CHAPTER 60

Preliminary studies of the action of efficient microorganisms (em) in the bioremediation of water from the vila bananeira dam in arapiraca-alagoas, brazil

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Esmeralda Aparecida Porto Lopes
PhD in Agronomy from the Federal Rural University of Pernambuco - UFRPE, adjunct professor at the State University of Alagoas - UNEAL, Tel - (82) 9 99905880
E-mail: eportolopes@yahoo.com.br

Janesmar Camilo de Mendonça Cavalcanti
PhD in Chemistry from the Federal University of Pernambuco - UFPE, full professor at the State University of Alagoas - UNEAL, Tel - (82) 9 96342655
E-mail: janesmar.cavalcanti@gmail.com

Noemia Cristina Gama dos Santos Cardozo
Undergraduate student in Biological Sciences at the State University of Alagoas – UNEAL, PIBIC/FAPEAL 2020 scholarship holder, Tel - (82) 9 96408505
E-mail: noemia_cristina_@hotmail.com

Giselle Silva de Souza
Undergraduate student in Biological Sciences at the State University of Alagoas – UNEAL, PIBITI/FAPEAL scholarship holder 2019, Tel – (82) 9 98133998
E-mail: giselle.silva908@gmail.com

Leonardo da Silva Santos
Undergraduate student in Biological Sciences at the State University of Alagoas – UNEAL, PIBITI/FAPEAL scholarship holder 2019, Tel – (82) 9 96948237
E-mail: leossantos.bio@gmail.com

ABSTRACT
Efficient micro-organisms (EM) are regenerative and completely natural, formed by lactobacilli, yeasts, photosynthetic bacteria and actinomycetes, which act on the physical, chemical and biological properties of the soil, being able to solve environmental problems such as, for example, wastewater treatment. The objective of the work was to analyze the effect of the EM inoculants on the bioremediation of water contaminated by animal waste as well as the physical-chemical and microbiological parameters of the fermented EM and contaminated water. The EM was captured and activated using the homemade method. The protocol for collecting contaminated water and EM samples was in accordance with Standard Methods for the Examination of Water and Wastewater. Total and thermotolerant coliforms of both water and EM were investigated, using the most probable number (MPN) technique, known as the multiple tube method. The physical-chemical parameters (pH, total solids, sodium, nitrite, nitrate, chlorides, total hardness, apparent color, conductivity, and turbidity) of the contaminated water and the EM were evaluated. To assess the bioremediation potential of EM, 10 tests were performed with different percentages of dilution of microorganisms, which followed the following concentrations: 0.3; 1.0; 3.0; 20; 25; 30; 35; 40; 45 and 50% of EM. It was possible to verify that the physical-chemical parameters analyzed meet the prerequisites of the Ministry of Health with regard to water quality, however, regarding microbiological parameters, 99% of the samples proved to be unfit for human consumption. The fermentation of efficient microorganisms is free of the coliform group. As for bioremediation using EM, low concentrations of 0.3 to 3 did not remedy the coliform group, however it was found that gradual concentrations of at least 25% of EM can remedy up to 78% of the coliform group.

Keywords: Total coliforms, thermotolerant coliforms, physical-chemical parameters, environmental pollutants.

1 INTRODUCTION

Among the natural resources, water is one of the most important on the planet and its use has intensified in recent decades with economic development and population growth, both in terms of the increase in the quantity demanded for a given use, as well as the variety of uses. its use (SILVA and ANDRADE, 2014).

According to Santos et al. (2010), maintaining water quantity and quality standards represents a challenge to society, since most problems related to water resources have as main problems the lack of planning and management, in addition to their contamination. This new look has been triggering actions and strategies to preserve the environment, land occupation and development of less harmful production processes (MORAES et al., 2014).

Among the biotechnologies used for the recovery of contaminated areas, efficient microorganisms (EM) consist of a combination of completely natural microorganisms (lactobacilli, yeasts, phototropic bacteria and actinomycetes), which when they coexist, present a synergistic effect that is greater than the sum of its individual members, being able, among many applications, to solve environmental problems such as, for example, treating wastewater. The use of microorganisms as tools for the remediation of contaminated environments is called bioremediation. This process can be carried out through one or more microbial consortia, indigenous or not, for the degradation of polluting organic contaminants (PEREIRA and LEMOS, 2003).

According to Tavares (2013), this is a technology that currently has a rapid growth, especially in collaboration with genetic engineering, used to develop strains of microorganisms that have the ability to

deal with specific pollutants. Under suitable conditions, microorganisms help or are responsible for chemical reactions that result in compounds that present little or no risk to living beings (MOREIRA and DOURADO, 2007).

In this context, the main objective of this work was to evaluate the effect of efficient microorganisms (EM) in the bioremediation of water contaminated by animal waste from the Vila Bananeira dam.

2 MATERIAL AND METHODS

Water samples for bioremediation evaluation were collected at the Vila Bananeiras Dam (9º 50'33.6 ”S 36º34'15.8”W) located in the rural area of the municipality of Arapiraca-AL, Brazil. The EM collection was carried out in a Private Reserve of Natural Heritage in Junqueiro – RPPN Madeiras (9º51'53.5”S 36º20'00.2”W) and its activation was carried out in the forestry resources laboratory, while the microbiological analyzes were carried out in the laboratory of microbiology, both from the Arapiraca Agro-Food Technological Pole of the State University of Alagoas. The physical-chemical analyzes were forwarded to the Analytical Central laboratory. The EM was prepared and activated through the homemade method of capturing and preparing the soil (SIQUEIRA et al., 2013). Cooked rice without salt was used for capture and placed on trays covered by a thin protective screen, the trays were left in virgin forest, covered with litter for approximately

15 days. For activation, molasses and water without chlorine were used, the mixture was placed in a container with a lid where it was fermented for 15 days.

The collection procedure followed the protocol according to Standard Methods for The Examination of water and Wastewater (HUNT and RICE, 2005). The collections took place in July 2019, using a stainless steel container and a polypropylene bottle to store water, all previously autoclaved at 121°C at 1kpa for 60 min, after which it was sent in Styrofoam boxes to the microbiological analysis laboratory.

2.1 EM DILUTIONS USED FOR BIOREMEDIATION:

The ME concentrations used to bioremediate the water from the Dam (BA) were: 99.7% BA + 0.3% EM; 99% BA + 1% MS; 97% BA + 3% MS; 80% BA + 20% MS; 75% BA + 25% MS; 70% BA + 30% MS; 65% BA + 35% MS; 60% BA + 40% MS; 55% BA + 45% MS; 50% BA + 50% EM.

2.2 MICROBIOLOGICAL ANALYSIS:

The methodology used was that of the American Public Health Association (APHA), total and thermotolerant coliforms were quantified using the technique of the most probable number (MPN) of the EM dam water and EM dilutions. After the analysis of the presumptive tests carried out in serial dilutions of 10^{-1}, 10^{-2} and 10^{-3} with LST Broth (Lauril Tryptose), of the tubes with a positive result , a survey was carried out in tubes with 10 ml of Bile Broth 2% Brilliant Green (to quantify total coliforms) that were incubated in a bacteriological oven at 35.5°C for up to 48 hours, and a rise in tubes with 10 ml of EC broth (*Escherichia coli*) (for thermotolerant coliforms) which were placed in a water bath at 45.5°C for up to 48 hours.

The positive results of the tests evaluated were observed by the formation of bubbles in the Durham tubes, through the fermentation of the broths. The results were compared with those established by the Brazilian Ordinance (Consolidation Ordinance) No. 5 of the Ministry of Health and with Resolution No. 357 of CONAMA, 2005.

2.3 PHYSICAL-CHEMICAL ANALYSIS

The methodology used for analysis by the Central Analytical laboratory was the Standard Methods for the Examination of water and Wastewater . Dam water and EM were evaluated with the following parameters for dam water – pH, total solids, sodium, nitrite, nitrate, chlorides, total hardness, apparent color, conductivity and turbidity.

3 RESULTS AND DISCUSSION

3.1 MICROBIOLOGICAL ANALYSIS OF DAM AND ME WATER:

It can be seen in table 1 that the water from the banana dam has a group of coliforms in the NMP value >1100/ mL showing 99% of contamination index. This result differed from Dornellas and Xavier (2008), who evaluated 6 collection points in the Piauí stream, one of them being the dam water, found 46.0 MPN/100mL. The dam was built 19 years ago with the purpose of generating jobs, income and leisure, but observing the results and according to CONAMA resolution nº 357, it does not fit into the class 1 of fresh water that can be destined to supply for consumption . human; to primary contact recreation such as swimming, water skiing and diving.

Table 1. Microbiological and pH assessment of the dam water and effective microorganisms, in July 2019.

	NMP/100mL	pH
BA	>1100	7.44
IN	<3.0	3.21

The NMP observed in the efficient microorganisms was <3.0/100mL, proving that it is free from the coliform group. *Escherichia coli is used all over* the world as a bioindicator of fecal contamination, in this sense it is a biological parameter that serves to monitor food, water, biofertilizers , etc. EM are widely used in agricultural practices and human health, few studies report microbiological analysis. The study shows that it can be recommended for all purposes as a soil conditioner, water decontamination and as a plant biofertilizer .

Darolt and Neto (2006), analyzing anaerobic and aerobic biofertilizers , also verified the absence of the fecal coliform group. The fermentation process that occurs to obtain the biofertilizer can be considered as one of the main factors that control the survival of pathogenic microorganisms, which may be related to the action of temperature, which possibly has been one of the determining factors in the destruction of fecal coliforms. . According to Gotaas (1956), and Kiehl (1985), these organisms do not survive at a temperature of 65°C, when exposed for 60 minutes. Another possibility would be the synergistic or antagonistic interactions between microorganisms (LOURES, 1988).

For the use of EM at concentrations of 0.3; 1 and 3% the values were >1100 NMP/100ml, showing that the water was not remedied, that is, the help of microorganisms in these percentages was not effective. In concentrations of 20; 25; 30; 35; 40; 45 and 50 the NMP result was: 15; 7.4; 7.2; 15; 3.6; <3.0; <3.0 for total coliforms, respectively. For thermotolerant coliforms, following the

same concentrations the values were: 27; 7.4; 7.2; 15; 3.6; <3.0 and <3.0 (table 2), showing within the required by CONAMA.

Table 2. Most likely number (MPN/100mL) of total and thermotolerant coliforms at concentrations of 0.3; 1; 3; 20; 25; 30; 35; 40; 45 and 50% of effective microorganisms.

BA+EM	NMP/100mL of total coliforms	NMP/100mL of thermotolerant coliforms
99.7% BA + 0.3% EM	>1100	6.1
99% BA + 1% EM	>1100	<3.0
97% BA + 3% EM	>1100	11
80% BA + 20% EM	15	27
75% BA + 25% EM	7.4	7.4
70% BA + 30% EM	7.2	7.2
65% BA + 35% EM	15	15
60% BA + 40% EM	3.6	3.6
55% BA + 45% EM	<3.0	<3.0
50% BA + 50% EM	<3.0	<3.0

Serial dilutions by the NMP technique were performed in aliquots of 0.1; 0.01 and 0.001

In bioremediation, living organisms are used technologically to remove and reduce environmental pollutants. It is an ecologically more adequate and effective alternative for the treatment of environments contaminated with organic molecules that are difficult to degrade, heavy metals, called recalcitrant, which may be of natural origin, synthesized by biological metabolism (SANTOS et al., 2009). The MS under study showed their ability and efficiency to be used as a bioremediator . According to Bonfim et al. (2011), the production of ME by family farming is an accessible and low-cost technology. As already pointed out, physical-chemical analyzes of both the dam water and the EM were also carried out, and only based on the results of table 3, the dam water would be satisfactory for human consumption, being within the recommended by the Brazilian Consolidation Ordinance 5 of the Ministry of Health, however, the work presented here is purely investigative and does not aim to accurately classify the potability of water from the banana dam, where it would also be necessary to measure the presence of other inorganic chemicals, as well as the heavy metals that can come from soil contaminated by pesticides, from local agriculture.

According to Lenzi et al. (2014), the acidity of natural waters comes from weak acids, such as dissolved carbon dioxide and even hydrogen sulfide, in addition to amino acids, fatty acids and conjugated acids of weak bases. Normally, $CO_{2\,(aq)}^{-}$ comes from the atmosphere or from the water body itself, caused by the action of aerobic microorganisms, which justifies the change in pH of BA water when compared to EM.

Currently, the banana dam water is used only for irrigation, however, due to the microbiological results, it cannot be used for this purpose.

At a concentration of 100%, EM, due to the physicochemical characteristics shown in table 3, cannot be used for human consumption or for irrigation. In general, it becomes complex to compare water with a solution of efficient microorganisms. Studies of physicochemical parameters at different dilutions of EM are required.

Table 3. Physicochemical parameters of contaminated water and efficient microorganisms (EM).

parameters	Water contaminated by animal waste	Efficient Microorganisms (EM)
pH*	7.44	3.21
Total solids (mg/L)	585.2	2988.3
Sodium (mgNa /L)	108.3	118
Nitrite in N, (mg/L)	0.06	0.32
Nitrate in N, (mg/L)	0.54	21.28
Chlorides (mgCl /L)	185.64	1115.84
Total hardness (mgCaCO3/L)	134.00	1400.00
Conductivity (µS/cm)	780	3984
Turbidity (NTU)	12.99	206.00

pH*- Hydrogenonic potential

4 FINAL CONSIDERATIONS

As preliminary results of the action of efficient microorganisms in the bioremediation of water, we have that the best MPN observed was <3.0/100mL, showing that it is free from the coliform group, an initially satisfactory result, despite the need for low concentrations of dilutions , requiring further economic feasibility studies *vs* production, since the remediation of contaminated water takes place in gradual concentrations of at least 25% of fermented effective microorganisms,

which can remediate up to 78% of the coliform group. The microbiological results showed that the water from the Vila Bananeira dam has a group of coliforms in the NMP value >1100/ mL showing 99% of contamination index, thus, according to CONAMA resolution nº 357, it does not fall into class 1 of water. sweets, making their use unfeasible for both irrigation and human consumption.

It is concluded that according to the physical-chemical parameters evaluated, the dam water is satisfactory for human consumption, being within the recommended by the Brazilian Ordinance (Consolidation Ordinance) nº 5 of the Ministry of Health, however, it is necessary to analyze other chemical constituents for total potability classification, in addition to further studies with EM dilutions, which will show the correlation of the formation of diluted weak acids as a result of EM action. Last but not least, we point out the need to complete the initial project of the Vila Bananeira dam, so that it will contribute to its social role and justify its investment of approximately R$ 20 million reais.

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