Monitoring degradation of restaurant wastewater by *Lysinibacillus sphaericus* C3-41

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**ABSTRACT**

This study investigates the biodegradation abilities of *Lysinibacillus sphaericus* C3-41 in restaurant wastewater. The extent of biodegradation was monitored using Fourier Transform Infrared Spectroscopy (FTIR) and Fatty Acid Methyl Ester (FAME) analysis. There was significant reduction in mineral content \((p < 0.05)\) of the wastewater after 6 days incubation while the highest growth (1.4 OD600) and lipase production (50mMol/min) was observed on the 5th day. About 2.5% (v/v) oil concentration and pH 8 were found to be optimal conditions for growth and lipase production, while a temperature of 30\(^{\circ}\)C, and 1% ammonium phosphate and glucose were detected as optimum nitrogen and carbon sources respectively. FAME analyses revealed the presence of some fatty acids such as palmitic acid (11.56 ppm), caprylic (11.22 ppm), caproic (11.19 ppm), lauric acid (9.95 ppm), undecanoic (4.77 ppm) and cyclopropane fatty acid (CFA, 4.34 ppm) in the fresh domestic wastewater. After biodegradation, there was an increase in the concentration of capric (0.39 ppm), lauric (0.33 ppm), palmitoleic (0.78 ppm), trans13 octadecanoic (1.00 ppm) and cyclopropanoic (2.53 ppm) in *L. sphaericus* C3-41. Also, reduction in concentration of some fatty acids components in the wastewater probably eventually resulted in their bioaccumulation in the bacteria cell. FTIR spectra revealed the changes in functional groups qualitatively and quantitatively with evidence of wastewater biodegradation by *L. sphaericus* C3-41. The prominent –NH stretching bonds at 3740 cm\(^{-1}\) from the primary and secondary amines completely disappeared after the 6th day, indicating chemical reduction of –NH bonds leading to liberation of NH\(_3\)(g). This study thus confirms the bioremediation potentials of *L. sphaericus* C3-41 for domestic and restaurant wastewater.

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1. Introduction

Wastewater discharges by food industries contain complex organic pollutants with high concentrations of suspended solids such as oil, food, dissolved organics and some surfactants (Pooja, Kumar, Singh, & Patil, 2020). Lipids are large and diverse group of naturally occurring organic compounds related by their solubility in non-polar organic solvents such as ether, chloroform, acetone and benzene (Guschina & Harwood, 2013). Hydrophobic organic pollutants such as lipids consume a large amount of soluble oxygen in water bodies, worsening the water quality and doing great damage to the aquatic ecological system (Elshorbagy & Chowdhury, 2013).

Bioremediation of lipid-contaminated sites is important because of the health and environmental threats posed by the contamination. Much attention has been paid to the removal of these organic compounds from wastewater through biological methods, owing to its economic and ecologic superiority. Biodegradation have been employed in wastewater treatment in a bid to find sustainable ways to clean up contaminated water (Unuofin, Okoh, & Nwodo, 2019). Biodegradation rate and the extent of removal of these organic substances partly depend on the characters of such substances in the waste, some of which have relatively high-water solubility, low acute toxicity and are easily degradable (Zheng, Li, Zhao, Zhou, & Fu, 2013).

In Nigeria, the indiscriminate disposal of lipid rich wastewater into the environment with inadequate waste management techniques have led to various forms of wastewaters constituting environmental pollution in the ecosystem (Odeyemi, Aderiye, & Bamidele, 2013). The scale of this problem and the recalcitrance of some lipids to degradation have necessitated the investigation into effective methods for biodegradation of lipids. Microorganisms especially bacteria play a significant role in the biological decomposition of materials. Several reports have explicated that biological...
treatment has become an important biotechnological application in the treatment of industrial and domestic wastewater containing recalcitrant organic pollutants (Canler et al., 2001; Silva-Bedoya et al., 2016). There is a need however to optimize culture conditions as biodegradation under aerobic conditions can be relatively inefficient and slow when applied to dense concentrations of lipid contaminants (Ibrahim et al., 2020).

*Lysinibacillus sphaericus* is a gram-positive, aerobic, sporulating, and functionally diverse bacterial species. Its biotechnological potential has been established in the areas of toxic metals bioremediation (Bafana, Chakrabarti, & Krishnamurthi, 2015; Rahman et al., 2015) and xenobiotics degradation (Bahuguna, Lily, Munjal, Singh, & Dangwal, 2011; Hu et al., 2014; Misal, Lingojwar, Lokhande, Lokhande, & Gawai, 2014). *L. sphaericus* has been found to degrade lipids that cause environmental problems and in previous investigations, have been found to possess potentials for bioremediation of restaurant wastewater (Aderiye & Sulaimon, 2017; Shah, Hasan, Hameed, & Ahmed, 2008).

The trending application of FTIR spectroscopy for the investigation of biological systems can be attributed to the rapid, sensitive, non-destructive and time saving features of the technique in detecting changes in wide range of functional groups and molecular structure, and providing information on the basis of chemical composition and physical state of the sample (Aderiye, Odusoga, & Adebayo, 2020; Ajayi et al., 2019). Since environmental pollution by lipid rich wastewater has become a serious issue, causing waste management problems, there is a need to develop a veritable bacteria strain that can be employed in bioremediation of restaurant wastewater (Aderiye & Sulaimon, 2017; Shah, Hasan, Hameed, & Ahmed, 2008).

The materials and methods section of the paper begins with a description of the source of wastewater and microorganism. Five hundred millilitres (ml) of domestic wastewater from Portofino eatery, Ado-Ekiti was collected in a sterile Eberbach (0379W28) 1-Litre sterile stainless-steel container, between 12:30 and 1:00 pm on each sampling day. The sample was conveyed to the laboratory in the Department of Microbiology, Ekiti State University for further work. *Lysinibacillus sphaericus* C3-41 previously isolated from domestic oil rich wastewater was used for this study (Odeyemi et al., 2013). A culture suspension with an optical density of 0.5 at 550 nm which equates 10⁶ CFU/ml was used as the population size except otherwise stated.

### 2. Materials and methods

#### 2.1. Source of wastewater and microorganism

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#### 2.2. Determination of inorganic mineral content (Na⁺, K⁺ and Ca²⁺ ions) of domestic wastewater using flame atomic absorption spectrophotometer

The inorganic mineral content (Na⁺, K⁺ and Ca²⁺ ions) of the domestic wastewater was estimated by flame atomic absorption spectrophotometer (model 760AA) using the method described by Keith (1996). Analysis of variance (one way ANOVA) was carried out to determine if there was significant reduction in the values after treatment.

#### 2.3. Gc-MS determination of fatty acids methyl esters (FAMEs) in domestic oil rich wastewater and *L. sphaericus* C3-41

Fatty Acid Methyl Ester reference standards, purchased from AccuStandard (Catalog Number FAMQ005) were used to prepare 4-point serial dilution calibrator standards (5, 10, 50, 100 ppm). After calibration, the samples were analyzed and the corresponding FAMES concentration obtained as previously analyzed by (Carvalho, 2005).

#### 2.4. Estimation of lipase activity and microbial growth

Five millilitres (5 mL) of culture suspension was centrifuged at 5000 rpm for 10 minutes. The optical density of the cells was measured with spectrophotometer at OD reading of 600 nm. Cell free supernatant was used as the crude enzyme for assay, and stored at 4°C until needed. Lipolytic activity was determined by colorimetric method based on the activity in cleavage of p-nitrophenyl palmitate (p-NPP) at pH 8.0. One unit of lipase activity was defined as 1 μmol of p-nitrophenol (p-NP) released per minute by 1 mL of enzyme (Khemika, 2012).

#### 2.5. Effect of environmental conditions on growth and lipase production by *L. sphaericus* C3-41

The environmental conditions investigated include (i) incubation time (hr.), (ii) oil concentration (iii) incubation temperature (iv) pH of medium (v) nitrogen source, (vi) carbon source and (vii) inorganic salts. In each case, 1 ml of bacteria culture which equals 10⁶ CFU/mL was added to the medium while estimation of growth and lipase activity were determined as previously described by Khemika (2012),
2.5.1. Growth and lipase production by L. sphaericus C3-41 at different incubation times

The method previously described by Guerzoni et al. (2001) was used. One millilitre (1 mL) of the cell suspension was inoculated into a 4 mL nutrient broth and incubated at 35°C for 6 days. Samples were collected every 24 h to check the production of lipase and determine growth. Five millilitres (5 mL) of the cell suspension were withdrawn each day and centrifuged at 10000 rpm for 30 min. The supernatant was used for measurement of lipase activity and the optical density of the cells.

2.5.2. Growth and lipase production by L. sphaericus C3-41 at varying oil concentrations

Different concentrations of sterile palm oil were tested on growth and lipase production by L. sphaericus C3-41 in Nutrient broth medium (2.5, 5.0, 7.5, 10.0, 12.5 and 15.0% w/v) as described by Guerzoni et al. (2001) and incubated at 35°C for 48 h. Five millilitre (5 mL) of the cell suspension was withdrawn and centrifuged at 10000 rpm for 30 min. The supernatant was used for lipase activity and cell mass estimation.

2.5.3. Effect of temperature and pH on growth and lipase production by L. sphaericus C3-41

The optimum temperature for lipase activity was determined at different temperatures at intervals of 10°C, between 20°C and 80°C for 24 h. Meanwhile, the test tube containing 10 mL each of mineral salts medium prepared in phosphate buffer of pH 6.0, 7.0, 8.0 and 9.0 was inoculated separately with loopful of 24 h old culture of the organism and incubated at 30°C (Aderiye et al., 2019). Growth and optimum lipase production were monitored at pH interval of 1. Bacteria cell mass and lipase activity were estimated as above.

2.5.4. Effect of various nitrogen and carbon sources on growth and lipase production by L. sphaericus C3-41

The effect of ammonium nitrate, ammonium nitrite, ammonium sulphate, ammonium chloride, ammonium carbonate and ammonium phosphate and some carbon sources such as glucose, fructose, galactose, maltose, sucrose and lactose each was examined on the growth and lipase production by L. sphaericus C3-41 in the broth medium using the method of Aderiye et al. (2019).

2.5.5. Effect of metals and salts growth and lipase production by L. sphaericus C3-41

The effect of metals such as calcium and magnesium ions and salts (calcium sulphate and sodium chloride) were examined on the growth and lipase production by L. sphaericus C3-41. Bacteria cell mass and lipase activity were estimated as above.

2.5.6. Biodegradation of wastewater at optimized conditions using L. sphaericus C3-41

From earlier experiments, one milligram per mole (1 mg/Mol) of ammonium phosphate (NH₄)₂PO₄, 2.5% v/v palm oil concentration, 0.5 mg/Mol of glucose, 1 mg/Mol of magnesium and 1 mg/Mol of calcium sulphate, which exhibited optimal growth parameters were mixed. One millilitre of the bacterial cell suspension was inoculated into a 9 mL medium and incubated for 5 days at 30°C. After the 5th day, 5 mL of the cell suspension was inoculated into 15 mL sterile wastewater in a 250 mL conical flask and later incubated for two, four and six days respectively. The wastewater sample (0.02 mL) from each day was analysed with Fourier Transform Infrared Spectroscopy (FTIR) as previously analyzed by Aderiye and Sulaimon (2017).

2.7. Fourier transform infrared spectroscopy analysis of wastewater, isolate and treated wastewater

The wastewater, L. sphaericus C3-41 and the inoculated wastewater samples were analyzed by an infrared spectrometer (Varian 660MidIR Dual MCT/DTGS Bundle with ATR. The spectra were recorded within a scanning range of 4000–400 cm⁻¹ at a resolution of 4 cm. The FTIR spectrum of the control was finally subtracted from the spectra of the various samples. Infrared spectrum was Fourier transformed and recorded in the absorption mode. IR solution software was employed for getting the spectrum as previously analyzed by Aderiye et al. (2019).

3. Results and discussion

The specific nutritional and environmental factors considered in this study for large scale production of lipase by L. sphaericus C3-41 agree with those of Sulaimon, Aderiye, and Odeyemi (2018). There was a significant reduction in concentration of Ca²⁺, K⁺.
and Na⁺ ions in the fresh wastewater by 20%, 33.64% and 6.8% respectively after 6 days of incubation with the bacteria isolate (p < 0.05) (Table 1). Sulaimon et al. (2018) reported a similar reduction in the mineral content of restaurant wastewater following degradation and attributed to bioaccumulation of the minerals by the organism and used as a cofactor in enzyme production. Aderiye and Sulaimon (2017) reported that assimilation of Mg²⁺ significantly stimulated enzyme production.

The optimization of the culture medium played a critical role in enhancing growth and lipase production by L. sphaericus. Ilesanmi, Adekunle, Omolaiye, Olorode, and Ogunkanmi (2020) proposed the need for optimization of culture conditions prior to enzyme production by microorganisms in fermenters as was done in this study. The effect of incubation time on growth and lipase production was determined and it was observed that the maximum lipase activity (50 mMol/min) was observed on the 5th day of incubation (Figure 1). A similar observation was reported by Sulaimon et al. (2018).

Lipases are generally produced in the presence of a lipid such as oil or any other inducer such as triacylglycerols, fatty acids, hydrolysable esters, bile salts and glycerol. Furthermore, maximum lipase activity (80 mMol/min) was achieved when the growth medium was supplemented with 2.5% palm oil (Figure 2). Misbah and Haq (2014) reported that 2% olive oil served as an inducer for lipase production by Penicillium fellutanum. However, increasing oil concentration brought about significant reduction in the growth of L. sphaericus C3-41 in this work. The maximum production of lipase at low oil concentration may be due to availability of sufficient oxygen for the organism to thrive in the basal medium. Oxygen becomes much available as an electron acceptor because L. sphaericus C3-41 thrives well wherever oxygen is available (Odeyemi et al., 2013).

One major physical parameter that influenced bacterial growth and lipase production was the pH of the culture medium. Optimum lipase activity by L. sphaericus C3-41 was also observed at pH 8.0 as shown in Figure 3. Bora and Kalita (2006) reported a similar pH for production of thermostable lipase by Bacillus sp. LBN 4 at 30 °C. L. sphaericus also grew best between 20 °C and 40 °C (1.2 at 20 °C and 1.65 at 30 °C OD₅₀₀) with the optimum temperature required for lipase production and maximum growth observed at 30 °C, with a value of 82 mM/Min and 1.65 at 30 °C OD₅₀₀ (Figure 4). Slight changes in growth temperature have been observed to affect enzyme production (Furini, Berger, Campos, van der Sand, & Germani, 2018). Amin and Bhatti (2014) noted that higher temperatures were detrimental to microbial growth and enzyme formation, due to large amounts of metabolic heat generated in the fermenting substrate. However, Ugras and Uzmez (2016) reported optimal lipase production by Acinetobacter sp. strain SU15 at 40 °C.

Generally, microorganisms produce high amount of lipase when organic nitrogen sources such as peptone and yeast extract are used for lipase production (Biniarz, Coutte, Gancel, & Łukaszewicz, 2018). Among the six inorganic nitrogen sources tested, optimum growth and lipase reduction (70 mMol/min) by L. sphaericus C3-41 was exhibited in ammonium phosphate (NH₄)₂PO₄ supplemented growth medium (Figure 5). Meanwhile, glucose supported optimum lipase activity (76 mMol/min) among the carbon sources used (Figure 6). This report agrees with Hasan, Shah, and Hameed (2006) who opined that the major factor for the expression of lipase activity has always been the carbon and nitrogen sources since lipases are inducible enzymes. These enzymes are generally produced in the presence of lipid or any other inducer. Awad, Mostafa, Danial, Abdelwahed, and Awad (2015) reported that lipase activity was enhanced in the presence of calcium and magnesium because these divalent ions often stimulate lipase activity in Bacillus cereus due to the formation of calcium and magnesium salts of long chain fatty acids. In this study, calcium sulphate encouraged maximum lipase activity (53 mMol/min).
The FAME analysis report of the concentrations of fatty acid components of the wastewater and the isolate are shown in Tables 2 and 3. In Table 2, Cis-10 heptadecenoic acid and Palmitoleic acid were not detected in the fresh wastewater but were detected after biodegradation (4.74 ppm and 6.60 ppm respectively). Odeyemi, Aderiye, Adeyeye, Donbraye, and Faleye (2014) opined that the new fatty acids formed after degradation must have been formed from other underutilized fatty acids that were broken down by L. sphaericus. Cyclopropane fatty acids (CFAs) contain a cyclopropane ring in the acyl group and usually present in low amounts in plants or bacteria Czerwiec et al., 2019). These FAs exhibit long-term resistance to oxidation and low temperature fluidity. Mass determination and MS spectra confirmed that these CFAs contain C17:0ca, and C19:0ca (Merlier, Imatoukene, Octave, Nicaud, & Thomasset, 2018) and correspond to the transformation of the C16:1 and C18:1 FA into C17:0ca and C19:0ca, respectively, through CFAs activity. The increase in palmitic acid after degradation is similar to the observation made by Sulaimon and Aderiye (2018) that some fatty acids increased in amounts compared to the initial amount before degradation.

Furthermore, they disclosed that the concentration of some saturated fatty acids (SFAs); C18:0, C20:0, C22:0 and C24:0 increased after degradation by the organism. Pereira, Pires, Mota, and Alves (2002) also reported the appearance of palmitic acid in wastewater after a biological treatment process and concluded that it is a by-product of biodegradation of oleic acid present in the wastewater. Sulaimon and Aderiye (2018) also observed that palmitic acid (C16:0) and stearic acid (C18:0) constituted the largest amount of the SFAs, approximately 34.69% and 8.07% respectively, while the concentrations of lauric acid, myristic acid, arachidic acid, behenic and lignoceric acids were very low (about 0.1071%, 1.7247%, 0.0230%, 0.0212%, and 0.0026% respectively). These results agree with Chipasa and Medrzycka (2006) who noted that the utilization rate of fatty acids differs as it is dependent on the length and degree of unsaturation of their carbon chains. The concentration of Cis-10 heptadecenoic acid initially in L. sphaericus C3-41 was 8.49 ppm and after degradation was 5.32 ppm which suggests that the bacterium must have lost 3.17 ppm of the fatty acid into the wastewater.

The decomposition of the domestic oil in the wastewater may be primarily dependent on the lipolytic ability of L. sphaericus C3-41 as suggested by Odeyemi et al. (2014). Some researchers have also reported that fatty acids accumulate in biological
wastewater treatment systems (Beccari, Majone, & Torrisi, 1998; Lalman & Bagley, 2001; Salminen, Rintala, Lokshina, & Vavilin, 2000). Therefore, since biodegradation and biosynthesis of fatty acids occur inside microbial cells, the increase in fatty acids content showed that they were released into the wastewater as microbial by-products (Kunau, Dommes, & Schulz, 1995) as shown in Table 3. At the same time, decrease in their contents show that fatty acids were subsequently consumed by the organisms as substrates. Fatty acids such as C20 (Arachidic acid), C22 (Behenic acid) and C24 (Lignoceric acid) were not detected in the organism. Matatkova et al. (2017) reported that microorganisms with high lipid content normally possess large amount of lipid with carbon chain between C12-C18 and very high palmitic acid. Palm oil has more palmitic acid and less of other fatty acids as compared to other vegetable oils (Odeyemi et al., 2014) showing that the persistent growth of L. sphaericus C3-41 during degradation might be due to the presence of some food debris in the wastewater which might have served as ready source of nutrients in spite of the presence of detergent, a deleterious substance for the microbe. Pallavi, Ravikumar, and Ram Reddy (2015) also reported that the presence of lipase catalyzed hydrolytic and inter esterification reactions depending on the source of lipase and reaction conditions.

FTIR spectrophotometer was used to assess the changes in the functional groups qualitatively and quantitatively. Evidence of biodegradation by L. sphaericus C3-41 on wastewater was monitored over a period of six days with intermittent FTIR analysis of the microorganism, freshwater and biodegraded wastewater after 2, 4 and 6 days respectively and the results are shown in Figures 7–11. The characteristic absorption bands indicative of different functional groups was employed to monitor the biodegradation of the wastewater at 2, 4 and 6 days respectively using the absorption bands observed for the microorganism and the fresh water as baseline, with the disappearance of bonds and the appearance of new ones as evidence of biodegradation.

Some of the prominent bonds are presented below:

- NH stretching bonds at 3740 cm⁻¹ from the primary and secondary amines completely disappeared after the 6th day, which is indicative of chemical reduction of −NH bonds leading to liberation of NH₃(g). Similarly, the prominent −NH stretch band at 3403 cm⁻¹ in the wastewater persisted till the 2nd day but disappeared completely

### Table 2. Concentration (ppm) of Fatty acid components of restaurant wastewater before and after degradation by L. sphaericus C3-41.

| S/N | Fatty acid component | Weight (ppm) before degradation | Weight (ppm) after degradation | Difference |
|-----|----------------------|---------------------------------|--------------------------------|------------|
| 1   | Caproic acid         | 11.19                           | 11.10                          | −0.09      |
| 2   | Caprylic acid        | 11.22                           | 11.23                          | 0.01       |
| 3   | Capric acid          | 10.87                           | 11.23                          | −0.36      |
| 4   | Undecanoic acid      | 4.77                            | 4.83                           | 0.06       |
| 5   | Lauric acid          | 9.95                            | 10.21                          | 0.26       |
| 6   | Tridecanoic acid     | 4.68                            | 11.86                          | 0.30       |
| 7   | Palmitic acid        | 11.56                           | 11.86                          | 0.30       |
| 8   | Hexadecanoic acid    | 4.51                            | 7.78                           | 3.27       |
| 9   | Stearic acid         | 9.78                            | 7.78                           | −2.00      |
| 10  | Trans13 octadecanoic acid | 4.21                          | 4.21                           | 0.01       |
| 11  | Cyclopropanoic acid (C17) | 4.34                          | 4.24                           | −0.10      |
| 12  | Cyclopropanoic acid (C19) | 4.29                          | 4.29                           | 0.00       |
| 13  | Palmitoleic acid     | 6.60                            | 6.60                           | 0.00       |
| 14  | cis-10 heptadecanoic acid | 4.74                          | 4.74                           | 0.00       |

### Table 3. Concentration (ppm) of Fatty acid (FA) components in L. sphaericus C3-41 before and after degradation of wastewater.

| S/N | (FA) component (ppm) | Before degradation | After degradation | Difference |
|-----|----------------------|--------------------|-------------------|------------|
| 1   | Caproic acid         | 11.26              | 11.27             | 0.01       |
| 2   | Caprylic acid        | 11.39              | 11.23             | −0.16      |
| 3   | Capric acid          | 10.75              | 11.14             | 0.39       |
| 4   | Undecanoic acid      | 5.60               | 4.65              | −0.95      |
| 5   | Lauric acid          | 9.85               | 10.18             | 0.33       |
| 6   | Tridecanoic acid     | 3.95               | 3.91              | −0.04      |
| 7   | Tridecanoic acid     | 4.63               | 4.51              | −0.12      |
| 8   | Pentadecanoic acid   | 2.45               | 2.45              | 0.00       |
| 9   | Palmitic acid        | 11.53              | 11.55             | 0.02       |
| 10  | Hexadecanoic acid    | 11.75              | 3.22              | −8.53      |
| 11  | Stearic acid         | 7.82               | 7.54              | −0.28      |
| 12  | Stearic acid methyl  | 4.46               | 4.46              | 0.00       |
| 13  | Trans 13 octadecanoic acid | 10.60            | 11.60             | 1.00       |
| 14  | Cyclopropanoic acid (C17) | 5.31              | 7.34              | 2.53       |
| 15  | Cyclopropanoic acid (C19) | 4.22              | 4.80              | 0.58       |
| 16  | Cyclopropanoic acid (C19) | 5.30              | 5.30              | 0.00       |
| 17  | Palmitoleic acid     | 8.49               | 5.32              | −3.17      |
| 18  | 11-hexadecanoic acid | 10.09              | 9.24              | −0.85      |
by the 4th and 6th day in the wastewater. It should be noted that the prominent bands at 2361 cm\(^{-1}\) (86.1\%) for N-H; and the 1574 cm\(^{-1}\) (36.6\%) associated with either the NH, C=\(\equiv\)C and or C=\(\equiv\)N plane bending vibrations of aromatic rings originated from the microorganism (\textit{L. sphaericus} C3-41) injected for the experiment and not from the wastewater. This accounted for their prominence on Day 2, Day 4 and Day 6 respectively. The presence of characteristic absorption peaks of peptides, lipids, polymeric substances, and carboxylic acids may be referred to presence of functional groups in the microorganism’s organelles and EPS (Extracellular Polymeric Substances) (Liu & Fang, 2002; Gulnaz, Kaya, & Dincer, 2006).

The -OH bonds are paramount and distinctive for the hydroxyl bonds in water, carbohydrates, alcohol and carboxylic acids. The -OH vibrational bands at higher wave numbers 3842 cm\(^{-1}\) (98.7\%) and 3476 cm\(^{-1}\) (89.2\%) subsisted after six days due to the strong hydroxyl bonds and H-bonded -OH (stretching) bonds. However, -OH (s) bonds at lower wave numbers disappeared completely at the sixth day. This applies to O-H (s) bond at 3154 cm\(^{-1}\) (92.4\%), -OH (bending) at 1845 cm\(^{-1}\) (94.4\%) and the 1043 cm\(^{-1}\) (87.8\%) which is due to the -OH bonds of carboxylic acid. The disappearance is a product of oxidation of the -OH bonds from the carboxylic acid and primary alcohols. Sheik, Chandrashekar, Swaroop, and Somashekarappa (2015) observed that

**Figure 7.** FT-IR spectra of fresh wastewater showing the elution peaks and its intensity.

**Figure 8.** FTIR spectra of \textit{Lysinibacillus sphaericus} C3-41.
these different changes have been attributed to hydrolysis and oxidation of bonds in the LDPE polymer by microorganisms.

The stretching vibrational bands of the carbonyl functional groups occurring at higher wave bands were completely degraded. For example, the prominent band at 1668 cm$^{-1}$ (81.8%) appearing on the second day was left with an ill formed band at 1743 cm$^{-1}$ (7.1%) after the sixth day. The absorption peaks in range 1754-1710 cm$^{-1}$ observed was connected with vibration of $\text{C}=$O bonds characteristic for carboxylic groups (Wharfe, Jarvis, Winder, Whiteley, & Goodacre, 2010). There was however the development of a poorly formed new $\text{C}=$O band (stretching) at 949 cm$^{-1}$ (39.2%). All $\text{C}=$O bonds were completely degraded after six days. Evidence of
4. Conclusion

This study reports the efficiency of *L. sphaericus* C3-41 as a good bioremediation agent for treating restaurant and lipid rich wastewater. Also, certain environmental factors such as pH, temperature, incubation time and aeration rate were observed to play major role during enzyme production and metabolic activities. Such studies are aimed at assessing the potency of microbial isolates which could be helpful in degrading lipid-rich wastewater. The GC/MS analyses revealed the bioaccumulation of some of the fatty acid components present in the wastewater as well as a selective removal of some fatty acid components. The results also succinctly revealed the use of FTIR technique in the characterization of chemical structures as well as in identification of various forms of fatty acids. It is also useful in monitoring quantitative changes in restaurant wastewater during biodegradation.

Disclosure statement

No potential conflict of interest was reported by the authors.

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