

Potential application of *Mytella charruana* in wastewater treatment. (Application of *M. charruana* in wastewater treatment)

Uso potencial del bivalvo Mytella charruana en el tratamiento de aguas residuales. (Aplicación de M. charruana en la depuración de aguas residuales)

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ABSTRACT

Field activities related to sampling *M. charruana* specimens and water samples in Las Quintas marsh in Cartagena de Indias were performed in order to evaluate the potential application of the species *Mytella charruana* in wastewater biodepuration systems from industries within the food sector. Laboratory bioassays were implemented in 20 liters' capacity aquariums where individuals were located with water from the environment, and the rate of biochemical oxygen demand (BOD_5) (%) and total volatile solids (TVS) (%) reduction in water were quantified. The results showed that the highest removal rate of BOD_5 (45.49 ± 30.8 %) and TVS (10.64 ± 6.27 %) occurs when the treatment involves the use of small organisms (< 2.5 cm) in water with high salinity (≥ 25). With the previous information, the conceptual design of two devices was developed: one with horizontal flow and one with vertical flow, both adaptable to space requirements, which is often a critical factor in the industry. It is concluded that *M. charruana* possesses a high application potential in biodepuration processes of water containing organic matter and volatile solids, which can be arranged in systems of vertical or horizontal flow, to reach a greater removal efficiency.

KEY WORDS: Biodepuration, *Mytella charruana*, water treatment.

RESUMEN

Con el propósito de evaluar la aplicación potencial de la especie *Mytella charruana* para su implementación en sistemas de biodepuración de aguas residuales procedentes de industrias del sector alimenticio, se realizaron actividades de campo vinculadas al muestreo de especímenes de *M. charruana*, y de muestras de agua, en la ciénaga de las Quintas en Cartagena de Indias. Los bioensayos de laboratorio se implementaron en acuarios de 20 L de capacidad, en los que se ubicaron organismos con agua del medio, y se cuantificó la tasa (%) de reducción de la demanda bioquímica de oxígeno (DBO_5) y de sólidos volátiles totales (SVT), presentes en el agua. Los resultados mostraron que la mejor tasa de remoción de la DBO_5 (45.49 ± 30.8 %) y SVT (10.64 ± 6.27 %), se dio en el tratamiento conformado por especímenes de menores tallas (< 2.5 cm) y agua con alta salinidad (≥ 25). La información posibilitó el diseño conceptual de dos sistemas de biodepuración de aguas, uno de flujo horizontal y otro de flujo vertical; ambos adaptables a las necesidades de espacio, que suele ser un factor crítico en las empresas. Se concluye que *M. Charruana* posee un alto potencial de aplicación en procesos de biodepuración de aguas que contienen materia orgánica y sólidos volátiles, la cual puede disponerse en sistemas de flujo vertical u horizontal, para alcanzar una mayor eficiencia de remoción.

PALABRAS CLAVES: biodepuración, *Mytella charruana*, tratamiento de agua.

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INTRODUCTION

Due to its content of polluting elements, wastewater must be treated before its final disposal. The treatment of these waters generally requires a phase of biofiltration (Romero, 2008; Lafont, Pessel & Gauthier, 2014), from natural phenomena such as degradation, metabolism and use of nutrients by plants, microorganisms, and another group of organisms with high removal capacity of xenobiotics present in the water column, such as bivalves (Pasqualino, Meneses, Abella & Castells, 2009; Binelli, *et al.*, 2014), which are characterized in that most of them have a high filtration rate of suspended material in the water column, especially organic material, which has given this group of animals a relevant role as regulators of organic material in suspension and primary productivity in the ecosystems that they inhabit (Yidi & Sarmiento, 2011).

It is important to clarify that besides the capacity of filtration, some species of bivalves are characterized by their tolerance to environmental conditions that are not tolerable for other groups of animals; this is due to the capacity they have to close their valves and maintain their metabolism through anaerobic processes, as occurs with the species *Mytella charruana*, which has been found in Las Quintas marsh and the Cartagena de Indias bay, where there is a reported high biochemical oxygen demand deficit (BOD_5) and total volatile solids in water (TVS), related to a high load of organic matter in the system (Puyana, Prato & Díaz, 2012; Tirado, Manjarrez & Díaz, 2010).

The aforementioned aspects have aroused interest for the application of bivalves in the biofiltration of organic material in effluents from aquaculture production systems in coastal areas, using mangroves as a substrate (Sánchez & Álvarez, 2008; Peña, 2009), as well as artificial devices in which these organisms are introduced and environmental variables

are controlled such as temperature, light availability, oxygen, salinity, type and speed of flow. This has given greater efficiency in the processes of removal of organic matter, heavy metals (Das & Jana, 2003; Perceval, *et al.*, 2006; Jorge, *et al.*, 2013), some polynuclear aromatic hydrocarbons (Thompson, Budzinski & Narbonne, 1999), bacteria, viruses and microalgae (Cerco & Noel, 2010; Zaldívar, *et al.*, 2011).

Due to the importance that the bivalves have had in aquatic biofiltration processes, the present study had the purpose of evaluating the application potential of *M. charruana* in unconventional systems for the treatment of wastewater coming from the industrial food sector, which is highly relevant to identify and evaluate native species of the region with great application capacity in the field of environmental biotechnology.

METHODOLOGY

Field phase

The specimens of *M. charruana*, used in this project, were obtained manually following the methodology proposed in the literature (Quirós & Arias, 2013), from the roots of a *Rhizophora mangle* patch located in Las Quintas marsh (10°24'44,20" N; 75°31'35,93" W y 10°24'22,91" N; 75°31'31,98" W), in Cartagena, Colombia (Figure 1). On the other hand, it is clarified that the specimens were kept submerged in water from the middle in a plastic basket, until their transfer to the laboratory.

Simultaneously, and with the help of a multi parameter analyzer, the value of the electrical conductivity of water ($\mu\Omega/cm$), dissolved oxygen (mg/L), salinity and dissolved total solids (mg/L) was recorded. The water required for the biofiltration tests in the laboratory was collected in the medium, with a 0.6 HP submersible electric pump, with which 576 L were collected (Figure 2).

Bioassays: removal of solids and organic matter

Prior to the start of the bioassays, in this continuity of the analysis with respect to the observed, the organisms were acclimatized for 72 hours, under similar conditions to those of the source medium: salinity (25), temperature (28 °C) and dissolved oxygen (4 mg/L). Subsequently bioassays were carried out following

a bi-factorial experimental design (Montgomery, 2004), having as factors of variation, the salinity of the experimental water, with two treatments (high: 25 and low: 12.5), and the size of the individuals of *M. charruana* (smaller size: ≤ 2.5 cm, and larger size: > 2.5 cm). Three aquariums with salinity water equal to the one of the ecosystem of origin, free of specimens of *M. charruana*, were used as control.

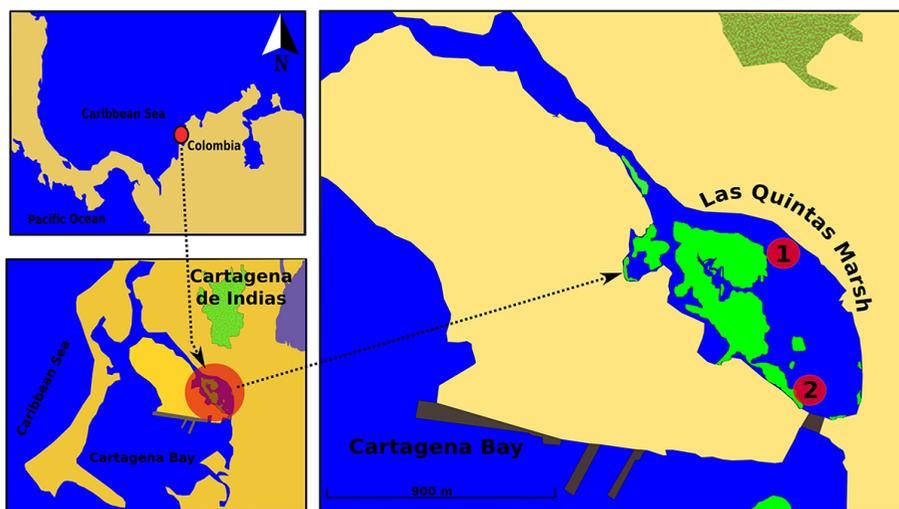


Figure 1. Location of Las Quintas marsh, in Cartagena de Indias. The arrows numbered 1 and 2 indicate the water sampling points and specimens capture of *M. Charruana* respectively. Taken and modified from Google Earth.



Figure 2. Collection of water sampling and capture of *M. charruana* in Las Quintas marsh, in Cartagena de Indias.

It is also important to indicate that as a response variable, the water removal rate (%) of BOD₅ and total volatile solids (TVS) was observed. To this end, the specimens were introduced into the aquariums (20 of larger size and 40 of smaller

size), into bags constructed in multifilament with a 500 μm mesh eye (Figure 3), at a density of 1.25 and 2.5 individuals/L of water, as recommended by the literature (Ramos, Vinatea & da Costa, 2008), as described in Table 1.



Figura 3. Experimental set-up of bioassays for the removal of BOD₅ and TVS.

Table 1. Bi-Factorial design for the combination of salinity and size variables of *M. charruana*.

Code	Salinity	Size of the specimens
G-A	High	Greater (> 2.5 cm)
G-B	Low	Greater (> 2.5 cm)
P-A	High	Smaller (< 2.5 cm)
P-B	Low	Smaller (< 2.5 cm)
Control	High	Without organisms

The design of the experiment followed the model indicated in the equation 1:

$$y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_{12}x_1x_2 + \varepsilon \quad (1)$$

Where:

y is the answer, the β are the parameters of the model, x_1 is the variable that represents the salinity, x_2 is the parameter that represents the size of the specimens of *M. charruana*, x_1x_2 is the interaction between both factors (salinity and size) of the specimens and ε is the random error.

For the appreciation of the results, the reading that is carried out is the one described hereinafter. During the experiment, 500 mL samples of water were taken in each aquarium, at 24-hour intervals for a period of 48 hours. Later in the laboratory of the Regional Autonomous Corporation of the Canal del Dique, certified under the Colombian Technical Standard NTC-

ISO/IEC 172005:2005, for each sample the BOD₅ (mg O₂/L); was sampled by the electrode-membrane method (APHA 2005a).

The rate of reduction of BOD₅ (TRDBO₅) was calculated with the equation 2:

$$TRDBO_5 = \frac{DBO_{5i} - DBO_{5f}}{DBO_{5i} * 100} \quad (2)$$

Where: DBO_{5i} = dbiochemical oxygen demand 5, at the beginning of the experiment; DBO_{5f} = biochemical oxygen demand 5, at the end of the experiment.

The measurement of TVS (mg/L) was performed by the gravimetric method, according to the methodology proposed by APHA (2005b) and WEF (2012). The reduction rate of these, was calculated with equation 3:

$$TRSVT = \frac{SVT_i - SVT_f}{SVT_i * 100} \quad (3)$$

Where: TRSVT= Removal rate of total volatile solids; SVT_i = Solids content at the start; SVT_f = Solids content at the end.

Processing and data analysis

With the information obtained from the bioassays, the descriptive statistics, arithmetic average and standard deviation were calculated, and an analysis of variance (ANOVA) was carried

out, one way for the combination of treatments used (water salinity and specimen size). Prior to the ANOVA, the assumptions of normality, independence and homoscedasticity of the data were tested (Montgomery, 2004). The totality of the statistics analysis were carried out with the computational program R 3.0.2.

Conceptual design of secondary wastewater treatment system through the use of *Mytella charruana* species

After obtaining the results of removal of the BOD₅ and SVT, a conceptual design of a wastewater treatment system was carried out, taking into account the conventional theories about this type of treatment, using living organisms such as bivalves in one of its stages (Romero, 2008).

RESULTS AND DISCUSSION

Reduction of BOD₅ and total volatile solids

During the development of the bioassays, similar conditions of physicochemical variables were maintained in all aquariums; the average dissolved oxygen in the water, pH and temperature are shown in Table 2.

With regard to the reduction of BOD₅, there were no statistically significant differences between treatments (p -value > 0.5, Table 3), however, when analyzing the behavior of the average, it was observed that the treatment of individuals with smaller size maintained in high water salinity, reduced BOD₅, the smallest reduction of this variable (23.78 ± 16.37 %) occurred in the treatment that had the smaller specimens and low salinity of the water; however, BOD₅ was not significantly affected in any of the treatments where larger specimens were used (Figure 4).

Table 2. Average physicochemical variables during the trials.

Variable	Average	Standard deviation
Dissolved oxygen (mg/L)	4.80	0.07
pH	8.45	0.05
Temperature (°C)	27.44	0.09

Table 3. ANOVA for the reduction of BOD₅, in both treatments and control.

Source	Sum of squares	Degrees of freedom	Mean square	F	P- Value
Between groups	695.381	4	173.845	0,39	0.8122
Within groups	4031.83	9	447.981		
Total (Corr.)	4727.21	13			

Total volatile solids also showed no statistically significant differences between treatments (p -value > 0.05, Table 4); however, it was observed that the organisms of smaller size, subjected to high salinity, were those that

showed the greatest reduction of TVS (10.64 ± 6.27 %); contrary to the behavior of larger organisms in the same conditions of salinity (Figure 5), whose average reduction rate was 3.23 ± 0.23 %.

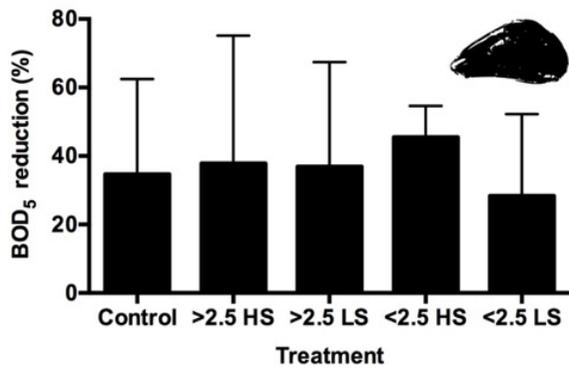


Figure 4. Comparison of the average reduction (%) of BOD₅, in each treatment, including control. <2.5: individuals with size less than 2.5 cm; > 2.5: individuals with a height greater than 2.5 cm; HS: High salinity; LS: Low salinity.

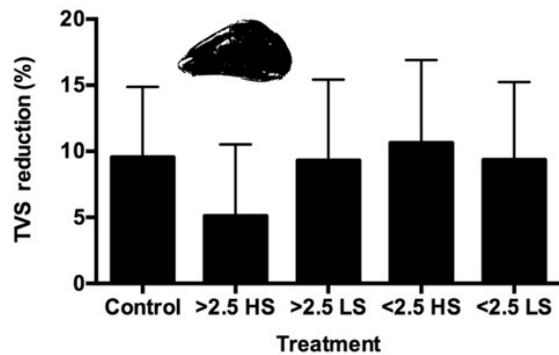


Figure 5. Comparison of average reduction (%) of total volatile solids (TVS) in each treatment, including control. <2.5: individuals with size less than 2.5 cm; > 2.5: individuals with a height greater than 2.5 cm; HS: High salinity; LS: Low salinity.

Table 4. ANOVA for the reduction of the TVS, in the treatments and the control.

Source	Sum of squares	Degrees of freedom	Mean square	F	P-Value
Between groups	90.5763	4	22.6441	0.71	0.06043
Within groups	286.357	9	31.8175		
Total (Corr.)	346.934	13			

Because *M. charruana* proved to be a species with the capacity to remove organic material present in water, at rates ranging between 23.78 % and 45.49 %, its use could be contemplated in systems of biofiltration of wastewater from industries dedicated to the production of food or organic compounds (Giffort, *et al.*, 2006), such as shrimp farms, fish farms, and companies producing organic fertilizers. Because in these cases it could remove a large part of the organic material that otherwise would reach the surrounding ecosystems (Dumbauld, Ruesink, & Rumrill, 2009; Thevenon, *et al.*, 2011; Gutierrez-Wing & Malone, 2006).

Taking into account the results, the use of organisms of smaller sizes (≤ 2.5 cm) is suggested to accelerate the removal process;

this situation is due to the fact that young bivalves have a much faster metabolism than adults (Bradley, 2009). In addition, better biofiltration results can be obtained, if organisms of less than 2.5 cm are used, maintaining a salinity in the water, equal to or greater than 25; since the reduction of salinity in the environment, generates in the specimens greater energy expenditure, to maintain osmoregulation processes (Shpigel, Avital & Jönsson, 2013).

Based on the analysis of the BOD₅ and TVS reduction values obtained in this research, the initial parameters were established to formulate the conceptual design of two devices that can be used for the treatment of wastewater from industries dedicated to food processing, that have problems due to the dumping of organic matter.

Conceptual design of the secondary wastewater treatment system through the use of the *Mytella charruana* species

Based on the results related to the behavior of the species *M. charruana*, with respect to the reduction of BOD₅ and TVS, two devices were designed that are proposed as new alternatives for the secondary treatment of wastewater.

Horizontal and vertical flow designs. The purpose of the designs of secondary wastewater treatment systems is the removal of organic load and volatile solids suspended in the body of water, through the use of five modules of aquarium-type

treatment, of rectangular geometry, where it is estimated that the totality of the modules will contribute with the removal of between 65 % and 85 % of the BOD₅ and the TVS. These designs will guarantee the continuous flow of water from one module to another, complemented by the principle of cascade flow, to ensure aeration and even distribution of water through a flow regulator, in each of these. In figures 6 and 7, the technical characteristics of the design of wastewater treatment systems are shown, making use of the bivalve *M. charruana*, which can be arranged in modules such as the one shown in Figure 8. The description of the components of both systems is summarized in Table 5.

Table 5. Description of the components of the conceptual design of wastewater treatment systems using *M. charruana*. *Not Applicable (NA)

Parameter	Value/Units	Structural proposal
Design flow	0,53 L/horur	NA
Residence time	15 hours	NA
Treatment modules	5 modules	Tempered glass, solid simple concrete and / or plastic
Module volume	8 L/module	NA
Module geometry	Base: 20 cm Longitude: 25 cm Height: 10 cm	NA
Mash	1 or 2 per module (Varies according to the efficiency you want to guarantee)	Fiberglass of low density, plastic meshes or artificial fabric fiber Metal supports in aluminum or plastic
Flow regulator	1 Gutter type regulator	PVC tube (polyvinyl chloride), chrome, aluminum or stainless steel
	4 Regulators adapted to the glass module, with holes 1 cm in diameter	Embedded in the holes of the glass module
Treatment Organisms	40 individuals/module Increase efficiency with 80 individuals/module	NA*
Final discharge	1 Channel	Simple concrete, polyvinyl chloride

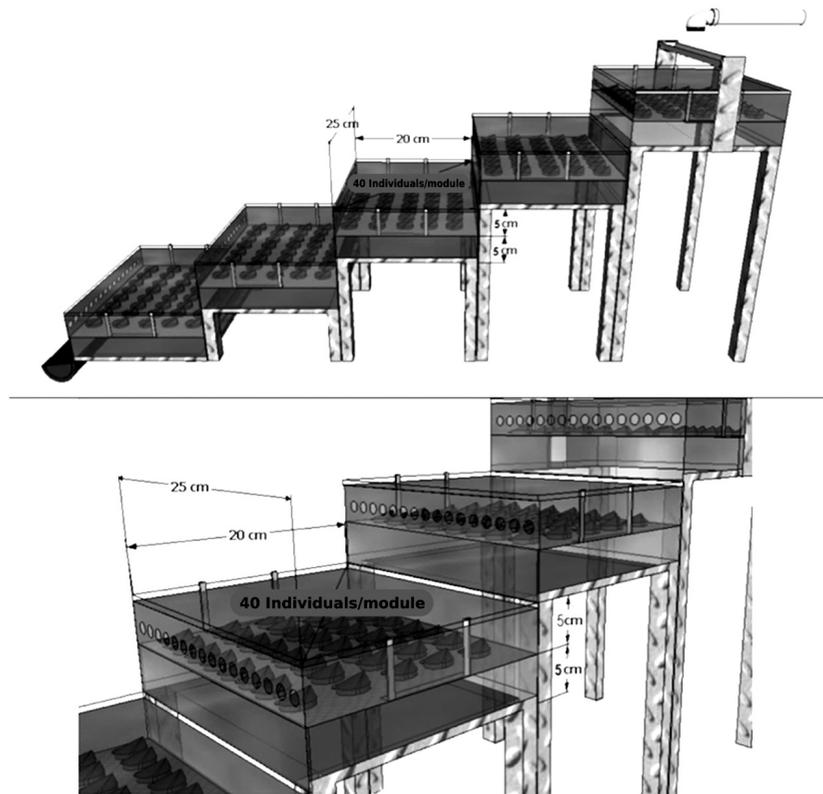


Figure 6. Conceptual design for the horizontal flow treatment system using bivalves.



Figure 7. Conceptual design for the vertical flow treatment system, with the use of bivalves.

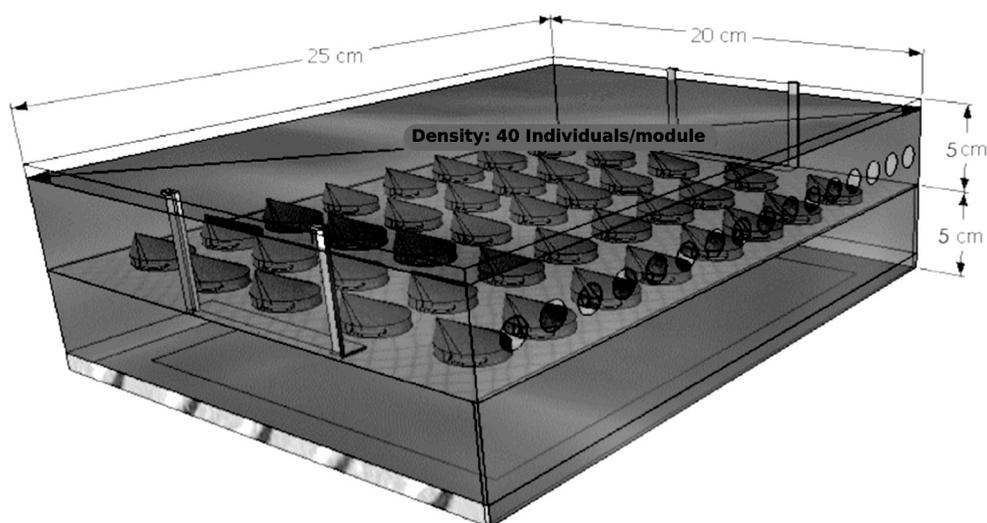


Figure 8. Module designed for the location of specimens in water treatment systems.

Comparisons of horizontal and vertical flow systems

In this part of the observation, it is possible to disaggregate, for example, that the implementation of the proposed devices depends, to a great extent, on the availability and use of space that each one demands. The system that operates horizontally requires more space, however, has lower height, which would reduce the use of pumping systems. On the other hand, the vertical design, having a greater height, would require a pumping system to drive water to the upper module; however, this system has a mechanism adaptable to any open or closed space.

As an essential consideration it is also important to indicate that the conceptual design of the wastewater treatment systems proposed in this research present similar characteristics among them, such as the design flow or flow rate to be treated, derived from the volume of water coming from the treatment modules, on which depends the residence time of the water and the contact with the specimens *M. charruana*. It is inferred that this last aspect is one of the critical parameters in the design of biological water treatment systems, because alterations in this parameter can significantly affect the removal efficiency of organic matter and volatile solids.

Each treatment system would consist of several units, similar to the aquarium-type modules used in the bioassays in this research, of rectangular type and with adaptable volume adaptable to the specific needs of the required treatment; likewise each treatment module would be equipped with a mesh for the adhesion of the specimens, held by metal supports at the ends of the module, to facilitate the cleaning and replacement of the organisms. In addition, a flow regulator (per module) is included in the design, which guarantees the continuous flow of the effluent that comes from the pre-treatment and that will then be filtered by the specimens. The final discharge structure would then monitor and control the parameters BOD_5 and TVS, in the final effluent.

Some artificial reactors have already been proposed to evaluate the biofiltration capacity of bivalve species such as *Tapes philippinerum*, and *Crassostrea gigas*; the first one allows to reduce between 57 and 97 % the concentration of chlorophyll present in the water, and between 64 and 97 % the turbidity; while the second species, can reduce between 69 and 93 % chlorophyll, and between 75 and 91 % turbidity (Shpigel, Avital and Eitan, 1997). For the removal of BOD_5 , from white shrimp farms, *Litopenaeus vannamei*, the species *Crasotrea rhizophorae* has been used, arranged in 50 L tanks, where it achieves a

maximum removal rate of 17.2 % of the BOD₅ (Ramos, Vinatea, and da Costa, 2008). When comparing these results with those found for *M. charruana*, it can be inferred that the species has a higher rate of removal of the BOD₅. Therefore, its implementation could be suggested in wastewater biofiltration processes, through the use of artificial devices such as the reactors proposed in the present investigation.

In addition, *M. charruana*, satisfies many of the established criteria for the selection of organisms with high application capacity in biofiltration processes, such as its high growth rate, relative sedentary lifestyle, high filtration capacity and wide range of resistance to changes of salinity (Giffor *et al.*, 2006).

As an alternative for handling organisms that have completed their cycle in the process of biofiltration, some authors suggest evaluating their potential use as organic fertilizers for direct application in agro ecological crops because of the dual role they can fulfill, as a source of nutrients and a regulator for the pH in the soil, due to the contribution of carbonates stored in its valves. However, prior to this application, it must be assured that these organisms contain a low concentration of critical pollutants such as heavy metals and poly-nuclear aromatic hydrocarbons, among others (Spångberg, Tidåker & Jönsson, 2013).

CONCLUSIONS

The results of this research allow us to conclude that the species *M. charruana* has a high potential for application in biofiltration processes of wastewater rich in organic matter and total volatile solids, reaching a greater efficiency when organisms with a size smaller than 2.5 cm are used, and water salinity greater than 25.

The species *M. charruana*, can be used in biofiltration processes by implementing horizontal or vertical flow devices, which help to save space and operability, constructed with low cost conventional materials, and easy to use.

It is necessary to implement studies aimed at the optimization of biofiltration by the species the pollutants evaluated, and other chemical elements

of environmental interest in order to expand the spectrum of application of the species in the field of environmental biotechnology.

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