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The impact of ruminal fluid lipolytic substances on the reduction of oils and fats in wastewater in the dairy industry and its influence on business decisions

Sofia Godoy[1], Gerardo Leon[2], Paulina Bolaños[3], Alexandra Procel[4]

[1]Polytechnic School of Chimborazo (ESPOCH) Riobamba-Ecuador, sofia.godoy@espoch.edu.ec

[234]Polytechnic School of Chimborazo (ESPOCH) Riobamba-Ecuador

Abstract

The dairy industry is known as one of the major contributors to wastewater generation in terms of organic load value and effluent volume. The microbial ecosystem of the rumen that in Ecuador is considered as a waste from the slaughter mainly of cows in the municipal beds comprises at least 30 predominant bacterial species. The purpose of this work was to determine the ability of lipolytic bacteria extracted from cow rumen for the degradation of wastewater oils and fats generated by the dairy industry. Experimental tests were performed for the isolation of lipolytic bacteria from the rumen from three culture media: tributyrin, lecithinase and tr ibutirine with 5 and 10% lecithinase under aerobic and anaerobic conditions. Tributyrin under anaerobic conditions was considered the most suitable culture medium for the massification of lipolytic bacteria from rumen fluid. The experimental units were conditioned also based on pH, temperature and Biochemical Oxygen Demand (BOD5). 3 pH regulating agents of the experimental units were tested: Sodium Carbonate, Sodium Bicarbonate and Bicarbonate. As pH regulating agent, sodium carbonate was used, which allowed reaching a value of 6.5 with only 5mL of dosage. Three moments of analysis were considered: the initial moment (or days), an intermediate moment (28 days) and the final moment (56 days). The ability of lipolytic bacteria to reduce the concentration of oils and fats in dairy industry wastewater in 56 days was 48.49% compared to the initial concentration, that is, 46.4 mg / L reduced to 22.5mg / L, so it was shown that its applicability is highly efficient in proportion to the research conditions. The high efficiency of these bacteria was directly related to the conditioning of pH and BOD5, based on the results that were generated by applying the correlation matrix and the Chi-square method. The analysis of the results obtained does not promote

the biotechnological treatment of industrial wastewater, but also promotes the use of waste as a source of degrading organisms.
Keywords: lipolytic bacteria; rumen fluid; wastewater oils and greases; dairy industry; tributyrin.

Introduction

A large amount of wastewater is generated after domestic and industrial uses, which is discharged directly or after a partial treatment close to water bodies that add a large amount of toxic pollutants(Mishra & Maiti, 2017). The dairy industry typically produces 1 to 2 m3 of wastewater per metric ton of processed milk. The main contributors of organic load to these wastewater are carbohydrates (lactose), proteins (casein) and lipids (fatty substances) from milk. (Kibena et al., 2013). The dairy industry is known as one of the major contributors to wastewater generation in terms of organic load value and effluent volume. Universal environmental pollution is a real situation that deteriorates our world step by step. The dairy factory spill is the second largest source of pollution in water streams. The environmental impact of these factories can be very high, especially due to the discharge of wastewater with high content of organic matter and nutrients(Kavaz & Öztoprak, 2017).

The nature and composition of dairy wastewater depends on the types of products produced, production methodology, season, processing capacity of the plant, the extent of pipelines, equipment and other industrial operations. Whey, a byproduct of dairy effluents, is greenish-yellow, liquid that is produced as a result of precipitation and removal of casein from milk during cheese making. Whey is considered an important contaminant in dairy products and wastewater due to its high volume and organic load. Depending on the treatment capacity, the total chemical oxygen demand (COD) of the effluent can be divided into two main components: biodegradable COD and non-biodegradable (inert) COD. Within the biodegradable components, only the biodegradable solubles can be easily used by microorganisms, considering that the biodegradable fraction of particles has to be converted into soluble fraction before it can be absorbed(Pandey et al., 2020)(Paçal et al., 2019)(Hu et al., 2011) .

The rumen is a fermentation chamber that contains a complex and diverse microbiota composed of different microbial communities, such as bacteria, archaea, protozoa and fungi. All of them involved in microbial fermentation. The rumen microbial ecosystem comprises at least 30 predominant bacterial species at a total concentration of 1010 to 1011/m L of (Darabighane et al., 2019) rumen fluid, about 40 species of protozoa (105 to 107/ml), and five species of fungi (< 105/mL), rumen bacterial species are considered more important than protozoa and fungi in determining the degree and rate of degradation. (Miron et al.,

2001) Yeasts and lactic acid bacteria (LAB) are produced as part of the natural microbial population in food fermentation and as starter cultures in food and beverage industry.(Shetty & Jespersen, 2006).

Wastewater from the dairy industry, containing valuable resources such as a high concentration of organic matter and a variety of carbon sources that are assimilable, can be recycled and used as a substrate for the cultivation of microorganisms. The values of these by-products for fortification of the food supply or for consumption as single-celled protein can be an alternative both to reduce the pollutant load and to solve the problem of global protein shortages(Arous et al., 2016). Whey is the main by-product obtained from the manufacture of cheese and the most polluting process due to its organic load composed of lactose, lactic acid, proteins and salts(Alfano et al., 2021). Due to the high content of lactose in addition to proteins, whey, very often in combination with other components of the medium, is also used as a substrate for the cultivation of various microorganisms(Alfano et al., 2021). Industries and restaurants face problems due to the inefficiency of existing anaerobic treatments and aerobic biological methods for the treatment of lipids containing wastewater due to their hydrophobic characteristics. Therefore, there has been constant research on the bioremediation of lipid-rich wastewater, either aerobically or anaerobically. The relatively low-cost "anaerobic-aerobic" biological system are treatment processes that are considered to be the most important process for wastewater treatment in the dairy industry(Saranya et al., 2014)(Yan et al., 2021).

The purpose of this work was to determine the capacity of lipolytic bacteria extracted from cow rumen for the degradation of wastewater lipids generated by the dairy industry in the rural area of Riobamba canton, Chimborazo province. The rumen was a waste from the slaughter of about one hundred cows from the municipal bed of the city of Riobamba, considering that in the stomach of these animals there is an average of 4 liters of rumen fluid, that is, 400 liters of this component are produced daily. This was intended to take advantage of a residue rich in microbial load, so that from this can be extracted by selective means lipolytic bacteria that when massified in the laboratory of Biotechnology and Animal Microbiology of the Polytechnic School of Chimborazo were inoculated in wastewater with high concentration of oils and fats thus evaluating the degradative capacity of organic components.

Materials and methods

Isolation of lipolytic bacteria from cow rumen

The rumen fluid samples were obtained from four cows previously slaughtered from the municipal bed of the Riobamba canton. The rumen

serves as an essential substrate for certain microbial species and is used in the synthesis of other branched-chain compounds such as amino acids and fatty acids.(Gleason et al., 2022)

For the isolation of lipolytic bacteria, experimental tests were carried out in three selective culture media: tributyrin, lecithinase and tributyrine with 5 and 10% lecithinase under aerobic and anaerobic conditions.

Knowing that Tributyrin is a triglyceride naturally present in butter and that itis an ester composed of butyric acid and glycerol that can act as a precursor of butyrate since butyrate can be cleaved by intracellular enzymes and that it has been found to play an important role in intestinal nutrient absorption and health were considered as a potential substitute for food additives antibiotics for the preparation of the culture media of this research(He et al., 2022).

For the preparation of the substrates conditions of 1 atmosphere of pressure and 121 ° C were needed. The requirements of the medium tributtirine were yeast extract, peptone, butter, agar agar, distilled water. The requirements of the medium lecitinase were peptone, disodium phosphate, sodium chloride, 0.5% w/v solution of magnesium sulfate, glucose, agar, distilled water.

With dilutions of up to 10-8 of the rumen fluid due to the high microbial load, 13 mL of each culture medium and 1mL of each rumen fluid solution were added in 8 petri dishes under aerobic conditions and 8 in anaerobic conditions incubated at 37 ° C for 48 hours.

After 48 hours of incubation, the bacteria in each of the media were identified microscopically and macroscopically. Microscopic identification was given by Gram staining, while macroscopic identification considered hydrolysis halos, color, texture, size and elevation of bacterial colonies. The bacterial massification was developed from the environment where a better growth of lipolytic bacteria was obtained both aerobic and anaerobiosis.

Table 1. Experimental design to determine Colony Forming Units (CFU) under aerobic conditions (UA)

	Experimental design			
	Colony forming units (CFU) under aerobic conditions (UA)			
Dilution	Tributirina (UAA)	Lecitinasa (UAB)	Tributirina+lecitinasa al 5% (UAC)	Tributirine+lecithinase 10% (UAD)
10^{-1}				
10^{-2}				
10^{-3}				
10^{-4}				

10^{-5}			
10^{-6}			
10^{-7}			
10^{-8}			

Table 2. Experimental design to determine Colony Forming Units (CFU) under anaerobic conditions (UN)

Dilution	**Experimental design**		
	Colony forming units (CFU) under anaerobic conditions (UN)		
	Tributyrin (UNA)	Tributirina+lecitinasa al 5% (UNC)	Tributirina+lecitinasa al 10% (AND)
10^{-1}			
10^{-2}			
10^{-3}			
10^{-4}			
10^{-5}			
10^{-6}			
10^{-7}			
10^{-8}			

Adaptation of experimental units for the degradation of oily compounds in waste water in the dairy industry

Initially, the respective physicochemical analyses of the wastewater of the dairy industry were carried out considering the parameters Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), pH, temperature, dissolved oxygen and fat and oil content and an initial microbiological analysis on tributyrin agar to identify organisms of lipid nature in the water quality laboratory of the Faculty of Sciences of the Polytechnic School of Chimborazo.

The pH of the wastewater was adjusted from an initial value of 3.8 to a pH of 6.5 which is the optimal condition for the growth of lipolytic bacteria. For this conditioning, three experimental units were developed in which 10% solutions of sodium carbonate, bicarbonate and sodium bicarbonate were prepared. Once these solutions were prepared, 9mL of each were placed and added in test tubes with 1mL of optimal nutritive broth incubated under aerobic and anaerobic conditions.

Table 3. Experimental design to adjust pH under aerobic (AU) and anaerobic (UN) conditions

pH adjustment in experimental units		
pH regulator	mL dosed	pH reached
Sodium carbonate		
Bicarbonate		
Baking Soda		

Identified the pH regulatory solution in each of the conditions raised proceeded to inoculate lipolytic bacteria isolated from rumen fluid in four experimental units, two of them in aerobic conditions and the remaining two in anaerobic conditions at 35 ° C and a pH 6.5 under the scheme of a greenhouse containing 12 liters of wastewater from the dairy industry. For each experimental unit, 162*10 8 CFU/mL of bacteria were inoculated. Unit UA1 and UN1 were considered reference experimental units to compare the changes presented in UA2 and UN2 Units respectively during 3 moments: initial moment (incubation day 0), intermediate moment (incubation day 28) and final moment (day 56) to evaluate the microbial growth curve and the corresponding physicochemical analysis that included the measurement of Temperature °C, pH, BOD5 (mg/L), Concentration of oils and fats (mg/L), CFU/mL

Table 4. Experimental design for evaluation of the reduction of oily compounds in wastewater under aerobic (AU) and anaerobic (UN) conditions

Experimental unit	Conditioning
UA1	12L of wastewater aerobic conditions at 35°C and a pH 6.5
UA2	12L of waste water+ 162*10 ^{8} CFU/mL of bacteria aerobic conditions at 35°C and a pH of 6.5
UN1	12L of wastewater anaerobic conditions at 35°C and a pH of 6.5
UN2	12L of wastewater + 162*10 ^{8} CFU/mL of bacteria anaerobic conditions at 35°C and a pH of 6.5

Statistical analysis of data

With a principal components design the data sets in the direct degradation of oils and fats from wastewater were determined, this statistical test was used to achieve a synthesis of information.

The Chi-Square test was also performed to contrast the observed values with those expected, comparing the distribution observed in the data studied with the expected distribution.

Results and Discussion

Isolation of lipolytic bacteria from cow rumen

Microbial growth in the culture medium tributyrin at 37 ° C under aerobic conditions

Determination of lipolysis on agar plates is a simple approach to determine the action of lipases derived from the action of inoculated lipolytic bacteria, but visual evaluation of lipolysis is frequently difficult in practice(Carrazco-Palafox et al., 2018). Emphasizing that this work did

not directly link the fatty acid profiles of rumen fluid and milk in the dairy industry with the diets of cows(Manzocchi et al., 2022) and from microbial growth tests with the culture medium tributyrin, sowing carried out at 37 °C in the presence of oxygen it was determined that in the first dilution (10-1) the highest microbial growth was developed, thus finding 7767 CFU/mL, however, it was from the dilution (10-6) where no growth could be evidenced.

The morphological characteristics of the cultivated colonies were recorded and isolated and preserved on tributyrin agar. The morphology of isolated organisms was studied. Lipolytic activity was tested in cell-free filtration of culture broth cells for each isolate

Table 5. Microbial growth in the culture medium tributyrin (UAA) at 37°C aerobic conditions

Dilution	Result Log UFC/mL
10^{-1}	7767
10^{-2}	6812
10^{-3}	5892
10^{-4}	4926
10^{-5}	3989
10^{-6}	0
10^{-7}	0
10^{-8}	0

Microbial growth in the culture medium tributyrine at 37 ° C anaerobic conditions

From the microbial growth tests with the culture medium tributhyrin, seeding carried out at 37 ° C in the absence of oxygen it was determined that in the first dilution (10-1) the greatest microbial growth was developed thus finding 10863 CFU / mL.

Table 6. Microbial growth in the culture medium tributyrin (UNA) at 37 ° C anaerobic conditions

Dilution	Result Log UFC/mL
10^{-1}	10863
10^{-2}	10000
10^{-3}	9017
10^{-4}	8091
10^{-5}	7135
10^{-6}	6193
10^{-7}	5477
10^{-8}	4653

It was in anaerobic conditions where greater bacterial growth could be evidenced, so it is defined that the lower the dilution factor, the greater the number of CFUs per mL of solution in the absence of oxygen.

Microbial growth in the culture medium tributyrine with lecithin at 5% aerobic conditions

When two specific media were combined: 5% tributyrin, lecithin under aerobic conditions at 37°C, no CFU/mL was evident in any of the eight experimental dilutions.

Table 7. Microbial growth in culture medium Tributyrin with 5% lecithin (UAC) at 37°C aerobic conditions

Dilution	Result Log UFC/mL
10^{-1}	0
10^{-2}	0
10^{-3}	0
10^{-4}	0
10^{-5}	0
10^{-6}	0
10^{-7}	0
10^{-8}	0

Microbial growth in the culture medium tributyrin with 5% lecithin anaerobic conditions

The first four dilutions allowed microbial growth in the absence of oxygen, the dilution factor (10-1) was the one that generated the greatest representativeness with 5301 CFU / mL, from the fourth dilution no growth was evidenced.

Table 8. Microbial growth in the culture medium tributyrinin with 5% lecithin (UAC) at 37 ° C anaerobic conditions

Dilution	Result Log UFC/mL
10^{-1}	5301
10^{-2}	4397
10^{-3}	3505
10^{-4}	0
10^{-5}	0
10^{-6}	0
10^{-7}	0
10^{-8}	0

Microbial growth in culture medium tributyrine with 10% lecithin aerobic conditions

When two specific media were combined: 10% tributyrin , lecithin under aerobic conditions at 37°C, no CFU/mL was evident in any of the eight experimental dilutions.

Table 9. Microbial growth in the culture medium tributyrine with 10% lecithin (UAD) at 37°C aerobic conditions

Dilution	Result Log UFC/mL
10^{-1}	0
10^{-2}	0
10^{-3}	0
10^{-4}	0
10^{-5}	0
10^{-6}	0
10^{-7}	0
10^{-8}	0

Microbial growth in the culture medium tributyrinin with 10% lecithin anaerobic conditions

Only the first dilution allowed microbial growth in the absence of oxygen, the dilution factor (10-1), with 3414 CFU / mL, from the second dilution no growth was evidenced.

Table 10. Microbial growth in the culture medium tributyrine with 10% lecithin (UAD) at 37°C anaerobic conditions

Dilution	Result Log UFC/mL
10^{-1}	3414
10^{-2}	0
10^{-3}	0
10^{-4}	0
10^{-5}	0
10^{-6}	0
10^{-7}	0
10^{-8}	0

Therefore, the overall results obtained were:

Table 11. Determination of Colony Forming Units (CFU) under aerobic conditions (UA)

Dilution	Experimental design		
	Colony forming units (CFU) under aerobic conditions (UA)		
	Tributirina (UAA)	Tributirina+lecitinasa al 5% (UAC)	Tributirine+lecithinase 10% (UAD)
10^{-1}	7767	0	0
10^{-2}	6812	0	0
10^{-3}	5892	0	0
10^{-4}	4926	0	0
10^{-5}	3989	0	0

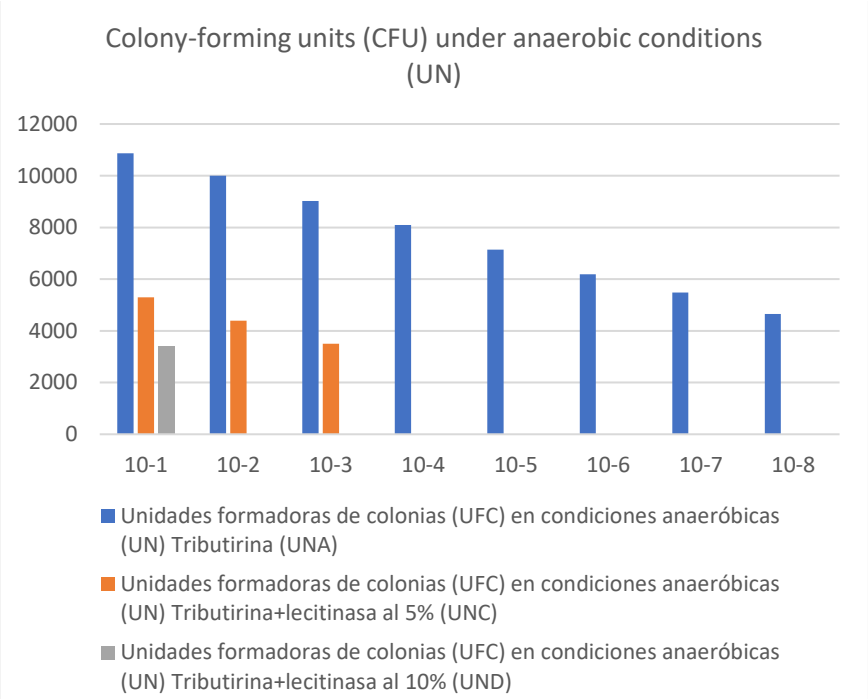
10^{-6}	0	0	0
10^{-7}	0	0	0
10^{-8}	0	0	0

Table 12. Determination of Colony Forming Units (CFU) under anaerobic conditions (UN)

Dilution	**Experimental design**		
	Colony forming units (CFU) under anaerobic conditions (UN)		
	Tributyrin (UNA)	Tributirina+lecitinasa al 5% (UNC)	Tributirina+lecitinasa al 10% (AND)
10^{-1}	10863	5301	3414
10^{-2}	10000	4397	0
10^{-3}	9017	3505	0
10^{-4}	8091	0	0
10^{-5}	7135	0	0
10^{-6}	6193	0	0
10^{-7}	5477	0	0
10^{-8}	4653	0	0

The best culture medium for CFU/mL growth was tributyrin. It was recognized that the lower the dilution factor there is a greater microbial load, which is why working with dilution factor 10-1 allows to have more lipolytic bacteria that, when massified in anaerobic conditions, will accelerate the process of degradation of oils and fats in cheese water with characteristics similar to that of the object of this study. Lecithin acted as an inhibitor of lipolytic bacteria. The results implied that tributyrin plays a role in regulating strain growth. This study provides novel insight into the metabolic cause of different acid production rates among lipolytic bacteria(Shen et al., 2022).

Figure 1. Determination of Colony Forming Units (CFU) under anaerobic conditions (UN)



The macroscopic and microscopic identification of lipolytic bacteria that were mostly easy to observe was in dilutions 10-5 to 10-8, with the presence of gram-negative bacilli (gram-negative coccobacilli), streptococci (gram-positive cocci), Staphylococcus (gram-negative bacilli), staphylococci.

Table 13. Macroscopic and microscopic identification of lipolytic bacteria under aerobic (AU) and anaerobic (UN) conditions

Dilution	Middle	Identification	
		Microscopic	Macroscopic
10^{-5}	Aerobe	Gram-negative bacilli Gram-negative coccobacilli	Creamy yellow colonies
10^{-7}	Aerobe	*Streptococci* Gram-positive coconuts	Creamy yellow colonies
10^{-8}	Aerobe	*Staphylococcus* Gram-negative bacilli	White rough colonies
10^{-5}	Anaerobic	Gram-positive coconuts Gram-negative	Creamy yellow colonies

		cocci *Streptococci*	
10^{-7}	Anaerobic	*Staphylococci*	White rough colonies
10^{-8}	Anaerobic	Gram-negative coccobacilli	White rough colonies

Adaptation of experimental units for the degradation of oily compounds in waste water in the dairy industry

The optimal pH for the growth of lipolytic bacteria is considered in the range of 6 to 7. By adding sodium carbonate, sodium bicarbonate and bicarbonate as pH regulating agents to the wastewater of the experimental units, it was possible to adjust this from an initial value of 3.8 to 6.5. Sodium carbonate was the most suitable compound to regulate pH conditions using 5 mL of dosage, while bicarbonate and sodium bicarbonate did so with a volume of 15 mL.

Table 14. Macroscopic and microscopic identification of lipolytic bacteria under aerobic (AU) and anaerobic (UN) conditions

pH adjustment in anexperimental it.

pH regulator	mL dosed	pH reached
Sodium carbonate	5	6.5
Bicarbonate	15	6.6
Baking Soda	15	6.5

Reduction of oils and fats in wastewater under aerobic (AU) and anaerobic (UN) conditions

In the UA1 and UN1 as reference units at a temperature between 34 and 36 °C and a pH between 6.20 and 6.40, parameters considered optimal for the development of lipolytic bacteria, BOD5 was outside the maximum permissible range with values between 1665 and 1700 mg / L as established in the Unified Text of Secondary Legislation of the Ministry of Environment of Ecuador that in the case of water discharges residual to a body of fresh water considers as a maximum permissible 100 mg / L thus representing an excessive amount of oxygen consumed by microbial activity by degrading the organic matter present in the medium under study.

In the case of oils and fats, current legislation indicates that as a maximum permissible value of this parameter must be 30 mg / L, however, as reference data it was had that the wastewater of the dairy industry presented concentrations of 46.40 mg / L, exceeding the range of permissibility, which allowed to consider that the water for experimentation was a contaminated water. There were no CFU/mL in UA1 and UN1 at 56 days of analysis indicating that the conditions of these were not sufficient to allow growth of autochthonous bacteria and, therefore, there could not be the expected degradation of oils and fats. In the UA2 and UN2 that were experimental units with the same

conditions of pH and Temperature as the reference units (AU1 and AN1), BOD5 at the end of study (day 56) in both UA2 and UN2 was outside the maximum permissible range with a concentration of 1649 mg / L and 785 mg / L respectively, however, this parameter had a significant decrease of 46.18% in UN2.

Table 15. Degradation of oily compounds in wastewater under aerobic (AU) and anaerobic (UN) conditions

***Degradation of oils and fats in wastewater under aerobic (AU) and anaerobic (UN)* conditions**						
Experimental unit	Conditioning					
	days	Temperature °C	pH	DBO5 mg/L	Oils and fats (mg/L)	Log UFC/mL
UA 1	0	34	6.20	1700	46.40	2.54
	28	36	6.40	1665	46.00	3.69
	56	36	6.60	1665	46.00	0.00
UA 2	0	34	6.20	1700	46.40	10.20
	28	36	6.30	1655	45.00	11.25
	56	36	6.20	1649	44.50	9.50
A 1	0	34	6.20	1700	46.40	3.51
	28	36	6.30	1700	46.40	0.00
	56	36	6.10	1700	46.40	0.00
A 2	0	34	6.20	1700	46.40	10.20
	28	36	6.40	1245	37.00	12.00
	56	36	6.80	785	22.50	15.00

Figure 2. Final concentration of oils and fats in the days of experimentation of AU and UN

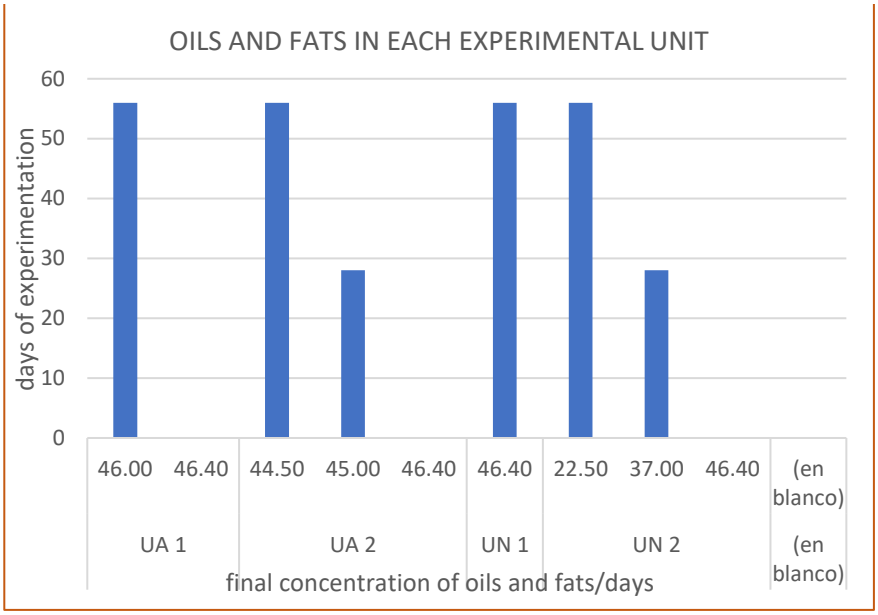
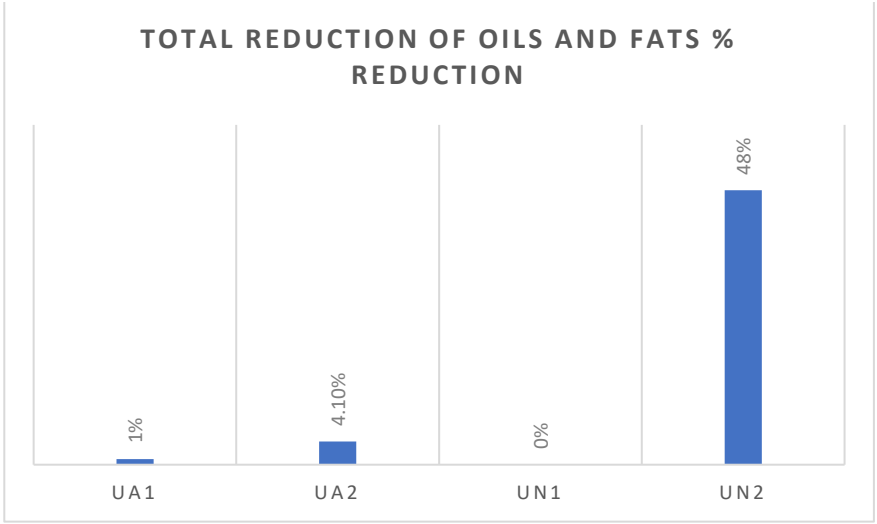
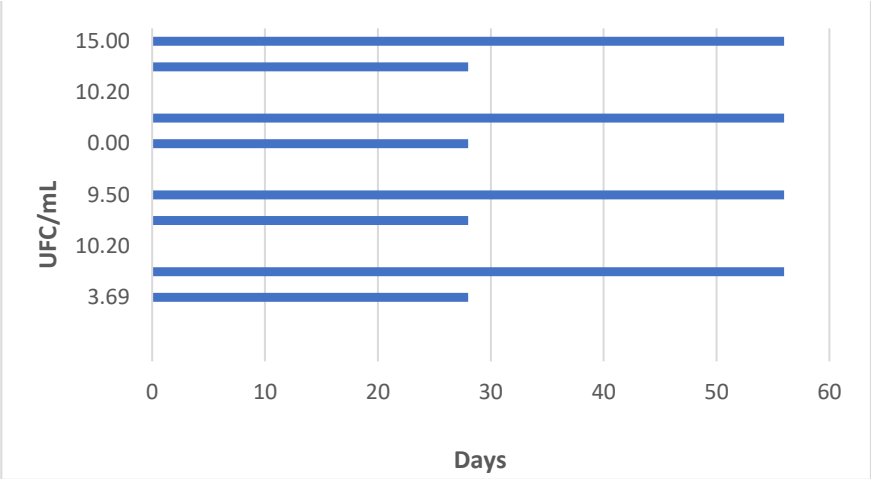


Figure 3. Percentage of total reduction of oils and fats in the days of experimentation of AU and UN



At day 56, the concentration of oils and fats in UA2 had decreased from 46.40 to 44.50 mg / L, while UN2 decreased from 46.40 to 22.50 mg / L, that is, it reduced 48.49% of the initial concentration.

Figure 4. Colony forming units (CFU) regarding the number of days of experimentation



During the time of inoculation in the UN2 from the first moment (day 0) to the final moment (day 56) there was an exponential growth of lipolytic bacteria of 68% which showed that, under anaerobic conditions, pH of 6.80, temperature of 36 ° C these manage to adapt and take as a source of carbon for their development to the oily substances of the wastewater studied.

Statistical analysis

Table 16. Matrix of correlations between parameters

Correlation	T°	pH	DBO_5	Fats and oils
T°	1.000	0.460	-0.339	-0.326
pH	0.460	1.000	-0.770	-0.765
DBO5	-0.339	-0.770	1.000	0.995
Fats and oils	-0.326	-0.765	-0.995	1.000

Because the determinant in the correlation test was 0.002 and a chi-square test result of p 0.301, there was a correlation between the control variables with the concentration of oils and fats that is directly proportional to the variability of BOD5 concentrations, pH value and temperature changes.

These findings could be used for future high-level lipase production using native isolate from lipolytic bacteria. In addition, this work demonstrated the feasibility of statistical methodology to develop an optimal composition of the medium for the efficient production of lipase using isolates de this type of microorganisms(Farmer et al., 2022).

Conclusions

Tributyrin was considered the most suitable culture medium for the massification of lipolytic bacteria from rumen fluid. Lipolytic bacteria are mesophilic microorganisms that under anaerobic conditions and a pH between the range of 6 to 7 indicate that the formulation provided in the experimental units with tributyrin constitute an excellent matrix to support the viability of the cells acting on mechanisms of degradation of oily compounds that can reduce pollution in wastewater with high concentrations of oils and fats of the dairy industry sector, which will allow producers to reduce the environmental externalities of their activity. Wastewater from the dairy industry has an acidic pH that originally prevents the development of lipolytic bacteria activity. The sodium carbonate allowed to regulate the pH stabilizing it to 6.5 which conditioned the optimal medium so that the bioaugmentation process could be carried out for the expected effect. The ability of lipolytic bacteria to reduce the concentration of oils and fats in dairy industry wastewater in 56 days was 48.49% compared to the initial concentration, that is, 46.4 mg / L reduced to 22.5mg / L, so it is shown that its applicability is highly efficient in proportion to the research conditions. The high efficiency of these bacteria was directly related to the conditioning of pH and BOD5, based on the results that were generated by applying the correlation matrix and the Chi-square method. The efficiency of lipolytic bacteria in the process of reducing oily compounds is an alternative for the use of rumen fluid as a primary source of these microorganisms, the rumen in Ecuador is currently discarded and discharged in most cases to the public sewer system or to receiving bodies without prior treatment. Lipases from lipolytic bacteria, especially those obtained from an extreme environment, have a large market in the food and detergent industries, (Bashiri et al., 2022) which not only promotes the biotechnological treatment of industrial wastewater, but also promotes the use of waste as a source of degrading organisms.

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