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OBTAINING A BIOFUEL FROM LACTOSE PRESENT IN CHEESE MANUFACTURING WASTE ON A PILOT SCALE IN A DAIRY COMPANY IN VALLEDUPAR

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Abstract: Biofuel was obtained from Saccharomyces as a fermentative agent using whey as a previously deproteinized substrate. To achieve this objective, it was necessary to characterize the physical (PH, Turbidity, Color), chemical (BOD, COD) and microbiological (NMS) parameters in which whey is found. Fermentation was carried out anaerobically and with independent variables such as glucose, from which the yeast obtains energy by dissociating the glucose molecules in the process, generating much more efficient results, and thiamine, which facilitates the start of fermentation and the implantation of the yeasts; The whole process was done in a homemade bioreactor, controlling all the conditions in the laboratory for 15 to 20 days. The refractometer measured the alcohol content, which in 500 ml measured that of 20 cc, performing the simple distillation where the vapors produced are immediately channeled to a condenser, which cools and condenses it so that the distillate is not pure, even so, when evaluating the efficiency in a glass container we tried to light the product and it was a success, the smell and the flames were characteristic of the presence of biofuel, releasing a high calorific value, it is the total amount of heat released in the complete combustion of a unit of mass of fuel when the water vapor originated in the combustion is condensed and, therefore, the heat released in this phase change is taken into consideration.
Keywords: Biofuel, Fermentation, Whey, Saccharomyces, Alcohol

INTRODUCTION

Whey is a liquid by-product obtained after the precipitation of casein during cheese making. It mainly contains lactose, proteins, minerals, vitamins and fats. The dairy sector in Colombia is an extremely important sector for the national economy.

It currently represents 2.3% of the national GDP and 24.3% of the agricultural GDP, in addition to generating more than 700,000 direct jobs (Pinto, 2017). On the other hand, the consumption of dairy products in Colombia is also an important figure. Due to the large quantities of cheese produced in Colombia, whey has generated an environmental pollution problem, since it physically and chemically affects the soil structure, which results in a decrease in the yield of agricultural crops and when it is discarded in water, it reduces aquatic life by depleting dissolved oxygen (Aider et al., 2009). To produce a biofuel from this by-product considered "waste" such as whey, is to give it a sustainable use by applying recycling to it and taking advantage of its potential, which in turn would generate additional income for the companies that apply it, and would also achieve a more effective reduction of its polluting impact, contributing to the achievement of improvements or modifications in the production process, so that it is more efficient and profitable, thus generating greater positive impacts on the environment. With the realization of this project, we seek to show in a practical way, on a pilot scale, that it is possible to reduce the various problems caused by not making good use of these by-products produced by dairy companies, since it is known that large quantities of whey are produced each year in our region due to the aforementioned fact that cheese is essential for families, and thus for every 100 liters from the production of approximately 10 kg of cheese, the equivalent of 2 L of gasoline in biogas could potentially be produced (Víquez Arias, 2012). The importance of generating a biofuel is to demonstrate the potential that bioethanol has in terms of social, environmental and technological dimensions; additionally, a confrontation is made with the limitations that occur when producing and using bioethanol.

If this is possible, we could show that this energy can replace conventional energy, which will also generate economic income, and in turn, it will create a stimulus and will be a sustainable investment alternative for companies, achieving an effective result to help the environment.

THEORETICAL FRAMEWORK

DAIRY WASTE

Whey is considered to be the fluid generated during the coagulation of milk and the separation of the curd in the production of different dairy products such as cheese, casein or similar products. Approximately 93 percent of whey is water and 7 percent are solids of different kinds (Klotz, 2014)

BIOCHEMICAL OXYGEN DEMAND (BOD)

The BOD of a liquid is defined as the amount of oxygen that microorganisms, especially bacteria (aerobic or facultative anaerobic: Pseudomonas, Escherichia, Aerobacter, Bacillius), fungi and plankton, consume during the degradation of the organic substances contained in the sample. It is expressed in mg/l. (Andreo, 2021)

CHEMICAL OXYGEN DEMAND (COD)

Chemical Oxygen Demand (COD) determines the amount of oxygen required to oxidize organic matter in a water sample, under specific conditions of oxidizing agent, temperature and time. Organic matter is calculated in terms of equivalent oxygen. (IDEAM)

PHYSICOCHEMICAL CHARACTERISTICS OF WHEY AND DIFFERENCE WITH MILK

Whey or whey has a high nutritional value, containing more than 50% of milk solids, including proteins, lactose, minerals and vitamins. Whey is mainly composed of lactose (75%), a relatively insoluble sugar with low sweetening power, which cannot always be absorbed by the human digestive system. The main proteins in whey are: beta-lactoglobulin (β-Lg) and alpha-lactalbumin (αLA), with a ratio of 3:1, while the immunoglobulins are serum albumin, peptone protease, lactoferrase and transferrin. Among the minerals in whey, potassium stands out, followed by calcium, phosphorus, sodium and magnesium. (Londoño et al, 2008).

TYPES OF DAIRY WASTE

There are several types of whey depending mainly on the elimination of casein. The first, called sweet, is based on coagulation by renin at pH 6.5. The second so-called acid results from the fermentation process or addition of organic acids or mineral acids to coagulate casein as in the production of fresh cheeses (Jelen, 2003)

USE OF WHEY

The level of use of this effluent is related to the feasibility of these products and the use of their components. According to Panesar et al., among the products that have been successfully accepted due to their low production costs, degree of nutritional quality and acceptable flavor, are soft drinks, fermented and alcoholic beverages, single-cell protein, biofilms, production of organic acids, protein concentrates, lactose derivatives, among others. From a process of reconstitution of whey through enzymatic hydrolysis of lactose, a hydrolyzed dairy drink with an orange flavor of acid pH was formulated with a simple production process, which was classified as an isotonic drink and with a wide acceptance by a sensory panel.

TYPES OF TREATMENTS FOR WHEY

Anaerobic digestion processes have been mainly applied to waste with a high organic load and wastewater, for example, effluents from wines, milk and their derivatives. This process has had advantages such as low sludge generation, reduced energy consumption and high methane production. The main disadvantage of anaerobic digestion is its slowness. (Sandoval et al., 2007).

Fermentation: In general terms, fermentation is described as an oxidation process in which the transformation of complex molecules into simple molecules leads to the generation of an organic final product with the release of energy; unlike common oxidation processes where oxygen or any oxidized inorganic compound acts as the final acceptor, the chemical energy in fermentation derives from a chemical phosphorylation process by which an electron transfer occurs that leads to the generation of an oxidized organic compound. (Nielsen, 2003).

Yeasts: Yeasts are non-filamentous unicellular fungi with a characteristic spherical or oval morphology widely distributed in nature, most yeasts form colonies of unicellular organisms that grow as the number of yeasts increases, this increase usually occurs by budding; in budding the parent cell forms a protuberance or bud on its external surface. Yeasts of the genus Kluyveromyces belong to the Ascomycotina division, this genus reproduces by multilateral budding, releasing spores when they reach maturity (its spores are spherical), it is one of the most abundant yeasts in dairy products, the species belonging to the Kluyveromyces genus produce β-galactosidases and are potent lactose fermenters. (Vargas Marín, 2017)

Influence of environmental factors on microbial growth: Bacterial growth is significantly influenced by the chemical nature or intrinsic factors (e.g. pH, water and nutrient activity) and physical nature or extrinsic factors (e.g. temperature, composition of the surrounding air or gas, or the presence of other bacteria) of its environment. Knowledge of these environmental influences allows us to control microbial growth and study the ecological distribution of microorganisms. (Vargas Marín, 2017)

Ethanol: Ethanol or ethyl alcohol is the oldest synthetic organic chemical used by man. It is presented as a colorless and flammable liquid with a boiling point of 78°C. Its chemical formula is CH_3-CH_2OH. It is the essential active component of alcoholic beverages. It is also one of the important raw materials for synthesis. It can be obtained through two manufacturing processes: fermentation or decomposition of the sugars contained in different fruits and distillation, which consists of purifying fermented beverages. (Vargas Marín, 2017)

BIOFUEL

Biofuel is understood to be fuels obtained from biomass. The term biomass, in the broad sense, refers to any type of organic matter that has had its immediate origin in the biological process of recently living organisms, such as plants, or their metabolic waste (manure); the concept of biomass includes products of both plant and animal origin. Today, this term has been accepted to refer to the group of energy products and renewable raw materials that originate from organic raw material formed by biological means. Therefore, fossil fuels or organic products derived from them are excluded from this concept, although they also had their biological origin in remote times. Today, different types of biomass can be differentiated.

Biofuels are biofuels such as alcohols, ethers, esters and other chemical products that come from organic compounds based on cellulose (biomass) extracted from wild or cultivated plants, which replace to a greater or lesser extent the use of gasoline in transportation or for producing electricity. (Salinas and Gasca, 2009)

TYPES OF BIOFUELS

There are two types of fuels

First generation. This refers to common biofuels obtained from sugar, starch or vegetable oil, and produced by fermentation, anaerobic digestion and transesterification.

Second generation. They are obtained from sustainable raw materials that can be converted into cellulose, such as woody vegetation.

Advantages of biofuels: So far, it seems that biofuels seem to be good substitutes for our conventional fuels that harm our environment due to the large quantities handled. Below we specify some of the advantages of biofuels:

- The cost of biofuels will be lower than that of gasoline or diesel once the technology with which they are generated is more widely available. In addition, the cost of raw materials will be practically zero since they are waste.
- The production processes are more efficient; they consume and pollute less.
- It is a source that is much less limited than fossil fuels, which take thousands of years to generate.
- Local employment is generated.
- Carbon and sulfur emissions are reduced.
- The amount of garbage decreases, since much of it can be used in the production of these biofuels.
- The level of safety is greater in terms of its handling and storage compared to fossil fuels.

Disadvantages of biofuels

Not everything was going to be advantages. Like everything, biofuels also have their bad side; their lesser-known side. In order not to give a biased view on the matter, here are some of the reasons why, for many, these sources of energy, despite being renewable, cannot be considered as "non-polluting":

Applications of biofuels

Biofuels are a useful source of energy in industrial or domestic human activities and can provide electricity and heat, but they mainly satisfy transportation needs. The use of these resources is increasingly frequent in large transportation industries due to a series of improvements that imply an economic and environmental level. For example, those involved in the design of racing cars take into consideration, biofuels such as methanol to improve the performance of the car.

Benefits of biofuels

Biofuels offer many benefits. By reducing the demand for oil, biofuels could make the supply of energy more secure. Their use would also reduce import costs to countries with energy deficits and would offer better trade and payment balances. All these developments would unfreeze the shortage of resources for other pressing needs.

Greenhouse gas emissions, carbon monoxide and particulate matter could be significantly reduced. And biofuels also improve vehicle performance; in fact, biodiesel's lubricity extends the life of diesel engines. (Arungu-Olende, 2007)

METHODOLOGY

According to the type of research, in this project an exploratory experimental research was carried out since we used previous secondary information and data present in different media on the subject to then analyze, reorganize and conclude with a type of general information, which helped us to carry out our project. The experimental design of our research work developed pure experiments since we must carry out different tests, analysis, trials to later compare them and analyze which one presents a better efficiency in the production of biofuel.

1. **First step:** The first stage consisted of collecting samples at the dairy company, taking a sample of approximately 1 litre, applying simple random sampling so that all possible samples have the same chance of being chosen from the total study population. The samples were characterised with physical, chemical and microbiological parameters, such as pH, turbidity, colour, COD, BOD and microorganisms present in the whey taken.

2. **Second step:** In this stage, Saccharomyces yeast was used since it has the capacity to generate ethanol during the fermentation process together with the lactose present in the whey. This process is carried out when this yeast is given conditions in a medium very rich in sugars and thiamine, it uses metabolic pathways that allow it to obtain a greater energy yield, being able to produce bioethanol. This process was carried out in a bio reactor where anaerobic digestion was produced for the fermentation of the whey and thus obtain bioethanol, water in greater quantities and other products.

3. **Third step:** The last stage of the research was to verify the efficiency of the biofuel, which was measured by its calorific value, represented as the amount of heat necessary to produce complete combustion of 1 m3 of biofuel under pressure and temperature conditions. To calculate the calorific value of a fuel, it will be enough to relate the amount of heat to the kilogram of the fuel being tested, as expressed in the following equation:

$$P = 4185{,}5\,\frac{Q}{m}\,\left(\frac{J}{Kg}\right) \quad (1)$$

where:
Q: Quantity of Heat
m: Mass

RESULTS AND DISCUSSIONS

The results and discussions of an article must be presented in a clear and organized manner, based on the data collected and the analyses performed during the study. Initially, the results must be presented in an objective and concise manner, using tables, graphs, and statistics, if appropriate, to highlight the main findings. Then, in the discussion section, the results are interpreted in light of the existing literature, highlighting similarities, differences, and implications for theory and practice.

In addition, limitations of the study and possible directions for future research are discussed. It is essential that both the results and the discussion are based on solid evidence and contribute significantly to the advancement of knowledge on the topic addressed.

STEP 1: CHARACTERIZE THE PHYSICAL (PH, TURBIDITY, COLOR), CHEMICAL (BOD, COD) AND MICROBIOLOGICAL (NMS) PARAMETERS OF WHEY, TO MEASURE THE CURRENT QUALITY CONDITIONS

- **Determination of Whey Samples:** To determine the whey samples to be used to obtain biofuel, the company's daily whey production data was used: ``*La Granja Del valle*`` and subsequently the number of samples was established by means of the following equation:

$$n = \frac{Z^2 * P * Q * N}{(N-1) * e^2 + Z^2 * P * Q} \tag{2}$$

In which:

n: Total number of samples = ?

N: Population size = 1350 liters of whey

Z: 95% confidence level = 1.96

P: Proportion of whey that favors the variable = 50%

Q: Proportion of whey that does not favor the variable = 50%

e: Precision or sample level = 50%

N = 3,83 ≅ 4 Liters of whey

The sample corresponds to 4 liters of whey per day, which will be collected during 3 different days of the same week in order to perform future physical, chemical and microbiological tests on the whey we are handling.

- **Recollection of the samples:** For the collection of samples, a specific monitoring was carried out, taking a volume of 2 liters, in previously sterilized plastic containers with caps and hermetically sealed to maintain the natural conditions of the waste product. Since whey is a product of cheese manufacturing, it always comes out of the production chain at high temperatures, so it was prudent to wait 1 hour for it to cool down to room temperature and then proceed to collect it.

- **Sample conditioning:** The whey collected on each sampling day was filtered by gravity with a non-woven polypropylene fabric as shown in Figure 1, in order to separate larger particles and possible foreign macroscopic agents unrelated to the cheese manufacturing process, trying not to separate the proteins from said whey, trying to maintain the purest possible conditions to proceed later to the analysis and start the fermentation of the same.

Figure 1. *Filtration of whey*

Source: authors.

PHYSICAL ANALYSIS OF WASTE LIQUID

- **pH Determination:** The pH was measured directly from the sample content with a pH meter, giving a result of 4.93, which is an acidic waste not optimal for discharge into surface water bodies or soils. Figure 2 shows the pH measurement setup and Table 1 presents its results.

Figure 2. *Determination of pH of the sample.*

Source: Authors

Sample number	pH of the sample
1	4,29
2	4,74
3	5,75
pH average	4,93

Table 1. *pH results of whey samples.*

Source: authors.

- **Turbidity Determination:** The turbidity of the whey collected at the La Granja del Valle Dairy Company was obtained using a digital turbidimeter, where a result of 9664.44 UNT was obtained, indicating that it is a waste with a large amount of organic load, which would generate a drastic deterioration in the water and soil ecosystems with the discharge of the volumes generated in the company and in the region. Figure 3 and Table 2 show the assembly carried out and the results obtained.

Figure 3. *Determination of sample turbidity*

Source: authors

Sample number	Turbidity Value (UNT)
1	10568,88
2	8610
3	9814,44
Turbidity Mean	9664.44

Table 2. *Actual Turbidity results of whey samples.*

Source: authors

- **Determination of lactose content:** The result of the determination of the lactose content in the sample is 6.8% BRIX or equivalent to 6.8 gr/dl, indicating the concentration of lactose present in the whey.

- **Determination of lactic acid concentration:** The determination of the lactic acid concentration of whey gives us a result of 20.4 mg/L, when converting by dilution the actual result of the lactic acid concentration of whey is 2040 mg/L. Whey is a suitable substrate for the production of lactic acid, but it is not suitable for fermentation in quantities greater than 3000 mg/L because it hinders the function of the yeast.

CHEMICAL ANALYSIS OF WASTE LIQUID

- **BOD determination:** Whey has a BOD of 50,453 mg/L, which means that it requires a high consumption of oxygen, generating contamination to the point of ending life in any ecosystem in which it is poorly managed and treated, since containing such high BOD values would cause asphyxiation in any living being that is in the presence of conditions of contamination by said effluent. According to resolution 0631 of 2015, the BOD for the production of food products is 400.00 mg/L O2 (Ministry of Environment and Sustainable Development). The BOD of whey is above these results with the regulations in force in Colombia.

- **Determination of COD:** A COD of 97,682 mg/L was obtained from whey, indicating that it can contaminate water sources and soil if discharged directly and without any treatment in accordance with resolution 0631/2015, because this COD value must be below 600 mg/L to avoid serious damage to ecosystems.

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- **Determination of fats and oils:** Average results of 10,642 mg/L were obtained, indicating high concentrations of G and A, which when discharged as whey into a water source or soil can generate contamination to any ecosystem, according to the Ministry of Environment and Sustainable Development, through resolution 0631 of 2015, which establishes that for oils and fats this must be below 20.00 mg/L. (Ministry of Environment and Sustainable Development)

- **Determination of Sedimentable Solids:** It was found that the whey contains approximately 0.3 ml/L of sedimentable solids, complying with the current legal regulations on discharge (0631/2015), with low concentrations and that for the required experiment, total suspended solids must have been determined, for better clarity of how much contamination we could cause due to the solids included in the whey.

- **Determination of Total Suspended Solids:** TSS concentrations of 1639 mg/L are presented and according to resolution 0631/2015 of the Ministry of Environment, they must be below 200 mg/L, which does not comply with this regulation. Table 3 shows the results of the chemical analysis obtained from the whey.

MICROBIOLOGICAL ANALYSIS OF WASTE WATER

- **Fecal Coliforms NMP/m:** The whey showed concentrations of fecal coliforms of >1100 NMP/ml, indicating health risks if the whey is discharged directly into water bodies or the sewage system, causing high levels of contamination and serious illnesses. (Resolution: 0631/2015. Min amb.2015). Table 4 shows a summary of the results of the microbiological analysis, the method used, and the date of its performance.

ANALYSIS	METHOD	DATE	RESULT
Fecal Coliforms NMP/mL	Most probable number	2022/10/05	>1100

Table 4. Results of whey NMP analysis.

Source: authors

STEP 2: PILOT-SCALE DEVELOPMENT FOR THE PRODUCTION OF BIOFUEL FROM WHEY WASTE FROM THE VALLEDUPAR DAIRY COMPANY

The fermentative agent in the present project was yeast: *Saccharomyces cerevisiae*, seeing that is the most easily available and cheapest commercial yeast, this poses a challenge in research. Yeasts: *kluyveromyces lactis* and *kluyveromyces marxianus* are ideal for accelerating the fermentation process of whey as a substrate, but since they are extremely expensive, we are forced to optimize the yeast as much as possible.: *Saccharomyces cerevisiae* and evaluate its performance, in order to obtain quality alcohol at low cost.

1. Fermentation Process: A first plate inoculation of the whey was carried out, where the yeast strains were supplied.: *kluyveromyces lactis* y *kluyveromyces marxianus*, carrying out as a first step a plate inoculation of the whey with dilutions of 10-1, 10-2, 10-3 and 10-4 in rose bengal agar for the selective isolation and enumeration of yeasts that could be present in the whey, in order to find a yeast that fulfilled the same functions as the yeasts: *kluyveromyces lactis* and *kluyveromyces marxianus* as lactose fermentation agents. This medium contains peptone, which acts as a source of carbon, nitrogen, minerals, vitamins and other nutrients essential for growth. Yeast growth was only observed when plate culture was done at a dilution of 10-2 and 10-3, so depletion culture was performed to obtain isolated yeast colonies for later evaluation of their performance as lactose fermentation agents and subsequent recognition of these.

ANALYSIS	METHOD	DATE	RESULT
Biochemical Oxygen Demand (BOD5) mg O2/L	SM 5210 B, Incubation 5 days	2022/10/06	50453
Chemical Oxygen Demand (COD) mg O2/L	Closed reflux	2022/10/07	97682
Fats and oils mg/L	INFRARED PARTITION METHOD C NTC 3362:2011 – Infrared	2022/10/07	10642
Sedimentable Solids mL/L	Imhoff cone	2022/10/05	0,3
Total Suspended Solids mg/L	SM 2540 D – Gravimetric	2022/10/06	1639

Table 3. *Chemical analysis of waste liquid.*

Source: authors

Figure 7. *Depletion seeding on rose bengal agar*

Source: authors

INOCULATION OF YEASTS PRESENT IN WHEY IN BROTH NUMBER 1

After growing yeasts by the depletion seeding method in the rose bengal agar culture medium, they were separated by assigning names to them depending on the color and macroscopic characteristics that could be observed, obtaining a total of three different yeasts which were assigned the names of "White", "Fuchsia" and "White Rose". These yeasts were seeded in a nutrient broth culture medium without inhibitors as shown in Figure 7, in order to grow the yeasts in mass found in the whey.

First, 10 ml of broth were taken for each seeding and a small amount of yeast was applied to each flask containing the nutrient broth with the help of a loop, taking care not to burn any microorganisms. This was repeated three times for each yeast found, giving a total of nine nutrient broth seedings. The broth seeding of each yeast is presented below.

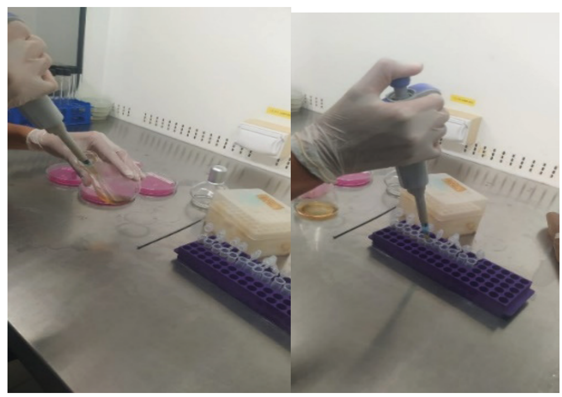

Figure 8. *Sowing in broth of each whey yeast*

Source: authors

SOWING OF YEASTS PRESENT IN WHEY IN BROTH NUMBER 2

As it can be seen in the previous procedure, a sowing of the yeasts present in the whey in nutrient broth was carried out for their growth in mass, but it was sown in very small quantities, so when this was noticed, a much larger sowing was carried out in order to be able to grow the yeasts in mass and subsequently carry out a fermentation with them and measure their performance.

This is how the same procedure is carried out as in the previous item, only this time a liter of nutrient broth will be prepared in order to make three 100 ml replicas each with each of the yeasts. This is how after the preparation of the liter of nutrient broth, it is divided into nine Erlenmeyer flasks, adding 100 ml to each one for a total of nine sowings of yeast. To three Erlenmeyer flasks the "white" yeast is added, to another three the "fuchsia" yeast and consequently to the last three the "white pink" yeast.

Two cultures of each of the yeast strains are left with agitation for 72 hours (Figure 8) and one of the cultures of each of the yeasts is left incubating at 35°C for the same time (Figure 9), this in order to recognize under which conditions there is greater yeast production in them and thus identify if the yeasts work better with or without agitation.

Figure 9. *Yeast culture in stirred nutrient broth*

Source: authors

Figure 10. *Yeast culture in nutrient broth during incubation*

Source: authors

SECOND PLATE PLATING OF WHEY

A surface inoculation is carried out by pouring the culture medium onto a Petri dish, then a little whey is placed directly on the surface with the help of a Drigalsky spatula, the inoculum (the whey) is spread until it is completely absorbed by the culture medium. This procedure was carried out with three selective media for the detection of yeasts, one of these media was the Bengal rose, this inhibits the growth of bacteria and limits the size and height of the fungi with accelerated growth, which allows the development and detection of other growing yeasts.

The second medium was the malt extract, which is suitable for yeasts due to its high concentration of maltose and other saccharides as an energy source. The acidic pH of the Malt Extract Agar is optimal for the growth of yeasts and molds, while it restricts the growth of other bacteria and the third medium is the Soy Tryptone, it is a source of carbohydrates that stimulate the growth of various microorganisms. The sowing in these three different culture media was done in order to find some yeast that was found within the whey that acted as effectively as: *kluyveromyces lactis* and *kluyveromyces marxianus* to ferment the lactose present in it.

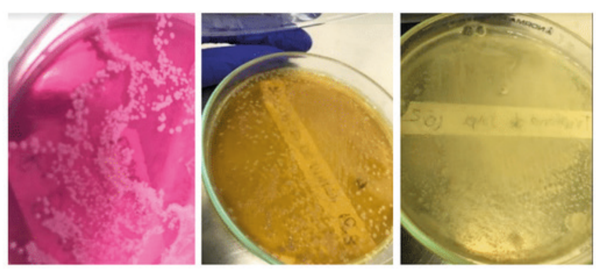

Figure 11. *Sowing on yeast plates on Rose Bengal Agar, malt extract and tryptone soy.*

Source: authors

INOCULATION OF YEASTS FOUND ON ROSE BENGAL AGAR, MALT EXTRACT AND SOY TRYPTONE IN NUTRIENT BROTH

After observing the microbial growth of yeast colonies on rose bengal agar, malt extract and tryptone soy, the development of only two yeast strains was identified in the three agars, so these two strains were sown in nutrient broth to achieve mass growth and subsequently use them as fermentation agents, in order to see the performance they have when consuming the lactose present in the whey. This is how the procedure of preparing 300 ml of nutrient broth is repeated to sow the yeast that we will call “yeast 1” in 100 ml of nutrient broth, sow the yeast that we will call “yeast 2” in 100 ml of nutrient broth and at this point we begin the tests with Saccharomyces cerevisiae which for the present study will be called “industrial yeast” so a small amount of industrial yeast was sown in 100 ml of nutrient broth to make them grow in mass.

This sowing was carried out with agitation for 72 hours in order to maximize the growth of these yeasts and obtain the maximum yield from them.

Figure 12. *Mass growth of industrial yeast in nutrient broth*

Source: authors

WASHING THE YEAST

The washing of the yeasts that were previously placed to grow in mass in nutrient broth is done in order to obtain the net biomass content obtained from each of the yeasts. This procedure begins by adding all the broth content together with the biomass in pendulum containers and taking them to the centrifuge at 4500 rpm for 3 minutes. Subsequently, all the nutrient broth that remains on the surface of the biomass is removed and distilled water is added to the edge of the pendulum container and the procedure is repeated, taking the containers again to 4500 rpm for 3 minutes, this procedure is repeated twice to finally remove the distilled water and leave only the biomass that was obtained from the mass growth.

The results of this procedure leave us with a very low microbial growth performance for yeast 1 and almost zero for yeast 2, so at this point the decision is made to start using only the yeast: *Saccharomyces cerevisiae* as a fermentative agent in the whey distillation process.

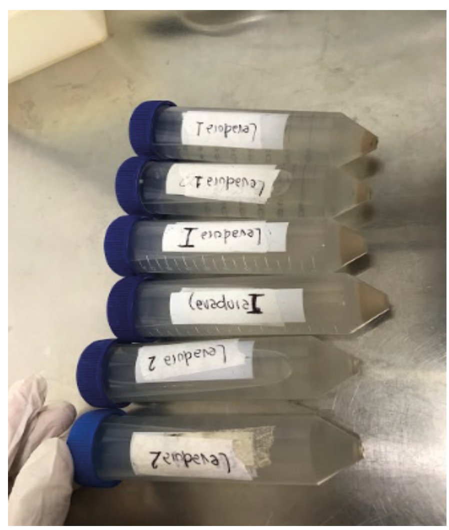

Figure 13. *Pendulum tubes with the yeast biomass after washing them*

Source: authors

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ALCOHOLIC FERMENTATION PROCESS

This process consisted of five fermentations which gave different results due to the variation of each of the independent variables in the process. What we were looking for was the best bioethanol yield in the process.

- **First alcoholic fermentation**

Therefore, three assemblies were made where the whey was fermented in completely sterilized containers with the yeasts identified as Rosa Blanca, Fuchsia and Blanca, and it was stored in a completely dark place. A set-up was carried out for each yeast in order to know its effectiveness for each one.

The anaerobic fermentation process in the absence of oxygen means that the final receptor of the electrons from NADH produced in glycolysis is not oxygen, but an organic compound that will be reduced in order to re-oxidize the NADH to NADH+ which would later result in ethanol, as shown in the following Figure (Fermentation).

Unfortunately, after 20 days of fermentation, we measured the alcohol content with an alcohol meter and it did not present any result in any of the three assemblies, which indicates that we obtained a totally null result in the three processes with each of the yeasts. Showing us that it was not the viable path and that we would need another yeast that meets the standards for carrying out a good fermentation process.

Figure 14. *First fermentation materials*
Source: authors.

- **Second alcoholic fermentation**

For the second fermentation we chose to take another route, which was to use yeast.: S*accharomyces cerevisiae* in the fermentation that due to its economy would be the most viable for this second experiment

Therefore, a fermentation was done with 500 ml of whey with 10 grams of yeast, but once activated, it was activated with a few ml of distilled water and a little sugar, the whey was completely raw and the second was with the same yeast but with deproteinized whey, this was obtained by sterilizing the whey at high temperatures and proceeding to pass it through a filter in order to separate particles by means of gravity filtration with a non-woven polypropylene fabric.

After 20 days, the whey was evaluated and did not present characteristics of a fermentation having occurred, it had the appropriate temperature, in the absence of oxygen, and in a place in the absence of light, forcing us to look for other alternatives and to improve the aspects that would help to carry out a fermentation in optimal conditions for notable results.

Figure 15. *Second fermentation*
Source: authors

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- **Third alcoholic fermentation**

Repeating the same procedure in the first and second fermentation but this time adding variables such as thiamine in a proportion of 60 mg, 50 ml of yeast extract as a carbon source, the yeast: *Saccharomyces cerevisiae*; all this in 1 L of raw sterilized whey. Yeast extract is a source of vitamin B and is composed of many valuable substances that help improve fermentation conditions as it helps yeast transform the sugar in the raw material into alcohol and carbon dioxide gas. Thiamine facilitates the start of fermentation and the implantation of the yeast, being very essential for the fermentation metabolism of the yeast.

In this third experiment, various factors were taken into account for the production of ethanol: an adequate temperature between 30 and 35 °C, with a pH of 4 to 5 for the best formation and work of the yeast, which with the help of thiamine was in good conditions for fermentation. Therefore, after 20 days, we took the respective measurements to measure the alcohol content, giving a result of 12 cc, indicating the presence of ethanol in the fermentation of a considerable value for the amount of whey; And if this happened it also tells us that energy was released for the yeast metabolism.

Figure 16. Third fermentation

Source: authors

- **Fourth alcoholic fermentation**

This way two experiments were carried out, one with yeast: *Saccharomyces cerevisiae* and thiamine and the other with *Saccharomyces cerevisiae* with thiamine and sugar, giving better alcohol levels than the one with sugar, although the one with thiamine was the same but in a smaller quantity. Thiamine is important for the growth, development and functioning of the yeast, seeing significant results with 18 cc, increasing the efficiency of the fermentation with glucose.

Figure 17. Fourth fermentation

Source: authors.

- **Fifth alcoholic fermentation**

For this fifth experiment, fermentation was done with yeast *Saccharomyces cerevisiae*, the enzyme lactase and thiamine in raw whey with the proteins, the other fermentation was with *Saccharomyces cerevisiae*, the enzyme lactase and thiamine, but here with the whey completely filtered, sterilized with the proteins.

Lactase is an enzyme of great biotechnological interest for nutritional and industrial reasons, since it is useful for the breakdown of lactose. The enzyme causes the hydrolysis of the β-1,4 bond of lactose, and breaks it down into its simple sugars, glucose and galactose, giving rise to a product with great sweetening power and with a great contribution to an alcoholic fermentation process.

Once the 20 days of fermentation had passed, it was clearly seen that the fermentation with *Saccharomyces cerevisiae*, enzyme lactase and thiamine and with the fully filtered whey, it presented the characteristics of having alcohol, a heterogeneous mixture was seen, where the alcohol with a lower density was found on the surface while that of the whey below this, due to the quantity of components that it presents is much denser than that of the produced alcohol, and that of the raw whey too but very little noticeable, in a smaller proportion; another characteristic was that it had a very distinguished smell like Varsol, in addition to having a transparent color.

Figure 18. *Fifth fermentation*

Source: authors

DISTILLATION PROCESS

For the distillation of the fermented whey in each of the aforementioned experiments, a 500 ml flask with a lateral release was used for the first four distillations and for the last one a 1 L flask with a lateral release was used. Likewise, in all of them a spiral condenser, a beaker or Erlenmeyer flask was used as a collection container and in the first four distillations a heating plate was used to heat the liquid to be distilled and in the last distillation a heating mantle was used.

It must be noted that the better the distillation setup, the better the yields will be and the fewer losses throughout the process. Table 5 below shows the bioethanol obtained in each distillation depending on the fermentation performed.

STEP 3: EVALUATE THE EFFICIENCY OF BIOFUEL FROM WASTE OBTAINED FROM THE VALLEDUPAR DAIRY COMPANY

The biofuel obtained in this experimental research project had particular characteristics as it had a strong Varsol smell, characteristic of hydrocarbons, and was quite greasy to the touch due to the raw material used for it. It had a low density and was highly flammable as it ignited instantly, making it difficult to handle under uncontrolled conditions.

- **Biofuel as a solution to environmental problems**

Bioethanol was obtained from cheese manufacturing waste, which can be found in industrial quantities anywhere in the world, so as it is a waste product it is not causing environmental damage as first generation biofuels do, which opt for deforestation in massive quantities of hectares, causing acceleration in global warming. In this case, the company Lácteos las granjas del valle produces around 1,350 liters of whey per day, so that the company alone may be producing approximately 492,750 liters of whey annually. If from one liter of whey we were able to obtain 33 ml of bioethanol, taking advantage of the entire production by the company (only speaking of Lácteos la granja del valle) we could obtain 4'295,635.7 gallons of bioethanol per year which would be used as an additive in engines that use gasoline, that is, any motor vehicle, obtaining profitable profits for the same company which would be taking advantage of its own waste and

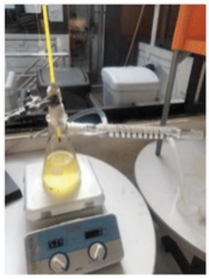
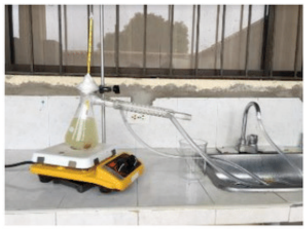
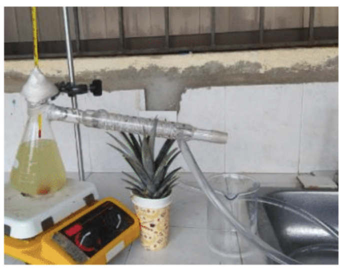
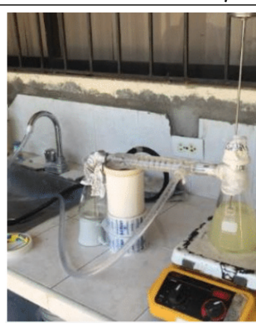
Fermentation	Mounting	Result
First Fermentation	 Figure 19. *First distillation assembly* Source: authors	The distillation process was carried out for about 2 hours and not a single drop of bioethanol could be obtained, so it is concluded that the use of yeasts that grow within the whey itself is not useful for the production of bioethanol using the lactose present in the whey as an energy source.
Second Fermentation	 Figure 20. *Second distillation assembly* Source: authors	In this case it was used only *saccharomyces cerevisiae,* as a fermentative agent both with deproteinized whey and raw whey and there were no promising results in this distillation, since after several hours in the process it was not possible to obtain bioethanol, this because *saccharomyces cerevisiae,* it is impossible for it to obtain energy from the lactose present in whey on its own.
Third Fermentation	 Figure 21. Third distillation assembly Source: authors	For this distillation, 12° of alcohol was present, which was measured by an alcoholmeter before distillation, but at the time of distillation no bioethanol was obtained, which could be due firstly to the fact that the quantity of alcohol was very low for the quantity of fermented whey and secondly the distillation setup was quite inefficient.
Fourth Fermentation	 Figure 22 . *Fourth distillation assembly* Source: authors	This corresponds to the distillation, here 19 ml of bioethanol were obtained with a characteristic smell of Varsol, a fuel with greasy characteristics which is due to the raw material used in the process, in turn showing that it was not totally pure but had a certain percentage of water.
Fifth Fermentation	 Figure 23 . *Fifth distillation assembly.* Source: authors	This corresponds to the distillation, here 33ml of bioethanol were obtained with a characteristic smell of Varsol, a fuel with greasy characteristics which is due to the raw material used in the process.

Table 5. *Distillation process*

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creating a product with greater added value on a low budget and high profitability, helping sustainable development and contributing to improving ecosystems.

- **Efficiency in bioethanol production**

Ethanol was obtained through various biological fermentation processes and then underwent a distillation process at temperatures of 70° C to 80° C. To evaluate whether the results were effective, it was necessary to test the distilled product. We added a small sample to a glass container and proceeded to light it to find out if it was ethanol, as seen in the following Figure:

Figure 24, Combustion of ethanol.

Source: authors

The figure above shows the color obtained by the distillation product, bioethanol; while it was burning, the smell of conventional hydrocarbons was perceived. Now, the volume of ethanol corresponds to that obtained after purification and the volume of whey used for fermentation, therefore, the yield was determined with the following equation:

% Yield= (Vol of ethanol) /(Vol of whey))*100

This way we will determine the yield of each of the fermentation processes, and the results are appreciated in figure 24 of this document

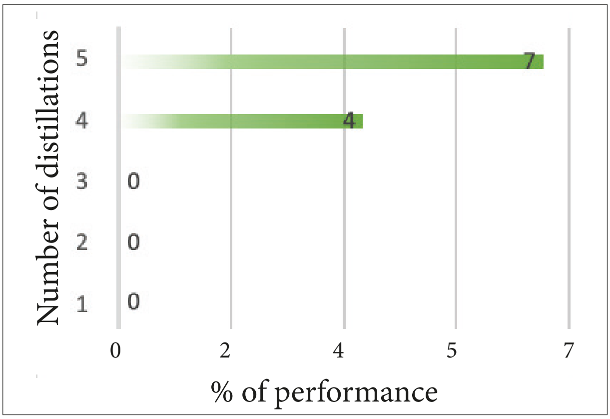

Figure 25. Calculation of the yield percentage for obtaining bioethanol.

Source: authors

From the figure above it can be observed that % yield was only obtained in distillation 4 and 5.

CONCLUSION

From this research project we can conclude that:

- In the characterization of the whey produced by the company Lácteos la granja del valle, it is known that the company generates approximately 1350 liters of whey per day and then with the realization of the physical, chemical and microbiological tests of the same we realize that we are facing acidic serums with high protein content, a considerable amount of lactose and microorganisms that seriously affect the ecosystem if said waste is dumped in an uncontrolled manner.

- In one liter of whey, 10 ml of activated Saccharomyces cerevisiae, 600 mg of thiamine and 1.5% lactase were added, which is an enzyme that helps the conversion of lactose into glucose and galactose, since yeast only manages to consume glucose to be able to produce energy. It is here where high yields of the yeast were obtained in the bioconversion, managing to produce 33 ml of high combustion bioethanol.

• The opportunity to produce bioethanol from whey, which is a waste product of dairy companies that produce cheese, turns out to be an innovative, effective, optimal and high-yield treatment that makes it possible to give added and useful value to a waste with low nutritional content.

• Whey, being a by-product of cheese manufacturing, contains large amounts of organic matter, so pouring it into the soil or surface water without prior treatment can cause great environmental damage, generating irreversible contamination. This is why the use of this whey to obtain a biofuel turned out to be an innovative treatment alternative, since it is observed that for each liter of whey fermented with "Saccaromyces" yeast, lactase enzyme so that this yeast better absorbs the sugar from the whey and thiamine, significant volumes of bioethanol can be obtained and this can be used to start up different combustion engines. And the recovered vinasse has properties suitable for the manufacture of fertilizers under specific conditions.

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REFERENCES

Aider, M., Halleux D., & Melnikova I, (2009). Skim acidic milk whey cryoconcentration and assessment of its functional properties: Impact of processing conditions. *Innovative Food Science and Emerging Technologies. 10,* 334-341.

Arumí, M. (2019). *Microbiología para Humanos.* Disponible en: https://microbiologiaparahumanos.wordpress.com/author/marcalarumi/

Arungu, s., & Olende. (2007). *Biocombustibles: Beficios y riesgo del tercer mundo*. Obtenido de scidev.net: https://www.scidev.net/america-latina/financiamiento/opinion/biocombustibles-beneficios-y-riesgos-del-tercer-m.html

Cargua, A & Eduardo, J. (2017). *DISEÑO DE UNPROCESO INDUSTRIAL PARALA OBTENCIÓN DE "BIOETANOL A PARTIR DE LACTOSUERO DESTINADO A LA ELABORACIÓN DE PERFUMES*. (Tesis pregrado, escuela superior politécnica de Chimborazo). http://dspace.espoch.edu.ec/bitstream/123456789/6996/1/96T00397.pdf

Castaño, E., Bernal, S. (2015). *Validación del método de ensayo de coliformes totales y fecales por la técnica de número más probable (NMP) en la calidad de queso fresco producido a pequeña escala.* Proyecto de grado presentado en la universidad libre seccional Pereira.

Castillo I., Delgado S., Hernadez E., Dominguez M., Baeza G., Cruz A., Carmona R., Morales C., Herrera F., Claveríe L., & Martínez M. (2012). APROVECHAMIENTO INTEGRAL DE LOS MATERIALES. *Revista iberoamericana de polímeros*.

Cota, C. (2011). *Producción continua de hidrógeno y metano en dos etapas a partir de la fermentación de suero de leche. (Tesis de posgrado,* Instituto Potosino de Investigación Científica y Tecnológica, A.C) https://repositorioslatinoamericanos.uchile.cl/handle/2250/2251693

De Jesus, E. Osorio, C. Sandoval, F. & Avalos, D. (2016). *Producción de bioetanol a partir de suero de queso proveniente de la región central del estado de Veracruz* . Revista sistemas experimentales. *3*(9), 42-50.

Fernández, C. Martínez, E. Morán, A. & Gómez, X. (2016.). *Procesos biológicos para el tratamiento de lacto suero con producción de biogás e hidrógeno.* Revista ION. *29*(1), 47-62.

Guerrero, W., Gomez, C., Gonzales, C., Castro, R. (2022) *LACTOSUERO Y SU PROBLEMÁTICA EN EL MEDIO AMBIENTE.* Congreso presentado en la universidad autónoma de estado de Hidalgo.

GONZALEZ, O., (2010). *ANALISIS SOBRE LA CONTAMINACÍON DE LAS AGUAS, PRODUCTO DE LAS EMPRESAS LACTEAS DEL MUNICIPIO DE UBATÉ. (Tesis de grado,* Especialización en Gerencia Ambiental) https://repository.unilibre.edu.co/bitstream/handle/10901/10729/PROYECTO%20DE%20GRADO%20%20OSCAR.pdf?sequence=1&isAllowed=y

Headon., Walsh. (2020) *Uso de enzimas para la fermentación alcohólica con lactosuero.* Proyecto de grado en la escuela Agrícola Panamericana, Zamorano Honduras.

Herrera B., Leyva S., Ortiz V., Cardenas J., & Garzon L. (2020). *Biocombustibles en colombia.* https://www.mincit.gov.co/mincomercioexterior/defensa-comercial/investigaciones-por-subvenciones/derechos-compensatorios-vigentes/alcohol-carburante-etanol.

Hidalgo. (2010). *Producción de Biocombustible apartir del lactosuero.* (Tesis de pregrado, Universidad de La Salle, Bogotá). https://ciencia.lasalle.edu.co/cgi/viewcontent.cgi?article=1065&context=ing_alimentos

IDEAM. (2007). *Demanda química de oxígeno por reflujo cerrado y volumetría.* Instituto de hidrología, meteorología y estudios ambientales en Colombia.

IDEAM. (2007). *SOLIDOS SUSPENDIDOS TOTALES EN AGUA SECADOS A 103-105 °C.* Instituto de hidrología, meteorología y estudios ambientales en Colombia.

Joaquín, A., Víquez A., (2012). *Conversión de suero Lácteo a Biogás.* Revista ECAG *12*(52), 13-19. http://users.df.uba.ar/carlosv/dov/biocombustibles/biogas-fisica+biologia/paper2.pdf

Lácteos La Granja del Valle. (2022). Antecedentes de la empresa Lácteos la Granja del Valle. Disponible en: https://www.facebook.com/photo/?fbid=565374085285217&set=a.565374038618555.

Lamothe-Abiet. (2022). *Tiamina.* Disponible en: https://lamothe-abiet.com/es/nutrientes/softan-power-5-2-3-3-3-2/

Lopez, G. (2022). *Agar Rosa de Bengala + Cloranfenicol.* Disponible en: https://instrumentalia.com.co/Instrumentalia-Informa/Enviar/agar-rosa-de-bengala-cloranfenicol.html.

LÓPEZ, V., (2010). *Composicion quimica de alimentos,* https://es.scribd.com/document/472335625/composicion-quimica-de-alimentos-parte-i-pdf

Marisa, A. (2021). *Demanda Biologica de Oxigeno (D.B.O).* https://webcache.googleusercontent.com/search?q=cache:LgXtag75iNsJ:https://www.mendoza.conicet.gov.ar/portal/enciclopedia/terminos/DBO.htm+&cd=10&hl=es-419&ct=clnk&gl=co

Ministerio de Ambiente, Vivienda y Desarrollo Territorial. (2007). *DEMANDA QUÌMICA DE OXÌGENO POR REFLUJO CERRADO Y VOLUMETRIA.* http://www.ideam.gov.co/documents/14691/38155/Demanda+Qu%C3%ADmica+de+Ox%C3%ADgeno..pdf/20030922-4f81-4e8f-841c-c124b9ab5adb

Parra, R., (2009). *LACTOSUERO: IMPORTANCIA EN LA INDUSTRIA DE ALIMENTOS.* http://www.scielo.org.co/pdf/rfnam/v62n1/a21v62n1.pdf

Pinto, A., (2017). Sector lechero en Colombia: Potencial desperdiciado. *Revista ANeIA.* https://agronegocios.uniandes.edu.co/2017/09/22/sector-lechero-en-colombia-potencial-desperdiciado/

Ricardo Adolfo Parra Huertas. (2009). LACTOSUERO: IMPORTANCIA EN LA INDUSTRIA DE ALIMENTOS. *Revista Facultad Nacional de Agronomía Medellín. 62*(1), 4967-4982.

Roldon, A. (2021). *La importancia del azúcar en la fermentación*. Blog. https://roldon.net/la-importancia-del-agua-la-sal-azucar-la-elaboracion-del-pan

Salinas, E., & Gasca, V., (2009). Los Biocombustibles. https://www.researchgate.net/publication/45087460_Los_biocombustibles

Vargas, X. . (2017). Evaluación de la producción de etanol a partir de lacto suero a nivel de biorreactor (bioflo 110) utilizando *Kluyveromyces marxianus* y *Kluyveromyces lactis* como agentes fermentativos. *(Tesis de pregrado, Colombia: Universidad de la Salle)* Evaluación de la producción de etanol a partir de lacto suero a n.pdf