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ENVIRONMENTAL AND SANITARY ENGINEERING

The use of Common Duckweed (*Lemna minor*) in the treatment of wastewater from the washing of sisal fiber (*Furcraea bedinghausii*)

INGENIERÍA AMBIENTAL Y SANITARIA

Lenteja de Agua (*Lemna minor*) para el tratamiento de las aguas residuales que provienen del lavado de la fibra de fique (*Furcraea bedinghausii*)

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Abstract

The production of sisal (*Furcraea Bedinghausii)* represents one of the main sources of income for many families in the different regions of Colombia. In the department of Cauca, around 12,000 families currently reap the rewards of sisal cultivation. Only 4% of the sisal leaf is actually used, the remaining 96% comprising juice and bagasse. Washing the harvested sisal is a stage in the transformation process that contaminates large volumes of water. This goes on to reach a natural water source, in this case the *Las Margaritas* river in Silvia, Cauca. With the above concern in mind, this study puts forward an alternative solution using Common duckweed (*Lemna minor*). Evaluation of the efficiency of the phytoremediation process on a pilot scale was carried out through the characterization of such physicochemical parameters as BOD_5, COD, TSS, nitrogen and phosphorus in the water. The variable used was hydraulic retention times (HRT), which were eight and twelve days. Results suggest that percentage removal by *Lemna minor* was highest with a HRT of eight days, reaching values of 79.6 % and 82.77 % for COD and BOD_5 respectively.

Keywords: *Lemna minor, biological treatment, natural wastewater treatment.*

Resumen

Una de las principales fuentes de ingreso de muchas familias de diferentes regiones de Colombia es la producción de fique (*Furcraea bedinghausii*), específicamente en el Departamento del Cauca, alrededor de 12000 familias se ocupan del cultivo del fique y se benefician del mismo. Actualmente sólo el 4 % de la hoja de fique es utilizada, el 96% restante corresponde a jugos y bagazo. Entre sus procesos de transformación se encuentra el lavado de la cabuya, actividad que contamina grandes volúmenes de agua que finalmente llegan a una fuente de agua natural, para este caso específico al río las margaritas en Silvia - Cauca. En este contexto, el presente estudio propone una alternativa de solución a este problema mediante el uso de la planta Lenteja de agua (*Lemna minor*). La evaluación del nivel de eficiencia en el proceso de fitorremediación a escala piloto se realizó mediante la caracterización de parámetros físico-químicos como DBO_5, DQO, SST, nitrógeno y fósforo en el agua. La variable considerada fue el tiempo de retención hidráulica (TRH), la cual fue de 8 y 12 días. Algunos resultados muestran que el porcentaje de remoción fue más eficiente con un TRH de 8 días, logrando valores para DQO y DBO_5 de 79,6 % y 82.77 % respectivamente.

Palabras Clave: *Lemna minor, tratamientos naturales de aguas residuales, tratamiento biológico.*

1. Introduction

It is widely known that water is one of the most important natural resources for human life, not only for consumption but for carrying out different industrial and agricultural activities. These very activities, however, are causing significant changes for the resource, resulting in a reduction in the quantity and quality of water available for humankind. According to a report by the United Nations in 2012, Colombia is ranked 24 in a list of 203 nations in water availability per capita. Although at first glance it would seem high on the list, it is troublesome because in the late twentieth century it was ranked Number 4 (Scientific and Technological Consultative Forum, A.C., 2012). This problem is largely due to agriculture, since it is one of the most widespread activities carried out in Colombia and since, according to studies, water consumption in this productive sector stands at 39,144 Mm^3/year (WWF, 2012). In Colombia, some agricultural processes, such as the processing of coffee and to an even greater extent, the washing of the fiber of sisal (*Furcraea bedinghausii*); local names include *fique, cabuya and penca*). The washing process demands large quantities of water. Cauca department is the leading sisal producer, with 9,430 hectares devoted to the production of 10,349 tonnes of the 23,959 tonnes produced nationally, some 43% of total production, with the greatest quantities in Cauca being grown in the municipalities of El Tambo, Silvia, Piendamo and Totoro (CONFIQUE, 2013). Sisal production involves everything from the sowing of the plant to extracting the fiber and is carried out by means of different steps. These correspond to the cutting, removing the spines, pulping, washing and drying. The process that causes greatest impact to bodies of water is the washing stage, since more than 1,000 liters of water are required to wash 126.5 Kg of sisal fiber (Dagua et al., 2008).

Regarding the use of floating plants for wastewater treatment, various phytoremediation studies have combined to establish the characteristics required by aquatic plants for use in wastewater treatment (Delgadillo et al., 2011). These include high productivity, high efficiency in removal of nutrients and pollutants, high predominance in adverse natural conditions and ease of harvest (Rodriguez et al., 2009). Studies carried out using *Lemna minor* show that it meets all of these requirements and because of this has been widely used in wastewater decontamination systems (Arroyave, 2004).

Sabine K. & al. (2003) conducted a pilot test in the laboratory on the performance of the duckweed plant in different types of wastewater in achieving nutrient removal, discovering it to be a promising macrophyte for this use. Furthermore, good results were observed in research using *Lemna minor* as having great potential for the removal of cadmium, selenium and copper in contaminated wastewaters with these elements (Zayed, 1998); likewise in the treatment of wastewater from pig farms in the Cauca Valley a decrease was reported in the biological oxygen demand along with a reduction in total suspended solids (Chara, 1998). For the removal of nutrients such as phosphorus, duckweed is able to efficiently reduce orthophosphates within short periods of between 2 and 8 days, according to a study under laboratory conditions (Obek & Hazar, 2002).

The main advantage of wastewater treatment systems involving aquatic plants as compared with conventional treatment systems is the low cost of construction and maintenance, as well as being simple to operate (Hidalgo & al, 2005). Up until now, despite searches of different scientific publishing media, no studies have been found that evaluate the use of duckweed as an alternative treatment for wastewater from washing sisal. Most studies focus on the physicochemical characterization of sisal waste water, as is the case of studies carried out by the Regional Autonomous Corporation of Cauca (CRC, from the Spanish acronym) and other studies that show that the juices from sisal processing are extremely toxic to fish and aquatic organisms (Martinez & Caicedo, 2002). With all this in mind, the present study proposes an alternative treatment for wastewater from sisal fiber washing using aquatic plants since, as mentioned above, they are considered as an efficient and economical alternative (National Planning Department, DNP, 2007).

To carry out the biological treatment, a pilot trial was conducted at a study site that took the wastewater from sisal washing in the village of Valle Nuevo in the municipality of Silvia (Cauca); the site was chosen because wastewater there is currently pouring into the Las Margaritas river, without any treatment, causing direct pollution, and some characteristics of eutrophication can be seen. The effect of hydraulic retention times (HRT) of 8 and 12 days on the efficiency of removal of biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total suspended solids (TSS), nitrogen and phosphorus was evaluated.

2. Methodology

To meet the project objective, a sisal fiber-producing farm was selected in the municipality of Silvia, Cauca, and a pilot plant (Figure 1) was built on-site. Beforehand, a physico-chemical characterization of the wastewater from the sisal washing process was carried out - a volumetric capacity that determined average discharge flow, pollutant load, and biodegradability index (COD/BOD5) to confirm whether or not the wastewater is biodegradable and can be subjected to a process of bioremediation using Common duckweed (*Lemna minor*). Sedimentation time was similarly determined, in order to consider a primary treatment in the pilot test assembly, specifically in tank 1 with a 1000 liter capacity (Figure 1), which would make it possible to control the presence of sedimentable solids present in this kind of wastewater, which eventually could be used in a composting process. Sedimentation efficiency was calculated using a sedimentability curve, shown in Figure 2, and applying Equation (1) (Perez, 2014),

$$E = 1 - C + \frac{Area\ of\ curve}{Sedimentation\ velocity} \qquad (1)$$

where: E: efficiency of removal and C: fraction remaining.

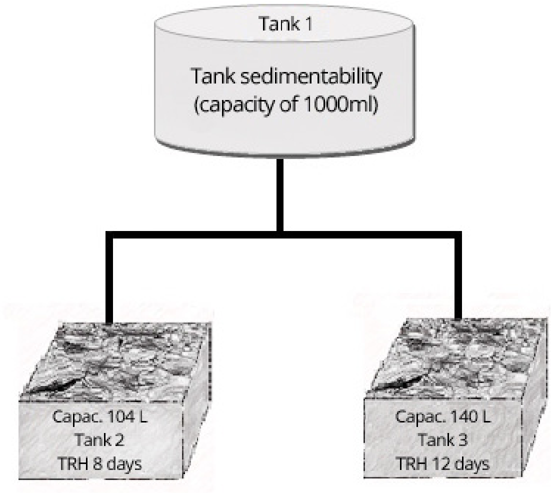

Figure 1. *Diagram of pilot plant.*

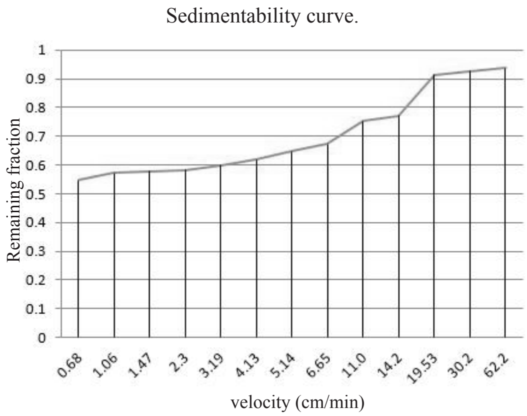

Figure 2. *Sedimentability curve.*

The values of sedimentation and fraction remaining desired to achieve a 60% removal were attained. A 60% removal exceed the requisites relating to permissable limits under Article 41 of Decree 1594/84. Then, settling time was calculated using Equation (2) (Pérez, 2014),

$$t\ settling = \frac{Htank}{Vs} \qquad (2)$$

where: H: Tank height and Vs: Sedimentation velocity.

Once the settling time was established, the flow rate to be used in the trial was determined. This was regulated by means of a constant head tank located in tank 1. The pilot test was implemented in the field and consisted of a 1000 liter tank into which the wastewater was deposited, acting as a sedimentor for a period of 45 minutes. A 1/2" pvc pipe was then fitted to the tank, through which the wastewater was transported with an inlet flow to each tank of 0.2 ml/s for a total volume of 69 liters (tank 2) and 104 liters (tank 3), according to the set HRTs of 8 and 12 days, determined by the authors taking account of the literature review that indicated those periods related to the greatest efficiency of the duckweed; the total capacity of tanks 2 and 3 was 104 and 140 liters respectively (tank 1: 30 cm wide, 70 cm long and 50 cm high; and tank 2: 40 cm wide, 70 cm long and 50 cm high). The dimensions were established according to the input and output flow. They were adjusted using a 1/2" pipe with a tap on the underside, for an output flow of 0.1 ml/s (Figure 1).

Once the pilot plant had been built with all the features mentioned above, planting of common duckweed (*Lemna minor*) in tanks 2 and 3 was carried out. Several conditions were necessary to enable the plant to grow well: water temperature between 5 °C and 30 °C, optimum pH between 4.5 and 7.5 (parameters which required only monitoring, since fique wastewaters fall within these ranges), and a mechanical control of plant growth in the removal of colonies to avoid eutrophication (Arroyave, 2004).

Following the sedimentation period, for each of the three samples taken, wastewater samples from the settling tank and from tanks 2 and 3 were collected, to establish the initial pollutant load of the water in the settling tank and the other tanks, according to the established HRTs. Using these values the removal efficiency for each treatment could be established. It should be pointed out that sisal fiber is produced, not on a daily basis, but whenever there is demand. For this reason, the replicate samples taken for sampling and measurement of the physico-chemical parameters in the pilot trial were separated by some 15 to 20 days. The performance of the system for each replicate sample was determined by the maximum HRT of 12 days.

Samples were taken at three sampling points - on the initial day of plant assembly and after 8 and 12 days.

Point 1: Outflow of wastewater from tank 1 (starting condition of wastewater)

Point 2: Outflow of wastewater from tank 2 (HRT of 8 days)

Point 3: Outflow of wastewater from tank 3 (HRT of 12 days)

The wastewater samples for the three replicates were collected in plastic containers and transported at 4 °C, in compliance with the protocol established for the collection and transport of samples in the standard method for the analysis of waters and effluents (APHA-AWWA-WEF, 2005). The physico-chemical analyses were carried out in the laboratory of the *Fundación Universitaria de Popayán* (FUP) and in order to check the veracity of the data obtained, these were taken to the certified laboratory of the CRC. The parameters to be evaluated were determined using respirometric, spectrophotometric, gravimetric and photometric measures: BOD_5 (SM5210B/SM4500-0G), COD (SM5220D, modified), ammonia nitrogen (SM4500-NH_3F modified), nitrites (NO_2 4500-SM-B), nitrates (SM 4500-NO_3 –B), phosphorus (SM 4500 Eg), SST (gravimetric), pH and dissolved oxygen (potentiometer) (APHA-AWWA-WEF, 2005). The results were analyzed using descriptive statistics. In the case of determination of pollutant load, it was calculated according to the regulations set out in Article 4 of Decree 3100 of 2003 for discharges into natural water sources, Eq. (3) (MAVDT, 2003),

$$Cc = Q \times C \times 0.0864 \times \frac{t}{24} \tag{3}$$

where: Cc: Pollutant Load (kg/day) Q: Average flow (l/s) C: Concentration of pollutant (mg/l), t: User discharge time (h), 0.0864 constant that indicates the unit conversion factor.

With regard to pollution load, efficiency of removal of pollutant load was calculated for each HRT setting, using Eq (4),

$$E = (So - S) / So \times 100 \tag{4}$$

where E: efficiency of removal of the system or one of its components (%) S: Outflow pollutant load (COD, BOD_5 or TSS) (mg/l), S0: inflow pollution load (COD, BOD_5 or TSS) (mg/l).

Percentage removal for nitrogen and phosphorus compounds were determined according to the provisions of Eq. 5

$$\%\ \text{Removal by Lemma minor} = \frac{(\text{Initial concentration} - \text{Final concentration})}{\text{Initial concentration}} \times 100 \tag{5}$$

3. Results and discussion

According to pollutant load data obtained (Table 1), it can be seen that each parameter sampled in the sisal wash wastewater from the pilot test exceeds the permissable discharge limits established by Article 8 of Resolution 0631 of 2015 (MINAMBIENTE, 2015) on natural water sources. Thus, the direct discharge of such wastewater would end up directly affecting the *La Margarita* river that is the end receiver of the water. The standard deviation found indicates little variation in the reported values with respect to the mean, i.e. the differences are not significant and therefore the results are reliable. It was further established that it is feasible the use Common duckweed as a biological alternative for treating sisal wastewater since the biodegradability index, in all cases, is greater than 0.5 (Table 2), indicating predomination of the presence of organic contamination, biodegradable in nature (Orozco et al., 2005).

Table 1. *Wastewater pollutant load.*

Parameter	Tank 1	Pollutant load Tank 1 (Kg/day)	Tank 2	Pollutant load Tank 2 (Kg/day)	Tank 3	Pollutant load Tank 3 (Kg/day)	Maximum permissable according to Resolution 0631/2015	Standard deviation
BOD_5 (mg/l)	1815.5	0.0627	1246	0.0108	1264	0.0119	200	0.0297
COD (mg/l)	2682.5	0.0927	2189.5	0.0189	2027	0.0175	400	0.0430
TSS (mg/l)	144	0.0049	64.6	0.0006	95	0.0008	50	0.0024
Flow rate (ml/s)	0.4		0.1		0.1		N.A	

Table 2. *Biodegradability index.*

Parameter	Tank 1	Index	Tank 2	Index	Tank 3	Index	Standard deviation
BOD_5 (mg/l)	1815.5	0.68	1246	0.57	1264	0.68	0.0635
COD (mg/l)	2682.5		2189.5		2027		

3.1 Physico-chemical analysis of wastewater

The data obtained from the analysis of BOD_5, COD and nutrients in the outflows of tanks 1, 2 and 3 are presented in Table 3.

The information provided in Table 3 indicates that high values of BOD_5 (1,815.5mg/l) and COD: (2,682.5 mg/l) were found in tank 1, causing redox processes and degradation of organic matter, possibly the reason why the dissolved oxygen value is in hypoxia conditions at 3.35 mg/l, reflected in such organoleptic aspects as undesirable color and odor. Once the biological treatment with aquatic plants is undergone, it can be seen in tank 2 (8 days HRT) that the values of BOD_5 and COD decrease from 1,815.5 mg/l to 1,246 mg/l and from 2,682.5 mg/l to 2,189.5 mg/l respectively. In addition, dissolved oxygen increases from 3.35 mg/l to 8.25 mg/l, indicating a decrease in the consumption of oxygen generated by processes of oxidation of nitrogen and organic matter. At this point, taking into account the values obtained in pollutant load, a percentage removal of 82.77% was achieved for BOD_5 and 79.6% for COD. In the tank 3 outflow, values measured for BOD_5 and COD were 1,264 mg/l and 2,027 mg/l, respectively, so that a percentage removal of about 81% was achieved for BOD_5 and COD; it could be seen that the percentage removal for HRT of 12 days was not significantly better compared to HRT at 8 days (Tank 2), given that the values of BOD_5 and COD began to increase again, while dissolved oxygen values fell again, dropping from 8.25 mg/l in tank 2 to 6.35 mg/l in tank 3. This was due to the duckweed suffering an increase in mortality of 80%, decreasing photosynthetic reactions and increasing dissolved oxygen consumption due to organic matter decomposition processes. This can be avoided by mechanically carrying out plant growth control by means of harvesting and thus avoiding alteration in the results at longer HRTs.

It should be taken into account that to eliminate nutrients such as nitrogen, the combination of two steps must be undergone: aerobic-anoxic and aerobic-anaerobic, where aerobic, anaerobic and anoxic processes (Ferrer & Seco, 2013) are involved. Table 4 shows a comparison of ammonia nitrogen, nitrites and nitrates with respect to the treatment applied. In addition, low standard deviation values indicate that the reported data are meaningful and reliable.

Table 3. *Average values of BOD_5, COD and dissolved oxygen.*

Parameter	Tank 1	Tank 2	% removal Tank 2	Tank 3	% removal Tank 3	Standard deviation
BOD_5 (mg/l)	1815.5	1246	82.8	1264	81.0	1.2728
COD (mg/l)	2682.5	2189.5	79.6	2027	81.1	1.0607
Dissolved oxygen (mg/l)	3.35	8.25		6.35		2.4705

Table 4. *Average values of ammonia nitrogen, nitrites and nitrates.*

Parameter	Tank 1	Tank 2	% removal Tank 2	Tank 3	% removal Tank 3	Standard deviation
Ammonia nitrogen (mg/l)	1.255	0.2	84.06	0.92	26.69	0.5391
Nitrites (mg/l)	0.125	0.015	88	0.02	84	0.0621
Nitrates (mg/)	16.6	15.65	5.72	14.2	14.45	1.2086

Removal of the nitrogen present in the wastewater was carried out by processes of nitrification and denitrification. It could be seen that values of ammonia nitrogen and nitrites are low in tanks 2 and 3, as they ought to show levels below 0.1 and 0.2 mg/l respectively according to Decree 3930 of 2010 (MAVDT, 2010). The concentration of ammonia nitrogen in the wastewater prior to treatment was above 1 mg/l, indicating that this aquatic environment contained contamination of organic origin (Tabares, CA, 2011). According to the data presented in Table 4 it can be seen that the ammonium and nitrite is readily biodegradable and the aquatic plants consume these nutrients in tank 2, generating a percentage removal of ammonium of 84.1% and of nitrites of 88% at 8 days of HRT; in tank 3, in which the same conditions as tank 2 are found but at 12 days of HRT it can be seen that the data for ammonium and nitrite, at 0.92 mg/l and 0.02 mg/l respectively, are higher compared to Tank 2, which may indicate that in tank 3 between 8 and 12 days the plants no longer assimilate the quantity of nitrogen nutrients present in the wastewater because they already reached their peak of cell growth and retention. It is important to consider the presence of nitrate in the water because the highest concentrations at 10 mg/l can cause diseases in infants, such as metahemoglobinemia (Sierra, 2011). In both treatment systems, high nitrate concentrations are found, indicating that the denitrification process is not being undergone, inhibited by the presence of oxygen as it is used as an electron acceptor before the nitrate and consequently inhibits the processes of release of phosphorus a nutrient that is required for plant development and on not being found in sufficient quantities in the system, would be a possible reason why mortality increased in the Lemna minor. (Ferrer, 2013).

Considering the results for phosphorus (Table 5), it can be seen that tank 1, the sedimentation tank where no treatment has yet been implemented, gives a value of 3.26 mg/l. Once the treatments are carried out in tank 2 and 3, a percentage removal of 57.8% and 58.2% was achieved, respectively; efficiency was not higher than that, possibly due to high values of nitrate found that prevent anaerobic conditions so that the processes of nitrification and denitrification required for the biological phosphorus removal were not able to reach completion (Ferrer & Seco, 2013).

The TSS show a drastic change from point to point (Table 6), considering that in tank 1, where a sedimentation treatment was undergone, an average value of 144 mg/l was obtained. After carrying out the biological treatment, in tank 2, an average of 64.6 mg/l was obtained, with a removal efficiency of 88% in relation to the pollutant load. Meanwhile, in tank 3 an average value of 95 mg/l was obtained, with an 83.8% removal. Based on these results, the HRT of 8 days was more efficient than that of 12 days.

***Table 5.** Average values of phosphorus and pH.*

Parameter	Tank 1	Tank 2	% removal Tank 2	Tank 3	% removal Tank 3	Standard deviation
Phosphorus (mg/l)	3.26	1.375	57.8	1.36	58.2	1.0927
pH	5.24	4.95		6.92		1.0636

***Table 6.** Average values of total suspended solids.*

Parameter	Tank 1	Pollutant load (Kg/day)	Tank 2	C Pollutant load (Kg/day)	% removal Tank 2	Tank 3	Pollutant load (Kg/day)	% removal Tank 3	Standard deviation
TSS (mg/l)	144	0.0050	64.6	0.0006	88.8	95	0.0008	83.8	0.0025

4. Conclusions

Common duckweed (*Lemna minor*) is a species that can be considered as an alternative for use in the biological treatment of wastewater from washing sisal, given that such water has biodegradable organic pollutants and that a pollutant load exceeding the permissible values for environmental regulations (Dec. 3930/2010) is being reported. The results showed that the best removal percentages for BOD_5, COD and TSS were obtained with a HRT of 8 days, taking into account that it is necessary previously to subject the wastewater to a primary treatment such as sedimentation. HRTs of 12 days may result only in similar performance percentages, so that it is unnecessary to extend these times should no greater efficiency in removal be guaranteed.

Although the duckweed is able to take advantage of the presence of nutrients such as nitrogen and phosphorus as a food source, is important to note that the presence of high levels of nitrates does not allow anaerobic conditions to take hold and may thus prevent an even more substantial removal of the phosphorus.

Biological treatment using *Lemna minor* is an alternative that can be beneficial for the farmers who process the sisal plant, from an economic and especially an environmental point of view, since it would minimize the high pollutant loads that this type of wastewater discharges into the various water sources. In addition, on completing the removal processes, the duckweed can also be used as fertilizer given the amount of nutrients that it would have been able to absorb.

It is recommended that other trials are carried out with Lemna minor, taking into account different variables such as climatic conditions and altitude, with the aim of verifying the efficiency of the plant as an alternative treatment for wastewater from the washing of sisal, due to the wide range of temperatures (5-30 °C) that it has in adaptation and functioning. It is important to note that hydraulic retention times do not require to exceed eight days, since it was observed in the pilot test that after this time, the duckweed does not show any substantial efficiency.

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