease trap waste was 65%. In the reported literature, almost similar moisture levels were observed as optimum moisture content for lipolytic fungi, which was 65% [14,47] and 50–55% [48].

The results indicated that at least three fungal isolates that were used in the present study were able to reach a degradation efficiency of around 50% for raw grease trap waste (Figure 7). The fungal isolates were identified as *Penicillium* sp., *Aspergillus* sp. and *Rhizopus* sp. As the full identity is not available, these were considered as level-II organisms. However, as spore inhalation of fungi can lead to allergic reactions, the use of personal protective ware is normally recommended.

The observed degradation efficiencies for raw grease trap waste were less than the values obtained under

optimum conditions for FOG degradation (Figures 6 and 7). Also, there is a significant potential to implement this developed low-cost biotechnological application in pilot/full scale due to its high degradation efficiency.

Conclusions

The study showed that there is a significant potential for implementation of this developed methodology as biotechnological application to degrade FOG in the food service industry. Therefore, in conclusion the following key points can be listed:

- Grease trap waste can be rapidly degraded using lipolytic fungi under low-moisture conditions by using SSD.
- Low-cost agricultural waste can be used as a bulking agent to ensure proper aeration.
- FOG degradation efficiencies around 50% can be achieved within 72 h with raw grease trap waste without adjusting the pH.

Disclosure statement

No potential conflict of interest was reported by the authors.

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