



Comparison of Biodegradation of Fats and Oils by Activated Sludge on Experimental and Real Scales

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Received: 25 April 2019; Accepted: 3 June 2019; Published: 20 June 2019



MDP

Abstract: Fats and oils are the most common pollutants in wastewater, and are usually eliminated through physical processes in wastewater treatment plants, generating large amounts of fats and residual oils that are difficult to dispose of and handle. The degradation of fatty wastewater was studied in a real wastewater treatment plant and a laboratory scale treatment unit. The wastewater treatment plant, located in Chile, was designed for a population of 200,000 inhabitants. It includes an aerobic digester that receives fat and oils retained in a degreaser and treats the fats and oils together with biomass. The biodegradation of fats and oils was analyzed in both wastewater treatment systems. 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 wastewater treatment systems. In the experimental activated sludge plant, the biodegradation of fats and oils reached levels in the range of 64% to 75%. For the wastewater treatment plant with an aerobic digester, the levels of biodegradation of fats and oils ranged from 69% to 92%. Therefore, considering the efficiency of the elimination of fats and oils ranged from 69% to 92%. Therefore, considering the efficiency of the elimination of fats and oils, the results indicated that physical treatment should be replaced with biological treatment so that the CO_2 generated by the biodegradation will be incorporated into the carbon cycle and the mass of fats and oils in landfills will be reduced.

Keywords: biodegradation; fats and oils; activated sludge

1. Introduction

Oils and fats are essential organic components of municipal and industrial wastewater, and their exact behavior in wastewater treatment processes is not well understood [1]. Although biological treatment of oily wastewater is not well developed, recent activities in this area have removed notable percentages of oil and grease from wastewater [2].

Many types of anaerobic digesters have been investigated for joint sludge treatment with oil and fat; for example, with a modified thermophilic digester [3], a co-digestion anaerobic of food waste, oils and fats with sewage sludge [4], an adaptation of the microbial community that influenced the conversion of long-chain fatty of oils and fat with municipal sludge [5], and similar aerobic experiences that analyzed the aerobic joint digestion of sludge with fats and oils [6].

Physical removal of fats and oils is a common procedure that is based on the lower density of fats and oils, between 920 g/L and 964 g/L, relative to water, allowing for flotation [7]. Flotation is used in the separation of immiscible or solid fluids and is common in wastewater treatment. Flotation can be achieved by air at atmospheric pressure, by dissolved air under pressure, or by induced air [8].

Cor

MLSS (kg/m³)

Fats and oils biodegradation (%)

Volume (m³)

Influent flow (m³/day)

Fats and oils are classified as slowly biodegradable substances. The cells initially store these substances in their cytoplasm and then use hydrolytic enzymes to convert fats and oils into an assimilating and biodegradable substrate [9].

This investigation started in an activated sludge experimental plant, where sunflower oil is the only carbon source in synthetic wastewater. Sunflower oil is easily accessible and is a standardized product, commonly domestically consumed in Chile. The chemical composition of the sunflower oil is shown in Table 1. The structure gives it the chemical identity. Prior to biodegradation, a hydrolysis stage is required that releases the fatty acids. The fat and oil tests include the fatty acids.

Table 1. Fatty acids of sunflower oil and specific stereo analysis [10]. Results are provided in % moles.

Fat/Oil	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	0.1
	2	1.3	1.1	21.5	76	
	3	9.7	9.2	27.6	53.5	

The Bío-Bío region of Chile has 90 activated sludge plants. The wastewater treatment plant of Los Angeles (WWTP-LA) is the only one that has an aerobic digester to treat fats and oils combined with biomass. In the WWTP-LA, fats and oils are retained by a degreaser and sent to the aerobic digester (Table 2).

Parameter	WWTP-LA	Activated Sludge Experimental	
Single substrate	Fats and oils	Sunflower oil	
ML ((kg COD/day)/kg MLSS)	0.3–1.5	0.15–1.1	
Structure	SBR	Conventional-continuous	
centration Fats and Oil of the influent	1000–2500	333–460	

Table 2. Conditions of design and systems operation.

COD: Chemical oxygen demand, WWTP-LA: Wastewater treatment plant Los Angeles, ML: Mass loading, MLSS: Mixed liquor suspended solids, SBR: Sequential batch reactor.

8.8 - 14

69-95

5128

1000

The biodegradation of organic matter in an activated sludge system is enhanced by the cultivation and development of dispersed bacterial biomass in the form of flocs, called activated sludge, dispersed in an aerated and agitated tank that is continuously fed by sewage with organic matter or sludge. The stirring of the tank aims to homogenize the mixture composed of the bacterial biomass and the residual water, avoiding sediments and zones of short circuiting ratios. Aeration is used to supply oxygen to the purifying bacteria [11].

In the parameter design and operation of an activated sludge treatment system, mass loading (ML) corresponds to the aeration tank, which is related to the daily feed mass of biodegradable organic matter entering the aeration tank and the degrading biomass contained therein [12]. The ML range was similar in both the experimental activated sludge plant and in the aerobic digester. This a fundamental parameter for this type of treatment for the bench scale activated sludge reactor and the real scale wastewater treatment plant (Table 2).

The process and behavior of lipid elimination through biological treatment systems have been exhaustively evaluated. The literature shows that lipids eliminated by biological treatment also inhibit microbial growth and are the cause of foaming, filamentous bacteria, and flocculation [1].

Plant

3.2 - 4.8

64–75 0.01

0.024

In an activated sludge biological treatment system, the efficient contact of the microorganisms present in the aqueous phase and oil is fundamental. This requires a considerable interfacial area, which can be increased by delivering energy to the system, either by mechanical agitation or by electric fields. Mechanical agitation is often used to increase the interfacial area between the aqueous phase and the oily or greasy phase [13].

Two important factors determine the level of emulsion in the full-mix stirred aeration tank and the dispersion level: (1) bubble size distribution and (2) dispersed phase fraction. The average bubble size is in the range of 150 μ m to 250 μ m [14], which is achieved with a suitable impeller and fine bubble diffusers. A suitable enzymatic concentration and the optimum interfacial area must be obtained between the aqueous phase and the oily phase to achieve mass transfer and the hydrolysis step [15]. Typically, lipase hydrolysis in oil to fatty acids occurs at the interphase between the oil and aqueous phases. The lipase enzymes can catalyze various types of reactions [16].

Biodegradation of fats and oils were evaluated in both plants from a mass balance perspective, for which the concentration of fats and oils in the influents and effluents, mass loading, and efficiency of biodegradation were monitored.

2. Materials and Methods

Two different wastewater treatment plant (WWTP) systems were analyzed, and therefore the applied methodology was different for each experimental situation studied. In both cases, a balance of matter was determined to estimate the fraction of fats and oils biodegraded.

2.1. Analytical Methods and Monitoring Parameters

The analytical methods and monitoring parameters applied are described below.

2.1.1. Total Suspended Solids (TSS)

TSS were determined by sample filtration of a known volume through 4.7 cm Whatman GF/C glass fiber filters and drying at 103–105 °C. The difference in weight of the filter before and after filtration was used to estimate the TSS [17].

2.1.2. Fats and Oils

For determination of fats and oils, groups of substances with similar characteristics were quantitatively measured on the basis of their common solubility in a suitable solvent using the gravimetric/Soxhlet test method [17].

2.1.3. Chemical Oxygen Demand (COD)

COD was determined using a variation of the potassium dichromate method [18]. This method uses a much smaller amount of sample and reagents. The sample is chemically oxidized through the action of potassium dichromate at a temperature of 150 °C for two hours. Silver sulfate is used as the catalyst and mercury sulfate is used to avoid potential chloride interference. Spectrophotometry measurements were recorded at 600 nm.

2.1.4. Monitoring Parameters

Description of parameters:

$$ML = (\text{kg COD applied/day})/(\text{kg of total suspended solids in aeration tank})$$
 (1)

$$ML = (Q \times COD)/(V \times MLSS)$$
(2)

where COD is chemical oxygen demand, (kg COD/m³); MLSS is mixed liquor total suspended solids (kgSS/m³); Q is influent flow rate, (m^3/h) and V is the volume of the aeration tank, (m^3) .

The biological treatment of wastewater with sunflower oil is carried out in a bench scale activated sludge reactor.

2.2.1. Description of Equipment

Experimental work was required to obtain information about the parameters describing the dynamics of the activated sludge process. The process depends on the fat and oil contents present in the incoming wastewater. The equipment used was a BIOCONTROL model MARK 2, described as follows (Figure 1).



Figure 1. The experimental equipment. A: Feed pump. B: Recirculation pump. C: Feed tank. D: Aeration tank. E: Sedimentation tank. F: Effluent collection tank. G: Block of oxygenation. H: Airflow meter. F0, C0: Flow and concentration of oil entering the feed tank, respectively. F1, C1: Flow and concentration of feed oil to the aeration tank, respectively. F2, C2: Flow and concentration of fats and oils are leaving the aeration tank, respectively. F3, C3: Flow and concentration of fats and oil of the purified effluent, respectively. F4, C4: Flow and concentration of fat and oil in the recirculation flow rate, respectively.

Unit Control

The unit control consisted of a main switch, a complete air cylinder with a flow meter, a flow control rate system, as well as a feed pump for wastewater to be purified, complemented by a control rate system. A timer was set for intermittent operations with an on–off switch and a pump that recycles the sedimentation tank sludge to the aerator tank (Figure 1).

Aeration Tank

The aeration tank was a transparent Plexiglas[®] cylinder (Vittadini, Milan, Italy) (38 cm high and 20 cm in diameter); it has outputs at various heights associated with different volumes: 7 L, 8 L, 9 L, and 10 L. The cylinder has two inputs: (1) the recirculation sludge, fed at the top of the cylinder and (2) the influent that flows to the lower level has, in its upper part, an outlet for the mixing liquor that flows to the secondary settler. Two ceramic diffusers are placed in the bottom to disperse the incoming air in tiny bubbles [19].

Sedimentation Tank

The sedimentation tank consisted of a transparent cylinder made of Plexiglas[®], and the basal part is cone-shaped to facilitate the sedimentation and thickening of the sludge. It receives the mixed liquor from the aeration tank by overflow. When this liquor reaches the clarifier, it creates a downward flow so that the sludge decants and is separated and recirculated through a pump. The clarified water also uses the mechanism of overflow to evacuate toward the accumulation tank (Figure 1).

2.3. Experimental Methodology

The activities included in the experimental methodology are described below.

Feed Preparation

The treatment system was fed with synthetic wastewater prepared in the laboratory according to the COD concentrations of sewage. The concentrations were in the range of medium to strong [20], with a C:N:P ratio of 100:5:1. Synthetic wastewater was prepared daily. Ammonium chloride, as the nitrogen source, and potassium hydrogen phosphate, as the phosphorus source, were added in accordance with the organic intake from the sunflower oil.

2.4. Determination of the Relationship Between COD and Fat

The relationship between pork fat, sunflower oil and COD was determined using the test already described in the methodology. The previously mentioned relationship was used to convert fats and oils to COD. Therefore, a fat and an oil were used to obtain a more representative conversion factor.

Operation Modes: Bench Scale Reactor

The synthetic wastewater was prepared daily and deposited in a 50 L storage and homogenization tank, where a stirrer was installed to disperse the oil or fat. Through a peristaltic pump, operated from the control unit, the feed was sent to the aeration tank. The oxygen supply and the recirculation flow rate were managed from the control unit. The effluent was collected in a 30 L tank, from which the samples were obtained for chemical analysis. The average temperature and dissolved oxygen range were 18 °C and 2–4 mg/L, respectively.

2.5. Real Scale Wastewater Treatment Plant

The biological treatment of wastewater with fats and oils is carried out in a real scale wastewater treatment plant.

Wastewater Treatment Plant: Los Angeles, Bío-Bío Region, Chile

The aerobic digester of the sludge line of the wastewater treatment plant in Los Angeles (WWTP-LA) was designed as activated sludge system at medium load with 0.6 kg BOD/day/kg MLSS. The plant has three similar modules built with a 5108 m³ aeration tank, and a 2400 m³ settler. The average cell residence time is 25 days, and biodegraded fats and oils are retained in a degreaser [21] (Figure 1). Therefore, the only substrate and source of carbon was fats and oils. This last process stage was compared with the experimental prototype (Figure 2).



Figure 2. The wastewater treatment plant (WWTP) in this study. 1: Wastewater elevating plant. 2: Sedimentation tank. 3: Skimmer/degreaser. 4,5,7: Reactors. 6: Aerobic digester. 8: Band filter. 9,10,11: Clarifiers. 12: Contact camera.

3. Results

3.1. Relationship of Fats and Oils with COD

The results show the relationship between pork fat and sunflower oil with chemical oxygen demand. From these relationships, it was possible to more accurately estimate the COD from the concentration of oils and fat in wastewater and the sunflower oil–COD relationship was used for the experimental work. The experimental values obtained for the correlation (COD to fats and oils) of vegetal and animal origin oil (Figure 3) were consistent with the data obtained by Henze et al. [22], and were used as the basis of calculation for the estimation of the COD and mass loading.



Figure 3. Relationship of (a) COD and sunflower oil and (b) COD and pork fat.

3.2. Mass Balance and Determination of Biodegradability of Fats and Oils in the Bench-Scale Activated Sludge Reactor

In this experiment, performed on the equipment shown in Figure 1, the water and oil were mechanically stirred and mixed in the feed tank. Part of the aggregated oil was accumulated in the feed tank so the oil fraction not going into the aeration tank was known. The influent contained sunflower oil and the concentration was gradually increased. The initial concentration of the biomass present in the aerobic reactor was about 4000 mg/L of TSS.

The mass of biodegraded sunflower oil was determined from the mass balance equation. The sunflower oil is part of the effluent fed to the treatment system. Initially, it is assumed that the system has no oil.

For the aeration tank:

$$\Delta M_1 / \Delta t = F_1 \times C_1 - F_2 \times C_2 - r_A \times V + F_4 \times C_4 \tag{3}$$

For the secondary sedimentation tank:

$$\Delta M_2 / \Delta t = F_2 \times C_2 - F_3 \times C_3 - F_4 \times C_4 \tag{4}$$

where M_1 corresponds to the oils and fats that are in the aerator tank, M_2 corresponds to the oils and fats that are in the sedimentation pond, V is the reactor volume and r_A is the biodegradation rate of vegetable oil.

The balance for the system as a whole is:

$$\Delta M_1 / \Delta t + \Delta M_2 / \Delta t = F_1 \times C_1 - F_3 \times C_3 - r_A \times V \tag{5}$$

where $r_A \times V$ corresponds to the vegetable oil that disappears per unit of time, i.e., the oil biodegraded by the microorganisms, such that:

$$r_A \times V = F_1 \times C_1 - F_3 \times C_3 - \Delta M_1 / \Delta t - \Delta M_2 / \Delta t \tag{6}$$

An important part of the oil floats and therefore does not biodegrade and passes to the 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 were measured, allowing us to determine the level of oil biodegradation in the influent.

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

$$M_1 + M_2 = F_1 \times C_1 \times \Delta_1 - F_3 \times C_3 \times \Delta_1 - r_A \times V \times \Delta_1 \tag{7}$$

where Δ_1 is the time elapsed.

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

Day 1:

$$M_{11} + M_{21} = F_{11} \times C_{11} \times \Delta_t - F_{31} \times C_{31} \times \Delta_t - r_A \times V \times \Delta_t \tag{8}$$

Day 2:

$$M_{12} + M_{22} = F_{12} \times C_{12} \times \Delta_t - F_{32} \times C_{32} \times \Delta_t - r_A \times V \times \Delta_t \tag{9}$$

Day n:

$$M_{1n} + M_{2n} = F_{1n} \times C_{1n} \times \Delta_t - F_{3n} \times C_{3n} \times \Delta_t - r_A \times V \times \Delta_t \tag{10}$$

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

$$\Sigma(M_{1n} + M_{2n}) = F_{1n} \times \Delta_t \times \Sigma(C_{1n}) - F_{3n} \times \Delta_t \times \Sigma(C_{3n}) - r_A \times V \times \Delta_t$$
(11)

Then, the biodegradability is:

$$B = r_A \times V \times \Delta_t / F_{1i} \times \Delta_t \times \Sigma(C_{1i})$$
(12)

$$B = (F_{1i} \times \Delta_t \times \Sigma(C_{1i}) - F_{3i} \times \Delta_t \times \Sigma(C_{3i}) - \Sigma(M_{1i} + M_{2i}))/F_{1i} \times \Delta_t \times \Sigma(C_{1i})$$
(13)

The results of the matter balance are shown in Table 3.

Length of Operation Including Balance (days)	Mass of Oil Fed (g)	Mass of Retained Oil (g)	Mass of Oil in the Effluent (g)	Mass of Biodegr-Adation (g)	Mass of Sedimentation Oil (g)	Biodegradation Efficiency (%)
1 to 8	79	12.3	5.4	54.8	6.5	75
9 to 16	120	28	8	65	19	71
17 to 23	157	70	10	56	21	64

Table 3. Mass balance of fats and oils in the bench-scale activated sludge reactor.

3.3. Mass Balance of Fats and Oils in a Real Scale Wastewater Treatment Plant

The mass balance of the wastewater treatment plant was calculated using the load of the influent corresponding to the entry of fats and oils. Therefore, the output load corresponds to fats and oils in the effluent of the WWTP. In the sludge line, the difference between the input and the output mass corresponds to the mass of biodegraded fats and oils in the aerobic digester (Table 4).

Table 4. Mass balance of fats and oils in real scale wastewater treatment plant.

Period	Influent (kg)	Effluent (kg)	Efficiency Elimination Water Line (%)	Sludge (kg)	Biodegraded Mass (kg)	Efficiency Biodegradation System (%)
1	46.604	2.072	96	264	44.268	95
2	19.005	1.456	92	676	16.872	89
3	25.572	3.164	88	85	22.323	87
4	10.083	1.045	90	110	8.927	89
5	16.562	4.725	71	439	11.398	69
6	11.031	1.446	87	118	9.467	86
7	9.269	987	89	0	8.282	89
Total	138.126	14.895	89	1.693	121.538	88

Figure 4 provides the flow diagram of the WWTP-LA. The mass balance at WWTP-LA is calculated as: Mass of biodegraded fats and oils = Mass of entry – Mass exiting in effluent – Mass in the sludge:

$$M_{GYA} = Q_{af} \times C_{af} - Q_L \times C_L - Q_{ef} \times C_{ef}$$
(14)

where Q_{af} is the flow of the influent, L/day; C_{af} is the concentration of fats and oils of the tributary, mg/L; Q_L is the flow rate from the digester to the sludge dehydrator, L/day; C_L is the concentration of fats and oils of the digester, mg/L; Q_{ef} is the outflow from the effluent, L/day; and C_{ef} is the concentration of effluent fats and oils, L/day.



Figure 4. General flow diagram of the Los Angeles WWTP.

3.4. Operation Parameters

The most relevant operational variables were monitored in both plants.

The Efficiency of Biodegradation of Fats and Oils

At the bench-scale plant, the sunflower oil concentration of the influent reached a constant level during three periods of eight days each. The oil concentration at the initial point was 333 mg/L, then increased to approximately 460 mg/L, and, for the last days of operation, the oil concentration in the influent was 500 mg/L. The sunflower oil, the only source of carbon, was eliminated by biodegradation and by flotation. Figure 5 shows the oil efficiency as it leaves the influent. The graph curves represent the oil concentration (mg/L) present in the effluent and its accumulation by sedimentation; that is to say, the non-biodegradable oil. The effluent and accumulated oil increased with the concentration (mg/L) of oil in the influent.



Figure 5. Changes in removal and biodegradation efficiencies of sunflower oil for the bench-scale activated sludge reactor.

In actual real-scale wastewater treatment reactor configurations, the fats and oils are retained in the degreaser and sent to the sludge digester. This is a significant structural variant concerning traditional solutions. The remaining part of the wastewater is treated by biological treatment of activated sludge in a conventional mode. Therefore, the residue of fats and oils not eliminated becomes part of the contaminants of the influent and the rest becomes part of the sludge leaving the filter.

The masses of fats and oils that leave the effluent are greater than the mass that leaves through the sludge (Figure 6).



Figure 6. Weekly changes in mass of fats and oils in real scale wastewater treatment plant.

Figure 6 shows the mass of fats and oils in the influent and effluent of the real-scale wastewater treatment and the sludge leaving the digester, which shows that approximately 90% of fats and oils were eliminated, which is a promising result for global-scale treatment. The fats and oils were retained in the degreaser and were subsequently subjected to biodegradation in the aerobic digester.

The biodegradation efficiency of fats and oils in the digester and the bench-scale activated sludge reactor indicates adequate acclimatization by the biomass. The only source of carbon and energy was fats and oils.

3.5. Mass Loading, Biodegradation, and Oil and Fat Removal

At the bench-scale activated sludge reactor, a considerable fraction of the sunflower oil accumulated in the secondary settler. The magnitude of this accumulation of oil mass is correlated with the oil concentration in the influent. The eliminated vegetable oil in the effluent shows that the sum of the biodegradation and oil flotation reached a high total elimination of vegetable oil. In addition, the biodegradation of oil does not depend on mass loading (Figure 7).



Figure 7. The efficiency of biodegradation and elimination as a function of mass loading in the experimental plant.

Considering the values of the mass loading, at the initial stage, the conventional regime was conducted, and later, a high rate regime was used (Figure 7). The value of the mass loading does not affect the quality of the effluent, which is coherent with the theory and the experience of wastewater treatment.

The vegetable oil removal percentage was over 90% which was mainly due to the biodegradation of vegetable oil. About 70% and 20% correspond to flotation, considering the mass balance shown in Table 4. This is comparable with the experiment with the activated sludge system, which achieved COD removals of 80% for wastewater from the olive oil refining industry [23].

For aerobic biological treatments, plants on a real scale were the subject of experimentation. An evaluation of 55 municipal water treatment plants was conducted in the USA: the concentration of fats and oils in the influent had average values lower than 80 mg/L and a BOD of 300 mg/L. The effluent had a concentration of fats and oils lower than 10 mg/L and a BOD less than 40 mg/L in the effluent. The elimination of fats and oils does not vary seasonally [24]. Our results are consistent with those of the literature.

The amount of accumulated oil increases with the oil concentration in the influent, affecting the quality of the effluent, which is corroborated by the results of the material balance, which established a biodegradability of 75% for a concentration of 340 mg/L, 71% for 460 mg/L, and 64% for 500 mg/L.

Aerobic experiments with granular sludge in a sequential batch reactor for the treatment of dairy effluents, rich in fats and oils, reached COD elimination levels of 90% after separation of the biomass from the blended liquor [25]. Another study was performed to characterize the transformation of lipids into activated sludge under aerobic conditions. The results showed that the total lipid content in the effluent would not be reduced to values below 300 mg/L from an initial 2000 mg/L [26].

Another experiment was conducted in an activated sludge experimental plant: 400 mg/L of saccharose was added in similar concentrations to fats and oils in the influent, i.e., in a concentration range of 100 mg/L to 850 mg/L. As a result, oil biodegradation ranged from 51% to 60% [27], showing the competitive effect of the saccharose substrate.

Anaerobic biodegradation experiments produced oil removal ranging from 81% to 97% [28], which was similar to the system shown here when flotation was considered.

Considering the experimental antecedents of the biological treatment of wastewater, fats and oils are physically eliminated, generating a significant amount of waste that must be disposed of in a landfill at a high economic and environmental cost. The degreaser discussed in this study eliminated 80% or more of the incoming fats and oils, resulting in concentrations in the effluent of the plant of 10 mg/L. This is a conventional treatment with high mass loading ranges and, therefore, a considerable variety of substrates. These diverse substrates compete with fats and oils advantageously as they have simpler biodegradation mechanisms. Both systems show high levels of biodegradation, since they work with a single substrate, fats and oils, which favor the acclimatization of the biomass [29].

In the Los Angeles real-scale wastewater treatment plant, there is an aerobic sludge digester that also removes fats and oils. Therefore, the volume of fats and oils physically retained in the degreaser were biologically treated in the aerobic sludge digester [21].

Figure 8 shows fat and oil removal efficiencies by biodegradation in the overall system, which varies from 70% to 95% with an average of 88%. The biodegradation efficiency was sustained in the sludge digester and is mainly explained by the high concentration of biomass therein, higher than the aeration tank of the bench-scale activated sludge reactor at a pilot scale (20% approximately). The mass loading changes within the conventional regime range from 0.3–1.5 kg COD/day/kg MTSS to high load. The presence of only one substrate stimulates the use of fats and oils for the construction of cellular tissue and energy source for the biomass.



Figure 8. The efficiency of biodegradation and elimination of COD/fats and oils as a function of the mass loading in a WWTP-LA.

The digestion levels of sludge, fats and oils in the aerobic digester is close to the levels achieved through the biological treatment of activated sludge with extended aeration for fats and oils when they are the only substrate present. Therefore, the method that eliminates fats and oils is an innovative process, with similar good results obtained in the bench-scale activated sludge reactor and real-scale

wastewater treatment plant. This innovative process does not generate fats and oils as the final residue, which eliminates the problem of their final disposal.

The mass loading range is a fundamental parameter to measure, and it was similar in both the bench-scale activated sludge reactor and the real-scale wastewater treatment plant with the aerobic digester.

4. Conclusions

In the bench-scale activated sludge reactor, biodegradation reached levels ranging from 64% to 75%. The fats and oils in the wastewater treatment process are removed by flotation and biodegradation mechanisms. When the methods were combined, the elimination of oil was above 85% in the bench-scale activated sludge reactor.

The higher biodegradation efficiency (75%) of the bench-scale activated sludge reactor and the biodegradation efficiency of the aerobic digester (92%) correspond to the lowest mass loading condition in both cases.

The biomass presents excellent adaptability to fat and oil substrates, favored in this case for the lack of competitiveness, with fats and oils being the only source of carbon in the influent.

Our results show that fats and oils are eliminated by biodegradation, with the bench-scale activated reactor eliminating more than 64% of the sunflower oil. Then, we compared this finding with a real scale wastewater treatment plant that serves 200,000 inhabitants in Los Angeles in the region of Bío-Bío, Chile that biologically treats the different fats and oils in their aerobic form, achieving similar results.

Fats and oils should be removed by biological treatment, replacing the flotation process, and at most using it as an intermediate process. Biological treatment is an efficient option to eliminate fats and oils and thus reduce the fatty residues that are difficult to dispose of and manage in WWTP.

Chilean wastewater treatment plants must replace or complement physical treatment with biological mechanisms to reduce waste in landfills and incorporate the CO₂ generated into the carbon cycle.

Author Contributions: Conceptualization, P.C.-O.; methodology, P.C.-O.; software,.; validation, P.C.-O., P.A.-A.; formal analysis, P.C.-O., P.A.-A.; investigation, P.C.-O.; resources, P.C.-O., P.A.-A.; data curation, P.C.-O.; writing—original draft preparation, P.C.-O.; writing—review and editing, P.C.-O., P.A.-A.; visualization, P.C.-O., P.A.-A.; supervision, P.C.-O., P.A.-A.; project administration, P.C.-O.; funding acquisition, P.A.-A.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

- 1. Chipasa, K.; Medrzycka, K. Behavior of lipids in biological wastewater treatment processes. J. Ind. Microbiol. Biotechnol. 2006, 33, 635–645. [CrossRef] [PubMed]
- Jamaly, S.; Giwa, A.; Hasan, S. Recent improvements in oily wastewater treatment: Progress, challenges and future opportunities. *J. Environ. Sci.* 2015, 37, 15–30. [CrossRef] [PubMed]
- 3. Li, C.; Champagne, P.; Anderson, B. Enhanced biogas production from anaerobic co-digestion of municipal wastewater treatment sludge and fat, oil and grease (FOG) by a modified two-stage thermophilic digester system with selected thermo-chemical pre-treatment. *Renew. Energy* **2015**, *83*, 474–482. [CrossRef]
- 4. Awe, O.; Zhao, Y.; Nzihou, A.; Minh, P.; Lyczko, N. Anaerobic co-digestion of food waste and FOG with sewage sludge–realising it's potential in Ireland. *Int. J. Environ. Stud.* **2018**, *75*, 496–517. [CrossRef]
- 5. Pintor, A.; Vilar, V.; Botelho, C.; Boaventura, R. Oil and grease removal from wastewaters: Sorption treatment as an alternative to state-of-the-art technologies. A critical review. *Chem. Eng. J.* **2016**, 297, 229–255. [CrossRef]
- 6. Ziels, R.; Karlsson, A.; Beck, D.; Ejlertsson, J.; Yekta, S.; Bjorn, A.; Svensson, B. Microbial community adaptation influences long-chain fatty acid conversion during anaerobic codigestion of fats, oils, and grease with municipal sludge. *Water Res.* **2016**, *103*, 372–382. [CrossRef]
- 7. Kirk, R.E. Encyclopedia of Chemical Technology, 5th ed.; John Wiley & Sons: Hoboken, NJ, USA, 2007.
- 8. Sainz, J. Separación de aceites de efluentes industriales. Ing. Quím. 2004, 409, 93–99.

- 9. Ronzano, E.; Dapena, J. *Tratamiento Biológico de Las Aguas Residuals*, 2nd ed.; Ed. Díaz de Santos: Madrid, Spain, 2002.
- 10. Belitz, H.D.; Grosch, W. Química de los Alimentos, 3rd ed.; Editorial Acribia S. A.: Zaragoza, España, 2009.
- 11. WEF; American Society of Civil Engineers. Design of Municipal Wastewater Treatment Plants. In *WEF Manual of Practice 8, ASCE Manual and Report on Engineering Practice 76;* Water Environment Federation: Alexandria, VA, USA, 1992.
- 12. Rittmann, B.; McCarty, P. Biotecnología del Medio Ambiente; McGraw-Hill: Madrid, Spain, 2001.
- 13. Weatherley, L.; Rooney, D.; Niekerk, M. Clean synthesis of fatty acids in an intensive Lipase-Catalysed Bioreactor. *J. Chem. Technol. Biotechnol.* **1997**, *68*, 437–441. [CrossRef]
- 14. Tsouris, C.; Neal, S.; Shah, V.; Spurrier, M.; Lee, M. Comparison of liquid-liquid dispersions formed by a stirred tank and elestrostatic spraying. *Chem. Eng. Commun.* **1997**, *160*, 175–197. [CrossRef]
- 15. Albasi, C.; Riba, J.; Sokolovska, O.; Bales, V. Enzimatic hydrolysis of sunflower oil, Characterization of Interface. *J. Chem. Technol. Biotechnol.* **1997**, *69*, 329–336. [CrossRef]
- Pongket, U.; Piyatheerawong, W.; Thapphasaraphong, S.; H-Kittikun, A. Enzymatic preparation of linoleic acid from sunflower oil: An experimental design approach. *Biotechnol. Biotechnol. Equip.* 2015, 29, 926–934. [CrossRef]
- 17. APHA-AWWA-WPFC. *Métodos Normalizados Para el Análisis de Agua Potable y Aguas Residuales*, 17th ed.; Editorial Díaz de Santos: Madrid, Spain, 1992.
- 18. Crespi, M.; Huertas, J. Determinación simplificada de la demanda química de oxígeno por el método del dicromato. *Tecnol. Del Agua* **1984**, *13*, 35–40.
- 19. Vittadini, G. Catálogo de Información de Equipamiento de Biocontrol; Vittadini Riferiment: Milan, Italy, 1991.
- 20. Tchobanoglous, G.; Burton, F.; Stensel, H. *Wastewater Engineering, Treatment and Reuse*, 4th ed.; Metcalf & Eddy, Inc.; McGraw-Hill Companies, Inc.: New York, NY, USA, 2003.
- 21. Degremont. Diseño, Construcción, Montaje y Operación Planta de Tratamiento de Aguas Servidas de Los Angeles, Chile; Degremont: Rueil-Malmaison, France, 2001.
- 22. Henze, M.; Harremoes, P.; Jansen, J.; Arvin, E. Wastewater, Volumes and Composition: Biological and Chemical Process, Wastewater Treatment; Springer: Berlin, Germany, 1995.
- 23. Brenes, M.; Garcia, P.; Romero, C.; Garrido, A. Treatment of green table olive wastewater by activated-sludge process. *J. Chem. Technol. Biotechnol.* **2000**, *75*, 459–463. [CrossRef]
- 24. Young, J. Removal of grease and oil by biological treatment processes. *J. Water Pollut. Control Fed.* **1979**, *51*, 2071–2087. [PubMed]
- 25. Schwarzenbeck, N.; Borges, J.; A, W. Treatment of dairy effluents in an aerobic granular sludge sequencing batch reactor. *Appl. Microbiol. Biotechnol.* **2005**, *66*, 711–718. [CrossRef] [PubMed]
- 26. Chipasa, K.; Medrzycka, K. Characterization of the fate of lipids in activated sludge. *J. Environ. Sci.* **2008**, *20*, 536–542. [CrossRef]
- 27. 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]
- 28. Fernandez, M.; Abalos, A.; Crombet, S.; Caballero, H. Ensayos de biodegradabilidad anaerobia de aguas residuales generadas en una planta refinadora de aceite de soja. *Interciencia* **2010**, *35*, 600–604.
- 29. Cisterna, P.; Gutiérrez, A.; Sastre-Andres, H. Impact of previous acclimatization of biomass and alternative substrates in sunflower oil biodegradation. *Dyna* **2015**, *82*, 56–61. [CrossRef]



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