

# BIODEGRADATION OF LECITHIN-BASED FATLIQUOR: OPTIMIZATION OF FOOD TO MICROBES RATIO AND RESIDENCE TIME

by

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## ABSTRACT

Oils are usually applied as aqueous emulsions in the fatliquoring process and they influence the physical characteristics of leather, such as tensile strength, extensibility, wetting properties, water-proofness and permeability to water vapour and air. During soybean oil processing, phospholipids are recovered from the degumming process to produce lecithin. The sulfonated phospholipids exhibit good hydrophilic properties, emulsifying properties and good permeability. They are therefore used in the tanneries as semi-synthetic fatliquor. Exhaustion of fatliquor was about 85 to 90% and the remaining was discharged in to the process water as wastewater. Grease traps fail to retain dissolved and emulsified fats efficiently, allowing them to enter the water treatment system. These lipids may then interfere with aerobic biological wastewater treatment processes by reducing oxygen transfer rates. The aim of the present study was to assess the rate of biodegradability of lecithin-based fatliquor containing wastewater generated from tanneries at various food to microbial (f/m) ratios of 0.35, 0.25 and 0.15 in aerobic batch reactors. Optimization of the f/m ratio and residence time was investigated in detail and the results established that a f/m ratio of 0.15 with residence time of 48 hours is needed for obtaining maximum biodegradation. Soluble Biochemical Oxygen Demand (SBOD) and Soluble Chemical Oxygen Demand (SCOD) removal efficiencies were 95.55% and 86.21%, which resulted in 28 and 240 mg/L in the treated effluent. It was evident from FT-IR and ESI-MS analysis that the phospholipids are degraded efficiently in the batch reactor.

## RESUMEN

Los aceites se emplean generalmente en forma de emulsiones acuosas en el proceso de engrase e influyen en las características físicas del cuero, tales como resistencia a la tracción, extensibilidad, propiedades de humectación, resistencia al agua y la permeabilidad al vapor de agua y al aire. Durante el procesamiento del aceite de soja, se recuperan fosfolípidos del proceso de desgomado para producir lecitina. Los fosfolípidos sulfonados presentan buenas propiedades hidrofílicas, propiedades emulsionantes y buena permeabilidad. Ellos son utilizados en las curtiembres como engrasante semi-sintético. El agotamiento del engrase fue aproximadamente 85 a 90% y el remanente fue descargado en el agua de proceso como aguas residuales. Las trampas de grasa no retienen eficientemente las grasas disueltas y emulsionadas, permitiéndoles entrar en el sistema de tratamiento de aguas. Estos lípidos pueden entonces interferir con los procesos biológicos aerobios del tratamiento de aguas residuales mediante la reducción de las tasas de transferencia de oxígeno. El objetivo del presente estudio fue evaluar la tasa de biodegradabilidad del engrasante basado en lecitina contenido en las aguas residuales de las curtiembres a distintas tasas alimento/microorganismos (f/m) de 0,35, 0,25 y 0,15 en reactores discontinuos aeróbicos. La optimización de la relación f/m y el tiempo de residencia fue investigado en detalle y los resultados establecieron que una relación f/m de 0,15 con un tiempo de residencia de 48 horas es necesario para la obtención de la máxima biodegradación. Las eficiencias de remoción de la demanda bioquímica de oxígeno soluble (SBOD) y la demanda química de oxígeno soluble (SCOD) fueron del 95,55% y 86,21%, lo que resultó en 28 y 240 mg/L en el efluente tratado. Fue evidenciado a partir de análisis FT-IR y ESI-MS que los fosfolípidos son degradados de manera eficiente en el reactor discontinuo.

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## INTRODUCTION

Leather processing consists of a series of operations that enable the conversion of the raw hide or skin, which is highly putrescible, into leather. Oils are usually applied in aqueous emulsions in the fatliquoring process while the leather is still wet from tanning.<sup>1</sup> The fatliquoring process influences the physical characteristics of leather, such as tensile strength, extensibility, wetting properties, waterproofness and permeability to water vapour and air.<sup>2,3</sup> Depending upon the source of the oils or fats used, fatliquor can be classified as natural (vegetable, animal and marine), synthetic and semi synthetic fatliquors.<sup>4</sup>

Fatliquoring is generally carried out after re-tanning and dyeing. The tendency is to use fatliquors excessively in order to ensure full penetration and complete reaction with leather fibres. As a result, unabsorbed fatliquors inevitably remain in the float, generating a high level of pollution. It is the largest amount of chemicals (10-20% based on wet blue leather weight) used in the leather making.<sup>5</sup> On an average, the exhaustion of fatliquor is around 85 to 90%, depending on the nature of raw material used i.e., skin/ hide. Hence, around 10 to 15% of un-exhausted fatliquor is discharged into the process water as wastewater.<sup>6</sup>

A large number of pretreatment systems (Grease-trap, Tilted Plate Separators, Dissolved Air Flotation Systems and physical-chemical treatment) are employed to remove oil and grease (O&G) from the lipid rich wastewaters prior to the main treatment process itself, which is generally of a biological nature. However, the cost of such treatments is high, the removal efficiency of dissolved and/or emulsified O&G is low and extremely problematic sludge is produced.<sup>7</sup> Applications of pressure-driven membrane processes in various wet operation steps of the leather industry were studied. The integrated membrane processes permits the recovery of by-products like protein, fats and chromium from the sectional streams<sup>8</sup>. The presence of fats, grease and oils in the effluent generated after fatliquoring process in the tanneries cannot be discharged without treatment since they may clog sewerage lines or pollute receiving streams leading to environmental concerns. Treatments include a hybrid separation process involving gravity settling, two step coagulation, nanofiltration and reverse osmosis<sup>9</sup>.

Soybean oil, extracted from the seeds of the soybean, is one of the least expensive vegetable oils. It is one of the most widely consumed cooking oils. As a drying oil, processed soybean oil is also used as a base for printing inks, oil paints formulation and also as one of the sources of vegetable oil used in the tanneries. During soybean oil processing, phospholipids are recovered from the degumming process to produce lecithin. Lecithin has emulsification and lubricant properties, and is

a surfactant. Commercial soybean lecithin is a complex mixture containing about 65-75% phospholipids, together with triglycerides and smaller amounts of other substances like carbohydrates, pigments, sterols and sterol glycosides.<sup>10</sup> Phospholipids constitute 0.3-0.6% of soybean seed, or 1.5-3.0% of crude soybean oil. Phospholipids are emulsified by sulfonation and sulfonated phospholipids exhibit good hydrophilic property, emulsifying property besides good permeability. This facilitates a wide range of its application as fatliquoring agent, flotation agent and emulsifying agent in leather, pharmacy and farm chemicals etc.<sup>11</sup> Biodegradability studies were carried out for rape oil based fatliquors prepared from different methods such as sulfited, sulfonated, phosphated and polymerized and the results were compared by Zhaoyang Luo et al.<sup>12</sup> The results indicated that the modification method which utilizes the double bonds or hydroxyl group of the oil decreased the biodegradability of the fatliquor.

Biological treatment plays a predominant role in wastewater treatment as it is the most effective and convenient method to degrade pollution loads such as Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) and to meet the discharge standards. A chemical that is easily biodegraded in the biological treatment system should be considered to be environment friendly. The environment friendliness of semi synthetic lecithin-based fatliquor was studied by undertaking aerobic biodegradation studies. Studies were carried out to optimize the food to microbial ratio (f/m) and residence time for effective biodegradation of lecithin-based fatliquor in batch aerobic reactors.

## MATERIALS AND METHODS

### Characterization of Lecithin-based Semi Synthetic Fatliquor

Anionic semi synthetic fatliquor containing lecithin was procured from a commercial company named Balmer Lawrie and Co. Ltd., India and was used for the study. The physical and chemical properties of phospholipids in lecithin are improved enormously by sulfonation, which results in an increase in the hydrophilic-lipophilic balance (HLB) from 1-2 to 12-16. The value of 12-16 indicates high emulsifying capacity of lecithin leading to good penetration and lubrication of the fibres<sup>11</sup>. Lecithin-based fatliquor is a multipurpose fatliquor designed for imparting silky and smooth grain on the leather. It produces very good gloss on Suede / Nubuck Nappa and it makes excellent lightweight leather ([http://www.balmerlawrie.com/lcd\\_grange.asp](http://www.balmerlawrie.com/lcd_grange.asp)). For preparation of synthetic wastewater, one gram of lecithin-based fatliquor was diluted in one litre of double distilled water. The fatliquor was characterized for physico-chemical parameters - pH (Part 4500-H<sup>+</sup> method B), Chemical Oxygen Demand (COD; Part 5220 method C), Biochemical Oxygen Demand (BOD as

BOD<sub>5</sub> at 20°C; Part 5210 method B), Hexane Extractable Matter i.e., oil & grease content (Part 5220 method B), Total Kjeldhal Nitrogen (TKN) (Part 4500<sub>org</sub> method -B), Phosphorus (Part 4500-P method E) as per Standard methods 20<sup>th</sup> edition.<sup>13</sup> Samples were analysed in triplicate and the average values are reported.

The principal characteristics of fatliquors are primarily determined in terms of iodine value and saponification value. Apart from routine physico-chemical characterization, the lecithin-based semi synthetic fatliquor was also analyzed for iodine value and saponification value. The iodine value specifies the amount of unsaturated compounds present in fatliquor, whereas saponification value specifies the amount of potassium hydroxide in mg required for neutralizing fatty acids in one gram of fatty matter. Iodine value was determined according to the ISO 3961 standard method.<sup>14</sup> The AOCS standard method Cd 13-60 was used to determine the saponification value.<sup>15</sup> Samples were analysed in triplicate and the average values are reported.

#### **Experimental Setup for Biodegradation Studies**

To assess the biodegradability of semi synthetic fatliquor, the experiments were performed in aerobic condition under ambient temperature (30 ± 2°C). For biodegradability tests, batch reactors are preferred because they are simple to operate and problems associated with reactor hydraulics are avoided. Hence biodegradability studies were carried out in batch reactors of 2.0 L capacity, with a working volume of 1.5 L. The batch reactors were fed with synthetic wastewater prepared in the laboratory using lecithin-based fatliquor, which is similar to the practice followed in a tannery. In order to maintain aerobic conditions, air was supplied at a rate of 1.5-1.6 L/h through two medium sized diffusers fitted to an aquarium-type pump. Complete mixing was provided to ensure the dissolved oxygen (DO) concentration of 2 - 2.5 mg/L throughout the duration of the experiment. The dissolved oxygen was measured using HACH portable DO meter. The activated sludge used in the experiment was collected from the aeration tank of a local sewage treatment plant situated in Chennai, India. The activated sludge was characterized for mixed liquor suspended solids (MLSS) concentration (Part 2540 method D) and mixed volatile suspended solids (MLVSS) concentration (Part 2540 method E) as per Standard methods 20<sup>th</sup> edition.<sup>13</sup> From characterization studies, the MLSS and MLVSS concentration were found to be 7500 ± 100 mg/L and 6000 ± 100 mg/L. The activated sludge was washed several times with distilled water and starved under aeration for 24 h to reduce the amount of dissolved substances present in the mixed liquor. The known concentration of washed activated sludge and the fatliquor were mixed in aerobic bioreactor for optimization of f/m ratio. COD:N:P ratio of 100:5:1 was maintained in the reactors with the addition of NH<sub>4</sub>Cl and KH<sub>2</sub>PO<sub>4</sub>.

#### **Optimization of Food to Microbial (f/m) Ratio and Residence Time**

It is necessary to maintain a proper f/m ratio in the aerobic bioreactors to provide a congenial environment to the aerobic microorganisms. According to literature on activated sludge systems with conventional aeration, the optimal f/m ratio falls between 0.15 and 0.4 kg BOD<sub>5</sub>/ kg MLVSS d.<sup>16</sup> The f/m ratio is frequently used for treatment plant control, and it is related to sludge volume index (SVI) and to settling characteristics of the sludge. At low f/m ratios, the amount of food present in the system is insufficient to maintain growth of the microorganisms and they are driven to endogenous respiration. At high f/m ratios, filamentous microorganisms predominate. The optimization of f/m ratio is therefore a crucial process parameter in aerobic bioreactors and it is substrate specific also.

In order to optimize the conditions required for biodegradation of lecithin-based fatliquor used in the tanning process, the aerobic bioreactors were operated with f/m ratio of 0.35, 0.25 and 0.15. Samples were collected in regular intervals of time and filtered through 0.45 μm filter. Filtrates were analyzed for soluble Biochemical Oxygen Demand (SBOD<sub>5</sub>) and soluble Chemical Oxygen Demand (SCOD). The BOD<sub>5</sub> testes were carried at 20°C for a 5 day period. In order to arrive at the optimum residence time for the removal of SCOD and SBOD, grab samples were collected from the reactors daily and filtered samples were analyzed for reduction in SBOD and SCOD. The test results indicated that the reduction rate was not appreciably increased after 72 h. Therefore for different f/m ratios, the reactors were operated for residence time of 72 h. For each f/m ratio, two batch aerobic bioreactors were operated to assess the reproducibility of the performance. Simultaneously, a control reactor was operated without addition of substrate i.e., fatliquor and the rate of biodegradation was assessed with respect to the control reactor.

#### **Fourier Transform Infrared Spectrometry (FT-IR) Analysis**

To assess the functional groups present in the semi synthetic fatliquor before and after biodegradation studies, the influent and the effluent samples (soluble) after aerobic biodegradation studies were analysed using Fourier Transform Infrared Spectrometry (FT-IR). The samples were lyophilized and the lyophilized samples were pelletized with potassium bromide (KBr) in the ratio of 1:50. The pellets were subjected to FT-IR analysis using transmission mode. The measurements were carried out in the mid-infrared range from 4000 to 500 cm<sup>-1</sup> with ABB make MB 3000 model FT-IR.

#### **Electrospray Ionisation Mass Spectroscopy (ESI-MS) Analysis**

In order to identify the molecular mass present in semi synthetic fatliquor before and after biodegradation studies, with the help of ESI-MS, the influent and the effluent (soluble)

samples after aerobic biodegradation studies were analyzed using LCQ Advantage ion-trap mass spectrometer (make: Thermo Fisher Finnigan). The ESI- MS was operated in the positive ion mode, with operating conditions as 275°C capillary temperature, isolation width of  $m/z$  1.0, mass range of  $m/z$  150-2000, nitrogen gas as auxiliary and sweep gas (helium gas) at the flow rate of 0.6 mL/min for collision induced dissociation (CID) of ions; spray voltage and capillary voltage 4.6 kV and 31 V respectively.

## RESULTS AND DISCUSSION

### Characteristics of Semi Synthetic Lecithin Based Fatliquor

The semi synthetic fatliquor was characterised for various physico-chemical parameters such as pH, BOD, COD, and Hexane Extractable Matter i.e., oil and grease content and the results are presented in the Table I. It was observed from the characteristics of fatliquor, the ratio of BOD to COD was 0.36. Generally, a compound is considered biodegradable when its  $BOD_5/COD$  value is higher than 0.35<sup>12</sup>. It was evident from this that the semi synthetic fatliquor containing lecithin is biodegradable. The unsaturated forms of oils are biodegradable as reported by Sun and Wakeham.<sup>17</sup> Saturated fatty acids are less soluble and less accessible to micro-organisms for the similar size of unsaturated fatty acids. The iodine value and saponification value of lecithin-based fatliquor was 54 and 122. Iodine value signifies the amount of unsaturated compounds (double bonds) in the fatliquor and saponification value signifies the amount of potassium hydroxide in mg required for neutralizing fatty acids in one gram of fatliquor. Iodine value and saponification value of vegetable oils like soybean oil is in the range of 124-133 and 188-195 (<http://www.veredlungschemikalien.basf.de>).<sup>18</sup> Based on the comparison between lecithin-based fatliquor and soybean oil, soybean oil has got more unsaturated bonds and hydroxyl groups. Zhaoyang Luo et al.<sup>12</sup> studied the biodegradability of rape oil based fatliquors prepared from different methods which consume double bonds or hydroxyl groups. The results indicated that presence of less number of double bonds or hydroxyl groups will decrease the biodegradability of fatliquors while presence of more double bonds or hydroxyl groups will increase the biodegradability. The rate of biodegradation of unsaturated fatty acids is much faster than saturated fatty acids.<sup>19</sup>

### Optimization of Food to Microbial (f/m) Ratio

Aerobic batch reactors were operated for different f/m ratios i.e., 0.35, 0.25 and 0.15, to optimize and to evaluate the efficiency of biodegradation of lecithin-based fatliquor in batch aerobic bioreactors. This is also in accordance with the literature for activated sludge systems with conventional aeration; the optimal f/m ratio falls between 0.15 and 0.4 kg  $BOD_5$ / kg MLVSS d.<sup>16</sup> The biodegradation efficiency was measured in terms of BOD and COD removal w.r.t. residence

**TABLE I**  
**Characteristics of the**  
**lecithin-based fatliquor.**

Sl. No.	Parameter	Average Value
1	pH	6.01
2	Chemical Oxygen Demand (COD), (mg/L)	1740
3	Biochemical Oxygen demand (BOD), (mg/L)	630
4	Hexane Extractable Matter i.e Oil and grease (mg/L)	680
5	Total Kjeldhal Nitrogen (TKN) as Nitrogen (mg/L)	12.5
6	Phosphorus as P (mg/L)	5.2
7	Iodine Value (g/100g of fatliquor)	54
8	Saponification Value (mg/g of fatliquor)	122

time in hours. The SBOD and SCOD removal efficiency of lecithin-based fatliquor for the f/m ratio of 0.35, 0.25 and 0.15 are presented in Figure 1. For the f/m ratio of 0.35, SBOD and SCOD removal efficiencies steadily increased and reached a value of 41.95 and 53.17 percent in 24 h of residence time. Increasing the residence time to 48 hours improved the SBOD and SCOD removal efficiencies to 65.51 and 73.01%. Further increasing the residence time to 72 hours resulted in marginal increase only. Two days residence time resulted in the SBOD and SCOD of the treated liquor at 170 mg/L and 600 mg/L. For the f/m ratio of 0.25, the SBOD and SCOD removal efficiencies increased steadily and reached a value of 66.66 and 59.19 percent in 24 hours of residence time and 82.53 and 75.28% in 48 hours residence time. SBOD and SCOD of treated liquor were 110 mg/L and 430 mg/L in 48 hours. Further increasing the residence time to 72 h resulted only in marginal increase in removal efficiencies of SBOD and SCOD. For the f/m ratio of 0.15, one day residence time, SBOD and SCOD removal was 79.36 and 75.86%. Two days residence time resulted in maximum SBOD and SCOD removal efficiencies of 95.55 and 86.21%, corresponding to 28 mg/L and 240 mg/L in the treated effluent, which meets the discharge standard limit prescribed for tannery effluent in India. Biodegradability of Bleached Kraft mill wastewater by activated sludge was evaluated by Diez et al.<sup>20</sup>. The f/m ratio was varied from 0.05 to 0.43  $gBOD_5/g$  MLVSS d during the overall reactor operation. Highest  $BOD_5$  removal (96%) was observed when the f/m ratio was varied between 0.12 to 0.23  $gBOD_5/g$  MLVSS d. When the ratio was increased beyond that, BOD removal efficiencies gradually dropped to 70% as the f/m ratio was raised to 0.43.<sup>20</sup>

Zhaoyang Luo et al.,<sup>12</sup> studied the biodegradability of rape oil based fatliquors prepared from different methods such as sulphated, sulfonated, oxidized sulphited, phosphated or copolymerized by utilizing either double bonds or hydroxyl groups of the vegetable oil. The studies indicated that the biodegradability decreased with reduction in number of double bonds or hydroxyl groups of the modified rape oil and this was indicated by reduction in iodine value and saponification value. Iodine value of lecithin-based fatliquor was 54 which denotes presence of lesser numbers of double bonds when compared with soybean oil's iodine value of 124-133, indicating presence of more number of double bonds and hence easily biodegradable. Reduction in lesser number of double bonds resulted in two days residence time for maximum removal of SBOD and SCOD at the f/m ratio of 0.15. It was observed that as f/m ratio decreased the SCOD removal efficiency was increased for treatment of stored DAF pre-treated high oil and grease pet food wastewater and raw wastewater.<sup>21</sup>

The results clearly indicated that high f/m ratio resulted in lesser removal of organic matter when compared with the low f/m ratio. Based on the removal efficiencies, the f/m ratio of 0.15 was taken as the optimum for effective degradation of lecithin-based semi synthetic fatliquor in aerobic batch reactor in two days residence time. Further increasing the residence time did not improve the removal efficiency. In order to identify the functional changes and compounds present in lecithin-based fatliquor, FT-IR and ESI-MS analyses were carried out for the optimized f/m ratio of 0.15 before and after aerobic treatment.

#### ***Evaluation of FT-IR Spectrum Before and After Aerobic Treatment***

FT-IR is an effective analytical tool for detecting functional groups and nature of the chemical bonding of the substance to be analyzed. FT-IR absorbance bands obtained for lyophilized wastewater containing lecithin-based-fatliquor before aerobic treatment are listed in Table II. Well-defined characteristic absorptions in the region of 3200-2700  $\text{cm}^{-1}$  are due to C-H stretching of carbon and hydrogen containing species. The presence of long linear aliphatic chain compound is identified by the presence of absorption band at 2857 and 2928  $\text{cm}^{-1}$  and also absorption at 1461.13 and 720.7  $\text{cm}^{-1}$ . Broad absorption band in the region 3459.35  $\text{cm}^{-1}$  signifies the presence of OH group. A major band in the region of 1850 - 1650  $\text{cm}^{-1}$  indicates the presence of a C=O group (carbonyl compound). Absorption band in the region 1750 - 1700  $\text{cm}^{-1}$  indicates the presence of ester, considering the triglycerides present in the fatliquor. Characteristic absorption band due to the presence of phospholipids was observed in the region of 1145-970  $\text{cm}^{-1}$  for the P-O-C stretching. Carbonyl group in conjugation with a double bond produces a second strong absorption between 1650 and 1600  $\text{cm}^{-1}$ . Based on the results, the semi synthetic fatliquor contains predominantly phospholipids of lecithin.

The presence of long linear aliphatic chain compound with unsaturation was identified in the phospholipid of soybean lecithin.<sup>22,23,24</sup>

Aerobic treated fatliquor shows several changes in the spectrum indicating the widening of the ester peak of C=O due to the formation of secondary oxidation products containing new carbonyl groups which resulted in shifting to lower wave number. Well-defined and characteristic absorptions in the region of 3200 - 2700  $\text{cm}^{-1}$  are due to C-H stretching of carbon and hydrogen containing species. The presence of long linear aliphatic chain compound was identified by the presence of absorption band at 2857 and 2928  $\text{cm}^{-1}$  and also absorption at 1461.13 and 720.7  $\text{cm}^{-1}$ . Broad absorption band in the region 3459.35  $\text{cm}^{-1}$  signified the presence of OH group. A major band in the region of 1850 - 1650  $\text{cm}^{-1}$  indicated the presence of a C=O group (carbonyl compound). Absorption band at 1638.73  $\text{cm}^{-1}$  indicated the presence of carboxylic acid.<sup>25</sup> The FT-IR results of the treated fatliquor showed the absence of the ester peak indicating that the phospholipid was hydrolyzed in the batch reactor and degraded. Presence of saturated carboxylic acid was indicated in FT-IR of the treated effluent.<sup>26</sup> Pablo et al.<sup>27</sup> reported the presence of saturated fatty acids at the end of aerobic treatment of oil bearing wastewater. As was evident from the present study that the lecithin-based fatliquor (containing predominantly phospholipids) biodegraded at the f/m ratio of 0.15 in the aerobic reactor, the presence of long chain carboxylic acid was confirmed from FT-IR spectra. FT-IR spectra of the lecithin-based fatliquor, before and after aerobic treatment, are presented in Figure 2.

#### ***Electrospray Ionisation Mass Spectroscopy (ESI-MS)***

##### ***Analysis of Lecithin-based Fatliquor***

Electrospray Ionisation (ESI) is one of the Atmospheric Pressure Ionisation (API) techniques and is well-suited to the analysis of polar molecules ranging from less than 100 to more than 1,000,000 Da in molecular mass. ESI-MS analysis was carried out on lecithin-based fatliquor, before and after biological treatment, to measure the molecular mass and structural changes after biological treatment. The ESI-MS spectrum of lecithin-based fatliquor is presented in Figure 3.

Commercial soybean lecithin is a complex mixture containing about 65-75% phospholipids which includes phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidyl inositol (PI), phosphatidic acid (PA), phosphatidylserine (PS) with smaller amounts of other substances like carbohydrates, pigments, sterols and sterol glycosides. All phospholipids contain a hydrophobic portion due to the presence of the fatty acid moieties and their complexity results from the two fatty acids on the 1,2-diacylglycerol portion. They also contain hydrophilic portions due to the presence of phosphate, choline, serine, ethanolamine, myo-inositol.

**TABLE II**  
**The main absorbance bands in FT-IR spectra before and after treatment of lecithin-based fatliquor.**

S.No	Group frequencies wave number (cm <sup>-1</sup> )	Nature of group	Frequencies for sample- before treatment (cm <sup>-1</sup> )	Frequencies for sample- after aerobic treatment (cm <sup>-1</sup> )
1	3570-3200	OH Stretch	3459.35	3452.92
2	2935–2915	Methylene (CH <sub>2</sub> ) C-H symmetric and asymmetric stretching	2927.66	2928.12
3	2865–2845	Methylene (CH <sub>2</sub> ) C-H symmetric and asymmetric stretching	2857.32	2857.74
4	2200-2450 2425–2325 2700–2100	Combination C–H stretching Phosphorus acid and ester P–H stretching Phosphorus acid and ester O–H stretching	2363.42	2361.71
5	1750-1725	Ester, C=O stretching	1740.98	
6	1600–1680	Carboxylate (carboxylic acid salt)	1638.44	1638.73
7	1485-1445	Methylene C-H bend	1461.13	1461.24
8	1200-1100	C-O-O-C symmetric stretching	1144.25	1139.26
9	950-1100 1000-1300	Second overtone O-H stretching, C-O stretching	1099.19	1100.60
10	750-720	Methylene-(CH <sub>2</sub> ) <sub>n</sub> - rocking ( $n \geq 3$ )	720.70	720.0

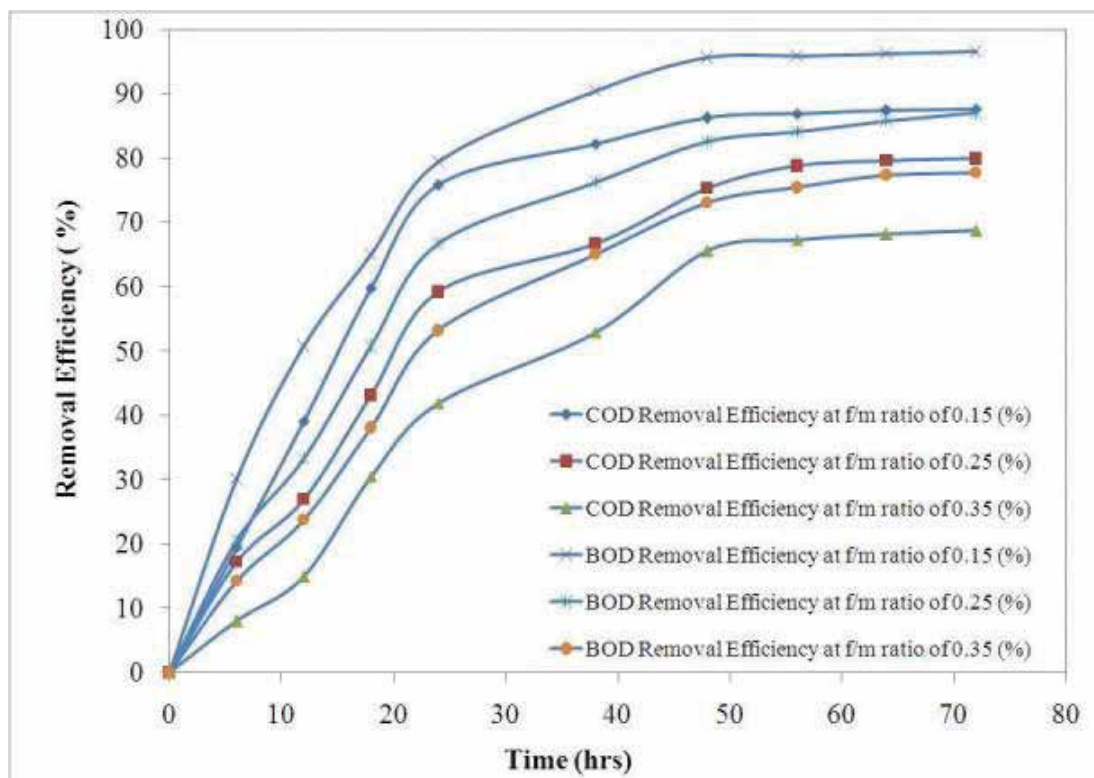


Figure 1. Biodegradation of lecithin-based fatliquor at f/m ratio of 0.35, 0.25 and 0.15.

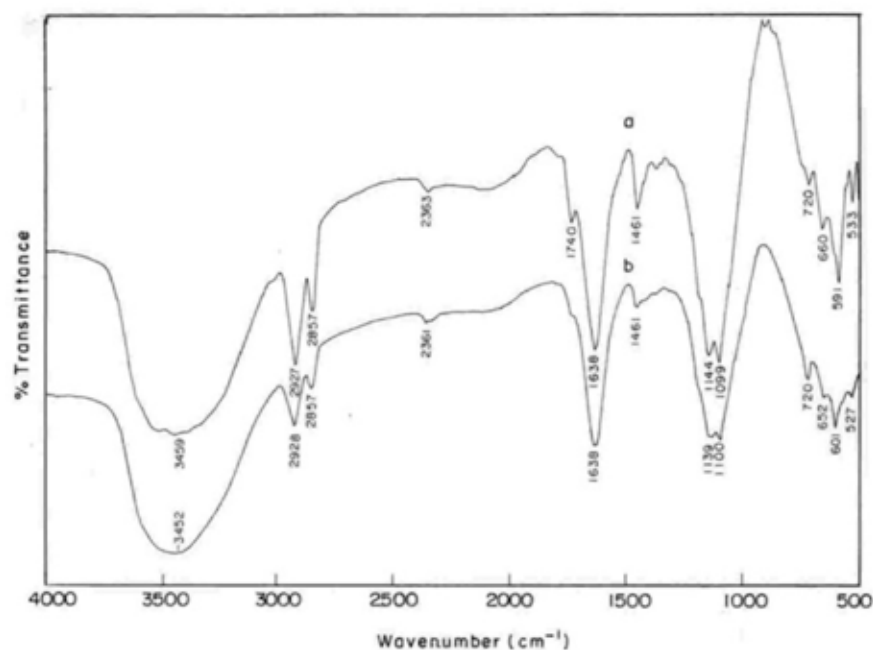


Figure 2. FT-IR spectra for lecithin-based fatliqor before and after aerobic treatment process.

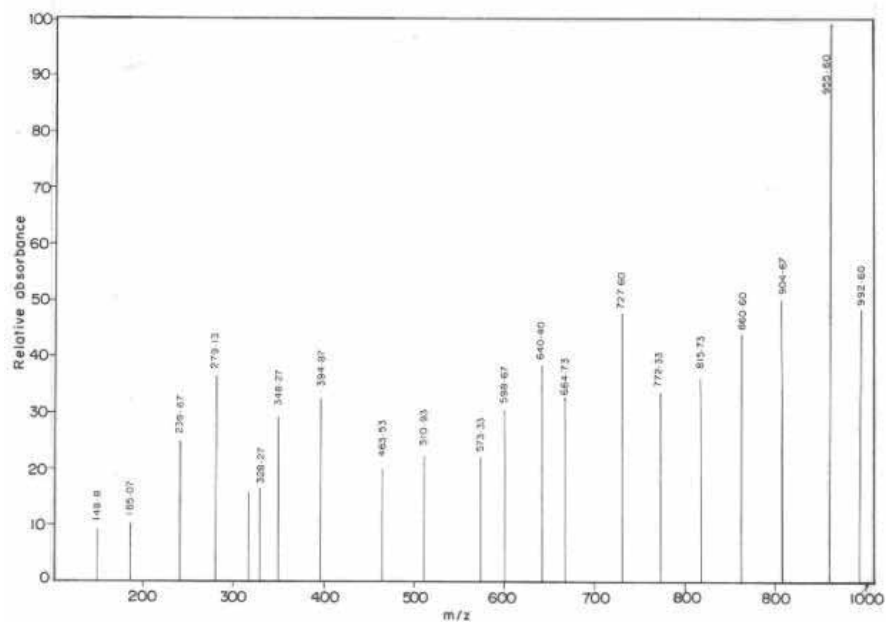


Figure 3. ESI-MS spectrum of lecithin based fatliqor before aerobic treatment

Figure 3. ESI-MS spectrum of lecithin-based fatliqor before aerobic treatment.

In positive-ion ESI, phospholipids typically exhibit an abundant protonated molecular ion  $[M+H]^+$  and several species-specific fragment ions through the cleavage of the phosphate-glycerol bond resulting in the elimination of the polar head group. Protonated and fragmented ions formed from phosphatidylcholine was observed in the  $m/z$  of 185.07,

279.13, 463.53 and 727.60. The presence of phosphatidylethanolamine in the fatliqor was observed as the protonated fragments at  $m/z$  of 148.8, 328.27. Signals at  $m/z$  573.33, 598.67 and 904.67 due to the protonated, fragmented ions of phosphatidylinositol were observed. Presence of phosphatidic acid was observed at  $m/z$  of 394.37, 279.13, and

640.4 by the fragmented ions. Signals due to the presence of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidic acid authenticated the presence of lecithin in the fatli liquor. These results are in accordance with the results obtained for the soybean lecithin analysis done for food application.<sup>28,29</sup>

Sterols and sterol glycosides have long been known to be present in soybean lecithin. All fatty acids normally in soybean oil were found in the acylated sterol glycosides, with increased amounts of saturated acids.<sup>10</sup> Presence of fragmented and protonated sterol glycoside was observed in the  $m/z$  signal of 148.8, 239.67, 394.37, 598.67, 860.60 and 904.67. The results confirmed the presence of phospholipids and sterol glycosides in the lecithin-based fatli liquor. Presence of increased amount of saturated fatty acids reduces the substrate utilization rate and this was reflected in long residence time of two days for getting maximum SBOD and SCOD removal at f/m ratio of 0.15.

#### ***Electrospray Ionisation Mass Spectroscopy (ESI-MS) Analysis on Aerobic Treated Lecithin-based Fatli liquor***

ESI-MS analysis was carried out after aerobic biological treatment of lecithin-based fatli liquor. From the biodegradation studies it was found that, f/m ratio of 0.15 was optimum value for maximum SBOD and SCOD removal efficiencies in the

batch reactor. The ESI-MS spectrum of treated fatli liquor is presented in Figure 4. It is evident from the figure that phospholipids are hydrolysed and degraded in the aerobic reactor. Fragmented, protonated, sodiated and adducts of phosphatidylcholine was observed at  $m/z$  of 258.07, 522.04, 653.33, 741.53 and 815.60. Fragmented, protonated ions of phosphatidylethanolamine were observed at  $m/z$  of 378.2, 467.33 and 691.53. Phosphatidylinositol ion signal was observed at  $m/z$  of 858. Oxidation products of squalene/or tocopherol was observed at  $m/z$  of 437.33. Fragmented ion from sterol glucosides was observed at signal  $m/z$  of 138.07 and 210.6. Presence of fragmented acyl chain of palmitic acid ion was observed at  $m/z$  313.07. At  $m/z$  of 255 fragmented ion of palmitic acid was observed. Palmitate ion of choline was observed at  $m/z$  258.07. The results indicated that lecithin was degraded in the aerobic reactor and presence of saturated acids was observed in higher quantity. These results are in agreement with the work of enzymatic hydrolysis of soybean lecithins and sunflower lecithin by Penci et al.<sup>30</sup> The enzymatic hydrolysis of lecithin revealed that about 20% of saturated palmitic acid and 4.5% of stearic acid was present in the hydrolysed products. Matsui et al.,<sup>31</sup> reported the findings of the work on oil containing wastewater treatment in immobilized fluidized reactor. The results indicated that long-chain saturated fatty acids are less degradable due to lower dispersion than unsaturated fatty acids.

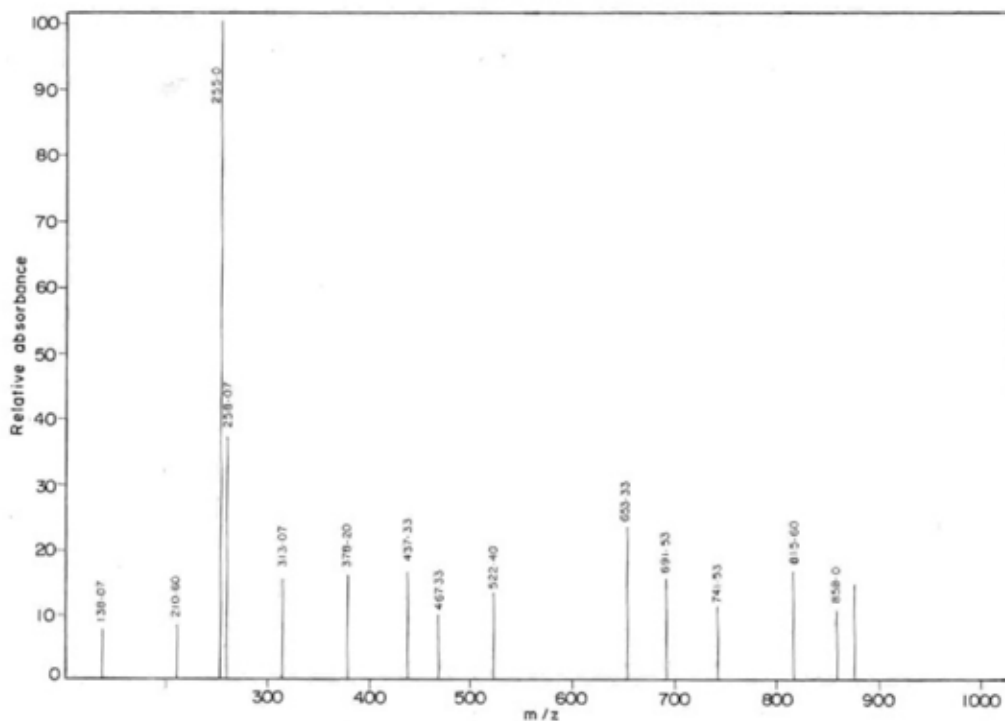


Figure 4. ESI-MS spectrum of lecithin-based fatli liquor after aerobic treatment.

The results of ESI-MS spectrum of semi synthetic fatliquor predominantly contain lecithin and this was confirmed by the presence of various forms of phospholipids. This result was in accordance with the FT-IR indicating the presence of phospholipids. Treatment by activated sludge process at the optimum f/m ratio of 0.15 resulted in hydrolysis and degradation of phospholipids in the fatliquor and presence of long chain saturated acid was established.

## CONCLUSION

The results clearly proved that most of the compounds present in fatliquor are degraded in the aerobic batch reactor. Lecithin-based fatliquor predominantly consists of soybean lecithin. BOD/COD ratio of 0.36 indicated that lecithin-based fatliquor is biodegradable. The results indicated that f/m ratio of 0.15 with the residence time of two days is needed to achieve the maximum SBOD and SCOD removal efficiencies of 95.55 and 86.21 percent, which resulted in 28 mg/L and 240 mg/L in the treated effluent. Two days residence time for the f/m ratio of 0.15 resulted in meeting the discharge standard limit prescribed for tannery effluent in India. FT-IR and ESI-MS analysis of lecithin-based fatliquor confirmed the presence of phospholipids. After aerobic treatment, the presence of long chain saturated fatty acid is confirmed by FT-IR and ESI-MS.

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