Chapter

Differential Impact of the Prior Mix by Stirring in the Biodegradation of Sunflower Oil

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Abstract

Fats and oils present in wastewater are usually eliminated by physical and biological processes. In this experience, the fatty wastewaters are treated biologically, and it assesses the impact of the mix in the fats and oils biodegradation and carried out the experiments in a laboratory scale unit. The biodegradation of fats and oils was analysed in two sceneries, with mix previous by mechanical agitation and without mix. Key parameters were monitored, such as the concentration of fats and oils in the influents and effluents, mass loading, and the efficiency of biodegradation. The mass loading range was similar in both sceneries. In the experimental activated sludge plant without mix, the biodegradation of fats and oils reached levels in the range of 28 to 42.5%. For the wastewater treatment plant with a previous mix by mechanical agitation, the levels of biodegradation of fats and oils ranged from 64 to 75%. Therefore, considering the efficiency of the biodegradation of fats and oils in both sceneries, the results indicated that the level mix is a high incidence.

Keywords: biodegradation, fats and oils, activated sludge

1. Introduction

Nowadays, the growing sensitivity with respect to environmental protection has led to increasing regulatory pressure on wastewater treatment, imposing severe limitations on pollutant concentrations before discharging them to the environment. In this context, one of the major challenges is represented by the biological treatment of oily wastewater [1]. Domestic and industrial wastewaters contain fats and oils. The fraction of lipids in urban wastewater is 30–40% of the chemical oxygen demand (COD) [2].

The biodegradation of lipids in activated sludge processes is not well known. The literature states that these can be treated by biological treatment, which eventually causes foam formation composed of filamentous bacteria and flocs that affect biodegradation [3]. Considering generic information, fats and oils are classified as slowly biodegradable substances. As for the biodegradation process, bacteria initially save these substances in their cytoplasm and later through the enzymatic process; it starts hydrolysis to produce an assimilable substrate that can be biodegraded [4].

There are three types of reactions catalysed by microbial enzymes: oxidative, hydrolytic, and synthesis. Hydrolytic enzymes are used to hydrolyse insoluble complex compounds, such as fats and oils, on simple components that pass through the cellular membrane by diffusion. These enzymes (i.e., oxidoreductases) act outside the cell wall [5]. The extracellular enzyme called lipases is the most common, releasing fatty acids as a consequence of enzymatic action [6]. Lipases break down molecules into simpler components, which appear as end products or intermediates that are consumed by microorganisms. If the solid substrate is sufficiently porous, the enzyme can diffuse and biodegradation can take place inside it; for low porosity material, such as oils where enzymes cannot diffuse, the reaction takes place on the outer surface of the particle [7].

Furthermore, a wider range of microorganisms biodegrades fatty acids from other microorganisms that do not produce extracellular lipolytic enzymes [8]. Wastewater with high lipid concentration inhibits the activity of microorganisms in biological treatment systems, such as active sludge and methane fermentation. To reduce such inhibitory effects, microorganisms capable of effectively degrading edible oils can be selected from different environmental sources [9]. There are many researches that study about elimination and biodegradation of lipids by biological treatment [10]. Biodegradation of fats and oils and other substrates, not soluble in water, is one of the greatest problems for the biological treatment of wastewater [11].

A wide variety of organic compounds such as carbon and energy are used as a source by microorganisms. When substrates possess low or zero solubility, the use of biosurfactants is recommended [12]. One of the main characteristics of emulsified mixtures is the presence of at least one hydrophilic polar liquid and at least one lipophilic; the simplest and most frequent case is when liquids are oil and water [13]. Depending on the emulsification process, the diameter of droplets in the internal phase ranges from 0.1 μm to 0.1 mm. Usually, there is a wide size distribution of bubbles; a narrow distribution can be considered when the ratio between the smallest and largest size droplets is 1:10. Emulsions of this class are normally thermodynamically unstable, so it is known the tendency to reduce the interface area between aqueous and oily phase, causing the oil droplets to coalesce. Coalescence of oil droplets can be reduced or even eliminated through stabilization mechanisms [13].

The unstable nature of an emulsion is because contact between oil and water molecules is not energetically favourable. Therefore, surfactants (emulsifiers) are added to the emulsion to improve the system stability; molecules inside are adsorbed to the surface of oil droplets during homogenization, providing a protective membrane that prevents flocculation and coalescence [14].

In a reactor, energy is delivered to the system, by mechanical agitation or electric fields, and the efficient contact of the phases increases the interfacial area and the transfer of matter. Mechanical agitation is simpler and of greater operational variety. Agitation speed is an important factor in the industrial application for the mixing efficiency to increase productivity [15]. The use of electric fields is more energetically efficient since the electric forces are applied on the interface of the fluids, unlike the mechanical agitation that delivers the energy to the bosom of the liquid and only a part of it is transferred to the interface [16]. A third technique to achieve an adequate mixture is ultrasound, which has mechanical and/or chemical effects, the first is due to the implosion of microbubbles, generating highly reactive free radicals, and the mechanical is caused by wave shocks during symmetric cavitation [17].

In biological treatment by activated sludge, the magnitude of the contact area of phases, water and oil is very important; therefore, a significant interfacial area is required. The interfacial area can be expanded by delivering energy to the system through mechanical stirring or an electric field. The increment of interfacial surface between the aqueous phase and the oily phase is often implemented by mechanical stirring [18].

It is known that the smaller the size of the emulsions, the more the biodegradation process is favoured, since the interface area increases and, therefore, the possibility that lipases in an aqueous medium can carry out the oil hydrolysis. As the stirring speed increases, the average droplet size decreases steadily [19]. It was observed that the enzymatic pre-hydrolysis under the influence of ultrasound drastically reduces the reaction time from 24 h to 40 min as compared to conventional stirring with improved yield [20].

Electrical potentials are also applied to reduce emulsion sizes and thereby increase the homogeneity of the oil and water system. There are studies, in which the following bubble sizes between 1 and 36 μm were obtained for a stirred system with two electrodes [21]. When electric fields are applied to increase the degree of dispersion of the discontinuous phase in the continuous one, the bubbles acquire a surface charge, which generates a self-rejection that leads to a reduction in the sizes of the bubbles [22].

The fatty acids produced during the course of the reaction act as surfactants, stabilizing the emulsions [18]. Biosurfactants are surface-active molecules that are produced by a wide range of microbes including bacteria, fungi, and yeast. They have several advantages over the chemical surfactants such as higher biodegradability, lower toxicity, better environmental compatibility, high selectivity, higher foaming, and specific activity under extreme conditions such as temperature, pH, and salinity [23].

Synthetic surfactants increase the desorption and solubilization of nonpolar compounds in soils and aquifer materials, but they cause environmental problems during their production, and they are resistant to biodegradation and can be toxic when they accumulate in the ecosystem [24].

Mechanical agitation is susceptible to improvement through the application of a non-stationary behaviour that consists of the displacement of the rotating propeller from top to bottom, which cancels the existence of segregated regions that are not affected by stable agitation due to the position of the propeller in the pond, and another mechanism is counter-rotation [25].

Mixing and corresponding dispersion is achieved in the aeration tank used; there are two important factors that largely determine the emulsion level: bubble size and distribution, and the fraction of the dispersed phase. The average bubble size is between 150 μm and250 μm [16], which can be obtained by means of a suitable booster and fine bubble diffusers. If there is a suitable enzyme concentration and the optimal interfacial area between phases: aqueous and oily, the mass transfer is solved and the hydrolysis stage starts [26]. Further studies on the effects of agitation speed from 0 rpm (static) to 200 rpm on tannase production showed that increasing agitation speed caused the fungal pellets to decrease in size but to increase in number per unit volume, increasing the interface area [27].

This work analyses and evaluates the differential impact caused by mixing by stirring on the behaviour of the biological treatment system for activated sludge on a laboratory scale when treating an influent containing fats and oils. In this experience, we work with vegetable oil.

2. Materials and methods

2.1 The choice of sunflower oil is explained based on the following criteria

- It is the most frequent oil used in Chile;
- It is well standardized, and
- It is a highly accessible product.

The chemical composition of sunflower oil is shown in **Table 1**.

Fat	Position	16:0	18	18:1(9)	18:2(9,12)	18:3(9,12,15)
Sunflower oil girasol	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	—

Table 1.
Fatty acids that make up sunflower oil and specific stereo analysis [18]. Results in % moles.

2.2 Physicochemical parameters and analytical methods

2.2.1 Chemical oxygen demand (COD)

The potassium dichromate method was used to evaluate COD levels. The method used is a variation of the standard method [28]; however, it maintains its basis. The variation used has the advantage it uses a significantly smaller sample and reagents. The sample is chemically oxidized through the action of potassium dichromate at 150°C for 2 h. Silver sulphate is used as a catalyst and mercury sulphate is used to avoid possible interferences with chloride. Afterward, determination by spectrophotometry at 600 nm is performed. Equipment and instruments are used to determine the various parameters to characterize the wastewater used.

2.2.2 Fats and oils

Determination of the fats and oils was used in Gravimetric Assay Soxhlet method. This method quantifies substances with similar characteristics, based on its common solubility in appropriate solvent, 213E method [29].

Total suspended solids (TSS) are determined by filtering a known volume of the sample on Whatman (Whatman plc., Maidstone, UK) 4.7 cm GF/C glass fibre filters and then drying it at 103–105°C. The difference in weight of the filter before and after filtration is used to estimate the TSS, 209C method [29].

Volatile suspended solids (VSS): The volatile suspended solids are determined by weight loss after calcination at 550°C, 208E method [29].

2.3 Continuous equipment

In the current investigation, an activated sludge plant at the laboratory scale is used to conduct biodegradability tests in wastewater with oils and fats. To meet these objectives, experimental work is required, with such parameter information describing the process dynamics regarding the aeration and sedimentation tanks regarding fat and oil content of the wastewater. For this purpose, BIOCONTROL-MARK 2 equipment was used. The details of the equipment are shown in **Figure 1**.

This experimental equipment consists essentially of the following parts:

- Control unit

Composed of the main switch, air cylinder, it is complemented with a flow meter and a flow regulation system. Additionally, the wastewater feed pump, that includes a flow rate regulation system, a timer for intermittent operations, and an ON–OFF switch allowing sludge recycling from the sedimentation tank to the aeration tank.

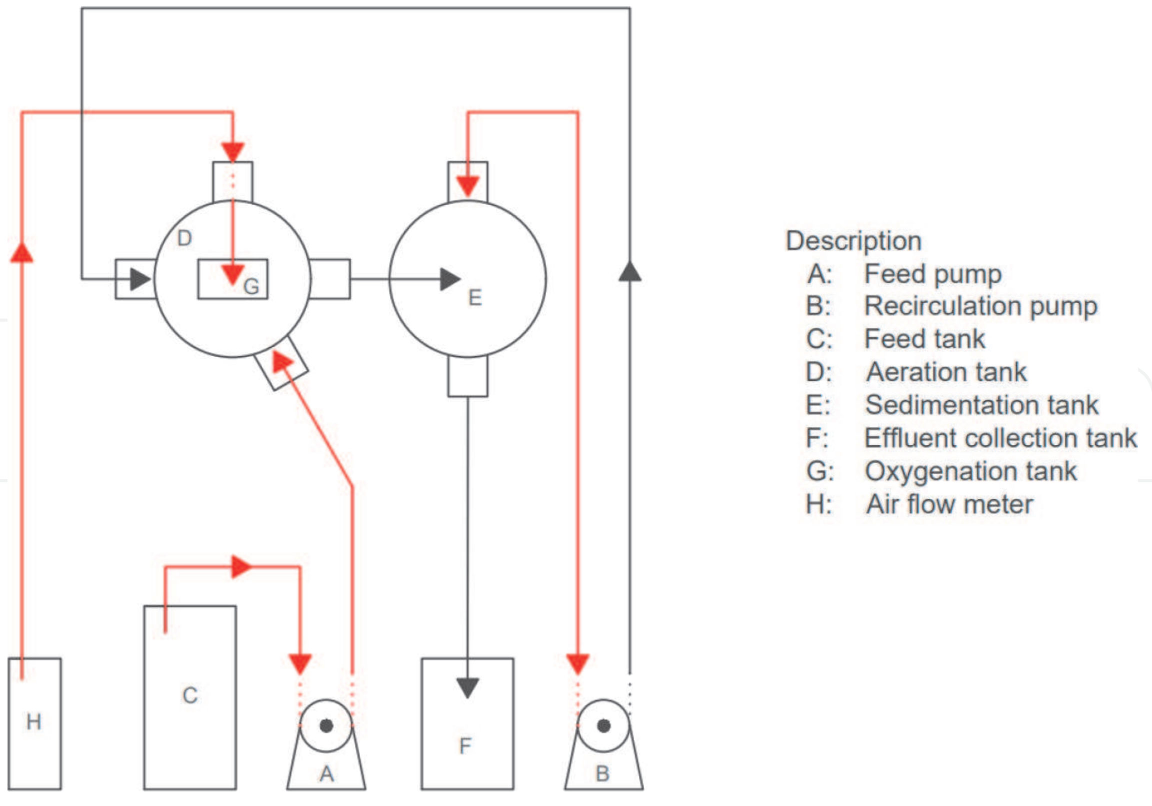

Figure 1.
Experimental equipment diagram.

- Aeration tank

It consists of a transparent Plexiglas® (Vittadini Riferimenti, Milan, Italy) cylinder with a height of 38 cm and a diameter of 20 cm, which has outlets at various heights associated with different volumes (7, 8, 9, and 10 l). There are two separated inlets allowing recirculation of sludge from the top. The influent to be treated is placed at the bottom. In addition, the system has two ceramic diffusers placed in the bottom in a way that they can disperse the air in tiny bubbles [30].

- Sedimentation tank

This consists of a transparent Plexiglas® (Vittadini Riferimenti, Milan, Italy) cylinder, where its lower part is cone-shaped to make sludge sedimentation and thickening easier.

The mixed liquor is fed from the aeration tank, which has an outlet in its upper part. This flow escapes by overflow, when it arrives in the sedimentation tank. The solid phase decantation gives a method to downward flow. Decanted sludge is separated and recirculated at the bottom through the pump towards the aeration tank. Treated water also uses the overflow mechanism to be evacuated to the storage tank.

2.4 Experimental methodology

a. Feed preparation

The treatment system was initially fed with synthetic wastewater prepared in the laboratory, according to strong urban wastewater typical characteristics of [31]. This wastewater has an approximate BOD of 400 mg/l, with the corresponding proportions of nitrogen and phosphorus in a relation to BOD: N: P = 100:5:1. Approximately 400 mg of saccharose, 20 mg of phosphate hydrogen of potassium, and 100 mg of ammonium chloride were added per litre of water. Measurements begin when sunflower oil is added, the concentration of this substrate gradually increases. Feed was prepared daily and nitrogen and phosphorus increased according to the organic input coming from fats and oils.

b. Operating modes

The synthetic wastewater was poured into a storage pond of approximately 50 l, where the stirring unit has been installed to disperse oil or fat. Through a peristaltic pump, controlled by the control unit, it drives the feed to the aeration tank. Oxygen feed and recirculation flow are controlled by the control unit. Process effluent is collected in a 30-l volume tank, where the samples are taken to be processed. The flow of synthetic wastewater is 25 l/day.

2.5 Mass balance: biodegradability determination

Experimental method is established in this protocol, which allows to carry out the material balance of fats and oils. From the process diagram, **Figure 2** shows flows and concentrations of inlet and outlet streams in different stages of the activated sludge process are indicated. To carry out material balance and estimate biodegraded oil in the activated sludge process, the oil in the feed tank must be estimated.

This experience works with influents that are biologically treated by active sludge, containing only vegetable oil. This retained fraction is an indicator of the mixing level reached in the feed tank, which corresponds to not emulsified oil, and therefore, it is not part of the influent entering the aeration tank; obviously, the size of this fraction indicates the mixing level of the system quantitatively.

Where:

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

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

F2, C2: Flow and concentration of fats and oil leaving the aeration tank = (Flow and concentration of fats and oils entering the secondary settler).

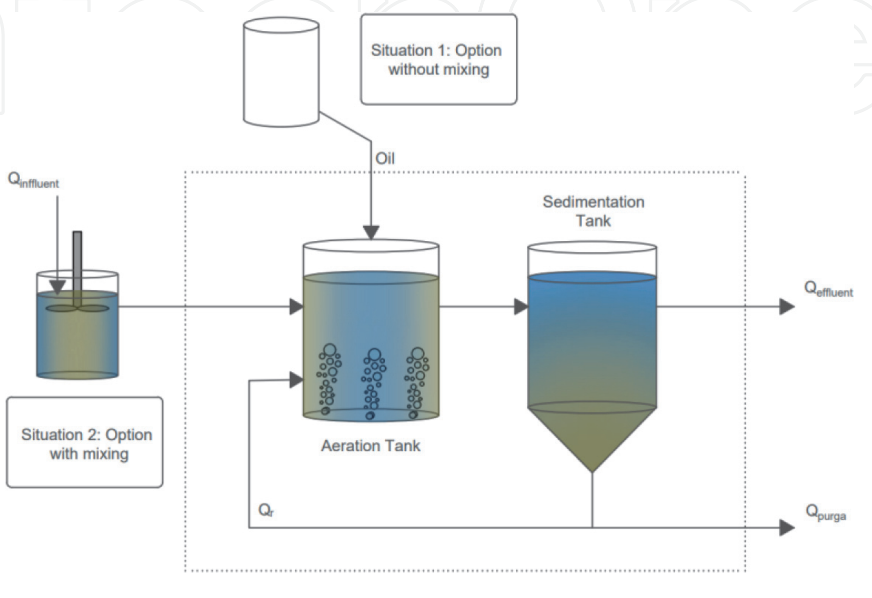

Figure 2.
Active sludge plant process diagram.

Operation days for balance sheet	Oil mass fed (g)	Retained oil mass (g)	Oil mass in effluent (g)	Accumulated oil mass (g)	Biodegraded oil mass (g)	Degradation efficiency (%)
1 a 8	79	12,3	5,4	6,5	54,8	75
9 a 16	120	28	8	19	65	71
17 a 23	157	70	10	21	56	64

Table 2.
Mass balance in active sludge for oily influent with prior stirring.

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

F4, C4: Flow and fats and 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 sedimentation tank and oil mass at the bottom of sedimentation tank by biomass attached.

Mass balance and biodegradability determination of fats and oils in bench-scale activated sludge reactor.

In this experiment made in equipment in **Figure 1**, water and oil were mechanically stirred and mixed in the feed tank. Part of aggregated oil was accumulated in the feed tank, so the oil fraction not going to the aeration tank was known. The influent containing sunflower oil and concentration was gradually increased.

Biodegraded sunflower oil mass was determined from the mass balance equation.

Sunflower oil is part of the influent fed to the treatment system.

Initially, it is assumed the system has no oil.

Results of matter balance are shown in **Table 2**.

From material balance, the mass of biodegraded sunflower oil is obtained, which is part of the influent fed to the system.

For the aeration tank:

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

For the secondary sedimentation tank:

$$\frac{\Delta M_2}{\Delta t} = F_2 \cdot C_2 - F_3 \cdot C_3 - F_4 \cdot C_4 \tag{2}$$

It must be noticed that, $F_6 = 0$.

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

M2: 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:

From this expression we have: $r_A \cdot V$, corresponds to vegetable oil that disappears per unit of time, it means the oil is clearly biodegraded by microorganisms, such that:

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

An important part of the oil floats and therefore does not biodegrade and passes to a secondary sedimentation pond in which it accumulates. For reasons of technical feasibility, the mass balance must be kept integral for some time. Fats and oils accumulated as a result of separation by flotation are measured, allowing to determine the oil biodegradation level in influent.

The equation for integral material balance for a certain period of time is as follows:

$$M_1 + M_2 = F_1 \cdot C_1 \cdot \Delta_t - F_3 \cdot C_3 \cdot \Delta_t - r_A \cdot V \cdot \Delta_t \tag{4}$$

Where is Δt the time elapsed.

Every day is identified by subscript as shown in the following examples:

Day 1:

$$M_{11} + M_{21} = F_{11} \cdot C_{11} \cdot \Delta_t - F_{31} \cdot C_{31} \cdot \Delta_t - r_A \cdot V \cdot \Delta_t \tag{5}$$

Day 2:

$$M_{1i} + M_{2i} = F_{1i} \cdot C_{1i} \cdot \Delta_t - F_{3i} \cdot C_{3i} \cdot \Delta_t - r_A \cdot V \cdot \Delta_t \tag{6}$$

Then, the mass balance for a given number of days of operation is as follows:

$$r_A \cdot V \cdot \Delta_t = F_{1i} \cdot \Delta_t \cdot \sum (C_{1i}) - F_{3i} \cdot \Delta_t \cdot \sum (C_{3i}) - \sum (M_{1i} + M_{2i}) \tag{7}$$

Where:

$$M_1 = \sum M_{1i} \, M_2 = \sum M_{2i} \tag{8}$$

Then, biodegradability is

$$B = r_A \cdot V \cdot \frac{\Delta_t}{F_{1i}} \cdot \Delta_t \cdot \sum (C_{1i}) \tag{9}$$

From the material balance calculation, the percentage of biodegraded oil of the influent is estimated. The flows and concentrations of the activated sludge process are indicated below.

The feed flow and the concentration of fats and oils (F0, C0) is known, and is set according to the conditions previously defined for the experimental phase.

F1, C1: The flow is determined according to the experimental conditions and regarding the concentration of fats and oils C1, and it is determined by the Soxhlet gravimetric method.

F3, C3: This flow is equal to inflow and, therefore, is given by predetermined conditions of the experiment, and the concentration of fats and oils, C3, is obtained by the Soxhlet method.

M1: This estimates the oil mass accumulated in a given period of time, and it is necessary to remove supernatant fats and oils, dry and weigh them.

M2: The mass of sunflower oil and derivatives accumulated in the secondary sedimentation tank in a determined period of time is determined. The upper or floating phase of the secondary settler is poured into an auxiliary tank, which is oil and its derivatives in humid conditions. Then, the remaining water is evaporated through a heat source and oil mass is obtained by gravimetric. Oil and derivatives accumulated in biomass in secondary settler are added to this mass, which is determined by the Soxhlet method for a 100-ml sample. With the concentration obtained, the oil retained in settled solids is estimated.

2.6 Incidence of agitation and biodegradation of sunflowers oil

In this experience, an influent that only has sunflower oil has been fed to the biological treatment system by active sludge, it is the only carbon source available for microorganisms under two sceneries, with and without previous agitation. Different operational parameters are monitored providing information on system behaviour. The oil biodegradation percentage is estimated by mass balance.

3. Material balance results: without agitation

Biodegradability is calculated from the mass balance in the system and concentrations and quantities of oil obtained. In this case, feeding is not subjected to a previous agitation, and this condition is achieved by introducing oil directly to the aeration tank by dripping, and the total volume entered into the system; it is measured every 24 h.

In the present experiment (**Table 3**), influent fed to active sludge system contains only oil and without stirring, and **Table 3** found that oil biodegradation levels range between 28.1 and 42.5%, which is considerable in spite of not being high. This is an interesting result because it opens the viability of biologically degrading substances, such as fats and oils, which evidently presents important comparative advantages when compared with the removal of fats or oils *via* flotation.

The above is explained by the lack of stirring, and it is worth noting that experience with olive oil production wastewater, a culture medium, with a concentration of 5% was used. Effects of agitation speeds were studied on the growth of species Scenedesmus microalgae, in a photobioreactor. A maximum specific growth rate of 0.031 1/h was obtained, using a speed of rotary impeller around 350 rpm, higher than that found in the absence of agitation 0.024 h^{-1} [32].

On the other hand, the synthesis of bio-lubricant from the effluent of palm oil plant, which is based on enzymatic hydrolysis. Effects of essential parameters were examined, which include stirring speed. The optimal hydrolysis rate (0.1639 mg/s.l) is achieved at 650 rpm, at 40°C, pH 7.0, 20 U ml of loading enzyme [33].

This biodegradation range achieved in this experience, although it is not an optimal result, it shows that oils and fats can be eliminated from influent through biodegradation process; it is also important to note this level of biodegradation can be increased with some modification processes.

The results for the oil removed by biodegradation are due to the fact that a considerable percentage of sunflower oil that floats in the aeration tank reaches the secondary settler, which is due to the very low solubility of substrate in water, which originates a two-phase system. This is increased by the oil tendency to float in water, which is favoured by the flow of air driven from the bottom of the aerobic tank.

Operation days for balance sheet	Oil mass fed (g)	Oil mass in effluent (g)	Accumulated oil mass (g)	Biodegraded oil mass (g)	Degradation efficiency (%)
1 a 22	160	11	81	68	42,5
23 a 28	95	14	52,5	28,5	30,0
29 a 33	90	5,22	59,5	25,28	28,1

Table 3.
Matter balance in active sludge for oily influent without previous stirring.

Figure 3.
Oil and grease accumulated in settling tank.

An increase in emulsification constitutes the vehicle that would generate more favourable conditions for the microbiological attack, since the hydrolysis of oil requires an oil-water interfacial area, which is favoured with the increase in the number of emulsions and with the decrease in their size. One of the causes of the results obtained with respect to biodegradability is the type of hydrodynamics of process reactor, since given the lower oil density in relation to the water and the injection of air from the bottom of the aeration tank, it generates conditions for that the oil floats and, therefore, does not remain in the treatment system during appropriate residence time. There is a part of the oil that is not biodegraded and is separated through flotation (**Figure 3**).

Among the already mentioned characteristics of oils and fats is their low solubility in water and their marked tendency to buoyancy, which is enhanced when the aeration tank is fed with air; as the oil floats, the coalescence process is favoured, reversing the emulsification achieved through aeration by the agitation caused a consequence of air injection into the aerobic pond.

It is noteworthy that the system worked for 50 days and the only carbon source was vegetable oil; with regard to toxicity, some authors [34] claim an inherent toxicity of fatty acids, since they are potential inhibitors of metabolic processes of cells due to their surfactant properties, and for this reason, it is considered they do not remain as such in cells; they are immediately converted into thioesters of their coenzyme A.

As already stated, the low solubility of fats and oils makes it difficult to disperse and distribute this substrate properly in the medium that supports microorganisms in water. In view of the aforesaid, the adequate size of the emulsions is not achieved, so contact surface with the mixing liquor, and therefore with bacterial flocs is limited by this operational factor.

Fats and oils are substances that do not favour the development and growth of bacterial colonies, due to the already explained, but when biomass is acclimatized for a considerable time to the new type of influent, it is found the biomass is adapted.

This is explained from the mutations caused by changes or chemical or physical agents, which change the DNA imparting new characteristics to the cell, thereby

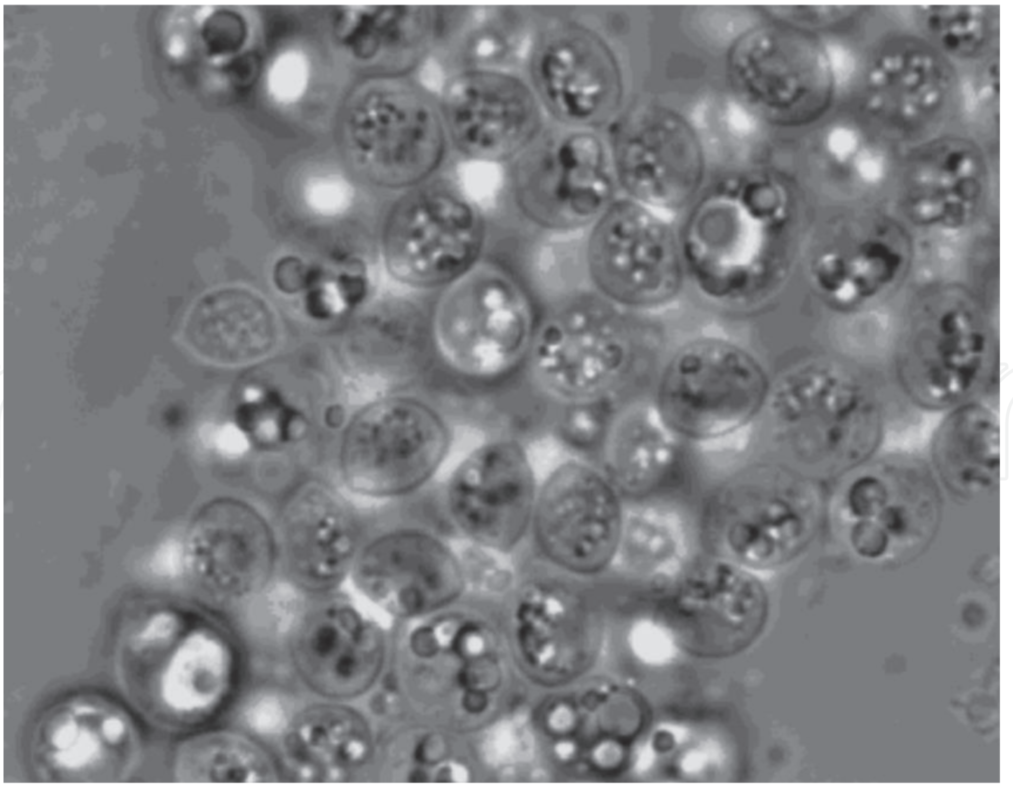

Figure 4.
Acclimatization of bacteria to oil particles.

allowing the cell to degrade a xenobiotic substance or facilitate biodegradation. Spontaneous mutations occur in one out of every 1,000,000 cells; however, the DNA molecule is capable of replicating [35]. **Figure 4** shows the environmental condition of the biomass in an oily medium.

The existence of an oily environment for the fat and oil-degrading biomass is an important factor and has to do with the above-mentioned. In kinetic monitoring experiences of the aerobic biodegradable processes of dairy fats and oils, we worked with native biomass from a lagoon that treated dairy water and commercial bioaugmentation inoculum, and the removal percentage was from 78 to 91% and from 82 to 95%, respectively. However, for high substrate concentrations, the native inoculum is more mineralizing than the commercial one [36].

- Oil concentration ratio in influent and effluent

The oil concentration in the effluent is not correlated with oil concentration in influent (**Figure 5**), which is mainly explained by the fact that an important part of the oil is retained in the sedimentation tank, instead of leaving through the effluent, given its buoyancy. There is no evidence that behaviour regarding the elimination of fats and oils in urban active sludge treatment plants is related to the concentration of fats and oils in influents in concentration ranges, which are the discharged influents usually present [37].

As the important part of oil floats is that it does not enter the interior mixed liquor of the active sludge system, this breaks the relationship that should exist between the concentrations of oil at the outlet and in the inlet, unlike other soluble substrates such as saccharose.

Stirring is the driving force that promotes the oil emulsification in the water, which depends on the flow and air pressure in the active sludge system.
The emulsified oil will be the most susceptible to biodegrade; it must be considered that the airflow also favours flotation, so both effects are opposed.

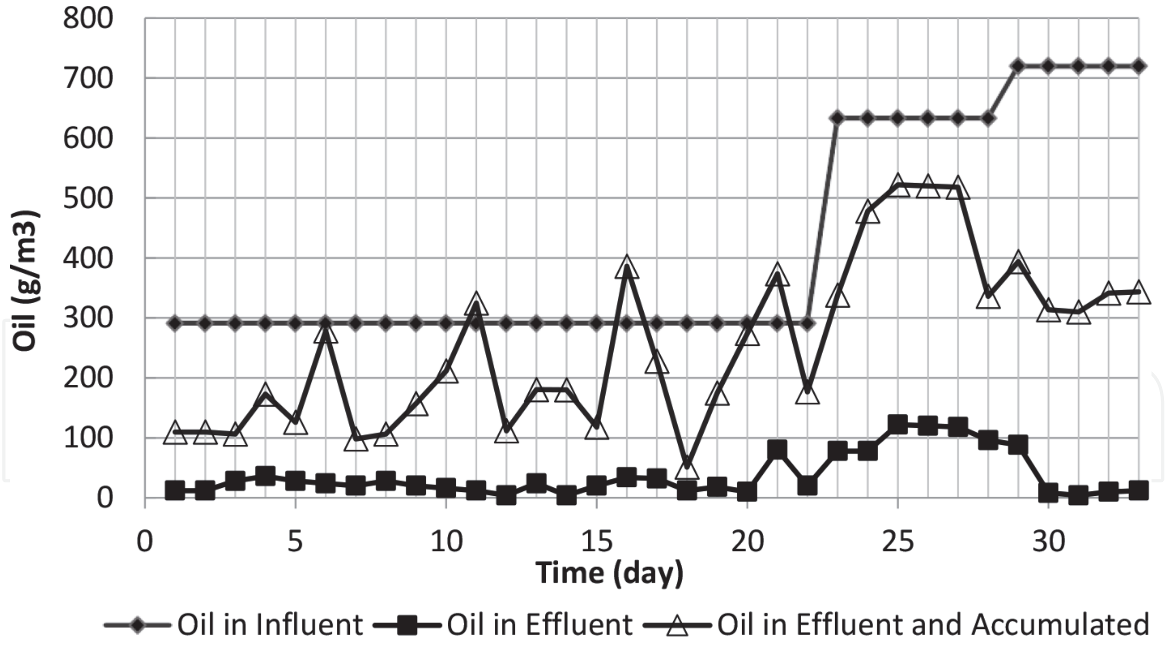

Figure 5.
Performance of oil concentration in influent, effluent, and effluent the most accumulated effluent, without previous stirring.

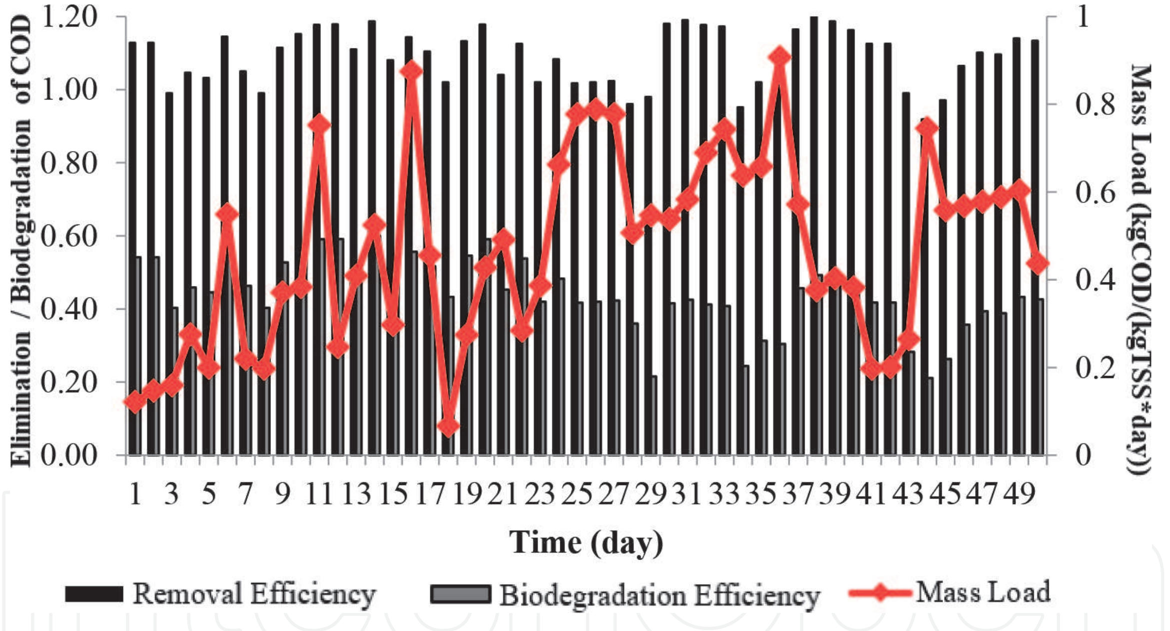

Figure 6.
Performance of COD removal and biodegradation efficiency and mass loading for sunflower oil feed without prior agitation.

Biomass constitutes an element of resistance from a hydrodynamic point of view to the flotation of the oil since these are particles that impede the flow of the air and on the other hand are a source of oil consumption, since they use it as a carbon source. The floating of the oil in the aeration tank is reduced according to the biomass concentration since it opposes resistance to the airflow.

Figure 6 shows that in this experience, removal and biodegradation efficiency do not depend on the mass load, and this indicates the blown air enhances flotation more than emulsification, given the initial system, condition, influencing without previous stirring.

The biodegradation efficiency is low, less than 40%, unlike the elimination that exceeds the average of 85%, which indicates that the flotation phenomenon prevails, it is worth noting that despite this pessimistic condition of mixture and size of emulsions, biodegradation level over 30% is also achieved.

4. Mass balance and biodegradability analysis

In this case, mass balance presents a difference with respect to the balance made for the case where the oil is directly added to the aeration tank, since in this experience the mixture of water and oil is previously prepared in the feed tank; part of the added oil remains accumulated in tank, so it is pertinent to estimate this fraction of oil does not enter the treatment system, as shown in **Figure 2**. In the following experiment, the mixture of water with oil is subjected to the previous stirring to evaluate the mixing effect on biodegradation and oil elimination, since a greater mixture stimulates the development of smaller emulsions and precisely treats to evaluate how this variable affects the biodegradation of fats and oils.

This experience worked with an influent that is subjected to previous mechanical stirring to evaluate the influence of and stirring on biodegradation of oil, since when stirring is carried out before entering the aerator tank, the level of emulsification of oil particles in the water is increased, and with this, the contact area between water-oil and oil-microorganisms is increased, which is closely related to the mass transfer phenomena that involve biodegradation of sunflower oil in active sludge treatment system.

Influents with concentrations of 300, 600, and 900 mg/l are prepared in a feeding tank, for a period of approximately one week, to measure the biodegradability levels achieved, and therefore compare the behaviour of system with results obtained where influent is not previously agitated. The operating conditions regarding airflow, residence time, recirculation ratio, and COD: N: P ratio at the feed level remain unchanged.

Biodegradation levels obtained improve considerably when a mixture of water and oil is subjected to mechanical stirring before being fed to an aeration tank; the agitation carried out is with a propeller-type mechanical stirrer. It should be mentioned that this type of stirring is limited which is significantly reflected in the oil that does not enter the system since it is not emulsified, which gives rise to the fraction of the oil that is retained.

Biodegradation values between 64 and 75% obtained with stirring are similar to those corresponding to aerobic biodegradation experience that eliminates fats and oils from the dairy industry, which uses a mixture of isolated and selected native bacteria as biomass, reaching 72% biodegradation efficiency [38].

Since the only substrate is oil, this system can be assimilated to the proposal for the treatment of fats and oils that is contemplated in the wastewater treatment project of the Los Angeles commune developed by DEGREMONT, which consists of treating the fats and oils together with the sludge in an aerobic digester; therefore, the only available substrate is the fats and oils collected in the primary treatment and that was dosed based on criteria of optimal distribution and mixing [39].

Recent researches have studied the behaviour and performance of aerobic thermophile bacteria for wastewater with a high oily organic content, verifying that between 55 and 58°C, the maximum growth rate is achieved [40]. Now, this is important given that at higher temperatures an increase in the dissolution of fats and oils is achieved, and as it is an aerobic digester, it is more feasible to reach temperatures in this range.

However, analysis of the first-order kinetic model constants showed that alteration in rotor speed resulted in an increase in the values of the kinetic constants (for instance, from 0.57 h^{-1} at 50 rpm to 0.84 h^{-1} at 75 rpm) [41].

It is important to note that the oil that enters the system is the one that has been emulsified and therefore the size of the oil droplets that enter the system reaches a comparatively much smaller size than in the previous case (without mixing), which allows a considerably greater interfacial area between the mixed liquor and the oil.

The bacterial mass is suspended in water, and therefore, contact level between microorganisms and oil droplets is considerably increased, which stimulates the production of the lipase by bacteria. Then, increasing the oil-water interfacial area, where occurs oil hydrolysis, the one that is increased and therefore the amount of fatty acids and glycerol, compounds that bacterial mass will biodegrade them; therefore, the increase of interfacial area allows the substantial increase in biodegradation levels.

4.1 COD elimination

According to observed, it is confirmed that an important part of organic matter corresponding to vegetable oil is accumulated in secondary settler and that the accumulated oil mass is clearly correlated with oil concentration in influent.

The COD values inform us that the sum of oil biodegradation and flotation phenomena makes it possible to achieve global elimination of vegetable oil, which is considerable. On the other hand, it is observed the biodegradation of oil does not depend on the mass load.

Considering the mass load values, it means an initial work with extended aeration and final stage in conventional type regime, which is consistent with theory and experience of wastewater treatment, since differences between both regimes are not observed in effluent quality.

In any case, the oil removal is mainly due to biodegradation of vegetable oil around 70%, while 20% corresponds to flotation (**Figure 7**).

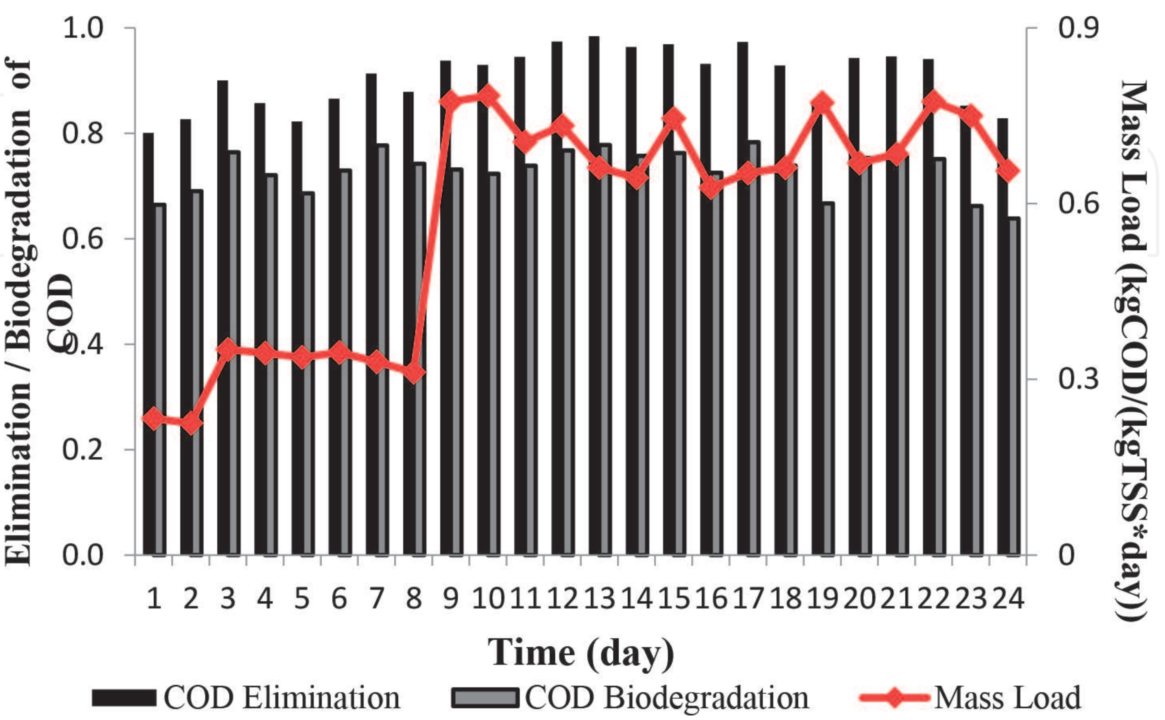

Figure 7.
Performance of COD removal efficiency and mass loading for sunflower oil feed with the previous stirring.

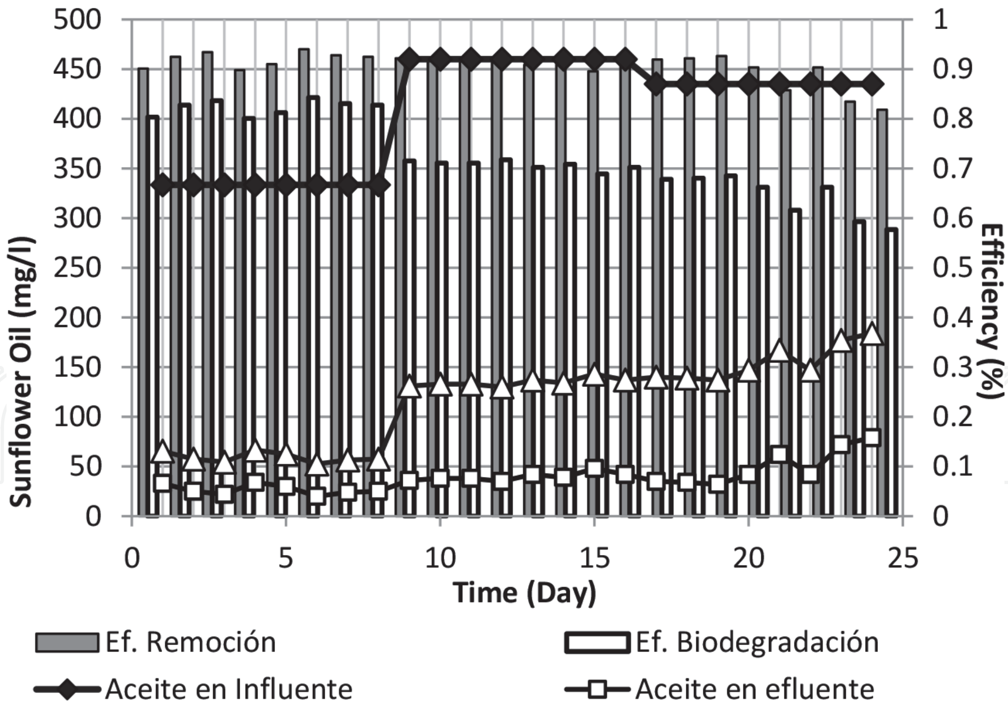

Figure 8.
The behaviour of sunflower oil concentration in influent, effluent, and effluent plus accumulated effluent and oil removal efficiency with prior agitation.

4.2 Removal of vegetable oil

The elimination of oil by biodegradation has a huge advantage over the physical and physicochemical processes currently used in the elimination of this substrate, since they generate a greasy residue that requires a final disposal and tends to accumulate, unlike the elimination by aerobic biodegradation that biologically oxidizes fats and oils to CO2, incorporate them into the carbon cycle.

From **Figure 8**, it can be seen that the concentration of sunflower oil of the influent is practically constant during a determined amount of approximately 8 days, three periods are distinguished, since the feeding during each period has different concentrations of vegetable oil in the influent. Initially, the oil concentration is 220 mg/l, later it increases to a value close to 470 mg/l and for the last days of operation, which corresponds to a concentration of vegetable oil in the influent close to 500 mg/l, this corresponds to a COD concentration above 1100 mg/l, and there is a slight increase in the COD of the effluent.

Regarding the elimination of oil, elimination levels that are around 90% are reached, where a considerable percentage of this elimination corresponds to oil that is biodegraded and the remainder is separated by flotation, as shown in **Figure 8**.

The amount of accumulated oil increases with the concentration of oil in the influent. It should be noted that the increase in oil concentration in the influent causes a decrease in the biodegradation performance of the activated sludge system, which is corroborated with the results of the material balance, such that the biodegradability of the oil decreases by 75% for vegetable oil concentrations of 220 mg/l and up to 64% when the concentration increases to 500 mg/l.

5. Conclusions

For influents with concentrations of fats and oils that range between 200 and 800 mg/l and that are not subjected to a previous mixing, the elimination by biodegradation of the same reaches 42.5 for the concentrations of smaller magnitude and for the concentrations of the highest rank decreases to 28%.

For influents with the concentrations of fats and oils ranging between 330 and 465 mg/l and that are subjected to a previous mixing, their elimination by biodegradation ranges from 64 to 75%, which has as a consequence a considerable reduction of greasy residues that accumulate and take up space to be disposed of.

From the results, it is concluded that the previous mixing is a relevant factor to increase the elimination by biodegradation of fats and oils in an oily influent.

The global elimination that includes biodegradation and flotation exceeds 80% at all events.

The biodegradation efficiency of sunflower oil increases through greater agitation, which is a contribution from the environmental point of view, since fats and oils are eliminated, transforming them into CO2 by the biological route and thus incorporating these residues into the cycle of carbon.

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