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Characterization and Degradation of Fatty Acid Methyl Esters Generated from Domestic Wastewater

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Abstract: *Fatty acids (FAs) are key component of lipids; and the physical, chemical and physiological properties of a lipid class depend primarily on its fatty acid composition. Fats and oils (FOs) in wastewater create problems including the production of foul odours, the blockage of sewer lines and may interfere with the proper operation of sewage treatment works. Removal of FOs from wastewater is thus critically important to ensure that wastewater is disposed of efficiently and economically. In this study, the fatty acids present in the domestic waste were characterized and enumerated. Ability of Lysinibacillus sphaericus C3-41 to degrade fat/oil under laboratory conditions was then investigated. Gas chromatography method was used for the identification of microbiological degraded fatty acids in the vegetable oils as methyl ester. The domestic oil analyzed for fatty acids revealed vast amount of fatty acids (FAs) including myristic, palmitic, stearic, oleic, linoleic, linolenic, behenic and lignoceric, which have specific carbon number and their values in approximate percentage are C14:0 (1.7), C16:0 (34.7), C18:0 (8.1), C18:1 (16.1), C18:2 (23.7), C18:3 (0.02), C22:0 (0.02) and C24:0 (0.01) respectively. Palmitic acid percentage was high in the oil which contained considerable amount of 34.7% whereas percentage reduction of oleic acid was highest among the fatty acids. The total reduction of fatty acid by Lysinibacillus sphaericus C3-41 after 24 hrs was 8.2%. Lipase from Lysinibacillus sphaericus C3-41 had potential for degradation of fatty waste. It could therefore be employed in environmental cleanup of oil spill site.*

Keywords: *Biodegradation; Domestic waste; Fatty acid; Lysinibacillus sphaericus*

1. INTRODUCTION

Extensive attention has been shifted to the management of environmental pollution resulting from toxic and hazardous materials release from municipal and restaurant effluents (Amina *et al.*, 2004). Municipal effluents are waste liquids that are composed of several organic and inorganic compounds [1].

Vegetable oils are oil extractions from plants and fruits such as palmnut, sunflower, soybean, coconut, rapeseed, canola olive, castor and corn [1]. Vegetable oils are harmful to the environment, like petroleum oils they produce similar environmental effects. Hence, it is a known fact that the vegetable oil causes the production of large quantity of vegetable mill waste as byproducts to the environment (Raskin and Ensley, 2000). These by-products are harmful to the environment, both terrestrial and aquatic (Al-Garru, 2005).

Many regions in Nigeria are great producers of vegetable oil, however, many other countries are now producers, others used to be high producers of oil and these countries had faced and present producers will continue to face the environmental problems generated by oil mill wastes.

Vegetable mill wastes could be treated physically, chemically, biologically or combination of the technologies. Biological treatment or bioremediation usually are considered the most environmental friendly and the least expensive [2]. Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site [3]. Bioremediation occurs either under aerobic or anaerobic conditions. A number of different species of bacteria, yeasts, molds and mushrooms have been tested in different conditions to treat olive mill wastes [4-6]. Many bacteria species produce lipases which hydrolyse esters of glycerol with long chain fatty acids. Fatty acids are usually made up of straight hydrocarbon chains with an attached carboxyl (COOH) group.

The chemical and physical properties of vegetable oil allow obtaining an effective separation in single chromatographic run (Gas chromatography) using fatty acid methyl esters (FAMEs), meaning that the fatty acids are converted to fatty acids methyl esters (FAMEs). The objective of this study was to characterize and degrade of fatty acid methyl esters generated from domestic wastewater using *Lysinibacillus sphaericus* C3-41.

2. MATERIALS AND METHODS

2.1. Estimation of Fatty Acids in Wastewater Samples

About 300ml of wastewater was sterilized under UV radiation at 60Hz (Model FG-Y15W). One hundred millilitre (100ml) of the sample was transferred into two sterile 250ml flasks and each labeled as sample A and sample B. Sample A was inoculated with *Lysinibacillus sphaericus* C3-41 and incubated at 30^0C while sample B was left un-inoculated and serves as control. Both samples were left for six days. The samples were warmed in sterile round bottom flask in a water bath (Model CS200) at 47ºC was then used for the estimation of the fatty acids (Dignac *et al*., 2003) The sample inoculated with *Lysinibacillus sphaericus* C3-41 was used to test its degradative capability (Odeyemi *et al*., 2014)

2.2. Extraction and Methylation of Oil from the Wastewater Samples

Fat was extracted from the wastewater samples by adding 60ml concentrated HCl to 60ml of the wastewater (Merck, 2002). This was then followed by the addition of 60ml diethyl ether and 60ml of n-hexane. The resulting mixture was thoroughly shaken and transferred to a separating funnel. It was then allowed to stand for 30min. The solvent layer was separated and removed from the fat at a low temperature (7ºC). The extracted oil from the wastewater samples was transferred into a test tube and 4ml of 0.5M methanolic sodium hydroxide (20g of NaOH in 1liter methanol) was added and heated on a steam bath for 5mins until it globulized into solution. Five millilitre of BF_3/MeOH (14% Boron trifluoride; 86% Methanol, 100 ml) was added onto the mixture and boiled for 2min. This mixture was transferred into a 250ml separating funnel and 30ml of petroleum ether (40-60ºC boiling range) was added. Twenty millilitre of saturated sodium chloride solution was later added and shaken vigorously. The lower layer of aqueous methanol was allowed to separate, drained off and discarded (Merck, 2002). The petroleum ether layer was filtered through a Whatman No.1 filter paper into a 50ml beaker. The solvent was evaporated on a steam bath to a final volume of 10ml. The methyl esters were used for HPLC analysis.

2.3. Fatty Acid Methyl Ester Analysis

Fifty (50mg) of the extracted fat content of the sample was saponified (esterified) for five (5) minutes at 95^0C with 3.4ml of the 0.5M KOH in dry methanol. The mixture was neutralized by using 0.7M HCl. Three (3ml) of the 14% boron triflouride in methanol was added (Merck, 2002). The mixture was heated for 5 minutes at the temperature of 90^0C to achieve complete methylation process. The Fatty Acid Methyl Esters were thrice extracted from the mixture redistilled n-hexane. The content was concentrated to 1ml for gas chromatography and 1µL was injected into the injection port of GC (Kolayli *et al*., 2011)

2.4. Characterization and Enumeration of Fatty Acids in the Methylated Oils

The AKTA basic 10/100 High Performance Liquid Chromatography (Amersham Pharmacia Biotech, Sweden) was used for the fatty acids determination. The analysis was performed by the modified method of (Kangala and Krystyna, 2008). The Fatty Acid Methyl Esters were separated using HP chem. station Rev A O 9.01 (1296) software and equipped with a flame ionization detector FID and Supercosil LC-18 column (25 cm x 4.6 mm x 5µm particles film thickness). The mobile phase consisted of acetonitrile: acetone (59:41; v/v) with a flow rate of 1.0ml/min and the fatty acids were detected using a UV (UV-900) detector. Ten microlitre sample of the fresh and biodegraded palm oil was injected for each run. The fatty acid components in the test samples were identified by comparing retention times to those of the standards (Merck, 2002). Each fatty acid in the test sample was expressed in percent of the total fatty acids present. The methyl esters of lauric, myristic, palmitic, stearic, oleic, linoleic, linolenic and arachidic acids were used as standards for identification, Merck (2002).

Calculation: % Fatty acid = $\frac{\text{Peak area of the sample x conc. of fatty acid x diluting factor}}{\text{Peak area of the standard}}$

2.5. Degradation of the fatty acids in the wastewater

This was carried out using a modified method of [Gogoi *et al*., 2007]. The Mineral Salt Medium consisting (g/L) of KH2PO4, 7.584; K2HPO4, 0.80; MnS04.4H20, 0.80; NaCl, 0.16; (NH4)2N03, 0.80; Fe2(S04)3, 0.08; and domestic oil (palm oil), 2.5%, pH maintained at 7.0. Hundred milliliter of the Mineral Salt Medium was dispensed into several 250 ml Erlenmeyer flasks. This medium in several flasks were autoclaved at 121°C for 15 minutes and allowed to cool. To each of the flasks was added 2 ml of olive oil sterilized through membrane pore filtration, as the sole carbon source. *Lysinibacillus sphaericus* grown in nutrient broth overnight was used in pure cultures to inoculate the flasks. Non-inoculated medium kept under the same condition served as control. The flasks were duplicated and were inoculated at room temperature on an orbital shaker Stuart SSLI at 180 rpm for 5 days. Residual oil in the culture media was extracted with n – hexane. This was carried out by pouring the content of the flasks into a separating funnel after which 100 ml n – hexane was added. After vigorous shaking and re-extracting of the aqueous phase, it was discarded while the organic phase was allowed to evaporate to a constant weight in a petri dish. The residual oil content was then analyzed by gas chromatography.

3. Results and Discussion

3.1. Degradation of Oil in the Domestic Wastewater by Lysinibacillus sphaericus C3-41

The fatty acids (FA) found in the sample ranged from C6:0 to C24:1 (with some of the FAs containing their isomers) (Table 1). Five FAs namely caproic, caprylic, capric, trans-11-vaccenic and Cis-5, 8, 11, 14 ecosenic were not detected in the sample. The fatty acids analysed and biodegraded by *Lysinibacillus sphaericus* C3-41 revealed vast amount of fatty acids (FAs) including its major types: for example, the saturated fatty acid (SFA), the mono-unsaturated fatty acid (MUFA), di-unsaturated fatty acid (DUFA) and poly-unsaturated fatty acid (PUFA). Some of the FAs i.e C18:0, C20:0, C22:0 and C24:0 and their unsaturated group members had negative values from degradation of the waste which simply showed that the excess might have come from under-utilized fatty acids that gave positive (+ve) balance under the same condition at the end of the reaction giving a balanced biochemical utilization of fatty acids, this is in line with (Odeyemi *et al*., 2014).

The saturated fatty acids (SFA) consist of seven (7) members and unsaturated fatty acids (USFAs), i.e in a range of ten (10) members of mono-unsaturated fatty acids (MUFAs), four (4) members of Di-unsaturated fatty acid (DUFAs) and seven (7) members of poly-unsaturated fatty acids (PUFAs). Among the saturated fatty acid (SFA), the palmitic acids (C16:0) and stearic acid (C18:0) were found to constitute the largest amount of approximately 34.69% and 8.07% respectively, while the concentration of lauric acid, myristic acid, arachidic acid, behenic acid and lignoceric was very low amount, about 0.1071%, 1.7247%, 0.0230%, 0.0212%, and 0.0026% respectively (Table 1).

The most abundant saturated fatty acid which was palmitic acid (C16:0), had a positive result of 0.6756 resulting in (0.0002%) degradation. Palmitic acid has the higher amount than any other saturated acid found in *Lysinibacillus sphaericus* C3-41 and just 0.6756 of the initial amount (34.6925) was degraded, this may be due to physiochemical properties of the waste i.e long chain fatty acids are easily degraded, solubility and absorption occur when the environmental temperature is high (Swicher, 2007). Swicher (2007) also reported that the rate of metabolism decreases as the chain length increases. Stearic, myristic, and lauric acid are also common though in a lesser constituent of the total fatty acid whereas C_{20} - C_{24} acids are found only in a minute quantity. This conform with Maitanelbarguren *et al.* (2014) who reported that microorganism with high lipidic content normally possess large amount of lipid at a carbon chain ranging from C_{12} – C_{26} and very high palmitic acid. Several authors have also reported the presence of some common FAs, for example Maitanelbarguren *et al.* (2014) also reported presence of archidic acid in *Pseudomonas aeruginosa*, behenic in *Corynebacterium diphtheriae* and *Lactobacillus acidophilus*. Palm oil has more palmitic oil and less of other fatty acids as compared to other vegetable oils, as noted in this study.

It was also noticed that the most abundant unsaturated fatty acid was mono-unsaturated oleic acids (Cis-6-elaidic) (C18: 1) and Cis-11-vaccenic acids (C18: 1) with high amount of 15.28% and 23.13% respectively which in total is approximately 38.4%. In addition, only linolenic had an appreciable amount of 16.71% among the DUFA group members. However, all the poly-unsaturated fatty acids (PUFA) found had low quantities ranging from 0.0030% to 0.0326%. Low fatty acids having chains less than C12:0 such as formic (C1), acetic (C2), propionic (C3), butyric (C4), caproic (C6), caprylic

(C8) and capric (C10) were not found in the sample while higher FAs constitute a much larger proportion of the total FAs in the cell because the fatty acids with short chains are readily soluble in aqueous system which might have been used up by the organism.

Branch chain (unsaturated) fatty acids have larger proportion among the higher fatty acids present. They constituted about 55.4% of the total FAs in the oil sample. Several authors have reported variations in the different type of fatty acids and their degradation. For instance, Berkeley *et al*. (2002) reported that a singularity of the *Bacillus* genus has the ability to degrade large amount of branched-chain FAs, with a predominance of branched chain iso and anteiso FAs containing 12–17 carbons. Song *et al*., (2000) also reported that branched-chain FAs also include ω-alicyclic FAs with or without modifications such as unsaturation and hydroxylation. The decomposition of the domestic oil derived from the wastewater may be primarily dependent on the lipolytic ability of *Lysinibacillus sphaericus* C3-41 (Odeyemi *et al*., 2014). The branched-chain (unsaturated) FAs exhibited high degradation compared to straight chains (saturated) and this may be accrue to the fact that branched-chain FAs usually display a lower melting point temperature than their equivalent straight-chain FAs and as a result their presence in the membrane is therefore expected to increase its fluidity (Song *et al*., 2000).

Seventeen out of 31 fatty acids had negative values when degraded by *Lysinibacillus sphaericus,* three of these had zero values at both initial and final readings. Nine of the FAs had positive balance given a balanced value i.e the differences of the initial amount of the FAs and the amount degraded. The positive results came from each of the three major types of fatty acids (FAs): the saturated fatty acid (SFA) of lauric acid (C12:0) had a positive result of 0.0256 resulting into 23.9% degradation, while myristic acid (C14:0) released out 0.7310 after degradation resulting into 42.63%.

After degradation, some fatty acids increased in amount compared to the initial amount before the inoculation of the organism which gave negative results which simply showed that the excess might have come from under-utilized fatty acids. The negative responses recorded for SFA, MUFA, DUFA and PUFA were as follows: SFAs of C18:0, C20:0, C22:0 and C24:0 had negative values of –3.0698 (30.04%), -0.0362 (61.14%), –0.0334 (61.17%) and -0.0041 (6.12%) respectively; MUFAs of myristic acid C14:1 (Cis-9) and palmitic acid C16:0 (Cis-9) had negative values of -0.0046 with (57.8%) and -0.0599 (0.002%) degradation respectively; the only DUFA of C22:2(Cis-13, 16) had a value of -0.032 (3.14%) while the PUFA, Linolenic C18:3 (Cis-6, 9, 12) released -0.0433 resulting in 61.07% degradation. The percentage degradation of the positive response showed a progressive increase from 12:0 to 18:0 (i.e from 23.9 to 74.23%) with exception of palmitic acid (C16:0) that gave 0.0002%. However, in case of the degradation of oleic acid, the mono-unsaturated fatty acid (MUFA) of C18:1 (Cis-6) and (Cis-11) gave a positive change of 0.3822 (2.5%) and 1.1622 (5.02%), while the di-unsaturated fatty acid (DUFA) of C18:2 (Cis-9, 13) and C18:2 (Trans-9, 12) gave values of 0.5222 (3.24%) and 0.0172 (74.23%) respectively. This showed that the percentage reduction of oleic acid was highest among the fatty acids.

The fate of fatty acid degradation in aqueous system like domestic wastewater may be complicated due to fact that there are numbers of water soluble and water insoluble salts. The predominance of calcium and magnesium ions in most waste waters normally leads to rapid formation of predominance relatively insoluble salts with the FAs therefore reducing their potentials to biodegrade. Knothe (2008) reported that the degree of saturation not only impacts the physicochemical characteristics of the FA, such as the melting point or the viscosity, but also the molecular shape of the lipid to which it is attached.

In addition, the degradation capability of *Lysinibacillus sphaericus* C3-41 may also be due to presence of some essential nutrients such as crude protein, active ingredients which might trigger the release of the lipolytic enzymes responsible for degradation. Odeyemi *et al.* (2014) also reported that the persistent growth of *Lysinibacillus 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 source of deleterious substances for the microbes. Meanwhile the non-availability of some essential nutrients and fatty acids such as caproic, caprylic etc in the palm oil made it just suitable medium for minimum/fair microbial proliferation. This observation is in line with the report of (Pallavi *et al*., 2012).

Microbial degradation and bioaccumulation of fatty acids content (%) by *Lysinibacillus sphaericus* C3-41 showed that most of the fatty acid methyl esters were degraded and utilized by the organism. The assimilation and utilization of the FAs also cut across all its major types (SFA MUFA, DUFA and PUFA) from the degraded contents. For example, about 23.9% of SFA C12:0 (Lauric acid) was degraded while about 65.3% was assimilated. It was observed that palmitic acid (C16:0) which was found to have the highest amount among the FAs was degraded and utilized in a minimal amount by *Lysinibacillus sphaericus* C3-41. Other SFA with higher carbon chains such as myristic (C14:0), palmitic (C16:0), stearic (C18:0), arachidic (C20:0), behenic (C22:0) and lignoceric (C24:0); the amount and percentage assimilated by the organism were 0.4783 (31.68%), 0.6756 (0.002%), 0.9718 (8.72%), 0.0213 (18.04%), 0.0334 (4.51%) and 0.0006 (6.12%) respectively. In MUFA of C14:1 (Cis-9), about 93.1% of the FAME was assimilated while PUFA C20:3 (Cis-11, 14, 17), only 16% of it was assimilated. In some of the FAMEs, the amount degraded was also the same as quantity accumulated (Table 2). This showed a progressive increase in the utilization of the fatty acids with increased number of branched chains.

The positive and negative values of the FAs under *Lysinibacillus sphaericus* C3-41 at 24h after degradation were almost of equivalent amounts showing that there was total utilization and high accumulation of the different FAs under *Lysinibacillus sphaericus* C3-41 microbiological degradation of the palm oil; the difference was just 0.0357% or overall percentage difference of 0.5%.

For biodegradation to occur on all the types of fatty acids, it shows that *Lysinibacillus sphaericus* C3-41 possess several enzymes for degradation. For example, isomerase acted only on free unsaturated fatty acids and not on esterified fatty acids, meanwhile almost all the unsaturated fatty acids were degraded indicating the release of isomerases by *Lysinibacillus sphaericus* C3-41. Pallavi *et al*. (2010) reported that the presence of lipase not only catalyzed hydrolytic reactions but also catalyzed inter-esterification reactions, depending on the source of lipase and reaction conditions. Because of the hydrophobic properties that lipids contain, they are able to form membranes within organisms. These lipids possess their hydrophobicity because of their fatty acids, Faber, (1992) also reported that the addition of organic solvents enabled and controlled the *cis-trans* conversion of unsaturated acyl chains of membrane phospholipids by the periplasmic isomerase. It can be deduced from this research that possible candidates for solvent-activated membrane component could be membrane phospholipases which, known to be strongly triggered by organic solvents. Van den Bosch, (2000) reported that membrane phospholipases hydrolyze phospholipids to free fatty acids that can subsequently serve as substrates for the periplasmic isomerase. The organic solvents that trigger activation of the enzyme could be associated with the fact that detergents are present in the domestic wastewater which may be bio-available for *Lysinibacillus sphaericus* C3-41 which in turn activate secretion of isomerase that catalyzes the degradation of unsaturated fatty acids. Odeyemi *et al*. (2011) had earlier reported that the presence of some food debris in wastewater created a favourable condition for the microbes as readily available source of nutrients. Similarly Odeyemi *et al*. (2013) noted that the rate of microbial secretions of enzymes was low for the utilization of limited amount of nutrients available in palm oil compare to wastewater.

The utilization of the domestic oil as substrate may be explained by fatty acids profiling of the extracted oil from the wastewater culture. After an incubation period of 24 h, it was observed that some of the fatty acids degraded were also utilized in by *Lysinibacillus sphaericus* C3-41 in similar amount. This is in agreement with the work Odeyemi *et al.* (2014) who reported that amount of fatty acids used up by the microbial cells of *Pseudomonas* sp, *Klebsiella* sp, *Staphylococcus* sp, and *Bacillus* sp was similar to the quantity released into the environment. Almost all the fatty acids originally found in the sample were degraded by *Lysinibacillus sphaericus* C3-41 while some were metabolized. Lepore *et al.* (2011) reported that for assimilation and metabolism to occur, if the fatty acids are the exogenous carbon source, after degradation the FAs will bind to transporter protein and as a result conformational change occur, a pore will then open for diffusion of fatty acid into the periplasm. *Lysinibacillus sphaericus* C3-41 utilized appreciable amounts of some fatty acids such as lauric, myristic, stearic, arachidic lignoceric but could not utilize palmitic acid. The fatty acids assimilated may serve as precursors for a variety of important building blocks such as phospholipids, sphingolipids, sterols, as secondary metabolites and as signaling molecules or attached to protein (Helge and Alexander, 2014). There is an indication that *Lysinibacillus sphaericus* C3-41 readily utilized the fatty acids and at the same time converting some that may be hazardous to the environment into non-hazardous substance.

In the case of both positive and the negative responses, the varied values cut across the three major types and other forms of FAs in *Lysinibacillus sphaericus* C3-41 with the total negative and positive values at -3.4823 and +3.5300 respectively; taking cognizance of these two arithmetical signs, the grand total value equal +0.050. When this one is placed all over 7.0123, the percentage value equals 0.70%. This percentage value of 0.7% is highly negligible and this is invariably the value of FAs that cannot be accounted for under the *Lysinibacillus sphaericus* C3-41 degradation activities in the palm oil at 24 h. Since 0.7% is so low that it can be said to be negligible, therefore, it could be said that, the negative and the positive values are visually of equivalent amount (Table 2).

In all the fatty acids analysed, the grand total of the fatty acids for the negative and positive values, each gave a grand total of virtually 0.00% of the fatty acid content (Table 2).

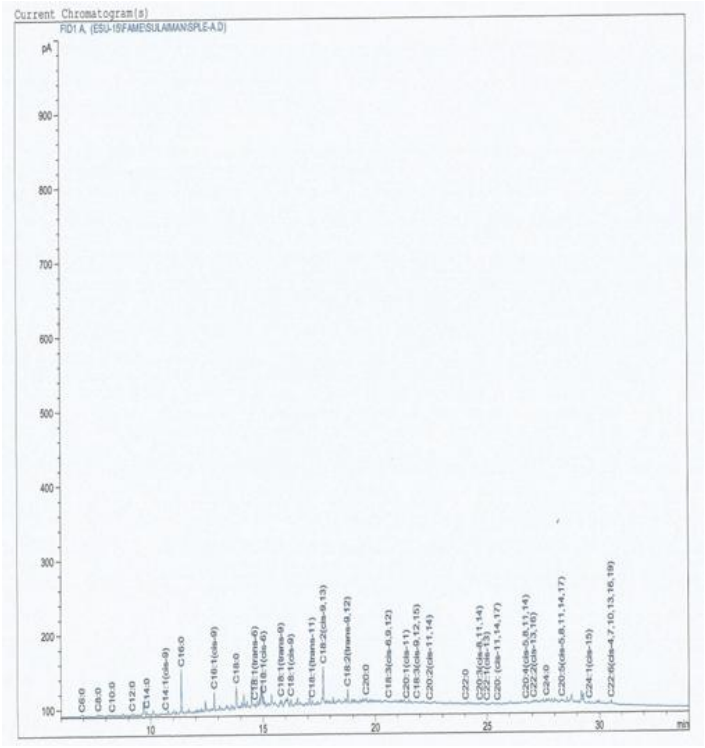

Figure1. *Chromatogram of the fatty acid methyl ester in the waste water before degradation*

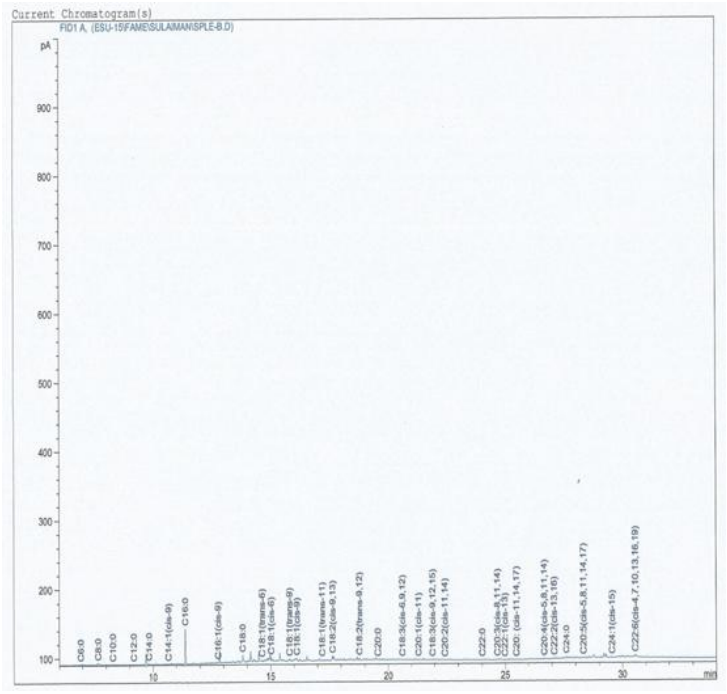

Figure2. *Chromatogram of the fatty acid methyl ester in the wastewater after degradation*

Table1. *Fatty acids content (%) and the amount degraded by Lysinibacillus sphaericus C3-41 in the domestic wastewater*

S/N	FATTY ACIDS	NOMENCLATURE	WASTEWATER A	B	C
1	C6:0	Caproic	0.0000	- 0.0000 =	0.0000
2	C8:0	Caprylic	0.0000	- 0.0000 =	0.0000
3	C10:0	Capric	0.0000	- 0.0000 =	0.0000
4	C12:0	Lauric	0.1071	- 0.0814 =	0.0256
5	C14:0	Myristic	1.7147	- 0.9837 =	0.7310
6	C14:1(Cis-9)	Cis-Myristelaidic	0.0029	- 0.0075 =	-0.0046
7	C16:0	Palmitic	34.6925	- 34.0169 =	0.6756
8	C16:1(Cis-9)	Cis-Palmitelaidic	0.0029	- 0.0628 =	-0.0599
9	C18:0	Stearic	8.0685	- 11.1383 =	-3.0698
10	C18:1(Trans-6)	Trans-6-Petroselenic	0.0083	- 0.0213 =	-0.0130
11	C18:1(Cis-6)	Cis-6- Elaidic (Oleic)	15.2814	- 14.8992 =	0.3822
12	C18:1(Trans-9)	Trans-Elaidic (Oleic)	0.0007	- 0.0019 =	-0.0012
13	C18:1(Cis-11)	Cis-11-Vaccenic(Oleic)	23.1399	- 21.9777 =	1.1622
14	C18:1(Trans-11)	Trans-11-Vaccenic(Oleic)	0.0000	- 0.0000 =	0.0000
15	C18:2(Cis-9,13)	Linoleic	16.7148	- 16.1926 =	0.5222
16	C18:2(Trans-9,12)	Trans-9,12-Linoladaidic	0.0097	- 0.0025 =	0.0072
17	C20:0	Arachidic	0.0230	- 0.0592 =	-0.0362
18	C18:3(Cis-6, 9, 12)	γ-Linolenic	0.0276	- 0.0709 =	-0.0433
19	C20:1(Cis-9)	Ecosenic acid	0.0386	- 0.0994 =	-0.0608
20	C18:3(Cis-9,12,15)	α-Linolenic acid	0.0214	- 0.0549 =	-0.0335
21	C20:2(Cis-11,14)	Eicosadienoic acid	0.0326	- 0.0084 =	0.0242
22	C22:0	Behenic	0.0212	- 0.0546 =	-0.0334
23	C20:3(Cis-8,11,14)	Dihomo-γ-linolenic	0.0271	- 0.0693 =	-0.0422
24	C22:1(Cis-13)	Cis-13-docosenic	0.0152	- 0.0390 =	-0.0238
25	C20:3(Cis-11,14,17)	Eicosatrienoic acid	0.0141	- 0.0362 =	-0.0221
26	C20:4(Cis-5,8,11,14)	Arachidonic acid	0.0000	- 0.0000 =	0.0000
27	C22:2(Cis-13,16)	Docosadienoic acid	0.0154	- 0.0384 =	-0.023
28	C24:0	Lignoceric	0.0026	- 0.0067 =	-0.0041
29	C20:5(Cis-5,8,11,14,17)	EPA	0.0016	- 0.0041 =	-0.0025
30	C24:1(Cis-15)	Nervonic acid	0.0026	- 0.0067 =	-0.0041
31	C22:6(Cis-4,7,10,13,16,19)	Tetracosahexaenoic	0.0030	- 0.0078 =	-0.0048
	Total –ve Total +ve Grandtotal		-3.4823 +3.5300 0.0357		

Key: A: *Original amount without L sphaericus C3-41,*

B: *Amount left in the sample after degradation by L sphaericus C3-41*

C: *Amount assimilation by L sphaericus C3-41*

Table2. *Fatty acids content (%) and the amount assimilated by Lysinibacillus sphaericus C3-41 in the domestic wastewater*

S/N	FATTY ACIDS	NOMENCLATURE	X	Y	Z
2	C8:0	Caprylic	0.0000	- 0.0000 =	0.0000
3	C10:0	Capric	0.0000	- 0.0000 =	0.0000
4	C12:0	Lauric	0.0231	- 0.0256 =	-0.0025
5	C14:0	Myristic	0.9837	- 0.7310 =	0.2527
6	C14:1(Cis-9)	Cis-Myristelaidic	0.0075	- 0.0046 =	0.0029
7	C16:0	Palmitic	34.0127	- 0.6756 =	33.3371
8	C16:1(Cis-9)	Cis-Palmitelaidic	0.0002	- 0.0599 =	-0.0601
9	C18:0	Stearic	11.1003	- 2.0980 =	9.0222
10	C18:1(Trans-6)	Trans-6-Petroselenic	0.0213	- 0.0130 =	0.0083
11	C18:1(Cis-6)	Cis-6- Elaidic (Oleic)	14.0209	- 0.3822 =	13.6467
12	C18:1(Trans-9)	Trans-Elaidic (Oleic)	0.0019	- 0.0012 =	0.0007
13	C18:1(Cis-11)	Cis-11-Vaccenic (Oleic)	20.8897	- 1.1620 =	19.7277
14	C18:1(Trans-11)	Trans-11-Vaccenic(Oleic)	0.0000	- 0.0000 =	0.0000

15	C18:2(Cis-9,13)	Linoleic	16.0872	-	0.5222	=	15.5650
16	C18:2(Trans-9,12)	Trans-9,12-Linoladaidic	0.0025	-	0.0072	=	-0.0047
17	C20:0	Arachidic	0.0230	-	0.0362	=	-0.0132
18	C18:3(Cis-6,9,12)	γ-Linolenic	0.0433	-	0.0015	=	0.0428
19	C20:1(Cis-9)	Ecosenic acid	0.0608	-	0.0071	=	0.0537
20	C18:3(Cis-9,12,15)	α-Linolenic acid	0.0335	-	0.0015	=	0.0320
21	C20:2(Cis-11,14)	Eicosadienoic acid	0.0084	-	0.0242	=	-0.0158
22	C22:0	Behenic	0.2265	-	0.0334	=	0.1931
23	C20:3(Cis-8,11,14)	Dihomo-γ-linolenic	0.0693	-	0.0422	=	0.0271
24	C22:1(Cis-13)	Cis-13-docosenic	0.0511	-	0.0238	=	0.0273
25	C20:3(Cis-11,14,17)	Eicosatrienoic acid	0.0367	-	0.0221	=	0.0146
26	C20:4(Cis-5,8,11,14)	Arachidonic acid	0.0000	-	0.0000	=	0.0000
27	C22:2(Cis-13,16)	Docosadienoic acid	0.0020	-	0.0230	=	-0.0250
28	C24:0	Lignoceric	0.0067	-	0.0041	=	0.0026
29	C20:5(Cis-5,8,11,14,17)	EPA	0.0031	-	0.0025	=	0.0006
30	C24:1(Cis-15)	Nervonic acid	0.0067	-	0.0041	=	0.0026
31	C22:6(Cis-4,7,10,13,16,19)	Tetracosahexaenoic	0.0002	-	0.0048	=	-0.0046
	Total –ve Total +ve Grandtotal		-3.4287 +3.5995 0.1708				

Key: X: Amount that remains from the degraded sample after removal of *L sphaericus* C3-41

Y: Amount assimilated by *L sphaericus* C3-41

Z: Amount left in the sample

4. CONCLUSION

Lysinibacillus sphaericus C3-41 and its cell free extract (enzymes) have been studied for their special characteristics applicability in various bio-processes such as degradation and assimilation of degraded saturated and unsaturated fatty acid methyl esters such as lauric, myristic, cis-myristelaidic, cis-palmitelaidic, trans-6-petroselenic, trans-elaidic, trans-9,12-linoladalaic and cis-13-docosenic which might be deleterious to the environment. *Lysinibacillus sphaericus* C3-41 whole cell containing hydrolytic enzymes have proven to have a significant potential in fatty waste degradation and as an agent remediation.

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