

**EFFECT OF THE USE OF MULTIPLYING STRAINS WITH EFFICIENT MICROORGANISMS IN THE BIOREMEDIATION OF AQUACULTURE AND WASTEWATER FROM PRODUCTIVE FISH-FARMING LAKES**

**EFEITO DO USO DE CEPAS MULTIPLICADORAS COM MICRORGANISMOS EFICIENTES NA BIORREMEDIAÇÃO DE ÁGUAS DE CULTIVO E RESIDUAIS DE LAGOS PRODUTIVOS PISCÍCOLAS**

**EFFECTO DEL USO DE CEPAS MULTIPLICADORAS CON MICROORGANISMOS EFICIENTES EN LA BIORREMIACIÓN DE AGUAS DE CULTIVO Y RESIDUALES DE LOS LAGOS PRODUCTIVOS PISCÍCOLAS**

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**ABSTRACT**

The intensification of aquaculture activity has increased the generation of organic waste and toxic compounds, such as nitrogen and phosphorus, threatening the quality of surface water bodies and environmental sustainability. This study aimed to evaluate the effectiveness of multiplying strains of efficient microorganisms (EM) in the bioremediation of culture water and wastewater from fish-farming lakes. The methodology consisted of physicochemical and microbiological characterization of the waters and the application of three experimental EM doses (1/110, 1/1000, and 1/3000) in 250-liter units, compared with a control over a 45-day period. The evaluated parameters included COD, total solids, nitrogen compounds (ammonium, nitrites, nitrates), phosphates, sulfates, and microbiological counts (coliforms and heterotrophs). The results showed that although there was an increase in dissolved oxygen over time, there were no statistically significant differences between the EM treatments and the control for most variables, including the removal of coliforms and nitrogen compounds. The exception was sulfate levels, for which a significant reduction was observed in the EM treatments. It was also observed that the highest dose (1/110) was counterproductive, inducing a eutrophication process and macrophyte growth that increased the organic matter and phosphate load. It is concluded that, under the studied conditions, EM were effective specifically for sulfate removal, and lower doses are recommended for future studies to avoid eutrophication.

**Keywords:** Bioremediation. Aquaculture. Efficient Microorganisms (EM). Wastewater Treatment. Sulfates.

**RESUMO**

A intensificação da atividade aquícola tem aumentado a geração de resíduos orgânicos e de compostos tóxicos, como nitrogênio e fósforo, ameaçando a qualidade dos corpos d'água superficiais e a sustentabilidade ambiental. Esta pesquisa teve como objetivo avaliar a eficácia de cepas multiplicadoras de microrganismos eficientes (EM) na biorremediação de

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águas de cultivo e águas residuais provenientes de lagos piscícolas. A metodologia consistiu na caracterização físico-química e microbiológica das águas e na aplicação de três doses experimentais de EM (1/110, 1/1000 e 1/3000) em unidades de 250 litros, comparadas a um controle durante um período de 45 dias. Os parâmetros avaliados incluíram DQO, sólidos totais, compostos nitrogenados (amônio, nitritos, nitratos), fosfatos, sulfatos e contagens microbiológicas (coliformes e heterotróficos). Os resultados demonstraram que, embora tenha havido aumento do oxigênio dissolvido ao longo do tempo, não foram observadas diferenças estatisticamente significativas entre os tratamentos com EM e o controle para a maioria das variáveis, incluindo a remoção de coliformes e de compostos nitrogenados. A exceção foi o parâmetro sulfatos, para o qual se evidenciou redução significativa das concentrações nos tratamentos com EM. Observou-se ainda que a dose mais elevada (1/110) foi contraproducente, induzindo um processo de eutrofização e crescimento de macrófitas, o que elevou a carga de matéria orgânica e de fosfatos. Conclui-se que, nas condições estudadas, os EM foram eficazes especificamente na remoção de sulfatos, recomendando-se a investigação de doses menores para evitar a eutrofização.

**Palavras-chave:** Biorremediação. Aquicultura. Microrganismos Eficientes (EM). Tratamento de Águas Residuais. Sulfatos.

## RESUMEN

La intensificación de la actividad acuícola ha incrementado la generación de residuos orgánicos y compuestos tóxicos, como nitrógeno y fósforo, lo que amenaza la calidad de los cuerpos de agua superficiales y la sostenibilidad ambiental. Esta investigación tuvo como objetivo evaluar la eficacia de cepas multiplicadoras de microorganismos eficientes (EM) en la biorremediación de aguas de cultivo y residuales provenientes de lagos piscícolas. La metodología consistió en la caracterización físicoquímica y microbiológica de las aguas y la aplicación de tres dosis experimentales de EM (1/110, 1/1000 y 1/3000) en unidades de 250 litros, comparadas frente a un control durante un periodo de 45 días. Los parámetros evaluados incluyeron DQO, sólidos totales, compuestos nitrogenados (amonio, nitritos, nitratos), fosfatos, sulfatos y recuentos microbiológicos (coliformes y heterótrofos). Los resultados demostraron que, si bien hubo un aumento del oxígeno disuelto en el tiempo, no existieron diferencias estadísticas significativas entre los tratamientos con EM y el control para la mayoría de las variables, incluyendo la remoción de coliformes y compuestos nitrogenados. La excepción fue el parámetro de sulfatos, donde se evidenció una reducción significativa de las concentraciones en los tratamientos con EM. Se observó además que la dosis más alta (1/110) resultó contraproducente, induciendo un proceso de eutrofización y crecimiento de macrófitas que elevó la carga de materia orgánica y fosfatos. Se concluye que, bajo las condiciones estudiadas, los EM fueron efectivos específicamente para sulfatos, y se recomienda investigar dosis menores para evitar la eutrofización.

**Palabras clave:** Biorremediación. Acuicultura. Microorganismos Eficientes (EM). Tratamiento de Aguas Residuales. Sulfatos.



## 1 INTRODUCTION

Microorganisms have a nutritional versatility that can be used for the biodegradation of contaminants. This type of process is called bioremediation and consists of the ability of certain microorganisms to convert, modify and use toxic pollutants for the production of energy and biomass in the process (Abatenh et al, 2017). Instead of collecting the contaminant and storing it, bioremediation is a microbiological activity that is applied to break down or transform pollutants into elemental forms and less toxic or non-toxic compounds.

Bioremediators are biological agents used to clean up contaminated sites, including bacteria, archaea, and fungi as the main agents (Strong et al, 2008). The application of bioremediation as a biotechnological process involves microorganisms to resolve and eliminate the dangers of many pollutants through the biodegradation of the environment. Microorganisms act as an important tool for removing contaminants in soil, water, and sediments; due to their advantage over other remediation protocols, they restore the original natural environment and prevent further contamination (Demnerova et al, 2005).

In the case of wastewater treatment, microorganisms play an important role. The rationale for using these strains of EM microorganisms is that they contain various organic acids due to the presence of lactic acid bacteria, which secrete organic acids, enzymes, antioxidants, and metal chelates (Okuda and Higa). Creating an antioxidant environment by MS helps improve solid-liquid separation, which is the basis for water cleanliness (Mandalaywala et al, 2017). One of the main benefits of using MS is the reduction in the volume of sludge, as the beneficial organisms present in MS they break down organic matter by converting it into carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), or using it for growth and reproduction. Effective microorganisms significantly reduce the volumes of wastewater sludge produced by eliminating associated odors (Shalaby, 2011). On the other hand, wastewater treatment carried out with conventional methods is more expensive because the chemical products used are of greater value in the market, so the implementation of a more economical and environmentally friendly technology is of greater interest (Cardona and García, 2008). In the Center's Bioinputs Unit there are multiplier strains that are used for the production of bioinputs, these strains in addition to having efficient microorganisms (EM) are enriched with other organic waste compounds that are produced in the unit and have had good results.

## 1.1 PROBLEM STATEMENT

The quality and quantity of the water supply worldwide and in the country is being threatened by the impact of industrial and agricultural activities. The degradation of water resources was initially due to contamination by organic waste and salinization of irrigation systems, later aggravated by the presence of suspended solids, heavy metals, radioactive waste, nitrates, organic micropollutants, acidification of lakes and streams and eutrophication of lakes and coastal waters (Salazar, 2008).

Intensive aquaculture is one of the productive activities that is increasingly booming in food security, which generates greater risks of environmental impact, like most human activities. Gondwe et al. (2012) and Vezzulli et al. (2008) indicated that aquaculture is one of the main activities contributing to the increasing levels of organic waste and toxic compounds. When not properly treated, aquaculture wastes can cause emerging diseases due to antibiotic resistance and harmful algal blooms (Hegaret, 2008; Rubert, 2008).

Wastewater effluent from the aquaculture industry contains nitrogenous compounds (ammonia, nitrite and nitrate), phosphorus and dissolved organic carbon that can lead to environmental deterioration at high concentrations (Ali et al., 2005). Ammonia ( $\text{NH}_3$ ) is the product of fish respiration and the decomposition of excess organic matter. Chemoautotrophic bacteria (*Nitrosomonas* and *Nitrobacter*) act by oxidizing ammonium ions ( $\text{NH}_4$ ) to nitrites ( $\text{NO}_2$ ) and nitrate ions ( $\text{NO}_3$ ). In nature, these ions are absorbed by aquatic plants, algae and bacteria, as they are assimilated into biomass as a source of nitrogen. Nitrogenous compounds present in excessive amounts are responsible for generating eutrophication, which disrupts the balance of the aquatic ecosystem and could lead to a massive mortality of aquatic fauna (Lananan et al, 2014). The conservation of water resources allows optimizing production in terms of profitability and sustainability for the fish farming industry, since it must be delivered again in the same or better conditions than those found at the time of capture, complying with quality standards (Botero and Jimenez et al, 2006), in addition to promoting the environmental sustainability of this resource, which is one of the most affected. Wastewater treatment carried out with conventional methods is more expensive because the chemicals used are of higher value in the market, so the implementation of a more economical and environmentally friendly technology such as the use of efficient microorganisms for bioremediation is of greater interest (Cardona and García, 2008).



## 1.2 HYPOTHESIS

Environmental water decontamination with the effective use of EM (efficient microorganisms) in aquaculture effluents, avoiding eutrophication in surface water bodies.

## 2 OBJECTIVES

### 2.1 GENERAL OBJECTIVE

To evaluate the effect of multiplier strains with efficient microorganisms in the bioremediation of the culture and wastewater water of the productive lakes of the fish farming unit at the "La Granja" Agricultural Center.

### 2.2 SPECIFIC OBJECTIVES

- Perform a physical, chemical and microbiological characterization of the multiplier strains that contain efficient microorganisms, produced in the bioinputs unit of the "La Granja" Agricultural Center
- Perform a physical, chemical, microbiological and phytoplankton characterization of the culture and wastewater water of the productive lakes of the fish farming unit.
- To evaluate the effect of different doses of multiplier strains on the treatment of culture and wastewater from the productive lakes of the fish farming unit.
- Analyze the quality of the water of the productive lakes of the fish farming unit and the wastewater of these productive areas in its initial state and after the application of efficient microorganisms, according to parameters established in the current regulations.

## 3 THEORETICAL FRAMEWORK ORIGIN OF AQUACULTURE IN COLOMBIA

Aquaculture in Colombia began at the end of the 30s of the twentieth century with the introduction of rainbow trout for restocking in waters for public use in natural lagoons located in highlands of more than 2,500 meters above sea level in the Andean Region; later, common carp (*Cyprinus carpio*) and *Tilapia rendalli* were introduced and culture experiments began with the tucunaré (*Cichla ocellaris*) to control the reproduction of tilapia in the ponds. Also at that time, studies began with native species, especially with the bocachico of the Magdalena river basin *Prochilodus magdalenae* and in 1968 experimental cultivation with mangrove oysters (*Crassostrea rizophorae*) began. (AUNAP-2014)

Continental aquaculture is represented exclusively by fish farming, mainly by the production of red and silver tilapia, cachama, trout and some native species, the first two being the ones that show a greater dynamic in production and market share; the native species with the highest participation are the white and black cachamas, the bocachico, the yamú (*Brycon amazonicus*) and other species such as catfish (*Pseudoplatystoma* sp). (AUNAP-2014)

Aquaculture must be framed within sustainable development criteria, defined by the FAO as "The management and conservation of the natural resource base and the orientation of technological and institutional change, in such a way as to ensure the continuous satisfaction of human needs for present and future generations". This sustainable development (in the agricultural sectors, 9 National Plan for the Sustainable Development of Aquaculture in Colombia AUNAP - FAO forestry and fisheries) conserves land, water and genetic resources plant and animal, does not degrade the environment and is technically appropriate, economically viable and socially acceptable." This is classified as a sustainable and beneficial practice for food security in supplying and supplying food demand, such small, medium and large-scale production requires an artificial or natural aquatic environment, inputs and raw material that generates waste that alters the physical, chemical and microbiological parameters of the water, however these aspects reveal that aquaculture is not sustainable and requires more planning and waste management that they discharge into surface water bodies.

### 3.1 ENVIRONMENTAL IMPACT ON AQUACULTURE

The level of environmental pollution caused by aquaculture is directly related to the farming production system (extensive, semi-intensive and intensive). The more the system is intensified, the more inputs and raw materials are used. For example: Commercial food leaves residues, part of which is not consumed and degrades in the water column producing sediments. (Elizabeth et al 2018) Residues are also produced by the accumulation of fish excreta, resulting from the food consumed and metabolized (Eley et al., 1972). This degradation of waste alters various physical and chemical parameters of the water, as well as stimulates bacterial production due to the high content of organic matter, thus changing the chemical composition, structure and functions of the water-sediment interface (Tsutsumi and Kikuchi, 1983) (Brown et al., 1987). Aquaculture is an activity closely related to the anthropogenic effects that are caused by this economic activity, which must be analyzed and

reconstructed in order to reach a balance between the support elements (soil, water, air, flora and fauna) and the producer.

The generation of aquaculture waste and dumping has become a global public health concern (Martins et al., 2010).

In general terms, there are two types of aquaculture/fish farming waste: dissolved solids. The former are considered to have the greatest environmental impact and are mainly made up of uneaten food and undigested substances. The main dissolved components are nitrogen and phosphorus (Dauda et al., 2019).

The water column is mainly affected in the discharge of nutrients from cultivated animals and the production process, if the flow of these compounds in particular concentrations of phosphorus and nitrogen are discarded into the environment and exceed the assimilation capacity of the ecosystem, it can cause severe impacts on both the water column and the benthos. such as eutrophication, oxygen depletion, and alteration of local biodiversity (Buschmann and Fortt, 2005). Similarly, the use and quantity of chemical substances and antibiotics depends on the species and the intensity of the crop; although they are used in a very dilute form, they are dissolved in the water and are accumulated in the sediments, affecting the quality and nature of these two factors (Read et al., 2003).

In the same sense, (Milthon et al. 2011) determine that, for fish farming activity, one of the main management concerns is focused on effluents, which, being rich in nitrogen and phosphorus, impact the receiving water bodies with an increase in primary productivity that accelerates eutrophication processes. As in other economic activities, aquaculture produces waste that must require other environmental services to be assimilated or recycled; when there is no control, sustainability is ultimately compromised (Buschmann, 2001).

Aquaculture produces sediments that are the product of nutrients, soil particles and concentrate that are not consumed in their entirety, all this is degraded producing sediments, all this generates a degree of contamination that is directly proportional to the size of the crop. (Elizabeth et al-2018)

It is found that organic matter in fish farms is generated by the accumulation of elements such as: food, fish feces, metabolic waste, benthos, plankton, fungi, bacteria and organisms of animal and plant origin in different stages of decomposition such as: carbon, oxygen, nitrogen and phosphorus, (González, 2015). since at the bottom the soil of a lake is in a constant exchange of compounds between the two environments, water and sediments,

thus producing effects on the composition of the water of the natural environment. (Boyd, 1995).

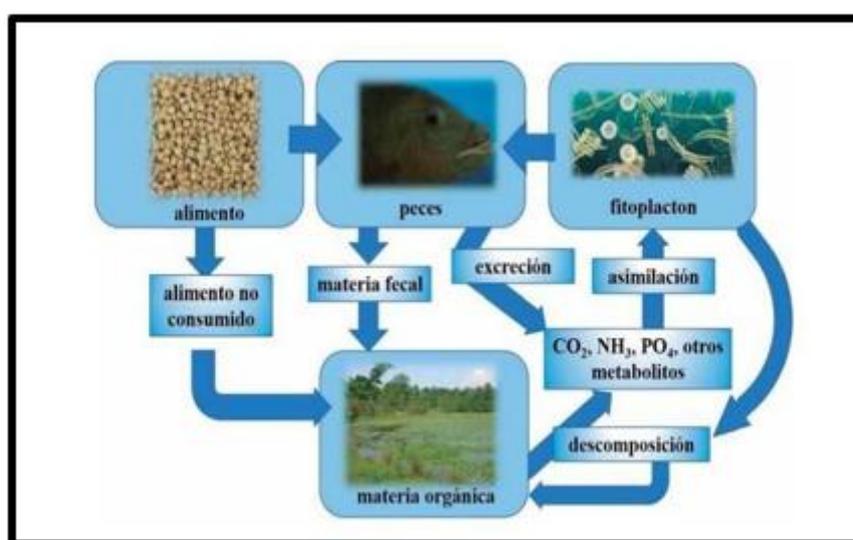
### 3.2 NEGATIVE ASPECTS OF (N)NITROGEN CONCENTRATION IN FISH EFFLUENTS

Nitrogen (N) is a contaminant present in wastewater that must be removed for multiple reasons:

Reduces dissolved oxygen in surface waters Is toxic to the aquatic ecosystem. It poses a risk to public health and together with (P). They are responsible for the overgrowth of photosynthetic organisms (eutrophication). (Aqualia-2019)

**Figure 1**

*Nitrogen cycle, organic matter that produces pollutants in aquaculture lakes. Taken from Aunap (2019)*



According to the above information, it can be mentioned that the culture of fish in confined environments biochemically alters the quality of the water in which they are produced; The most important alterations are related to the increase in organic matter produced mainly by excretions, by the feed supplied and by the contributions of the inputs added in the culture ponds; which generates variations in the concentration of oxygen (DO), increase in the concentration of suspended solids (TSS), increase in the biological oxygen demand (BOD), increase in the chemical oxygen demand (COD), increase in nitrogenous compounds (ammonium, nitrite and nitrate), increase in carbon dioxide and phosphate,

exaggerated growth of algae and eutrophication. Studies have shown that BOD<sub>5</sub> from effluents from aquaculture ponds normally has a range between 5 and 20 mg/l. (Corpoamazonia-2018)

Likewise, it is important to highlight that the contamination of surface waters by nutrients can induce adverse effects on human health, especially the ingestion of nitrites and algal toxins, because they can cause diarrhea, vomiting, gastroenteritis, among others (Camargo & Alonso, 2006). In addition, the accumulation of phytoplankton, the death and decomposition of plant material in the receiving sources, can generate unpleasant odor and taste, reducing its use as a recreational and consumption resource (Figueruelo & Marino, 2004).

### 3.3 BIOREMEDIATION PRACTICES

It consists mainly of the use of different organisms such as (plants, yeasts, fungi, bacteria, etc.) as a medium to neutralize toxic substances, either transforming them into less toxic substances or making them harmless to the environment and human health. One of the most widely used biocorrective measures in the use of microorganisms for the decontamination of natural resources, this decontamination system is based on the absorption of organic substances by these microorganisms, which use them as a source of carbon necessary for their growth and energy for their metabolic functions. (Duilio Torres Rodriguez-2003).

#### 3.3.1 Bioremediation in aquaculture

Wastewater management in aquaculture ecosystems is essential to maintain the good health status of animals, as well as counteract negative impacts on the environment. Bioremediation, which is nothing more than the application of microorganisms (BACTERIA) to remove hazardous waste, is a very helpful tool for the on-site treatment of contaminated wastewater and sediments. For the bioremediation of nitrogenous compounds, bacteria have to perform the function of nitrification and denitrification (Chávez-Crooker & Obrequé-Contreras, 2010). this biological process is carried out by ammonium oxidizing bacteria (BOA) and nitrate oxidizing bacteria (BON). Although a wide range of bacterial species are capable of to perform nitrification or denitrification, not all species are applicable for bioremediation products. The strains *Paracoccus* sp. and *Thiobacillus* sp. have recently gained great interest

due to their degrading abilities. On the other hand, Bacillos sp can also carry out several functions in water cleaning (Nakano et al., 1998)

### 3.4 DENITRIFYING BACTERIA

#### 3.4.1 Nitrification

It is an aerated biological process where ammonia is oxidized until its conversion to nitrate, it is divided into two stages, the first is defined by the oxidation of  $\text{NH}_4^+$  to nitrite ( $\text{NO}_2^-$ ) and the second stage defined by the oxidation of (nitrites)  $\text{NO}_2^-$  to (nitrates)  $\text{NO}_3^-$ .

#### 3.4.2 Denitrification

It is a biological process that takes place in the absence of oxygen in which nitrate is transformed into gaseous nitrogen that is incorporated into the atmosphere.

**Figure 2**

*Nitrogen denitrifying bacteria cycle*



Note. nitrification and denitrification process. Taken from (Saubot, 2002)

The process of decomposition of organic matter, leads to greater oxygen consumption and the generation of  $\text{CO}_2$  and anoxic environments (Magni et al 2008).

#### Origin of Efficient Microorganisms in the Use of Bioremediation

The term "effective microorganisms" (EM) is used to denote specific mixed cultures of known beneficial microorganisms, which are effectively employed as microbial inoculants

(Higa and Parr, 1994). E.M is a technology developed by Dr. Teruo Higa in the eighties in Okinagua, Japan and has been used in different fields such as agriculture, industry, animal, environmental remediation, among others, and is currently widely distributed (Sangkara, 2002).

### 3.5 MAIN EFFICIENT MICROORGANISMS (EM)

**Figure 3**

*Efficient microorganisms*



Note. view of efficient microorganisms. Taken from Fundases laboratory

#### 3.5.1 Photosynthetic bacteria (*Rhodospseudomonas* spp)

They use natural light and the heat of the sun as a source of energy, organic matter and harmful gases, decompose offensive gases such as ammonia and hydrogen sulfide into gases with non-offensive odors (Fernando et al, 2018).

Regarding Colombia, Roldán et al., (2007) found that after the application of EM, both in domestic and synthetic wastewater, significant decreases in the content of total coliforms were evidenced.

#### 3.5.2 Photosynthetic bacteria in agriculture

Phototropic bacteria are a group of independent, self-sustaining microbes. These bacteria synthesize useful substances from root secretions, organic matter, and/or harmful gases (e.g., hydrogen sulfide) with the use of sunlight and soil heat as energy sources. These useful substances include amino acids, nucleic acids, bioactive substances, and sugars, all of which promote plant growth and development. The metabolites made by these microorganisms are absorbed directly by plants and act as a substrate for the population

increase of beneficial microorganisms. For example, in the rhizosphere, vesicular and arbuscular mycorrhizae (VA) are increased thanks to the availability of nitrogenous compounds (amino acids) that are secreted by phototropic bacteria. VA mycorrhizae in response increase the solubility of phosphates in the soil and therefore provide phosphorus that was not available to plants. VA mycorrhizae can also coexist with azobacter and rhizobiums, increasing the ability of plants to fix nitrogen from the atmosphere.

### **3.5.3 Lactic acid bacteria (Lactobacillus Spp)**

Lactic acid bacteria (lactobacillus) were found in yogurt, suppress pathogenic microorganisms, are a strong sterilizer, attack pathogens, accelerate the decomposition of organic matter, prevent putrefaction and bad odors (Fernando et al 2018).

### **3.5.4 Lactic acid bacteria in agriculture**

Lactic acid bacteria produce lactic acid from sugars and other carbohydrates, produced by phototropic bacteria and yeast. For this reason, some foods and drinks such as yogurt and pickles have been made with lactic acid bacteria since ancient times. However, lactic acid is a strong sterilizing compound that suppresses harmful microorganisms and aids in the breakdown of materials such as lignin and cellulose by fermenting them, removing undesirable effects from unbroken organic matter. Lactic acid bacteria have the ability to suppress diseases including microorganisms such as fusarium, which appear in continuous culture programs. Under normal circumstances, species such as fusarium weaken plants, exposing them to diseases and large populations of pests such as nematodes. The use of lactic acid bacteria reduces nematode populations and controls the spread and dispersal of fusarium, thereby inducing a better environment for crop growth.

### **3.5.6 Yeasts (Saccharomycetes spp.)**

They synthesize or ferment the substances secreted by PHOTOTROPHIC bacteria, they produce proteins and sugars that are taken up by LACTIC BACTERIA. (Fernando et al 2018).

### **3.5.7 Yeasts in agriculture**

Yeasts synthesize antimicrobial and other useful substances required by plants for their growth from amino acids and sugars secreted by phototropic bacteria, organic matter

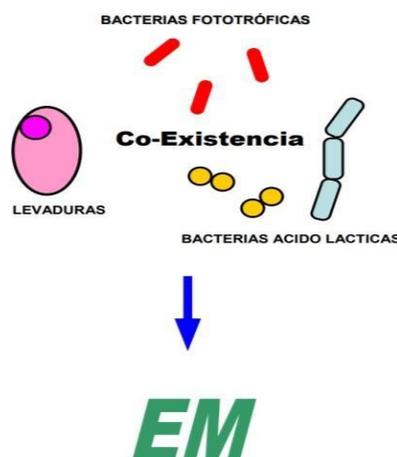
and plant roots. Bioactive substances such as hormones and enzymes produced by yeast promote active cell and radical division. These secretions are also useful substrates for MS such as lactic acid and actinomycetes bacteria. The different species of effective microorganisms (Phototropic bacteria, lactic acid and yeast) have their respective functions. However, phototropic bacteria can be thought of as the core of MS activity. Phototropic bacteria reinforce the activities of other microorganisms. This phenomenon is

called "coexistence and co-prosperity". The increase in MS populations in soils promotes the development of beneficial microorganisms existing in the soil. Since the microflora of the soil becomes abundant, and therefore the soil develops a well-balanced microbial system. In this process, specific microbes (especially harmful ones) are suppressed, in turn reducing disease-causing soil microbial species. In contrast, in these developed soils, EM maintains a symbiotic process with plant roots next to the rhizosphere. Plant roots also secrete substances such as carbohydrates, amino acids, organic acids, and active enzymes. The MS uses these secretions for growth. In the course of this process, MS also secretes and delivers amino acids, nucleic acids, a wide variety of vitamins and hormones to plants. This means that the EM in the rhizosphere coexists with plants. Therefore, in soils dominated by EM, plants grow exceptionally well.

### 3.6 BENEFITS OF USING EFFICIENT (ME) MICROORGANISMS

**Figure 4**

*Relationship between beneficial bacteria*



Note. Taken from (Tencio, 2011)



Efficient Microorganisms are almost all facultative in terms of their respiration. (which grows in both the presence and absence of oxygen).

They are saprophytic, feed on inert organic matter and are not adapted to live in the pantries of living beings; It is not harmful to the health of plants, animals and man.

The species contained in MS were selected for fulfilling one or more of the following functions

Solubilize nutrients (N, O, P, K, MG, CA, FE, ZN, CU, CO, SI, S, C) contained in the clays, in the parent material distributed in the soil profile.

Agricultural use.

Microbial inoculant, they restore the microbiological balance of the soil, improving its physical conditions. Chemicals, increase crop production and protection.

Animal production use.

It reduces bad odors (ammonia) and populations of pest insects, as a result of the fermentation process of excreta in situ.

To collect excreta and urine, reducing the frequency of water use.

Reduces the requirement and utilization of disinfectants and production and maintenance costs.

Organic solid waste management.

Reduces bad odors from manure and urine Eliminates pathogenic microorganisms and weed seeds

It prevents the decomposition of organic matter by oxidation in which gases generated by bad odors (sulfurous, ammoniacal and mercaptans) are released

It prevents the proliferation of insect vectors, such as flies, it does not find a suitable environment for its development

It speeds up the composting process to a third of the time of a conventional process.

Wastewater management.

Transforms and synthesizes organic matter and COD (non-biodegradable organic matter contained in water)

Increases oxygen values

Reduces sludge production in conventional treatment systems Reincorporates wastewater as irrigation water

Effectively removes more than 99% of total and fecal coliforms. (Fernando et al 2018).

Parameters and optimal values for life resolution 631 of 2015

**Table 1**

*Parameters and optimal values for life*

Parameter	Units	Maximum Limit Values Permissible
Temperature (T°)	°C	40 °C
Dissolved Oxygen (DO)	mg/l	Analysis and reporting
Ph	Units of Ph	6.00 to 9.00
Chemical oxygen demand (BOD)	mg/l O <sub>2</sub>	150,00
Biochemical Oxygen Demand (BOD <sub>5</sub> )	mg/l O <sub>2</sub>	50,00
Total Solids (TS)	mg/l	Analysis and reporting
Sulfates (So <sub>4</sub> )	mg/l	250,0
Sulfides (S <sub>2</sub> )	mg/l	1,00
Ammonium (Nh <sub>4</sub> )	mg/l	Analysis and reporting

**Table 2**

*Parameters and Optimal Values for Life Resolution 2115 of 2007*

Parameter	Expressed as	Maximum Acceptable Value (mg/l)
Nitrites	No <sub>2</sub>	0,1
Nitrates	No <sub>3</sub>	10
Phosphates	PO <sub>4</sub>	0,5
Total Coliforms	CT	Analysis and reporting



Eschericia Coli	E. Coli	Analysis and reporting
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#### 4 STUDY AREA

The research project was carried out in the bio-inputs and fish farming unit of the "La Granja" Agricultural Center of the National Learning Service (SENA), Espinal Tolima.

#### 5 METHODOLOGY

To evaluate the effect of different doses of the multiplier strains with effective microorganisms, on the quality of the waters of the productive lakes of the fish farming unit and the wastewater of the same (productive area – Lakes) of the Agricultural Center "La Granja", the analysis of some physicochemical and microbiological parameters, the process of characterization of the water of the lakes, of wastewater and multiplying strains, tend to identify in the first instance the initial microbial and physicochemical loads to establish monitoring indicators according to concentrations, protocols and techniques of parameter analysis, standardization of doses of microorganisms, in general establish removal capacity in adequate ranges.

Physicochemical and microbiological characterization of the water of fish farming lakes. The physicochemical parameters to be measured were: COD, Total Dissolved Solids (TDS), NO<sub>3</sub>, NO<sub>2</sub>, NH<sub>4</sub>, PO<sub>4</sub>, SO<sub>4</sub>, Dissolved Oxygen and pH. Likewise, the microbiological characterization of the samples was carried out to establish the microbial concentration of both fecal contamination indicators (total and fecal coliforms), total heterotrophs and microorganisms present in MS on specific culture media.

##### Evaluation of different doses of multiplier strains in wastewater treatment

Three doses were used to evaluate the effect of the doses of the multiplier strains in wastewater treatment: 1/110, 1/1000, 1/3000 (vEM/vAR). As an experimental unit, cans with a capacity of 250L were used. For both treatments and control (without MS doses), three replications were performed for a total of 12 experimental units. On the other hand, the samplings were carried out on the first day, at 15, 30 and 45 days after the application of the doses and finally the results were analyzed taking into account the current regulations.

**Figure 5**

*Assembly of experimental units*



## 5.1 STATISTICAL ANALYSIS

Descriptive statistics of the data were performed, then the analysis of Shapiro-Wilks ( $p < 0.05$ ) and Q-Q graphs to observe the distribution of the data. It was determined whether the data present a normal distribution to establish whether parametric or non-parametric statistics are used, because they did not present a normal distribution, non-parametric tests

were used for all analyses. To establish which treatments there were statistically significant differences, the LSD Fisher comparison test was performed. Finally, a principal component analysis was performed to observe the relationship of the variables studied. The InfoSTAT package was used for statistical analysis.

## 6 RESULTS AND DISCUSSION

### 6.1 INITIAL CHARACTERIZATION OF WASTEWATER

**Table 3**

*Initial microbiological characterization of wastewater*

Microorganisms	Count (CFU/ml)
Lactobacilli	0
Phototrophic	0
Yeasts	3 x 10 <sup>4</sup>
Heterotrophs	1.2 x 10 <sup>5</sup>
Coliforms	Countless

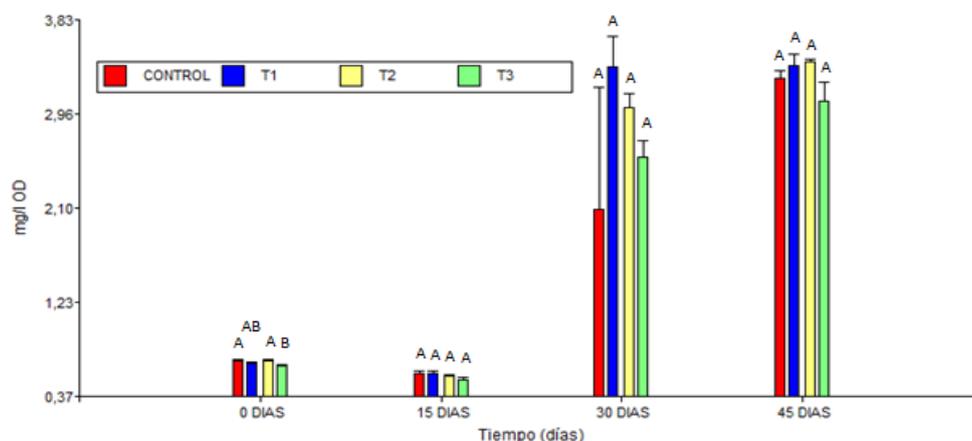
**Table 4**

*Initial physicochemical characterization of wastewater*

Physicochemical parameters	Concentration
<b>COD</b>	
<b>Total solids</b>	173
<b>Ammonia</b>	0,38
<b>Sulfate</b>	27,6
<b>Phosphate</b>	1,43
<b>Nitrate</b>	0,63
<b>Nitrite</b>	0,0028

**Figure 6**

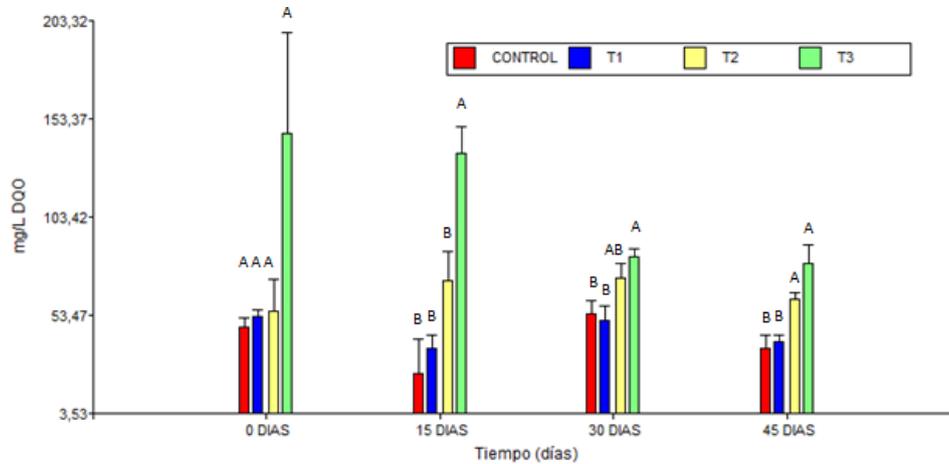
*Dissolved oxygen concentration in treatments*



Dissolved oxygen concentrations increased over time, with significant differences at 30 and 45 days of study; however, no statistically significant differences were observed between control and treatments. Dissolved oxygen concentrations in the first days were between 0.58 and 0.70 mg/L, which are low values considering that a minimum of 5 - 6 mg/L O<sub>2</sub> is required to maintain aquatic biodiversity (APHA, 2005). For the COD parameter, no significant differences were observed, except with treatment 3, whose results in COD concentration were higher compared to the other treatments and control, due to the eutrophication process that occurred in this treatment. The results obtained coincide with what has been reported, since as the easily degraded compounds are exhausted and the microorganisms begin to consume other more complex substances, there comes a certain point at which the microorganisms do not have the enzymes or nutrients required to continue with the process that allows the reduction of COD, this, added to the lack of oxygen, limits degradation, as observed in the similar value where there is no significant change for COD due to the presence of compounds whose complex chemical structure does not allow their easy degradation (Cardona & García, 2008).

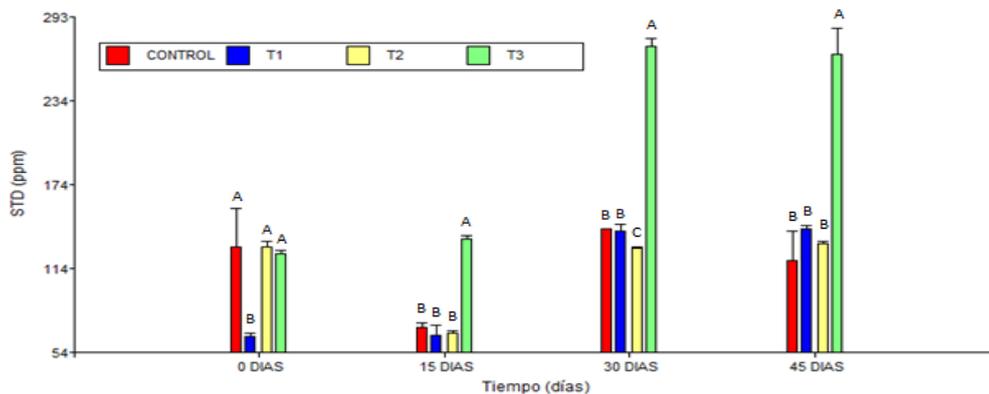
**Figure 7**

*Chemical oxygen demand in the treatments analyzed*



**Figure 8**

*Total solids dissolved in treatments*



In general, there were no significant differences between the control and the treatments for the dissolved total solids variable. For this reason, it is stated that there were no significant differences in STD concentrations after the application of MS in the wastewater. The concentration of STDs increased over time in treatment 3, due to the presence of aquatic plants, which is consistent with the increase in ammonium and phosphate concentrations.

## 6.2 PH

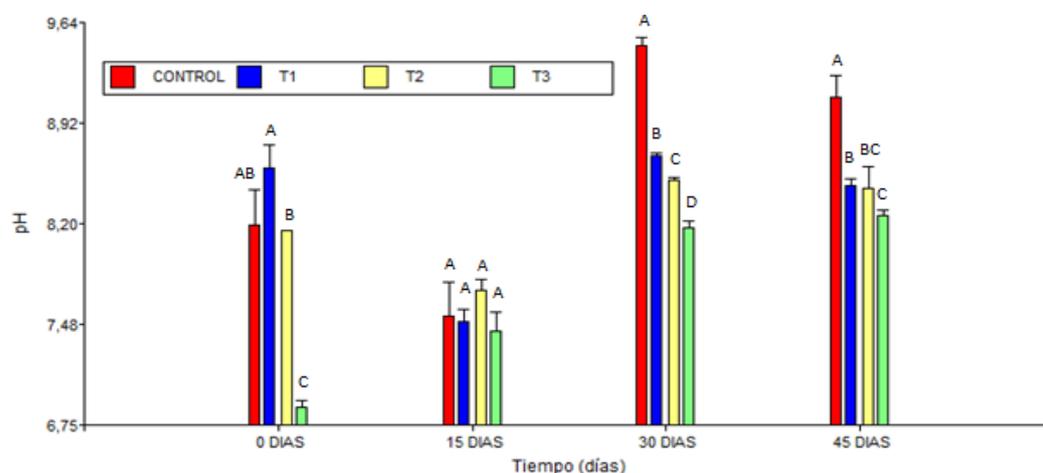
In the initial phase, the pH tended to alkalinity; however, for treatment 3 it was more neutral (6,88). Fifteen days after the application of the treatments, there were no significant differences between them, but there was a decrease to neutrality with respect to the initial day. For control, alkalinity increased considerably by day 30 (9.47) and 45 days (9.10).

The trend towards alkalinity does not coincide with the findings of Fioravanti (2005) who evaluated efficient microorganisms as stabilizers of wastewater, presenting a decrease from 6.3 to 4.5, despite the fact that in the present study such a drastic decrease in pH was not observed, comparing the treatments with the control if there was a significant decrease in the last days of treatment.

In the study carried out by Roldán et al. (2007), the pH values did not present statistically significant differences, likewise, Gómez and Galindo (2008) did not observe statistically significant differences in this variable.

**Figure 9**

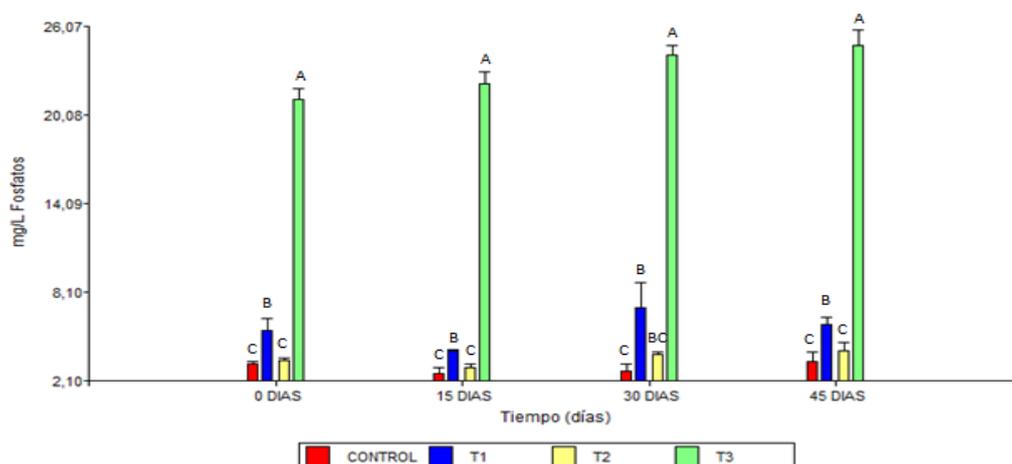
*Hydrogen potential in treatments*



### 6.3 PHOSPHATES

**Figure 10**

*Phosphate concentration in treatments*



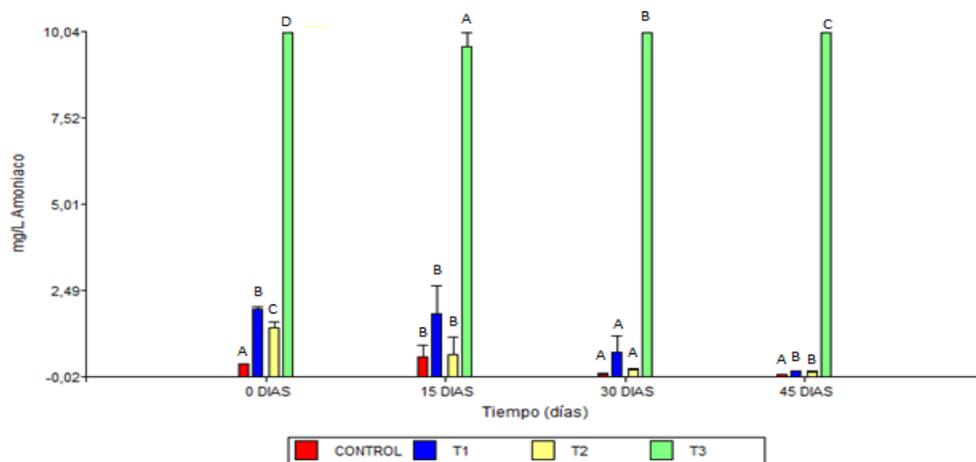
There were significant differences with treatment 1 and 3 compared to treatment 2 and control and a slight increase in concentration over time. Phosphate is one of the main compounds that contributes to the pollution of water bodies.

According to the classification of Metcalf and Eddy (2003), wastewater with a high concentration of organic phosphorus is considered to be that with more than 5mg/L, so the concentration presented in the treatments is high, especially in treatment 3, possibly this was due to the fact that in the experimental units with this treatment there was the growth of aquatic plants and this promoted the eutrophication process. In this variable, control was better than treatments.

#### 6.4 NITROGENOUS COMPOUNDS

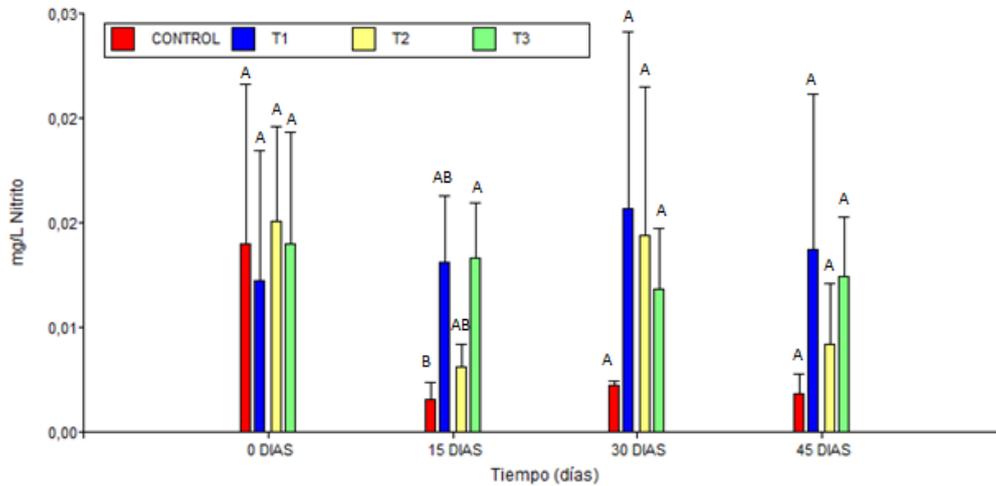
**Figure 11**

*Ammonia concentration in treatments*



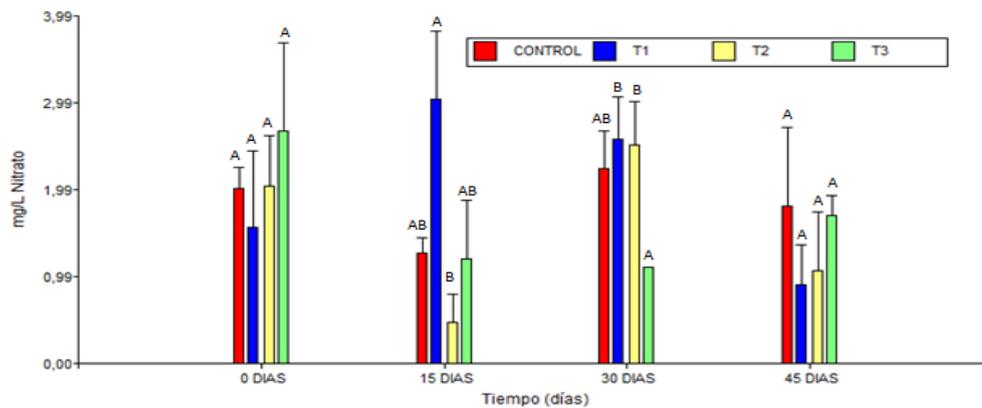
**Figure 12**

*Nitrite concentration in treatments*



**Figure 13**

*Nitrate concentration in treatments*



Nitrogenous compounds formed by reduction reactions such as ammonium showed a tendency to decrease over time, with the exception of treatment 3 which presented high concentrations due to the growth of aquatic plants.

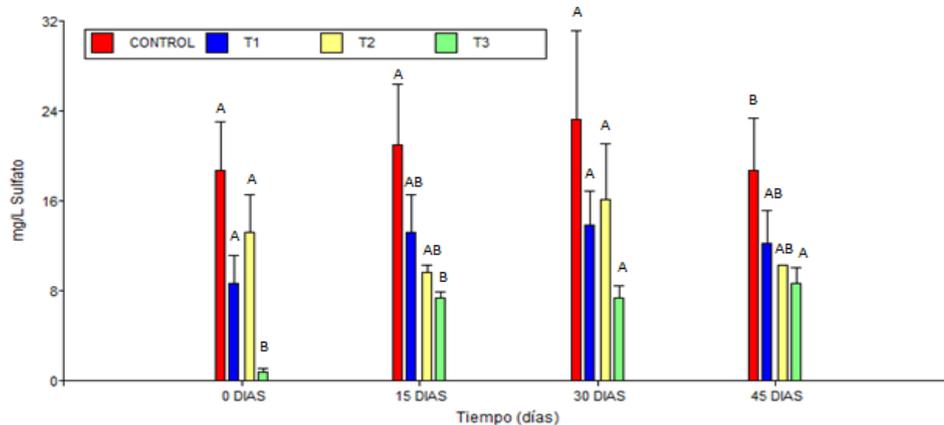
The nitrite and nitrate compounds had no statistically significant differences between treatments and control, nor over time.

Cardona and García (2008) and Romero and Vargas (2017) in their research observe results similar to those obtained in this work, the non-regular behavior of nitrogenous compounds. The authors mention the situations regarding the variability of the  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  ions with the use of EM on wastewater, in close relation to the concentration of oxygen, which was low in the first days and which in a certain way is responsible for the nitrification and denitrification processes that occur in wastewater.

## 6.5 SULFATES

**Figure 14**

*Concentration of sulfates in treatments*



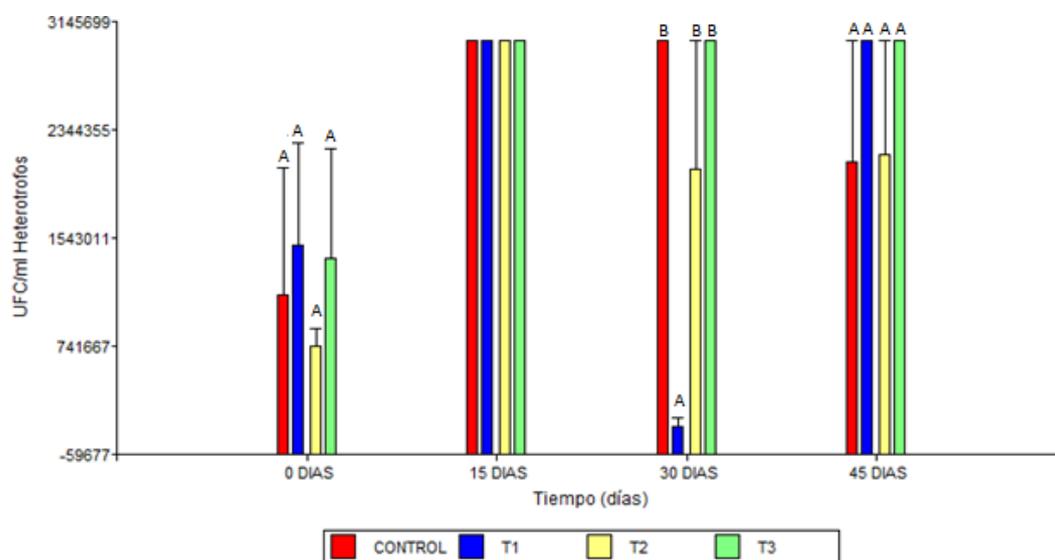
Sulphate was the only compound that presented significant differences between the control and the treatments, with lower sulfate concentrations in the treatments. It has been

It has been reported that phototrophic bacteria that are present in efficient microorganisms remove sulfur compounds, which was evidenced in the present study (García, 2006). Microbiological parameters

No significant differences were observed between treatments and controls in relation to total heterotroph and total coliform counts in sampling events between control and treatments. Therefore, after the application of MS to the wastewater, no significant treatment effect was observed. The behavior of heterotrophs was potentially related to the other microbial groups evaluated in the study.

**Figure 15**

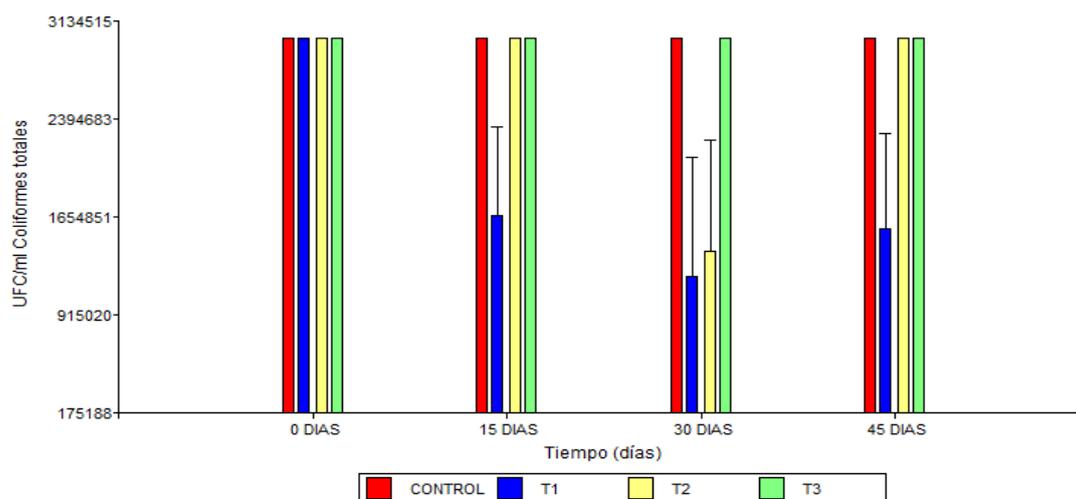
*Heterotroph colony-forming units in treatments*



For heterotrophs, there were no statistically significant differences between control and treatments, except at 30 days for treatment 1 where there was a significant decrease in heterotrophs; however, over time there was an increase in this microbial population over time.

**Figure 16**

*Total coliform colony-forming units in treatments*



The concentration of total coliforms did not present statistically significant differences between the control and the treatments or over time, however, there was a decrease in the concentration of these microorganisms with treatment 1. Although no statistically significant differences were observed over time, the decrease in colony-forming units in the culture

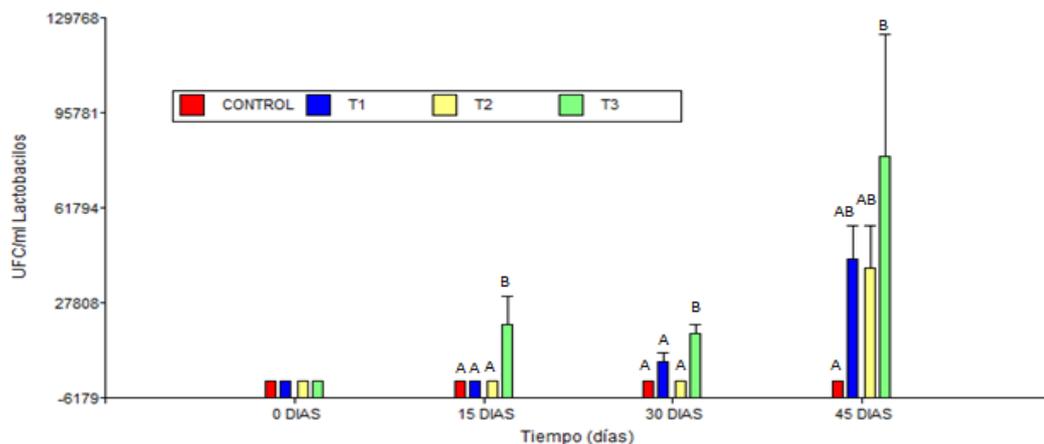
media was visually evident, possibly a higher dilution should have been used in stocking because the total coliform populations in fish wastewater are very high.

In a study conducted by Roldán et al. (2007), the effect of the application of ME (1/3000 v/v) on wastewater was evaluated for 60 days, in which a significant reduction in total coliform counts was obtained in both cases on day 30 of the study. This is because the metabolites produced by the microorganisms present in MS such as acids, ethanol and bacteriocins, inhibit the populations of pathogens of fecal origin that are

They are found in contact with these microorganisms, possibly because they lower the pH of the medium and/or limit their growth by destabilizing transport across the membrane by blocking receptors or generating changes in the ionic balance (Miklota et al, 2004).

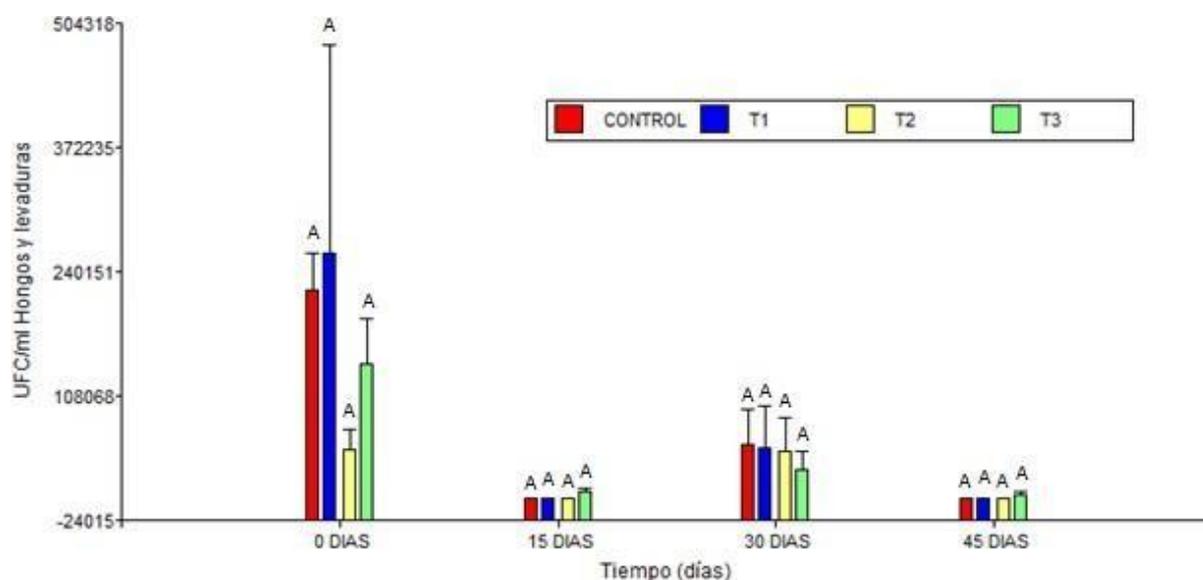
**Figure 17**

*Lactobacilli colony-forming units in treatments*



**Figure 18**

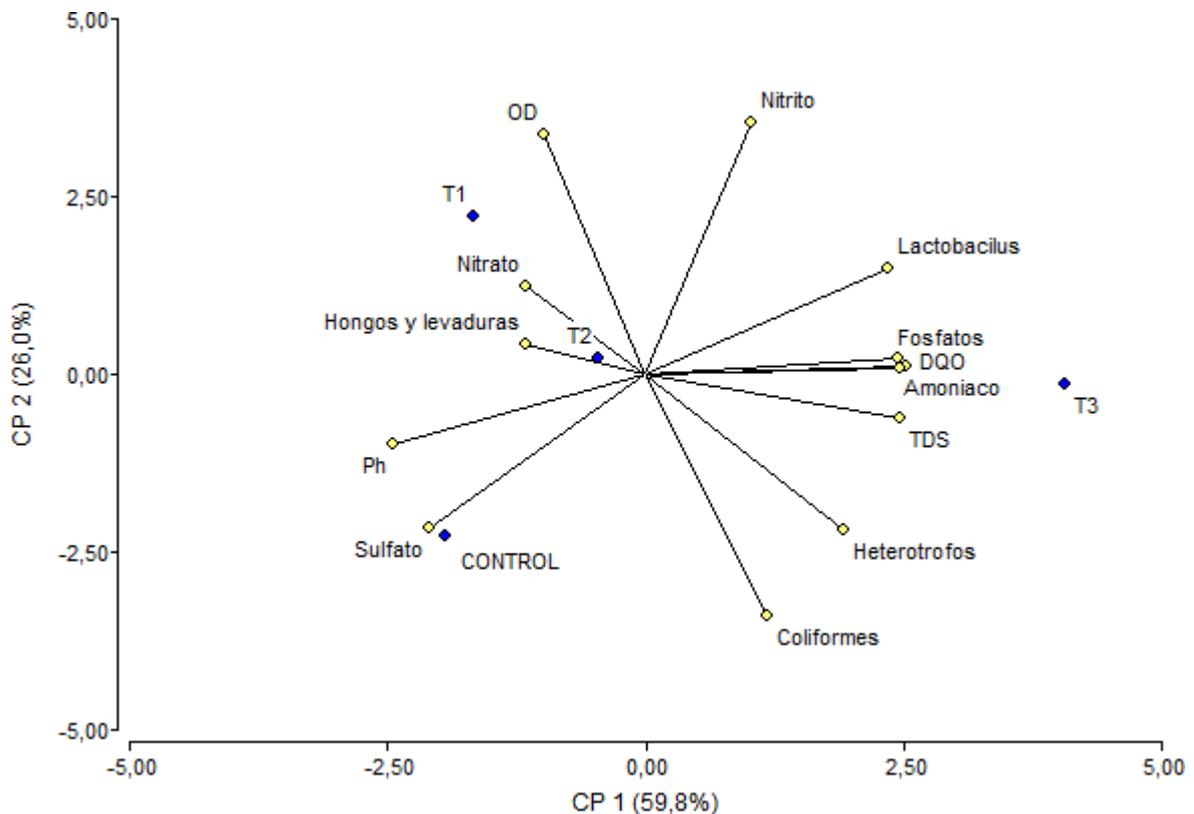
*Colony-forming units of fungi and yeasts in treatments*



The concentration of fungi and yeasts did not present significant differences between treatments, while a decrease was observed over time. On the other hand, lactobacilli increased over time.

**Figure 19**

*Principal component analysis of all the variables studied*



The principal component analysis allowed a summary analysis of all the variables studied. Component 1 explained 59.8% of the total variability of the data and component 2 explained 26% of the total variability. Each vector corresponds to each of the parameters evaluated. Vectors that are presented in opposite directions with an angle of 180 degrees, have a negative correlation and those that are in the same direction very close have a positive correlation, for example, the concentration of COD is correlated with the high presence of phosphates, ammonia and total solids.

In treatment 3 there were high concentrations of COD, phosphates, ammonia and total solids, the control treatment with high concentrations of sulfate and treatment 1 with dissolved oxygen and nitrates.

## 7 CONCLUSIONS

For most of the parameters evaluated, no statistically significant differences were observed between the control and the treatments, with the exception of sulfate, which presented lower concentrations in the treatments with efficient microorganisms. In treatment

3, which had the highest concentration of EM, the growth of aquatic plants occurred, which caused a process of eutrophication of the water so that nitrogenous compounds and phosphates increased. It is recommended to perform an analysis of the effect of efficient microorganisms in the removal of these compounds at lower concentrations than those used in this study.

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