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Differential Impact of the Biodegradation Sunflower Oil, Particulate Substrate, Caused by the Presence of Saccharose, Soluble Substrate, on Activated Sludge Treatment

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Abstract: This research studies the biodegradation of sunflower-type vegetative oil in two proposed activated sludge systems, the first one to biologically treat an influent containing only vegetative oil and the second one to treat a mixture of vegetable oil plus saccharose. The purpose of these analyses is to evaluate the differential impact caused by the soluble substrate saccharose on the removal of vegetative oil. Vegetative oil biodegradation in both systems was studied and quantified via integral mass balance, and relevant operating parameters were monitored. This experimentation based on the mass balance estimation of biodegraded vegetative oil serves as a reference to understand the effect of soluble substrates present in mixed wastewater on oil biodegradation. Information was generated on the performance of the two activated sludge treatment systems. Both influents were pre-stirred before they entered the bench-scale activated sludge plants. The working range for sunflower oil concentration was 120 to 520 mg/L for the influent with sunflower oil and 180 to 750 mg/L for the influent with sunflower oil and saccharose. Biodegradation was in the order of 56 to 72% and 47 to 67%, respectively. The removal of sunflower oil in biodegradation and flotation was in the order of 90% in both scenarios.



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Keywords: biodegradation; vegetative oil; activated sludge

1. Introduction

Recent methods have been developed for the treatment of oily wastewater. In the past, effluents from the vegetable oil industry were discharged directly into the soil or groundwater. For this reason, various treatments are applied, in which contaminants such as vegetative oil and inorganics present in these waters are eliminated, thus avoiding damage to the environment [1,2].

Among the treatments, the biological treatment of petrochemical wastewater has demonstrated its ability to reduce the concentration of pollutants prior to discharge into the environment [3].

There is much research studying the removal and biodegradation of lipids via biological treatment [4]. However, the literature indicates that biological treatment can lead to foaming with filamentous bacteria and flocs, which affects biodegradation [5]. In this context, vegetative oils are classified as slowly biodegradable substances [6]. Biodegradation is initiated through a process of hydrolysis of insoluble compounds such as vegetative oil, which is an enzymatic action. The extracellular enzyme called lipase is the most common enzyme that releases fatty acids as a consequence of enzymatic action [7]. In this sense, microbial lipases are attracting industry attention due to their stability, broad substrate specificity, high yield and easy availability.

On the other hand, it is known that a high concentration of lipids in wastewater inhibits the activity of microorganisms in biological treatment systems. Microorganisms capable of biodegrading edible lipids reduce these effects [8]. The elimination of vegetative

oil can be achieved by preparing a lipolytic biological culture, which takes the fats present in the waste, metabolizes them and takes advantage of the nutrients, minerals, carbohydrates and proteins [9–12].

Currently, the biodegradation of vegetative oil and non-water-soluble substrates is one of the major problems for the biological treatment of liquid waste [12]. When these substrates have low or zero solubility, the use of biosurfactants to emulsify the mixture is recommended [13]. Normally, such emulsions are thermodynamically unstable, so there is a known tendency to reduce the interface area between the aqueous and oil phases, leading to coalescence of the oil droplets. The coalescence of oil droplets can be reduced or even eliminated by stabilization mechanisms [14]. The unstable nature of the emulsion is due to the fact that the contact between oil and water molecules is not energetically favorable, so surfactants (emulsifiers) are added to the emulsion to improve the system stability [15].

In biologically activated sludge treatment, the magnitude of the contact area in water or oil is important, as a large interfacial area is required. This area can be expanded by providing energy through mechanical stirring or an electric field [16]. As the stirring rate increases, the average droplet size decreases steadily [16]. With an adequate enzyme level and optimum interfacial area between the aqueous and oily phases, the mass transfer is resolved, giving rise to the hydrolysis stage [17].

New research has evaluated the use of hydrolyzed lipids extracted from algal biomass mixed with sugar (saccharose) factory wastewater as a low-cost nutrient and carbon source for the growth of microalgae *Ettlia* sp. Lipid productivity increased markedly from 5.8 to 95.5 mg when 20% sugar (saccharose) factory wastewater was added to the culture medium. A remarkable increase of 20% was verified for the C16 and C18 fractions, present in the form of methyl esters of fatty acids, under the mentioned conditions using gas chromatographic analysis [18,19].

It can be summarized that the biodegradation of vegetable oil by microorganisms is feasible and has adequate removal efficiency. It reduces management time, is environmentally friendly and easy to apply, is disease-free and is low-cost because it reduces chemical oxygen demand and the amount of suspended lipids compared with thermal methods [20].

According to the reports to date, the performance of vegetative oil removal via biodegradation has not been studied in terms of evaluating the impact of soluble substrates on the removal of sunflower oil. In this context, a study was designed to analyze and evaluate the differential impact caused by the presence of saccharose on the performance of the activated sludge biological treatment system at bench scale when treating an influent containing vegetative oil. In this scenario, an experiment was carried out with artificial wastewater containing only sunflower oil and a second experiment was carried out with artificial wastewater containing sunflower oil and saccharose.

Vegetative oils are usually treated with physical processes; however, in this experiment a biological treatment was applied to eliminate them. The first motive was to open the treatment window for this substrate, and the second was to evaluate how the soluble substrates that accompany them impact the elimination of vegetative oils. The quantitative determination of the biodegradation of fats and oils is a topic that presents considerable difficulties, which was resolved with the applied methodology based on the material balance, which allowed us to reach the results we obtained through experimental work.

2. Materials and Methods

2.1. Utilized Substrate

The substrate used was sunflower oil, which has the following composition (see Table 1).

Table 1. Composition of fatty acids of sunflower oil and spatial disposition [21]. Results are in % moles.

Fat	Position	16:0	18	18:1 (9)	18:2 (9,12)	18:3 (9,12,15)
Sunflower oil	1	10.6	3.3	16.6	69.5	-
	2	1.3	1.1	21.5	76.0	-
	3	9.7	9.2	27.6	53.5	-

2.2. Physicochemical Parameters and Analytical Methods

The potassium dichromate method was used to evaluate chemical oxygen demand (COD) levels. The method used is a variation of the standard method [22], and maintains the basis of it. However, the variation applied uses a significantly smaller sample and fewer reagents. The sample was chemically oxidized through the action of potassium dichromate at 150 °C for two hours. Mercury sulfate was used to avoid possible interferences with chloride, and silver sulfate was used as a catalyst. Subsequently, a value of 600 nm was determined using spectrophotometry.

Vegetative oil determination was carried out using:

- The gravimetric assay Soxhlet method and the 213E method [23].
- Total suspended solids (TSS) (209C method [23])
- Volatile suspended solids (VSS) (208E method [23])
- For the sludge volume index, the 213E method was used [23].

2.3. Continuous Equipment

This work involved analyzing for 22 days the water treated in a bench-scale activated sludge plant (Figure 1) containing sunflower oil in the first step, with saccharose added in a second experiment.

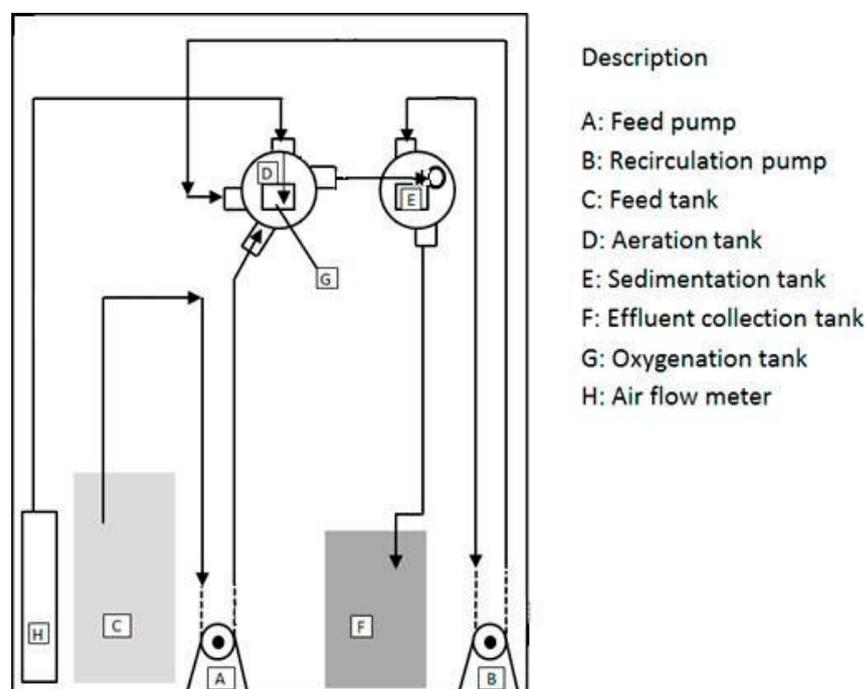


Figure 1. Experimental equipment diagram.

This experimental equipment essentially consisted of the following parts:

- Control unit:

The control unit is composed of an air pump and a feed pump, complemented with a flow meter. It also has a timer for intermittent operations and a sludge recycling system from the sedimentation tank to the aeration tank [24].

- Aeration tank:

This is a 12 L transparent Plexiglas[®] cylinder with outlets corresponding to different volumes (7, 8, 9 and 10 L). It has two inlets, one for the influent and one for activated sludge recirculation. In addition, the aeration tank has two ceramic diffusers located at the bottom [24].

- Sedimentation tank:

This is a transparent Plexiglas[®] cylinder, in which the upper half is cylindrical and the lower half is conical, which favors sedimentation and activated sludge thickening. The sedimentation flow comes from the aeration tank. The decanted activated sludge is separated and recirculated in the lower part by being pumped to the aeration tank.

2.4. Material Balance: Biodegradability Determination of Fats and Oils

The percentage of vegetative oil removed via biodegradation and flotation was estimated through mass balances. The process diagram, Figure 2, shows the flows and concentrations of the inlet and outlet streams in the different stages of the activated sludge process.

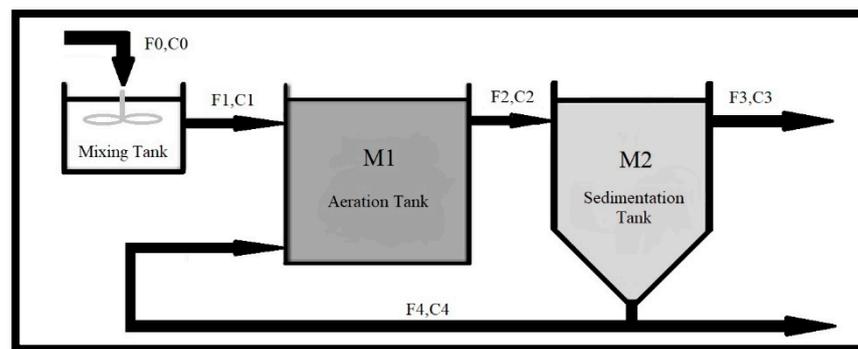


Figure 2. Activated sludge plant process diagram.

To achieve the aforementioned balance, the remaining oil in the feed tank corresponding to the non-emulsified oil must be estimated and, therefore, it is not part of the influent entering the aeration tank. The size of this fraction quantitatively indicates the level of mixing in the system. In this experiment, we worked with two types of influents, one of which contained only vegetable oil; the other contained saccharose in addition to oil.

The Figure 2 shows the following:

F0, C0: Flow and concentration of oil entering the mixing tank.

F1, C1: Flow and feed oil concentration moving to the aeration tank.

F2, C2: Flow and concentration of vegetative oil leaving the aeration tank (flow and concentration of vegetative oil entering the secondary settler).

F3, C3: Flow and concentration of vegetative oil from purified effluent.

F4, C4: Flow and vegetative oil concentration in recirculation flow.

M1: Oil mass contained in the mixing liquor of the aeration tank.

M2: Oil mass floating in the upper part of the sedimentation tank and oil mass at the bottom of the sedimentation tank with biomass attached.

In this experimental work, water and sunflower oil were mixed in the feed tank. Some of the added sunflower oil accumulated in the feed tank and did not enter the treatment system. The mass of biodegraded sunflower oil was determined from the mass balance equation. Initially, the system was assumed to be oil-free.

For the aeration tank:

$$\frac{\Delta M_1}{\Delta t} = F_1 \cdot C_1 - F_2 \cdot C_2 - r_A \cdot V + F_4 \cdot C_4 \quad (1)$$

For the secondary sedimentation tank:

$$\Delta M_2 / \Delta t = F_2 \cdot C_2 - F_3 \cdot C_3 - F_4 \cdot C_4 \quad (2)$$

It must be noticed that $F_6 = 0$

where M_1 corresponds to the oils and fats in the aerator tank.

M_2 corresponds to oils and fats in the sedimentation tank.

V is the volume reactor.

r_A is the vegetable oil biodegradation rate.

The balance for the system as a whole is as follows.

Expression number 3 corresponds to vegetable oil that disappears per unit of time; this means the oil is clearly biodegraded by microorganisms, such that

$$r_A \cdot V = F_1 \cdot C_1 - F_3 \cdot C_3 - \frac{\Delta M_1}{\Delta t} - \frac{\Delta M_2}{\Delta t} \quad (3)$$

The fats and oils separated by flotation and accumulated in the aeration tank and in the clarifier were measured, as well as the fats and oils in the influent, which allowed the level of biodegradation to be determined. Considering that the sunflower oil accumulated in the feed tank is measured, the equation for the integral balance of matter for a given period of time is

$$M_1 + M_2 = F_1 \cdot C_1 \cdot \Delta t - F_3 \cdot C_3 \cdot \Delta t - r_A \cdot V \cdot \Delta t \quad (4)$$

2.5. Operating Modes

The bench-scale activated sludge plant was fed with synthetic wastewater, where the feed was prepared daily considering the appropriate proportion of organic load (fats and oils have a concentration range of 100 to 800 mg/L and saccharose 400 mg/L when present) and nutrient, phosphorus and nitrogen load according to the ratio C:N:P = 100:5:1 [25]. The measurements started when sunflower oil was added, and the concentration of this substrate gradually increased. The feed was prepared daily, and nitrogen and phosphorus increased as a function of the organic input from fats and oils. Initially, the system was started up and when the increasing amount of sunflower oil was fed through the influent to the system, the measurement and recording of the process variables began. The feed was prepared daily considering the appropriate proportion of organic load and the load of nutrients, phosphorus and nitrogen. The synthetic wastewater was prepared in a 50 L storage tank, which was equipped with an agitator to adequately distribute the sunflower oil in the water. By means of a peristaltic pump, the influent was fed to the aeration tank, which is activated from the control unit. The oxygen feed and recirculation flow are controlled by the control unit (dissolved oxygen concentration: 2 mg/L). The effluent was accumulated in a 30 L volume tank, from which the samples were taken for testing. The synthetic flow rate was 25 L/day. It should be mentioned that the process was carried out at room temperature in a range between 10 and 20 °C.

3. Results and Discussion

3.1. Mass Balance and Biodegradation Rate

In this work, the prepared wastewater with vegetable oil as a substrate was subjected to a preliminary agitation and the same procedure was applied for wastewater containing a mixture of substrates, such as saccharose and vegetable oil. In both experiments, the influent was subjected to mechanical stirring prior to entering the aeration tank, thus increasing the level of emulsion of the oil particles in the water, which increased the contact

area between the oil, the water and the microorganisms. This is closely related to the mass transfer phenomena required for the biodegradation of sunflower oil in treatment systems for active sludge.

The mass balance for sunflower vegetable oil developed in the protocol allows for estimating the biodegraded oil in both work scenarios. The oil entering the aeration tank is biologically treated by activated sludge, and the oil removed is different from the biodegraded oil since part of the incoming oil is retained via flotation.

(A) Biodegradation and removal of vegetable oil as the sole substrate

In this case, the removal and biodegradation of vegetable oil were studied; vegetable oil was the only substrate present in the wastewater influent and, therefore, the only carbon source in the biomass present in the tank. The following is the balance of matter (Table 2) for the aeration tank according to the protocol indicated above. The results indicated in Table 2 show biodegradation rates between 64 and 75%. In this experiment, the work was carried out with mechanical stirring of the influent in the aeration tank, which generates smaller oil emulsions, thus producing a better mix and distribution of substrates present in the influent fed to the system. Considerable levels of biodegradability were achieved (between 56 and 72%), demonstrating the effect of mechanical agitation, which improved the contact area in the mixture and reduced the level of oil emulsion, thus increasing the interfacial area and, therefore, the biodegradation of the oil.

Table 2. Mass balance for oily influent with previous agitation.

Operation Days, Balance Included	Oil Mass Fed (g)	Oil Mass Retained (g)	Oil Mass in Effluent (g)	Oil Mass Accumulated (g)	Biodegraded Mass (g)	Biodegradation Efficiency (%)
1 a 7	39.5	22.6	4.35	0.5	11.9	71
8 a 15	88	23	6.4	11.8	46.8	72
16 a 22	108	38	9.7	21	39.3	56

(B) Biodegradation and removal of vegetable oil for mixed influent

In the second experiment, a mixed influent containing saccharose and oil as a carbon source was treated. The aeration tank contained activated sludge that had previously degraded sunflower oil and saccharose, confirming that the biomass had suffered a previous acclimatization with respect to the oily substrate. Table 3 shows oil biodegradation values ranging from 47% to 66%, which are below the values obtained for an influent containing only oil.

Table 3. Mass balance for mixed influent with previous agitation.

Operation Days, Balance Included	Oil Mass Feeding (G)	Oil Mass Retained (G)	Oil Mass in Effluent (G)	Oil Mass Accumulated (G)	Biodegraded Mass (G)	Biodegradation Efficiency (%)
1 a 5	30	8	3	4.5	14.5	66
6 a 12	101	8	8	23	62	67
13 a 16	86.5	10.5	5.5	35	35.5	47

Biodegradation values between 56 and 72% were obtained with agitation for the oil-only influent, which is similar to the results of an experiment on aerobic treatment to eliminate vegetative oil in the dairy industry with a mixture of native bacteria as a biomass, which reached an efficiency of 72% in the biodegradation of fats and oils [26–28]. The high level of wastewater from the food industry often leads to pollution of the environment. The activity of microorganisms in biological wastewater treatment systems is prevented. In this work, 75 strains were separated from activated sludge, which purified wastewater

containing edible oil. Eight isolated bacteria grew in liquids with edible oil as the sole carbon source, confirming their high capacity for oil degradation [29–31]. As the only substrate is oil, this system can be compared with the fats and oils treatment proposal considered for the wastewater treatment project of the commune of Los Angeles, Chile, developed by DEGREMONT. This project involves treating vegetable oil together with activated sludge in an aerobic digester so that the only available substrate is the fats and oils collected in the primary treatment, which are dosed based on optimal distribution and mixing criteria [32–34].

Treatment with biological grease became widespread in France. In 1998, about sixty plants designed with a volumetric load of 2.5 kg COD/m^3 per reactor per day were recorded. Different areas were selected to represent and study them [35,36].

Recent research has involved studying the behavior and performance of aerobic thermophile bacteria for wastewater with a high oily organic content, showing that the maximum growth rate is reached between 55 and $58 \text{ }^\circ\text{C}$ [37]. This is consistent with the hypothesis on the direct correlation between temperatures and the dissolution of vegetative oil. On the other hand, laboratory studies have been conducted for commercial supplements, namely multispecies-type bioenhancers, and they have been found to increase the removal of vegetative oil from 37% to 62%, which has some similarity to the biomass acclimation effect, which is a biological measure [38–41]. With the above information, it is evident that the operating parameters are significant to the performance of the system.

In this context, influents with concentrations of 300, 600 and 900 mg/L were prepared in the feed tank for a period of approximately one week, in order to measure the levels of biodegradability achieved and to compare the behavior of the system with respect to the results obtained when the influent was not previously agitated. The operating conditions in terms of air flow, residence time, recirculation ratio and C: N: P ratio at the feed level were the same.

3.1.1. Mass Load and COD Elimination

Figure 3 shows the COD removal efficiency and COD biodegradation efficiency achieved by the activated sludge treatment system. The elimination given by the COD biodegraded by the biomass present in the aeration tank should be added to the flotation of vegetative oil, which is retained in the respective system tanks. For lower concentration levels of sunflower oil, there was a greater proximity between the COD removal and the biodegradation, as the COD removal via flotation was marginal, but not for high concentration ranges, where a considerable increase in vegetable oil removed via flotation was observed. Figure 4 shows that COD removal was not affected by mass loading, as it remained relatively constant over the range analyzed, because variations associated with vegetable oil biodegradation were compensated for by an increase in its removal via flotation. On the other hand, this parameter did affect the levels of biodegradation achieved. For the biodegradation of oily organic matter, a relationship was established between the applied mass load and the biodegradation efficiency, decreasing for mass load ranges greater than $0.6 \text{ (kgCOD)/(kgTSS}\cdot\text{d)}$ that corresponded to a high load regime, so there was a higher biodegradation level for the conventional regime and with extended aeration.

As can be observed, it is confirmed that an important part of organic matter corresponding to vegetable oil accumulates in the secondary settler and correlates with the oil concentration in the influent. The COD values indicate that the sum of oil biodegradation and flotation phenomena of the oil allows a considerable overall removal of the vegetable oil, over 80%. Considering these mass load values, it is recommended to work with low mass loads and prolonged aeration and, subsequently, in a conventional regime at a high load. It is pertinent to mention that oil removal is mainly due to biodegradation of vegetable oil, about 70%, while 20% corresponds to flotation.

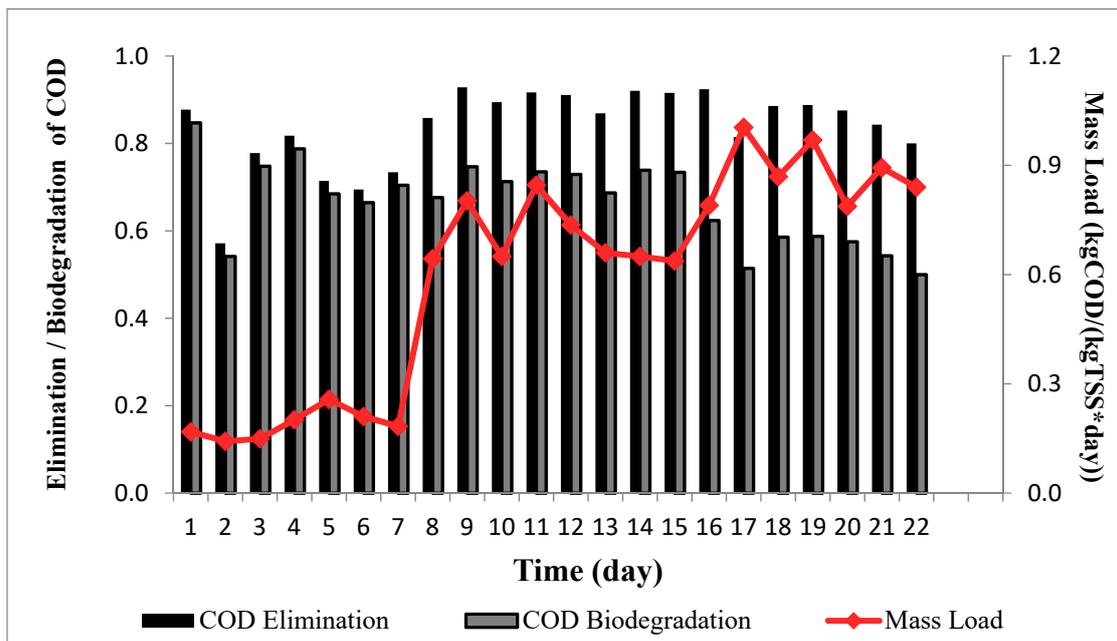


Figure 3. COD removal efficiency and mass loading for oily influent.

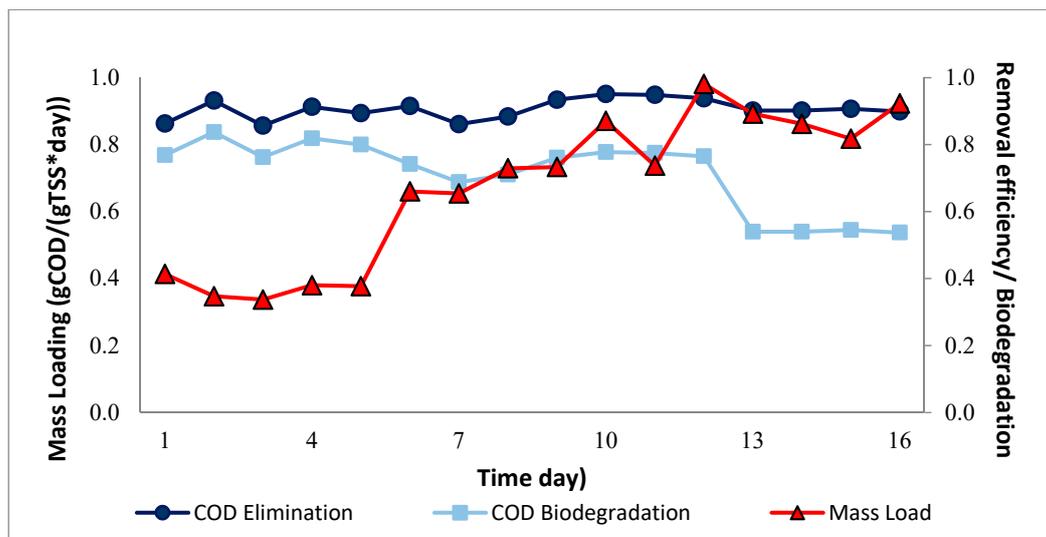


Figure 4. COD removal and biodegradation efficiency and mass loading for mixed influent.

Figure 4 shows that for mass loads, COD elimination levels reach around 80% for mass loads greater than 0.8 (g COD/(g TSS* d)), which correspond to conventional-type processes and high load. However, COD removal via biodegradation is considerably reduced, reaching values close to 56%. It should be noted that regardless of the decrease, a removal via biodegradation of more than 55% is a remarkable result.

Figure 5 shows that at higher concentrations of organic matter in the sunflower oil influent, there is an increase in effluent COD. In this case, the concentrations of sunflower oil are considerably high (approximately between 180 and 640 mg/L), so that the COD concentrations, including the contribution of saccharose and vegetable oil, initially present approximately 750 mg/L and reach values above 2100 mg/L, which is the main cause of the progressive increase in the concentration of organic matter in the effluent.

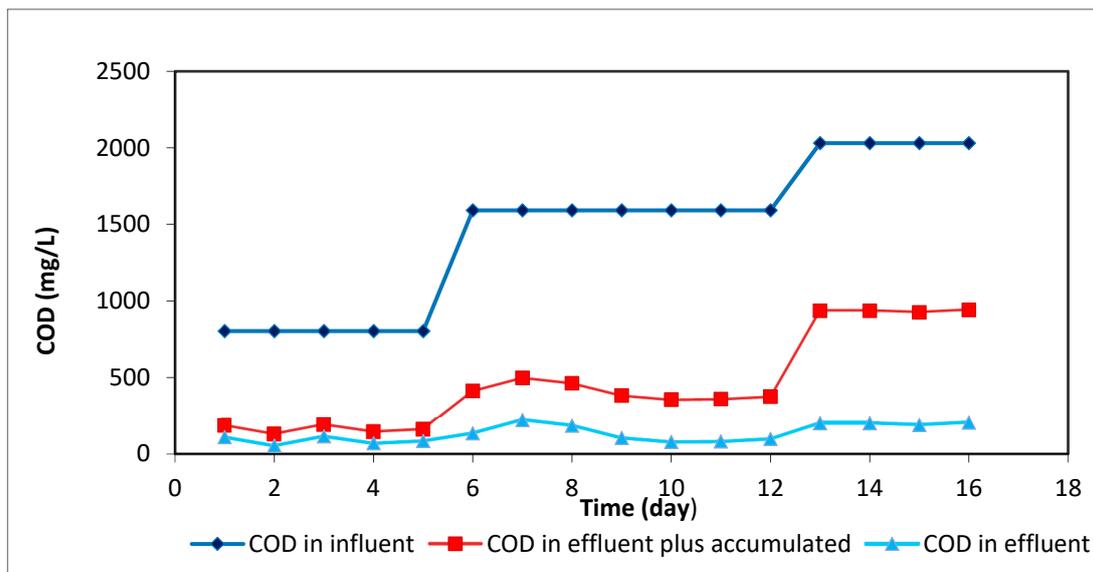


Figure 5. COD behavior in mixed influent, effluent and effluent plus accumulated effluent.

This experiment showed that the removal for this range of concentrations of sunflower oil decreases with biodegradation. It was observed that given the high concentration of sunflower oil in the influent, an important fraction was retained via flotation and accumulated in the tanks of the system and especially on the surface of the secondary settler. The accumulation of sunflower oil and fatty acids in the secondary settler favors the presence of these substrates in the effluent and is the physical cause of the increase in COD in the effluent. These results are consistent with those observed previously.

In order to verify the above assumptions, Figure 6 shows three periods, each with its corresponding vegetable oil concentration in the influent. For the first period the oil concentration was 120 mg/L, then it increased to a value close to 400 mg/L and in the last 7 days of operation, a concentration of vegetable oil in the influent close to 500 mg/L was observed. On the other hand, the sunflower oil concentration in the effluent was practically constant. Regarding oil removal, the elimination levels reached around 90%, where a considerable percentage corresponded to biodegraded oil; a complementary fraction was separated via flotation, as can be observed in Figure 6, in the first two weeks.

The accumulated sunflower oil level increases with oil concentration in the influent. It should be noted that the vegetative oil concentration increase in the influent inhibits biodegradation, which is corroborated by the results of the balance of matter, where biodegradability of over 70% was reached in moderate concentrations of vegetable oil and decreased to 56% when the concentration increased. This decrease in sunflower oil elimination via biodegradation is explained because the biomass had been fed with a strictly oily influent, which generates an overload of sunflower oil and derivatives due to the slow biodegradation of this substrate.

The anaerobic processes are known to inhibit long chain fatty acid methanogens (LCFAs) in fat-rich wastewater. Reactors often fail due to excessive LCFA accumulation in the slurry. For this reason, long-term acclimation processes are a strategy to improve the methanogenic conversion of these compounds. Anaerobic sludge previously exposed to LCFA for more than 100 days converted a specific associated biomass substrate of $(3.2 \pm 0.1) \text{ kg} \cdot \text{kg}^{-1}$ with very short delay phases (<1 day), whereas non-acclimated sludges showed lag phases of 11–15 days when metabolizing $(0.06 \text{ to } 0.08) \text{ kg} \cdot \text{kg}^{-1}$. It is clearly shown that long-term acclimatization sludge for LCFAs is essential for high-rate methanogens of LCFAs [42].

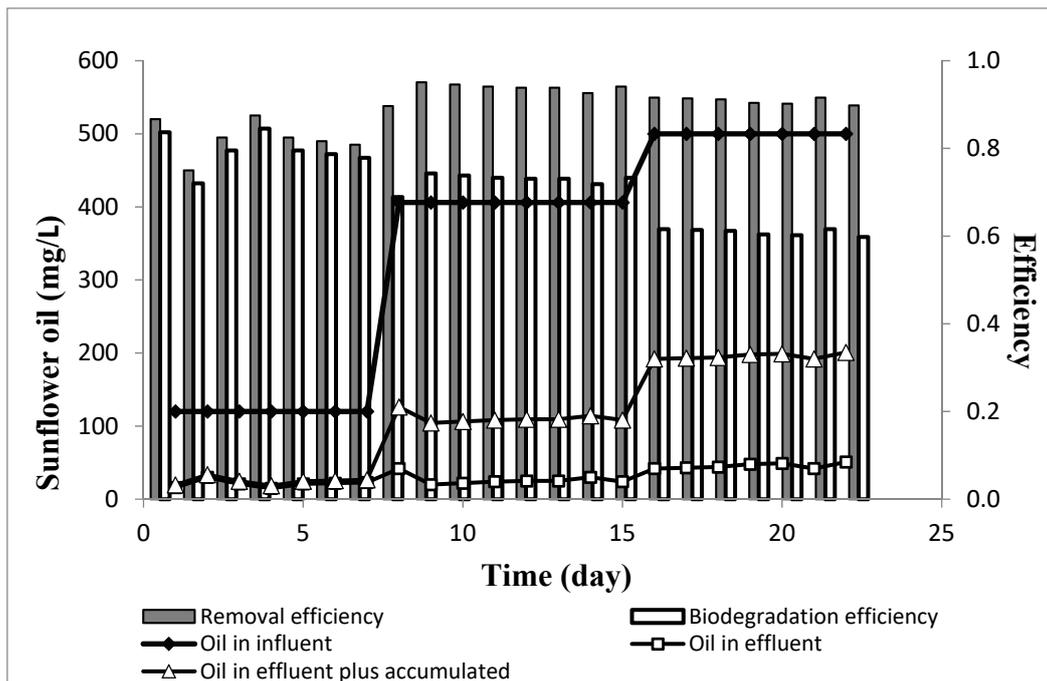


Figure 6. Feeding without saccharose. Sunflower oil concentration in influent, effluent and effluent plus accumulated oil and oil removal and biodegradation efficiency.

3.1.2. Oil in Influent and Biodegradation Efficiency

Figure 7 shows that the accumulated sunflower oil for low concentration ranges in the influent was marginal and the significant biodegradation efficiency was about 66%. It can be observed that from the fifth day of the experiment, there was an increase in the oil concentration in the influent, which increased the accumulated sunflower oil. With this scenario, not only is there a higher mass of biodegraded oil, but there is also a higher mass of sunflower oil retained via flotation in the secondary settler of the biologically activated sludge treatment system.

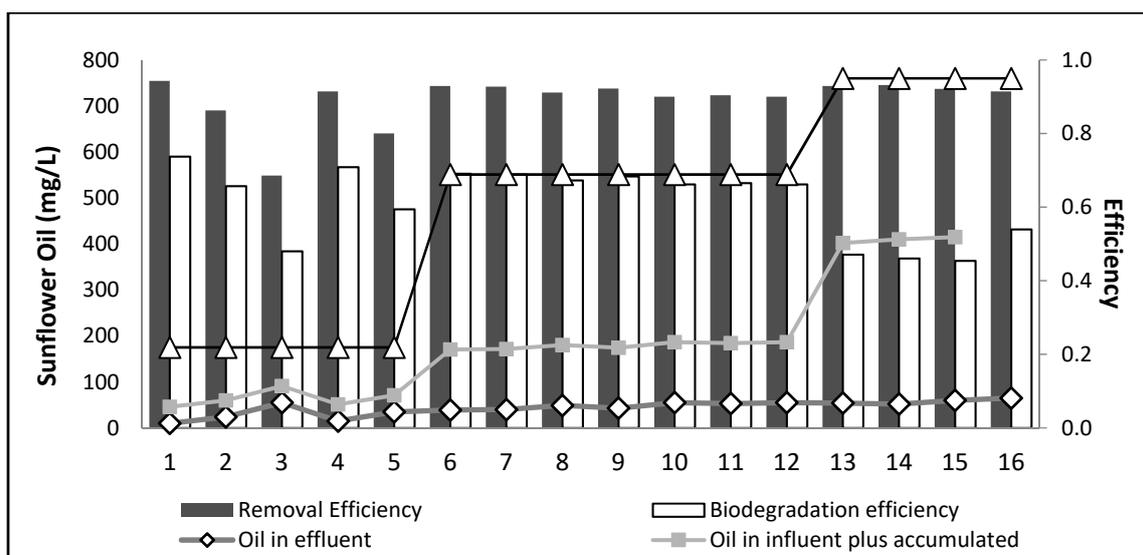


Figure 7. Concentration of mixed influent, effluent and effluent plus accumulated effluent.

The second relevant accumulation event recorded in this experiment was observed on day 13, which corresponds to influent sunflower oil concentration of 750 mg/L. Therefore,

the accumulation due to the increase in sunflower oil in the affluent was confirmed. In addition, a decrease in the removal via biodegradation of sunflower oil was observed. Therefore, the accumulation was more noticeable in this case and was mainly due to two factors: increased concentration of sunflower oil in the influent and less biodegradation.

The decrease in sunflower oil biodegradation indicates that it is inhibited at high concentrations of sunflower oil, despite the acclimatization to which the biomass was previously subjected. It should be noted that the saccharose concentration remained constant, corresponding to the mass of readily biodegradable substrate, and it can be seen from Figure 7 that for the range of sunflower oil concentrations above 750 mg/L, biodegradation decreased to about 40%, but a removal of sunflower oil above 90% was also achieved because the decrease in biodegradation was compensated for by the flotation mechanism. This analysis made it possible to evaluate the differential impact caused by the presence of saccharose on the performance of the bench-scale activated sludge biological treatment system regarding treating an influent containing only vegetative oil.

3.1.3. Biomass Behavior

Figure 8, which shows the evolution of mixed liquor total suspended solids (MLSSs) and mixed liquor volatile suspended solids (MLVSSs), evidences a decrease in both at the beginning of the experiment, in an activated sludge system with unacclimatized biomass treating an influent containing sunflower oil. Subsequently, the system maintained a relatively stable concentration of suspended solids around 3000 mg/L, which indicates that the evolution of solids is independent of the concentration of sunflower oil in the influent. It was observed that values obtained for the sludge volumetric index (SVI) varied according to the influent oil concentration level. The SVI values for sunflower oil concentrations of 120 mg/L in the influent, which are close to 160 mL/g, are high values explained by the low amount of substrate entering the system generating very low mass load conditions and causing the deflocculating phenomenon, which hinders sedimentation and biomass production. Furthermore, deflocculating increases effluent suspended solid concentration. When the influent sunflower oil concentration was increased to 406 mg/L and subsequently 580 mg/L, the SVI value was reduced, reaching values lower than 100 mL/g, since under these conditions the bulking phenomenon is controlled because of the considerable concentration of a particulate substrate that requires hydrolysis [43].

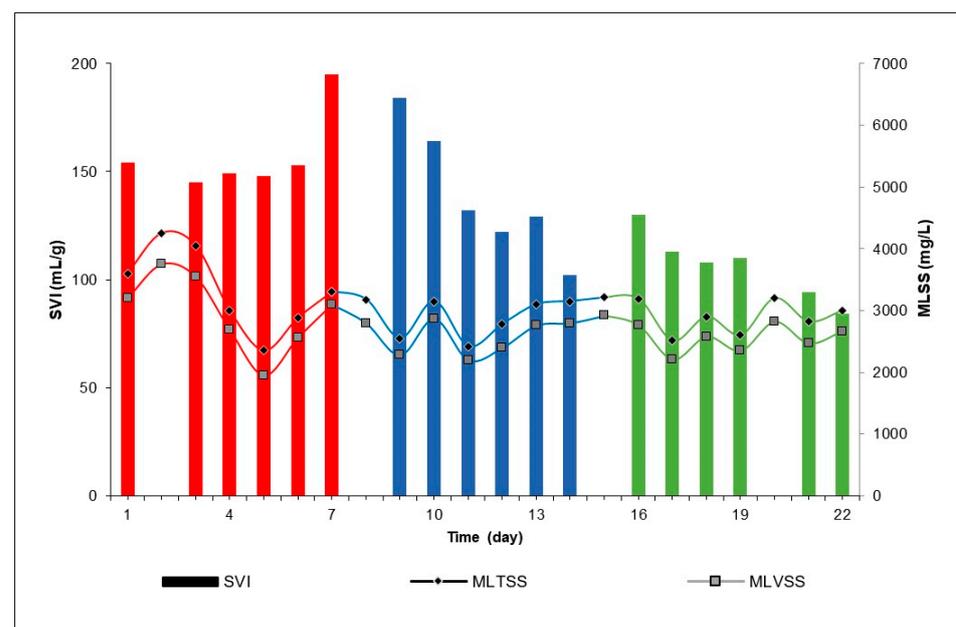


Figure 8. Feeding without saccharose. Biomass behavior, MLSSs, MLVSSs and SVI evolution.

The Oxygen Uptake Rate (OUR) Behavior

Through the application of this test, the level of biomass activity in both scenarios and the incidence of the type of substrate were studied. In the case of the system fed only with sunflower oil, Table 4 shows that the higher the concentration of sunflower oil, the lower the OUR and therefore the activity of the biomass, since it consumes less oxygen. The decrease in the OUR is explained by the increase in the concentration of sunflower oil, a substrate that is difficult to biodegrade. In this case, the only factor that affects the oxygen consumption and the biodegradable activity of the biomass is the concentration of the sunflower oil. Evidently, the rate of biodegradation of this substrate is affected by its increased concentration in the aeration tank, which stimulates the coalescence of oil emulsions and progressively hinders the biosorption process by the microorganisms.

Table 4. Evolution of the OUR.

Sunflower Oil Influent (mg/L)	OUR (gO ₂ /(gMLVSS·h))
120 (Red)	0.032
406 (Blue)	0.011
580 (Green)	0.007

The OUR values (Table 4) are in the range of other experiments for slowly biodegradable organic substrates [44].

Figure 9 shows the behavior of the MLSSs and MLVSSs. In this case, the biomass was fed with a mixed influent, saccharose and sunflower oil, and at the beginning there was a higher concentration of saccharose; therefore, the biomass had more substrate available for easy biodegradation, which changed over the course of the experiment, since the ratio [saccharose mass/oil mass] progressively decreased: [2.2], [0.7], [0.5].

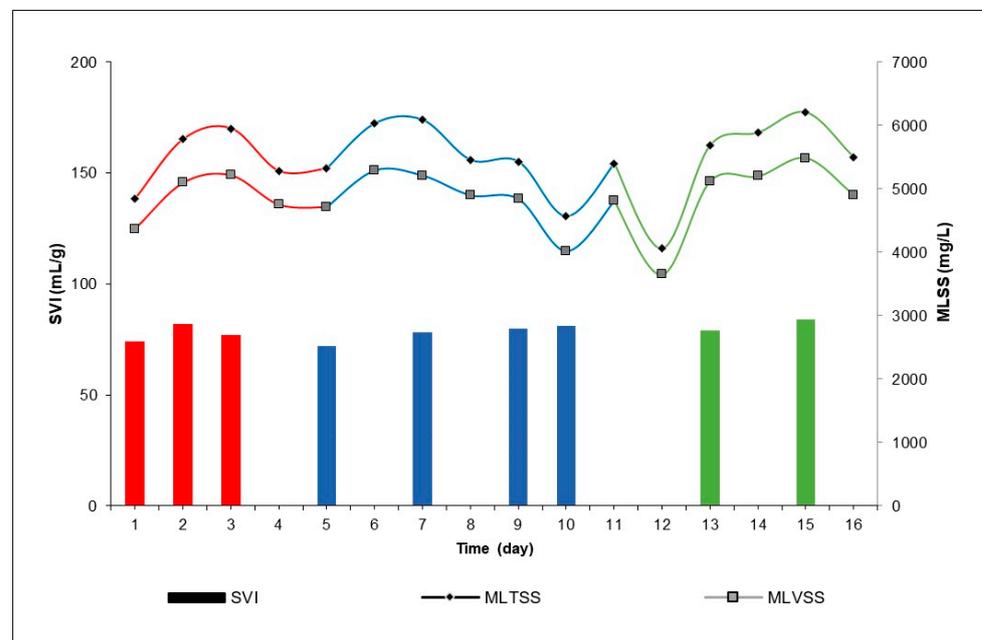


Figure 9. Feeding with saccharose. Biomass behavior, MLTSS, MLVSS and SVI evolution.

This is because regardless of the increase in total substrate concentration, the overall biodegradability of the substrates decreased, resulting in a compensation effect between these two factors that affected biomass growth. This is explained by the fact that in spite of increasing the overall substrate load, the overall biodegradability of the substrates decreased, producing a compensation effect between these two factors that affected biomass

growth, with a consequence being that the concentration of MLSSs and MLVSSs remained relatively constant. In the analysis of the evolution of the SVI, the values obtained indicate adequate sedimentation, which is consistent with the actual behavior that occurred in this experiment. The reason for this good response is due to the work involving sludge that had already been fed with fats and oils prior to this experiment. Finally, it can be deduced that under conditions of wide load range and composition, activated sludge is a viable treatment for effluents with fats and oils. It should be noted that better results are obtained with a higher percentage of the most easily biodegradable component.

The Oxygen Uptake Rate (OUR) Behavior

Table 5 shows that the concentration of sunflower oil decreases the OUR value. Biomass activity levels are higher in the situation where saccharose has a considerable proportion with respect to oil, which is conditioned by its biodegradability.

Table 5. Evolution of the OUR with saccharose.

Sunflower Oil Influent (mg/L)	OUR (gO ₂ /(gMLVSS·h))
175 (Red)	0.048
550 (Blue)	0.008
760 (Green)	0.007

4. Conclusions

The results of this work demonstrate that it is possible to remove fats, oils and grease from wastewater using biological processes. For influents with sunflower oil concentrations ranging from 120 to 580 mg/L, removal via biodegradation reaches 72% for the lower concentration range, and for the higher concentration range, it decreases to 56%. For influents with sunflower oil concentrations ranging from 175 to 760 mg/L and containing a soluble substrate, saccharose, their removal via sunflower oil biodegradation reaches 67% for the lower-magnitude concentration range, and for the higher-range concentrations, it decreases to 47%. In addition, a relationship between the applied mass loading and the biodegradation efficiency was evident, with the latter decreasing for mass loading rates higher than 0.6 (kgCOD/(kgTSS·d)). This increase in sunflower oil concentration in the influent inhibits biodegradation, as the biodegradation rate is lower than the incoming oil load.

From these results, it is concluded that the presence of a soluble substrate is a factor affecting the biodegradation of vegetative oil in an oily influent, causing a decrease in its biodegradation rate. The overall removal of sunflower oil via biodegradation and flotation is close to 90% in both scenarios. Increasing the sunflower oil concentration decreases the rate of oxygen consumption by the biomass; this decrease is less prominent when the oil is the only substrate. This is due to the fact that a higher total substrate load generates a lower overall biodegradability of the substrates, producing a compensation effect between these two factors that affect biomass growth. Finally, a higher SVI is observed when sunflower oil is the only substrate, as the sludge acclimatizes to it and adequate compaction levels are reached, which is verified by SVI values that are below 150 mL/g.

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References

1. Jamaly, S.; Giwa, A.; Hasan, S.W. Recent Improvements in Oily Wastewater Treatment: Progress, Challenges, and Future Opportunities. *J. Environ. Sci.* **2015**, *37*, 15–30. [[CrossRef](#)]
2. Un, U.T.; Koparal, A.S.; Ogutveren, U.B. Electrocoagulation of Vegetable Oil Refinery Wastewater Using Aluminum Electrodes. *J. Environ. Manag.* **2009**, *90*, 428–433.
3. Capodici, M.; Cosenza, A.; Di Trapani, D.; Mannina, G.; Torregrossa, M.; Viviani, G. Treatment of Oily Wastewater with Membrane Bioreactor Systems. *Water* **2017**, *9*, 412. [[CrossRef](#)]
4. Chipasa, K.B.; Mdrzycka, K. Characterization of the Fate of Lipids in Activated Sludge. *J. Environ. Sci.* **2008**, *20*, 536–542. [[CrossRef](#)] [[PubMed](#)]
5. Chipasa, K.B.; Mdrzycka, K. Behavior of Lipids in Biological Wastewater Treatment Processes. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 635–645. [[CrossRef](#)] [[PubMed](#)]
6. Rodríguez, L.F.; Muñoz, Y.D.O.; Chaparro, H.N.; Garcia, H.E.; Montaña, V.H. Ensayos de Eficiencia Con Macrófitas Para La Remoción de Carga Contaminante En Aguas Residuales de Hatos Lecheros Para Un Subsector de La Laguna Fúquene. *Rev. Científica* **2006**, *8*, 130–156. [[CrossRef](#)]
7. Kurashige, J.; Matsuzaki, N.; Makabe, K. Modification of Fats and Oils by Lipases. *J. Dispers. Sci. Andtechnol.* **1989**, *10*, 531–559. [[CrossRef](#)]
8. Sugimori, D.; Utsue, T. A Study of the Efficiency of Edible Oils Degraded in Alkaline Conditions by Pseudomonas Aeruginosa SS-219 and Acinetobacter Sp. SS-192 Bacteria Isolated from Japanese Soil. *World J. Microbiol. Biotechnol.* **2012**, *28*, 841–848. [[CrossRef](#)]
9. Otálora, M.F.; Peña, J.L.; Martínez, M.M.; Varela, A. Evaluación de La Capacidad Degradadora de Aceite Por Bacterias Lipolítica En El Lodo Residual de La Extracción de Aceite de Palma. *Palmas* **2000**, *21*, 283–294.
10. Chou, K.W.; Tan, S.W.; Morad, N.; Tow, T.T.; Kadir, M.O.A.; Ismail, N. Aerobic Post-Treatment of Different Anaerobically Digested Palm Oil Mill Effluent (POME). *Int. J. Environ. Sci. Dev.* **2016**, *7*, 511. [[CrossRef](#)]
11. Azbar, N.; Yonar, T. Comparative Evaluation of a Laboratory and Full-Scale Treatment Alternatives for the Vegetable Oil Refining Industry Wastewater (VORW). *Process Biochem.* **2004**, *39*, 869–875. [[CrossRef](#)]
12. Volkering, F.; áM Breure, A.; Van Andel, J.G. Effect of Micro-Organisms on the Bioavailability and Biodegradation of Crystalline Naphthalene. *Appl. Microbiol. Biotechnol.* **1993**, *40*, 535–540. [[CrossRef](#)]
13. Jiménez Islas, D.; Medina Moreno, S.A.; Gracida Rodríguez, J.N. Propiedades, Aplicaciones y Producción de Biotensoactivos: Una Revisión. *Rev. Int. Contam. Ambient.* **2010**, *26*, 65–84.
14. Schubert, H.; Armbruster, H. Principles of Formation and Stability of Emulsions. *Chem. Ing. Tech.* **1989**, *61*, 701–711. [[CrossRef](#)]
15. Capek, I. Degradation of Kinetically-Stable o/w Emulsions. *Adv. Colloid Interface Sci.* **2004**, *107*, 125–155. [[CrossRef](#)] [[PubMed](#)]
16. Weatherley, L.R.; Rooney, D.W.; Niekerk, M. V Clean Synthesis of Fatty Acids in an Intensive Lipase-catalysed Bioreactor. *J. Chem. Technol. Biotechnol. Int. Res. Process Environ. Clean. Technol.* **1997**, *68*, 437–441. [[CrossRef](#)]
17. Albasi, C.; Riba, J.P.; Sokolovska, I.; Bales, V. Enzymatic Hydrolysis of Sunflower Oil: Characterisation of Interface. *J. Chem. Technol. Biotechnol. Int. Res. Process Environ. Clean. Technol.* **1997**, *69*, 329–336. [[CrossRef](#)]
18. Moon, M.; Kim, C.W.; Farooq, W.; Suh, W.I.; Shrivastav, A.; Park, M.S.; Mishra, S.K.; Yang, J.-W. Utilization of Lipid Extracted Algal Biomass and Sugar Factory Wastewater for Algal Growth and Lipid Enhancement of *Ettlia* sp. *Bioresour. Technol.* **2014**, *163*, 180–185. [[CrossRef](#)]
19. Al-Bahry, S.N.; Al-Wahaibi, Y.M.; Elshafie, A.E.; Al-Bemani, A.S.; Joshi, S.J.; Al-Makhmari, H.S.; Al-Sulaimani, H.S. Biosurfactant Production by Bacillus Subtilis B20 Using Date Molasses and Its Possible Application in Enhanced Oil Recovery. *Int. Biodeterior. Biodegrad.* **2013**, *81*, 141–146. [[CrossRef](#)]
20. Belitz, H.D.Y.G.W. *Química de Los Alimentos*, 2nd ed.; Editorial Acribia SA.: Zaragoza, Spain, 1997.
21. Rittmann, B.E. *Biología Del Medio Ambiente*; McGraw Hill Madrid: Madrid, Spain, 2001.
22. Cisterna, P. Biological Treatment by Active Sludge with High Biomass Concentration at Laboratory Scale for Mixed Inflow of Sunflower Oil and Saccharose. *Environments* **2017**, *4*, 69. [[CrossRef](#)]
23. APHA-AWWA-WPCF. *Métodos Normalizados Para El Análisis de Aguas Potables y Residuales*; Versión En Español; Ediciones Díaz de Santos, S.A., Ed.; Dialnet: Madrid, Spain, 1992; p. 188.
24. Vittadini, G. *Catálogo de Información de Equipamiento de Biocontrol*; Vittadini Riferiment: Milan, Italy, 1991.
25. Metcalf, L.; Eddy, H.P.; Tchobanoglous, G. *Wastewater Engineering: Treatment, Disposal, and Reuse*; McGraw-Hill: New York, NY, USA, 1991; Volume 4.
26. Loperena, L.; Ferrari, M.D.; Díaz, A.L.; Ingold, G.; Pérez, L.V.; Carvallo, F.; Travers, D.; Menes, R.J.; Lareo, C. Isolation and Selection of Native Microorganisms for the Aerobic Treatment of Simulated Dairy Wastewaters. *Bioresour. Technol.* **2009**, *100*, 1762–1766. [[CrossRef](#)]
27. Loperena, L.; Ferrari, M.D.; Saravia, V.; Murro, D.; Lima, C.; Fernández, A.; Lareo, C. Performance of a Commercial Inoculum for the Aerobic Biodegradation of a High Fat Content Dairy Wastewater. *Bioresour. Technol.* **2007**, *98*, 1045–1051. [[CrossRef](#)]
28. Nahib, I.; Ambarwulan, W.; Sutrisno, D.; Darmawan, M.; Suwarno, Y.; Rahadiati, A.; Suryanta, J.; Prihanto, Y.; Rudiastuti, A.W.; Gaol, Y.L. Spatial-temporal heterogeneity and driving factors of water yield services in Citarum river basin unit, West Java, Indonesia. *Arch. Environ. Prot.* **2023**, *49*, 3–24.

29. Gao, L.-L.; Lu, Y.-C.; Zhang, J.-L.; Li, J.; Zhang, J.-D. Biotreatment of Restaurant Wastewater with an Oily High Concentration by Newly Isolated Bacteria from Oily Sludge. *World J. Microbiol. Biotechnol.* **2019**, *35*, 179. [[CrossRef](#)] [[PubMed](#)]
30. Ren, J.; Fan, B.; Huhetaoli; Niu, D.; Gu, Y.; Li, C. Biodegradation of Waste Cooking Oils by *Klebsiella Quasivariicola* IUMR-B53 and Characteristics of Its Oil-Degrading Enzyme. *Waste Biomass Valorization* **2021**, *12*, 1243–1252. [[CrossRef](#)]
31. Lobos-Moysa, E.; Bodzek, M. Application of Hybrid Biological Techniques to the Treatment of Municipal Wastewater Containing Oils and Fats. *Desalination Water Treat.* **2012**, *46*, 32–37. [[CrossRef](#)]
32. Cisterna-Osorio, P.; Arancibia-Avila, P. Comparison of Biodegradation of Fats and Oils by Activated Sludge on Experimental and Real Scales. *Water* **2019**, *11*, 1286. [[CrossRef](#)]
33. Dueholm, T.E.; Andreasen, K.H.; Nielsen, P.H. Transformation of Lipids in Activated Sludge. *Water Sci. Technol.* **2001**, *43*, 165–172. [[CrossRef](#)] [[PubMed](#)]
34. Cisterna Osorio, P.E.; Faundez-Miño, B. Differential Impact of the Prior Mix by Stirring in the Biodegradation of Sunflower Oil. In *Biodegradation Technology of Organic and Inorganic Pollutants*; IntechOpen: London, UK, 2021; pp. 155–173. ISBN 1839688963.
35. Canler, J.P.; Royer, C.; Duchene, P. Aerobic Biological Treatment of Grease from Urban Wastewater Treatment Plants. *Water Sci. Technol.* **2001**, *44*, 219–226. [[CrossRef](#)] [[PubMed](#)]
36. Wakelin, N.G.; Forster, C.F. An Investigation into Microbial Removal of Fats, Oils and Greases. *Bioresour. Technol.* **1997**, *59*, 37–43. [[CrossRef](#)]
37. Sürücü, G. Growth Requirements of Thermophilic Aerobic Microorganisms in Mixed Cultures for the Treatment of Strong Wastes. *Water Sci. Technol.* **1999**, *40*, 53–60. [[CrossRef](#)]
38. Brooksbank, A.M.; Latchford, J.W.; Mudge, S.M. Degradation and Modification of Fats, Oils and Grease by Commercial Microbial Supplements. *World J. Microbiol. Biotechnol.* **2007**, *23*, 977–985. [[CrossRef](#)]
39. Shon, H.K.; Tian, D.; Kwon, D.Y.; Jin, C.S.; Lee, T.J.; Chung, W.J. Degradation of Fat, Oil, and Grease (FOGs) by Lipase-Producing Bacterium *Pseudomonas* Sp. Strain D2D3. *J. Microbiol. Biotechnol.* **2002**, *12*, 583–591.
40. Schneider, I.; Topalova, Y. Bioaugmentative Approaches for Dairy Wastewater Treatment. *Am. J. Agric. Biol. Sci.* **2010**, *5*, 459–467. [[CrossRef](#)]
41. Silva-Bedoya, L.M.; Sánchez-Pinzón, M.S.; Cadavid-Restrepo, G.E.; Moreno-Herrera, C.X. Bacterial Community Analysis of an Industrial Wastewater Treatment Plant in Colombia with Screening for Lipid-Degrading Microorganisms. *Microbiol. Res.* **2016**, *192*, 313–325. [[CrossRef](#)] [[PubMed](#)]
42. Silva, E.J.; Almeida, D.G.; Luna, J.M.; Rufino, R.D.; Santos, V.A.; Sarubbo, L.A. Use of Bacterial Biosurfactants as Natural Collectors in the Dissolved Air Flotation Process for the Treatment of Oily Industrial Effluent. *Bioprocess. Biosyst. Eng.* **2018**, *41*, 1599–1610. [[CrossRef](#)] [[PubMed](#)]
43. Cisterna-Osorio, P.; Calabran-Caceres, C.; Tiznado-Bustamante, G.; Bastias-Toro, N. Influent with Particulate Substrate, Clean, Innocuous and Sustainable Solution for Bulking Control and Mitigation in Activated Sludge Process. *Water* **2021**, *13*, 984. [[CrossRef](#)]
44. Drownowski, J. The impact of slowly biodegradable organic compounds on the oxygen uptake rate in activated sludge systems. *Water Sci. Technol.* **2014**, *69*, 1136–1144. [[CrossRef](#)]

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