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Bioflocculant from *Leuconostoc Pseudomesenteroides* for the Treatment of Waters from the Moche River

De La Cruz-Noriega, Magaly [1]; Luis A. Cabanillas-Chirinos[1]; Waldo Salvatierra-Espinola[2]; Karol Mendoza-Villanueva[3]

[1] Institutos y Centros de Investigación. Universidad Cesar Vallejo, Trujillo 13001, Perú;
mdelacruzn@ucv.edu.pe lcabanillas@ucv.edu.pe
[2] Escuela de Enfermería, Facultad de Ciencias de la Salud, Universidad Cesar Vallejo, Trujillo, 13001, Perú
aldose0508@gmail.com
[3] Programa de Investigación Formativa, Universidad César Vallejo, Trujillo 13001 mendvillan@gmail.com

Abstract **-** The exponential increase in the contamination of our water resources is alarming. Water is essential for human life, so it is necessary to recover it through ecological treatments, such as bioflocculants. In this context, the present study aimed to evaluate the bioflocculant activity of dextran from *Leuconostoc pseudomensenteroides* in treating water from the Moche River.For this purpose, sugarcane juice was used and sown on potato sucrose agar (PSA) in a selective medium, where characteristic colonies of the genus Leuconostoc sp. were observed, with a shiny, rubbery appearance. Molecular identification confirmed 100% that it was the Leucnostoc pseudomensenteroides strain. The flocculating activity of dextran was evaluated using doses of 5, 20, and 40 ppm. During the process, a fast speed of 180 rpm and slow speeds of 50 to 70 rpm were applied. In addition, we observed that the maximum flocculation activity was obtained at a pH of 9 and a concentration of 40 ppm of dextran, with a fast-stirring speed of 180 rpm for 5 min and a slow stirring speed of 70 rpm for 15 min. This procedure achieved an 88.0% removal of the turbidity in the waters of the Moche River.

Keywords**:** bioflocculant, *Leuconostoc pseudomesenteroides*, dextran, Moche River waters, sucrose.

1. Introduction

Water is one of the main supports of human life, being used in various anthropogenic activities. The population has increased, and these activities have generated harmful effects on the environment, such as the contamination of water resources. This situation has been accentuated in the waters of the Moche River, which is why the treatment of these waters has become complicated. They contain metals, organic and inorganic particles, fine suspended solids, and other impurities, which are difficult to eliminate due to their size and surface charge [1,2]. For this reason, it has been necessary to develop and implement various water and wastewater treatment methods, such as membrane processes, ion exchange, and other effective techniques [3]. In addition, coagulation and flocculation processes have been widely used in water treatment to agglomerate colloids and fine particles in water into larger particles, known as flocs, facilitating the separation of solids and liquids. Although chemical flocculants are preferred in different industrial processes for their cost-effectiveness and high efficiency, such as the use of aluminum sulfate, ferric chloride, and polyacrylamide, they generate negative impacts on both humans and the environment due to the toxicity and environmental pollution generated by conventional treatment processes, which often discharge sludge into water bodies and represent a potential risk [4-6].

In this context, alternatives such as biologically based flocculants, known as flocculants, emerge, which play a crucial role in reducing turbidity and eliminating other contaminants present in the water through flocculation processes. These can be synthesized by plants such as beans, moringa, and cacti, as well as by microorganisms such as fungi, bacteria, algae, and actinomycetes [7-10]. These bioflocculants are metabolites secreted by these organisms during their growth phase. These microorganisms are selected based on their morphology and ability to produce extracellular polysaccharides. These biopolymer-based flocculants have significant flocculation and biodegradation activity, which makes them a potential alternative to chemical flocculants.[7]. Some of these microbial flocculants are extracellular biopolymers derived from natural sources such as polynucleotides (DNA, RNA), polypeptides (proteins), and polysaccharides (carbohydrates), obtained from a variety of microorganisms, used for the removal of contaminants from water and wastewater [11,12]. Their application has considerable potential for both conventional water purification, biodegradable sludge in agriculture, and effluent treatment; they can be applied in subsequent processes in the fermentation industry [13]. In this regard, Baranwal et al. (2019) reported a bio flocculant-producing bacterial strain belonging to the Citrobacter genus, Gram-negative coliform bacteria of the Enterobacteriaceae family [14]. Likewise, Abu et al. (2018) investigated a bioflocculant produced by *Bacillus salmalaya* for wastewater treatment; and reported a removal efficiency of 93% in Chemical Oxygen Demand (COD) and 92.4% in Biochemical Oxygen Demand (BOD) respectively, these results indicate that this bioflocculant has promising applications in wastewater treatment [15].

Similarly, Agunbiade et al. (2018) investigated the production of bioflocculant by a strain of Streptomyces platensis, grown under specific conditions, having glucose as the only carbon source and peptone as the nitrogen source. These conditions contributed to the high effectiveness of the flocculant. The results showed that the optimal dose for the clarification of a kaolin clay suspension was 4 g/L; on the other hand, this bio flocculant contributed to the decrease of COD and the reduction of turbidity by 84.3 and 75.6% of river waters and wastewater, respectively. Based on this, the bacteria S. platensis, having a high flocculation rate, can represent a valuable tool in bioremediation for water treatment [16]. De La Cruz et al. (2022) argue that the bacterium *Leucnostoc mesenteroides var. mesenteroides* produces a bioflocculant whose best flocculant activity is at a concentration of 40 ppm of dextran, with a fast-stirring speed of 150 rpm for 5 min and a slow stirring speed of 50 rpm for 15 min at pH 9, achieving 77.7% removal of turbidity from the effluent of the sugar industry [17].

For this reason, the present research aimed to evaluate the bio flocculant activity of dextran from Leuconostoc pseudomensenteroides (molecularly identified) in treating water from the Moche River. For this purpose, the turbidity and BOD measurements were carried out at different concentrations of bioflocculant, for which dextran was obtained, a biopolymer from the fermentation of the Mayeux medium supplemented with 10%. These bioflocculants emerge as a promising alternative to inorganic coagulants and synthetic organic flocculants. This is due to the increasing attention they have garnered for their high flocculating activity, biodegradability, low toxicity, and various environmental applications, such as wastewater treatment [9].

2. Material and method

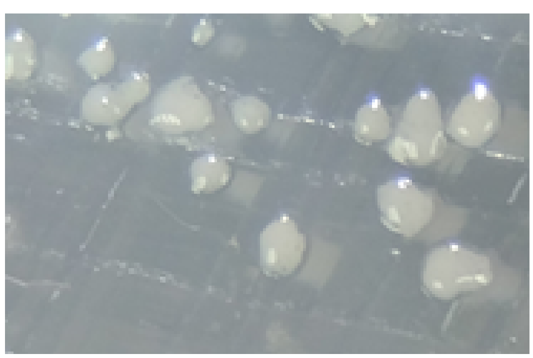
2.1.- Collection of Water Samples from the Moche River

Two liters of water sample were obtained from the Moche River in Víctor Larco Herrera, Trujillo, La Libertad, Peru. The samples were preserved at 4°C and transported to the Institute and Research Centers of the Cesar Vallejo University for processing.

2.2.- Isolation and Molecular Identification of L. pseudomesenteroides

Residual sugarcane stalks in a state of decomposition with the presence of a gummy substance, approximately 1 kg, were collected. They were then washed with distilled water and cut into 10 cm pieces. They were then passed through an Oster FPSTJE316W extractor to obtain approximately 100 mL of sugarcane juice.

A sample of sugarcane juice was taken and inoculated on Potato Sucrose Agar (PSA) plates, which composition is: 1 liter of potato broth, 10% sucrose, using the streaking technique. The plates were incubated at 30°C for 72 hours. After this time, colonies were selected based on their morphological characteristics (translucent, shiny, convex surface, and gummy) (see Fig 1) [17,19].



Molecular identification was performed by Ecobiotecnology SAC Laboratory. A pure culture was sent for DNA extraction and sequencing of a PCR product specific for the 16S ribosomal gene region specific to bacteria [20]. The sequenced regions analyzed in the BLAST program obtained a 100% identity percentage corresponding to the species L. pseudomesenteroides (Table 1).

Fig 1. Translucent, shiny, convex, and gummy colonies observed in PSA medium.

Table 1. Molecular identification of a bacteria isolated from cane juice

Identified species	pb	Identity (%)	BLAST Accession number
Leuconostoc pseudomesenteroides	1470	100%	LC306846.1

2.3- Production and Extraction of Dextran

To obtain the inoculum, Mayeux Broth (900 mL) with sucrose (100 g/L) and 100 mL (10% v/v) of the bacterial inoculum (1.6 × 10^8 CFU) were prepared. This was added to a 500 mL bioreactor, hermetically sealed, initiating the fermentation process under the following conditions: pH of 7.0 ± 0.2 at 30°C, 0.5 volume of air/medium unit per minute, and 200 rpm for 76 hours under sterile aeration conditions. For the extraction of dextran, the method of Pinchi [2017] [21] was used.

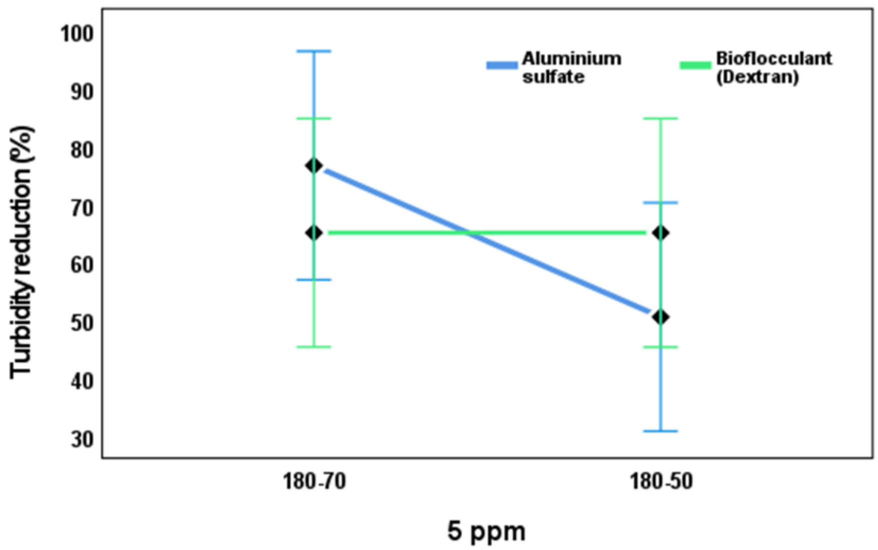
2.4- Determination of Dextran Flocculation Activity at Different Concentrations Using Physicochemical Methods

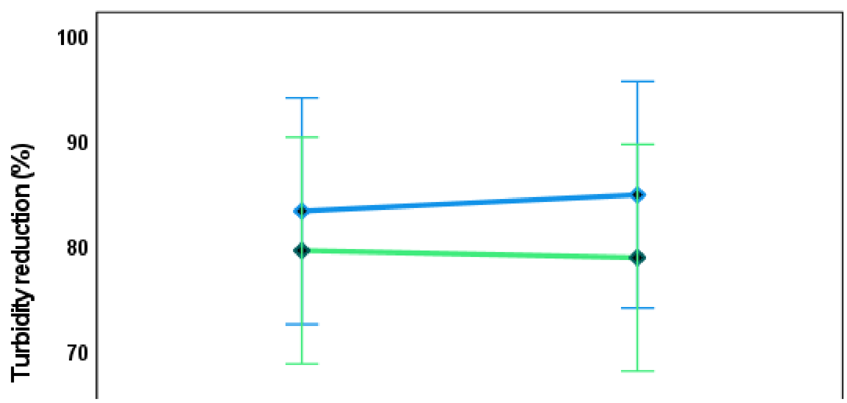
A randomized experimental design with three replications was used. The experiment aimed to evaluate the effect of different concentrations of the biopolymer dextran (5, 20, and 40 ppm) as a bioflocculant in the effluents of the Moche River waters.

In 250 mL flasks, 200 mL of Moche River water with a turbidity range of 110 – 175 ppm was added, followed by the bioflocculant (dextran) at different concentrations for each trial with rapid agitation (180 rpm) for 5 minutes and slow agitation (50–70 rpm) for 15 minutes at pH 9. After this period, the agitation was cautiously stopped to avoid breaking the flocs, and the solution was allowed to settle. Immediately after sedimentation, turbidity was measured for each trial, evaluating the quality of the flocculant formed during sedimentation and the precipitated flocs (considering their consistency and compaction), as well as the possible presence of flocs in suspension. Turbidity analyses were carried out using nephelometry, with a turbidimeter (Thermo Scientific, Orion Model AQ 3010) [22].

3.-Results and Discussion

Regarding the results of the flocculant activity of the chemically-based polymer (aluminum sulfate) and the bioflocculant (dextran) at different doses (5, 20, and 40 ppm), at different times of rapid agitation (180 rpm) and slow agitation (50–70 rpm), the following results are shown:


Aluminium sulfate
Bioflocculant (Dextran)
Turbidity reduction (%)
100
90
80
70
60
50
40
30
180-70
180-50
5 ppm


Turbidity reduction (%)
100
90
80
70

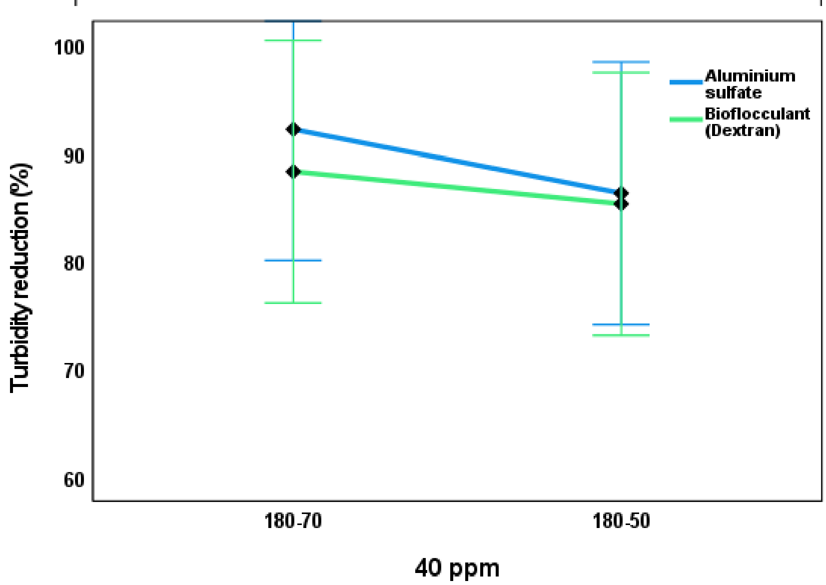

Aluminium sulfate
Bioflocculant (Dextran)
Turbidity reduction (%)
100
90
80
70
60
180-70
180-50
40 ppm

Fig 2. Profile graph of the flocculation process using aluminum sulfate and a bioflocculant (dextran) at three different doses.

In Fig 02, it is shown that there is a reduction in turbidity when increasing the doses of the flocculant up to 40 ppm of aluminum sulfate and the bioflocculant (dextran), with reduction percentages of 92% and 88% respectively. The performance of aluminum sulfate differs from what was reported in another study where it was determined that, for an initial concentration of 75 ppm of aluminum sulfate, turbidity removal efficiencies range from 50% to 90%; that is, high concentrations of chemical flocculant are required to reduce turbidity; therefore, toxicity effects may occur [23]. On the other hand, the performance of the dextran biopolymer is similar to another study where an average turbidity removal of between 17% and 80% was found using effective doses from 20 ppm of the biopolymer chitosan; indicating that low doses are required for efficient turbidity reduction; demonstrating a lower risk in its use due to being less toxic. The two flocculants exhibit different reduction patterns; as it is observed that they increase unevenly, indicating that both could follow different adsorption and neutralization mechanisms [24]. Similarly, in Wang et al.'s study [25], a dose of a dextran-based bioflocculant is mentioned as being effective for flocculating (98.2%) a kaolin suspension used as wastewater. In the flocculation process, the destabilization of free particles can occur; in the case of the chemical coagulant, trapping is a possible mechanism; while the flocculation-promoting efficiency of the biopolymer is related to its physicochemical characteristics, the composition of the components, the structure, and the functional groups present [26].

Table 2. Non-parametric contrast in the levels of dose and type of coagulant variables.

Variable	M1-M2 (ppm)	Range difference	Sig.
Dose	5-20 *	-11,083	0,03
	5 -40*	-17,917	0,00
	20-40*	-6,833	0,336
Flocculants	Aluminium Sulfate Biofloculant**	-2,34	0,506

Note: Statistics used for contrast: *Kruskal-Wallis; **Mann-Whitney U. α=5%

In Table 2, the pairwise comparison at each level of the tested variables (Dose and Type of flocculant) on the percentage reduction in turbidity is shown. For the dose variable, the differences are significant ($p<0.05$) when working at 5 ppm, with no difference ($p>0.05$) in turbidity results when using doses of 20 and 40 ppm. Regarding the type of flocculant used, no significant difference ($p>0.05$) was found in turbidity reduction, indicating favorable flocculant properties of the biopolymer for water treatment applications.

Table 3. Effect of biopolymer and Aluminum Sulfate on the reduction in Biochemical Oxygen Demand (BOD) in waters of the Moche River (Trujillo, Peru).

Biopolymers	Dose (ppm)	Agitation (rpm)	DBOi (mgO_2/L)	DBOf (mgO_2/L)

Bioflocculant (Dextran)	5	180-70	20	5
Bioflocculant (Dextran)	20	180-50	20	4
Bioflocculant (Dextran)	40	180-70	20	13
Aluminum Sulfate	5	180-70	20	5
Aluminum Sulfate	20	180-50	20	3
Aluminum Sulfate	40	180-70	20	3

In Table 3, it was determined that a 20-ppm concentration of a microbial dextran-based bioflocculant, applied under agitation at 180-50 rpm, reduced biochemical oxygen demand (BOD) in the waters of the Moche River, reaching a minimum value of 3 mg O_2/L. In contrast, aluminum sulfate at concentrations of 20 and 40 ppm showed BOD values of 3 mg O_2/L. Both flocculants showed similar efficiency in BOD reduction, with a slight advantage for aluminum sulfate at 20 ppm, as its lower value can be attributed to its faster and more aggressive action compared to the bioflocculant. At low doses (5 and 20 ppm), both flocculants showed similar efficiency in BOD reduction, with a slight advantage for aluminum sulfate at 20 ppm. At higher doses (40 ppm), aluminum sulfate maintains its efficiency, while dextran shows a decrease in its effectiveness, which could be due to the formation of excessively dense structures that inhibit proper flocculation.

It has been observed that aluminum sulphate maintains its efficiency even at higher concentrations due to its strong coagulation capacity, which is less prone to saturation compared to biopolymers. Therefore, the literature suggests that chemical flocculants such as aluminum sulphate have high efficiency in removing contaminants at various concentrations due to their ability to form dense and stable flocs [27]. In contrast, biopolymers such as dextran may show a decrease in effectiveness at higher concentrations (40 ppm) due to the formation of excessively dense structures that may inhibit proper flocculation. This phenomenon may be due to excessive particle agglomeration, which reduces the surface area available for flocculant-particle interaction and decreases the overall efficiency of the flocculation process [28].

Microbial biopolymers show promising results as indicated by Agunbiade et al. [29]. The results obtained differ from those described by Du et al. [30], who found that at 40 ppm and between 40-150 rpm, dextran was able to remove up to 88% of water turbidity, which also implies a significant reduction in COD. This efficiency is due to the structure of dextran, which forms bonds and complex networks capable of trapping organic and inorganic particles.

According to Abu et al., chemical coagulant could significantly rely on the aggregation of suspended particles as the main mechanism; while the efficacy of biopolymer in the coagulation process is influenced by its physicochemical profile, including molecular composition and functional groups, to interact with suspended particles and promote coagulation [31].

Overall, both coagulants show different reduction patterns, suggesting variations in their increase, indicating possible different mechanisms of adsorption and neutralization. However, the biopolymer shows the ability to achieve efficient turbidity reduction at low doses, suggesting a lower risk in its use due to its lower toxicity, as mentioned by Zhou et al. [27].

4. Conclusion

This research was successfully carried out. The Leuconostoc pseudomensenteroides bacteria was isolated and molecularly identified. It can produce dextran, a biopolymer with a rubber-like characteristic. The maximum dose of dextran as a bioflocculant was reached at 40 ppm at a fast-stirring speed of 180 rpm for 5 min and a slow stirring speed of 70 rpm for 15 min at pH 9, reducing turbidity by 88.0% from a volume of 250 mL of water from the Moche River. Using the 20-ppm dose of dextran as a flocculant, the BOD decreased from 20 to 3 mg O2/l.

However, future studies are needed to evaluate additional parameters such as heavy metal removal, total coliform count, and oils and fats. Bioflocculants present a promising alternative for wastewater treatment, demonstrating efficacy in removing contaminants and environmental sustainability due to their biodegradability and low toxicity. Despite their higher production cost, they can result in long-term savings and improve public health by reducing pollution. In the context of the

Moche River, their implementation could offer a sustainable and beneficial solution for both the environment and local communities.

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