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Impact of previous acclimatization of biomass and alternative substrates in sunflower oil biodegradation

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Abstract
The aim of this work is to evaluate the incidence rate of the previous acclimatization of biomass and the presence of any other easily biodegradable substrates, such as sucrose. This experiment will be undertaken with sunflower oil biodegradation in a batch reactor with suspended biomass. It used a biomass concentration of 5000 mg/l in batch reactors, and in every condition the elimination of oil by means of biodegradation was achieved with levels ranging from 10 to 90 % It was noticed that sludge acclimation substantially improves biodegradation efficiency by increasing the average biodegradation from a value of 30% to 80%. When adding sucrose to an acclimatized biomass stage, biodegradation sunflower oil is reduced, from an average value of 80% to 60%.

Keywords: Biodegradability; Fat and oils; Acclimatization.

Incidencia de la aclimatación previa de la biomasa y sustrato alternativo en la biodegradación de aceite girasol

Resumen
El objetivo de este trabajo es evaluar la incidencia de la aclimatación previa de la biomasa y la presencia de otro sustrato, de fácil biodegradación como es la sacarosa, sobre la biodegradación del aceite girasol en un reactor discontinuo con biomasa en suspensión. Se trabajó con concentraciones de biomasa del orden de los 5000 mg/l en los reactores discontinuos y en todas las condiciones se logró eliminar aceite por la vía de la biodegradación desde niveles que van de un 10% hasta un 90% aproximadamente y observándose que la aclimatación de los lodos mejora sustancialmente la eficiencia de biodegradación aumentando la biodegradación promedio de un 30% a un valor de 80% y al agregar sacarosa en un escenario de biomasa aclimatada se reduce la biodegradación del aceite girasol, de un valor medio de 80% a un 60%.

Palabras-clave: Biodegradabilidad, Grasas y Aceites, Aclimatación.

1. Introduction

Fats and oils are an important component in both domestic wastewater and some types of industrial wastewater. The amount of lipids in municipal wastewater is approximately 30 to 40% of the organic matter, which is measured as Oxygen Chemical Demand [14].

However, the exact behavior of lipids in these processes is not well understood. The literature generally states that lipids and fatty acids are removed by biological treatment methods that inhibit microbial growth, and cause foam formation, which is the growth of filamentous bacteria and floating floc [9].

Currently, Flotation is used in the separation of immiscible fluids or fluids, while solids are increasingly used in sewage treatment. There are three different types of flotation characterized by the use of air at atmospheric pressure, dissolved air and induced air. The difference lies in the way of introducing air into the wastewater [28].

Physico-chemical treatment is applied to highly emulsified and dispersed systems, and the size of fat particles is less than 20 microns. It has been found that when using a

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DAF system to treat waters in the oil industry the efficiency of the process increases from 50% to 88% [13] with the addition of chemicals. A more recent alternative, electro coagulation, has shown high effectiveness in destabilizing such emulsions and the subsequent removal of oils and fats [7].

As for the biodegradability of fats and oils, there is generic information on which of these are classified as slowly biodegradable substances. As for their degradations, cells initially save these substances on their cytoplasm and later, through an enzymatic process, they perform hydrolysis to produce an assimilable substrate that can be biodegraded [27]. The results of the biodegradability obtained were between 0 and 40 % for mineral oils and from 60 to 90% [8] for vegetable oils and diesters.

This research studies the biodegradation of sunflower oil, for which tests are carried out in a discontinuous system with a behavior that approaches suspended biomass. The sunflower oil choice was based on the following criteria:

- It is the most frequent oil used in Chile.
- It is a product that is easy to access as well as being a standardized one.

Among the oils supplied in Chile, the most common is fish oil, and to a lesser extent, sunflower oil, canola and corn.

Many of the crude oils are imported, mainly soybean and sunflower [12].

Sunflower oil removal by means of biodegradation in a batch type reactor was studied and a comparative analysis of the behavior of sunflower oil biodegradation with acclimated and non-acclimated sludge was undertaken.

Other experiments that added sucrose as a different substrate were also undertaken as this provides competition phenomena that must be taken into account.

It is known that oils are formed by the condensation of glycerol and fatty acids.

1.2. Biological treatment of oils and fats

The specific problem of fatty and oily influent in the activated sludge treatment (the most commonly used method) is related to the release of free fat in the aeration stage. This limits and reduces the BOD5 removal during the early stage and also floating floc in the clarification stage.

Anaerobic treatment is commonly preferred to treat strong effluent with high organic content, in which fat and oil are the most common substrates from the food industries. The assessment for treatment usage is usually based on factors such as cost of aeration, sludge treatment, and disposal, etc.

Table 1.
Fatty acids that constitute sunflower oil [3].

String Type	Common Name	Percentage
C16	Palmitic	6,5
C18	Stearic	5,0
C20	Arachidic	0,5
C18:1(9)	Oleic	23
C18:2(9,12)	Linoleic	63
C18:3(9,12,15)	Linolenic	0,5
C20:1(3)	Arachidonic	1

Source: Adapted from Belitz, H.D. and Grosch, W., Química de los alimentos, 1997.

It is presumed that these meet the requirements of organic matter in the effluent. This consideration is important for the selection of an appropriate treatment technology [15]. Regardless of which type of biological treatment is chosen, acclimatization of degrading biomass and the presence of other substrates affect the biodegradation of fats and oils.

The composition and the particle substrate's size also determine the hydrolysis and biodegradation speed and mechanism in wastewater treatment system [24].

There have been some experiences, when treating industrial effluents vegetable oil, of anaerobic treatment preceded by aerobic treatment having been applied. These processes are affected by several problems such as the development of micelles, and the excess of biomass due to a high coefficient of performance, etc. Therefore, the addition of a physicochemical pretreatment, implemented to selectively remove biorefractory organics before anaerobic treatment has been implemented, yielding satisfactory results [30].

The process of biodegradation of fats and oils requires the involvement of enzymes in the hydrolysis. Enzymes are proteins which act as catalysts during reactions. They have the particular characteristic that is used for a specific reaction and specific substrate type. Typically the degree of specificity of an enzyme is associated with the biological role played. [22].

Hydrolysis begins with the biodegradation of fats and oils. This refers to the breakdown of the primary organic substrate present in the original wastewater into smaller products that can be captured and subsequently biodegraded by bacteria [33].

Microbial enzymes catalyze three reactions types: Hydrolitic, Oxidatives and those of synthesis. The hydrolytic enzymes are used to hydrolyze insoluble complex compounds on simple components that are able to pass through the cellular membrane by diffusion.

These enzymes can act outside the cell wall, meaning that they can be intracellular or extracellular enzymes, such as oxirreductasas, which operate within the cell [26].

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Fats and oils are attacked by a wide range of organisms in a process in which extracellular enzymes called lipases are used to free fatty acids [20]. In this case the fatty acids can be biodegraded by a broader range of microorganisms. Those that do not produce lipolitic extracellular enzymes are also included in the process [25].

One of the tools that is most commonly used is the acclimatization of biomass, a procedure which allows the pollutants to be removed biologically. [26]. Due to the complex nature of the sensitive microbial ecosystems, they dynamically provide answers regarding environmental conditions such as temperature, pH, availability and substrates type. [26].

In this context, acclimatization it is associated with a type of substrate that is to be used as an energy source; therefore, biomass carries out the biodegradation thereof.

Also, in this context, the importance of the acclimatization of sludge to a type of substance that was initially described as harmful is confirmed. When considering the existing literature, fats and oils are substances that do not favor the development and growth of bacterial colonies. As sludge is acclimatized for a considerable time to the new type of influent, it appears that the biomass adapted to it. This is explained by mutations, or changes caused by chemical or physical agents, which change the DNA and impart new features to the cell, thereby allowing the cell to degrade xenobiotic substances or ones that are a result of biodegradation. Spontaneous mutations occur in one of 106 cells. However, the DNA molecule is capable of auto-repeating [6].

In recent years, use has been made of commercial microbial supplements that are bioincreasers as they remove of fats and oils, and make a distinction between those with a variety of bacteria from those that contain a specified one [7].

The nature of organic, soluble or particulate substrates as well as the concentration of nutrients, which dissolve oxygen in the reactor and the organic load, and the sludge age are some of the main factors affecting competition [1].

Given the possibility that heterogeneous communities of bacteria simultaneously metabolize individual components of mixtures of carbon compounds, they are dependent on the concentration of the respective substrates [29].

Finally, in the case of mineral oils, the OECD current standards require both a knowledge of the chemical structure to calculate the theoretical value of the rate of oxygen consumption (OUR) and CO2 evolution, and an experimental determination of the value for these parameters, which is closely associated with ecological impacts [5]. In fact, pollution from oil spills in marine ecosystems and reserves accelerates the repair of the damaged ecosystem through bioremediation techniques. These are based on the application of microbial metabolic activity to degrade petroleum hydrocarbons [2], which is also based on the principles of acclimatization.

2. Methodology

2.1. Batch Team

The batch reactors are beakers of 250 m, which are filled with bacterial biomass generated from the biodegradation of sucrose, which has a suspended solids concentration in the range of 4000 to 6000 mg/l as well as a stirring device.

2.2. Food Preparation

The batch reactor was fed with synthetic wastewater, prepared according to the typical characteristics of a strong urban wastewater [23] This has a COD of about 1000 mg/l, with the proportions of nitrogen and phosphorus, which are given by the COD: N: P=100:5:1. In addition to the carbonaceous substrates, potassium hydrogen phosphate and ammonium chloride were added.

2.3. Analytical Testing

Equipment and instruments used to determine the various parameters to characterize the wastewater were used. The following parameters with their corresponding methods were measured:

- Total Suspended Solids (TSS),

TSS is determined by filtering a known volume of sample on Whatman 4.7 cm GF/C glass fiber filters and then drying it at 103-105°C. The difference in weight of the filter before and after filtration is used to calculate the content SST, 209C method [1].

- Fats and Oils

When determining the fats and oils, the Gravimetric Assay Soxhlet method is used, which quantifies substances with similar characteristics on the basis of their common solubility in an appropriate solvent, 213E method [1].

The experimental design of this work starts with a comparative experience, using acclimatized and non-acclimatized biomass, with different concentrations of TSS in an exploratory phase in order to verify the effect of acclimatization on the biodegradation of fats and oils.

In the second experiment, when working with the additional substrate –sucrose– in all scenarios, the same concentration of TSS was used with acclimatized and non-acclimatized biomass.

3. Results

The biodegradation sunflower oil tests were performed in an incubator at a temperature of 30°C and an agitation of 200 (rev/min).

3.1. Acclimated sludge

The results obtained are reported in terms of the biodegradation that is left by the removal of the oil type, 0.92 g, using two different concentrations of non-acclimatized biomass, 4000 and 6000 mg/l.

A relationship is observed between the levels of biodegradation and the time that the substrate remains in the reactor. This is a consequence of the specific features that the oil has in terms of its solubility and buoyancy. These features result in a random situation regarding the meeting of microorganisms with the oil molecules. It is observed that biodegradation levels range from 8% for 1 day to 43% for 4 days in terms of residence time, Table 2. The increase in biomass concentration is not a relevant factor.

In order to develop microbial wastewater treatment, contaminated edible oils were selected that contained microorganisms capable of quickly biodegrading edible oil. The screening study showed a co-culture of yeast strain that was comprised of Rhodotorula pacifica ST3411 and ST3412

Table 2.
Oil Biodegradation in batch systems with acclimatization.

Oil (g)	Vol. System (ml)	Conc. Sludge (mg/l)	Efficiency Biodegrade (%)	Period (day)
0,92	225	6000	27	2
0,92	225	6000	24	3
0,92	210	4000	8	1
0,92	210	4000	18	2
0,92	210	4000	43	3
0,92	210	4000	37	4

Source: Authors' own.

Table 3. Oil Biodegradation in batch systems with acclimatization.

Oil (g)	Oil remnant (g)	Vol. (ml)	Conc. Sludge (mg/l)	Effici. Biodeg. (%)	Period (day)
0,92	0,037	225	5000	96	3
0,92	0,295	225	5000	68	2
0,92	0,37	225	5000	61	1

Source: Authors' own.

strain Cryptococcus laurentii. The degradation rate in 24h 3,000 ppm of mixed oils (salad oil / butter / beef tallow, 1:01 w/w) at 20°C was 39.8%. Faster degradation was observed at 20°C and pH 8. In a larger scale experiment, the salad oil was rapidly degraded by a cocultivation of 67152.0 to 14396.7 ppm in 24 hours, and the degradation rate was 79.4% [32].

3.2. *Acclimated sludge*

In order to measure the acclimatization effect of the sludge, experiments with acclimated sludge (C/A) on a batch reactor were developed. The following results were obtained:

In the above results the importance of acclimation of sludge in biological treatment can be observed. Conversion levels between acclimated sludge reactors were compared with ones that had not been acclimatized, and the differences noted. For the cases with acclimated sludge, biodegradation levels over 60% produced values of up to 98%, and in the case of sludge without acclimatization values ranging from 8 to 42% were obtained. An important factor is the acclimatization of the sludge, which plays an important role. High concentrations of lipids emulsified, about 400 mg/l are absorbed quickly and efficiently when they are brought into contact with activated sludge previously acclimated to low concentrations of lipids of 30 mg/l.[18]

These results are very similar to those obtained by other research on biodegradation of olive oil in a batch activated sludge system using both acclimated and non-acclimated sludge. For non-acclimated sludge, levels of biodegradability obtained between day 1 and day 4 were from 6% to 68%, while for acclimated sludge, results vary from day 2 to day 5 by 58% and 96% [34].

The feasibility of sewage sludge co-digestion using intermediate waste generated inside a wastewater treatment plant, i.e. trapped grease waste from the dissolved air flotation unit, has been assessed in a continuous stirred lab reactor operating at 35 °C with a hydraulic retention time of 20 days. The results indicate that a slow increase in the grease waste dose could be a strategy that favors biomass acclimation to fat-rich co-substrate, which increases long chain fatty acid degradation and reduces the latter's inhibitory effect. [31].

Moreover, there are laboratory studies for commercial supplements -multi bioaugmenters type species- that have verified the increase in the removal of fats and oils from 37% to 62% [6]. This bears some similarity to the effect of acclimatization of sludge: also being a biological type.

The use of bio augmenters was applied to bakery wastewater, which is rich in oils and fats. During this process, the pH, and the aeration system of an external mix bioreactor was properly adjusted to optimize the efficiency of treatment tank and the biological filter. The main function of the external mix bioreactor is producing and injecting the improved biomass into the main system. A large reduction of fats and oils 1.5g / 0.03g / l was achieved. This improvement was been observed over a period of 20 months [19].

In other cases that are based on acclimatization, the bioremediation of a contaminated soil was compared with a gasoline-diesel fuel mixture on a laboratory scale, to evaluate biostimulation against natural attenuation and bioaugmentation. The reduction of Total Petroleum Hydrocarbons (TPH) concentration over the course of three months was 52.79% for natural attenuation, 60.45% for biostimulation and 64.92% for bioaugmentation. A bacterium with the capacity to degrade hydrocarbons identified as Bacillus sp. was isolated during inoculation in the bioaugmentation treatment [16].

A similar study for Yarrowia lipolytica W29 that was immobilized by calcium alginate was considered. In this case the main objective was to degrade the chemical oxygen demand (COD) and oil. Biodegradation by immobilized cells (cell density of 6.65×10^6 CFU/ml), was able to remove up to 2000 mg/l of oil and up to 2000 mg /l of COD in 50 hours of treatment at 30°C (pH 7, 150 r/min). Similarly, using free cells, it was observed that the resulting biodegradation efficiency was 80% of the previously cited figures [21].

3.3. *Effects of Joint Food*

Experiences of biodegradation in batch systems for two types of influent, one with mixed feeding and another that only has oil were measured in order to assess the effects on the biodegradation of an additional substrate in the influent.

A non-acclimated biomass

When comparing the results shown in the above tables that correspond to levels of biodegradation obtained in a batch reactor, both strictly oily sucrose and mixed influent, the effect of acclimatization is confirmed. Higher levels of biodegradation are checked for acclimated sludge. This was undertaken at a temperature of 30°C and 200 (rev/min).

These results allow the effect of sucrose to be evaluated on the biodegradation of oil, which is manifested at a reduced level of oil biodegradability. This is explained by the fact that microorganisms have an alternative carbon source and competitive phenomena occur. It can be said that the presence of sucrose causes an inhibitory effect on the biodegradation of oil.

Table 4.
Biodegradation of oil in batch systems without acclimatization and sucrose.

Oil (g)	Oil rem. (g)	Saccharin (g)	Conc biom (mg/l)	Vol. Biom (ml)	Efficie Biode (%)	Period (day)
1	0,37	0,5	5000	150	63	1
1	0,38	0,5	5000	150	62,0	2
1	0,23	0,5	5000	150	67,0	3
1	0,26	0	5000	150	74,0	1
1	0,12	0	5000	150	78,0	2
1	0,19	0	5000	150	81,0	3

Source: Authors' own.

Table 5.
Biodegradation of oil in batch systems with acclimatization and sucrose.

Oil (g)	Oil rem. (g)	Saccharin (g)	Conc biom (mg/l)	Vol. Biom (ml)	Efficie Biode (%)	Period (day)
1	0,41	0,5	5000	150	59,0	1
1	0,29	0,5	5000	150	71,0	2
1	0,26	0,5	5000	150	74,0	3
1	0,29	0	5000	150	71,0	1
1	0,16	0	5000	150	84,0	2
1	0,04	0	5000	150	96,0	3

Source: Authors' own.

In this case, sunflower oil biodegradation was studied in a batch-type system for both types of influent, over a period of three days, with the same concentration of biomass, at a temperature of 30°C and 200 (rev/min).

As can be seen from the results, for the cases where the influent only has oil and the biodegradation growth level achieved ranges from 71% to 96%, for acclimatized biomass with levels between 73,6 to 81%, and for non-acclimatized biomass, a higher level of biodegradation is observed. For the influent case in which biomass was acclimatized at between 62.2 to 66,7%, and for non-acclimatized biomass that has sucrose as well as the oils, biodegradation ranges from between a 59% and 74%, and the reaction times are from one to three days.

In similar experiences, considering the same amount of biomass (500 mg/l), where only vegetable oil is present in the effluent, a range of biodegradation is observed between 74% and 81% for the non-acclimated biomass. These results are clearly lower than the range observed for acclimated biomass (71 to 96%)

The intake besides oil also contains saccharin. The biodegradation reaches ranges of between 62% and 67% for the non-acclimated biomass and 59% and 71% for the acclimated biomass.

In both experiments the processing time ranges from between one to three days. Similarly, in both cases, with and without acclimation, it is observed that the procedure improves the oil biodegradation level, and the alternative substratum (saccharin) also competes with the oil reducing in both cases its degradation levels.

This confirms the previously obtained results regarding acclimatization. It also appears that the concentration of TSS is not relevant between the 4000-6000 mg/l range.

The experiments conducted characterized the transformation of lipids in the activated sludge under aerobic conditions. The results show that the total lipid content in the effluent could not be reduced to values below 300 mg/l from an initial concentration of 2000 mg/l [9]. This is a value that is consistent with those obtained in this investigation, which reached 98% within three days of residence time, larger than that of activated sludge.

This study investigates the COD removal of two-phase anaerobic digestion to treat a mixture (1/5 v/v) of used vegetable oil waste and pig manure. It uses two semi-continuous digesters that were operated at a mesophilic temperature. One of the experiments was conducted at a hydraulic retention time (HRT) of 4 days in the first stage (acidifier) and at a HRT of 20 days in the second stage (methanizer). The CODs removal efficiency were 44% and 79,5% respectively [17].

4. Conclusions

Under all conditions it was possible to remove oil biodegradation pathways with levels ranging from 10% to about 90%.

It was found that acclimation of sludge substantially improves the efficiency of biodegradation, increasing it from an average value of 30% with no biomass acclimated to an average of 80% over a period ranging from 1 to 3 and 1 to 4 days.

The presence of other more biodegradable substrates, such as saccharine, reduces the efficiency of biodegradation of sunflower oil, from an average value of 80% to 65%.

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