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Research Article

Municipal Wastewater Treatment using Fluidized bed Bioreactors with Biofilms Fixed on Natural Supports Derived from Cacti

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In recent years, the population in Western Guatemala has grown, increasing the demand for water and basic services, alongside a rise in waste production. Notably, wastewater generation has had a significant environmental impact on water bodies, leading to eutrophication and other harmful effects that negatively influence the quality of life and health of people. This project focused on assessing the technical feasibility of using fluidized bed bioreactors under aerobic and anaerobic conditions, employing natural supports derived from cactus materials native to Guatemala as an improvement over conventional plastic support. The research was divided into four stages: 1. Characterization of wastewater from the basin of interest. 2. Collection, treatment, and characterization of two cactus materials (*Stenocereus* spp. & *Opuntia* spp.) to be used as supports in the fluidized reactors. 3. Evaluation of treatment in fluidized bed bioreactors under aerobic and anaerobic conditions. 4. Development of mathematical models to describe the experimental data. The main results of the research included the removal of chemical oxygen demand (COD) in synthetic wastewater, achieving a range of 50% to 63% using fluidized bed reactors. During the research period, the Samalá River showed average COD values of 96.38 and 277. Biological oxygen demand (BOD) averaged 68.38 and 129.40. Nitrogen levels averaged 2.16 and 17.40. Phosphorus levels averaged 3.55 and 11.11. The most efficient natural support and treatment method was *Stenocereus* spp. with aeration, achieving a 86.76% reduction in COD. The reaction exhibited zero-order kinetics with an equation of $K = -1.75 \cdot 10^{-4} \pm 0.15$.

Keywords: Wastewater, bioreactor, biofilms, cacti, aerobic, anaerobic

1 Introduction

It is estimated that 99% of Guatemala's surface water bodies are polluted (INSIVUMEH, 2017; IARNA-URL, 2012). This has serious implications for the quality of life of the population due to exposure to disease risks. In response to mitigating environmental pollution, environmental policies such as the National Water Policy (2011), Governmental Agreement 236-2006, and similar legislation have been implemented to promote improvements in surface water quality in the country.

Guatemala has three major watersheds: the Pacific watershed, the Caribbean Sea watershed, and the Gulf of Mexico watershed. Within the Pacific watershed lies the Samalá River basin, which extends across 35 municipalities in five departments of Guatemala. The Samalá River is a naturally occurring, permanently flowing body of water with a short current. It originates in the departments of Quetzaltenango and Totonicapán in the Sierra Madre, flows through the southwestern region of the country, and empties into the Pacific Ocean. The Samalá River basin is divided into three sub-basins:

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lower, intermediate, and upper, with the upper basin being the largest.

Additionally, the upper basin is highly relevant for the country as it contains Guatemala's second most important city, Quetzaltenango, as well as the municipality of Zunil, one of the country's largest producers and exporters of vegetables. According to the Municipal Development Institute (INFOM), most municipalities lack wastewater treatment plants. Those that do have them are not all operational, and the majority lack a biological treatment process.

A study characterizing domestic effluents determined the average concentrations of the following parameters: temperature 22.6 °C, pH 7.44, color 403.7 (UC), BOD_5 253.6 $mg.L^{-1}$, COD 422 $mg.L^{-1}$, total solids 640.8 $mg.L^{-1}$, suspended solids 282.8 $mg.L^{-1}$, total nitrogen 40.5 $mg.L^{-1}$, total phosphorus 11.6 $mg.L^{-1}$, total coliforms 9.7×10^{11} MPN.100 mL^{-1}, fecal coliforms 6.8×10^{11} MPN·100 mL^{-1}, with a BOD_5/COD ratio of 0.57 (Peralta Salgado, 2015). These parameters exceed the maximum levels established by Governmental Agreement 236-2006.

The need for wastewater treatment is undeniable, both for improving human health and reducing environmental impacts. It is essential to implement wastewater treatment processes for domestic discharges in Guatemala to purify effluents and mitigate potential environmental effects. The most widespread wastewater treatment processes are biological, of which two historically stand out: the traditional aerobic activated sludge process and the anaerobic biodigestion process. The difference between these methods is that the former uses oxygen in the consumption of organic matter, while the latter does not – causing a significant difference in treatment time and residual sludge generation.

Therefore, in recent years, there has been increased research into the development of treatment technologies that can degrade both ordinary loads and recalcitrant pollutants while remaining cost-effective. One such technology is the fluidized bed reactor (FBR), which uses biofilm reactors fixed on carriers that remain "free" within the reactor, either in aerobic or anaerobic conditions. The freedom of movement of these carriers presents several advantages compared to conventional biofilm reactors on fixed supports (Alfredo et al., 2013; Pen & Jose, 2008).

The FBR technology has been demonstrated to be effective (Tisa et al., 2014) as a biological process (Zou et al., 2016). Some characteristics of FBR include low operating cost (Ahmadi et al., 2015), high resistance to system alterations (Brackin et al., 1996), high mass transfer rates, and uniform mixing (Andalib et al., 2014). Many researchers have studied FBR applications in wastewater treatment, particularly in advanced oxidation processes (AOP) and biological processes (Lin et al., 2010). Although AOP applications for FBR are relatively new, large-scale FBR systems have been widely used in biological wastewater treatment.

The findings of this research will have a national impact on discharges into receiving bodies, as it will provide characterization data for wastewater in a vulnerable basin like the Samalá River Basin and evaluate innovative biotechnology solutions to determine adequate and relevant treatment options.

2 Material and methods

2.1 *Wastewater Characterization – Sampling and Composition*

Sampling was conducted at the unification point between the Xequijel River and the Samalá River, located in the jurisdiction of Cantel, Quetzaltenango, where the Quetzaltenango City Wastewater Treatment Plant is planned to be built. The approximate coordinates are 14° 50′ 38.9" N 91° 28′ 49.0" W. This point is easily accessible from CUNOC.

In situ parameters were verified, and composite sampling was carried out over 8-hour periods using an automated American Sigma 1,350 device. Subsequently, the samples were transported, and aliquots were preserved for analyses that do not need to be conducted immediately.

Samples were taken over three different days at 10-minute intervals for 4 hours to ensure greater representativity of the water. Analysis was conducted on 10 parameters, including biochemical oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen (Ntot), total phosphorus (Ptot), settleable solids, color, pH, turbidity, nitrites, nitrates, and total suspended solids. The following standard methods were used for analysis: SM 5210-D, SM 5220-D, SM 4500-NO3-E, SM 4500-NO2-B, SM 3540F, SM 2120(3-200), SM 4500H+, SM 2130, SM 4500E, SM 4500B, and SM 2540-D.

As part of the statistical analysis, mean and standard deviation were calculated. The main sources of error include variations in river water due to rainfall, random methodological errors, and human error.

2.2 *Collection and Treatment of Natural Supports for Biofilm Fixation*

The methodology consisted of identifying sites where cactus species were found in semi-arid areas of municipalities in Baja Verapaz, as well as collecting samples. For this, leather gloves, machetes, and storage sacks were used.

The results are described as follows: The coordinates of collection sites were recorded in case additional samples of the same material were needed. Each collected sample was identified, indicating the collection site, type of plant, collected plant part, collection date, sample number, and other relevant data.

The arms, which have a cylindrical structure, were first brushed to remove most of the solids that are not part of the structure itself. Then, they were cut into small pieces of approximately 7 cm in length and 1 cm in width. Following this procedure, the pieces were washed, soaked for one week, rinsed again, and boiled in distilled water. Subsequently, they were sterilized in an autoclave at 121 °C with a pressure of 0.1034 MPa.

After this process, the pieces were dried in a forced convection oven at 100 °C for 8 hours, handled with tongs to prevent contamination. Before use, they were weighed after drying. The mass/volume ratio for the bioreactors was determined experimentally. Before being put into operation in the reactors, the pieces were rehydrated for three days with distilled water to prevent absorption of synthetic experimental wastewater. After the three-day period, any unabsorbed water was decanted. In this way, the reactors will be ready for batch or medium-term tests. This methodology was based on Martínez (2008).

2.3 Composition of Synthetic Wastewater

In the study, two types of bioreactors were used, which are the following:

Upflow Anaerobic Sludge Blanket (UASB) Reactor has a medium efficiency; organic load removal with low sludge production; higher efficiency with "packing" (material for biofilm growth); low energy demand (though it operates better at temperatures above 30 °C), and sensitive to sudden changes.

On the other hand, the Fluidized Aerobic Reactor has a high efficiency; Organic load removal; greater efficiency with "packing" (material for biofilm growth); high energy demand for aeration, and adaptable to sudden changes.

The experiments were conducted using materials obtained in the second stage, specifically cactus pieces that were cut, washed, sterilized, and rehydrated. The mass/volume ratio of the bioreactor was determined experimentally.

The bioreactors were inoculated with 1 litter of activated sludge from a treatment plant located in San José Chacayá, Sololá. Additionally, synthetic wastewater from the previously indicated formula was used. The supports and synthetic wastewater were gradually added and operated under these conditions for one week. The wastewater was mixed with the inoculum water in increasing proportions to allow gradual acclimatization, with controlled retention times using peristaltic pumps. Daily sampling was performed during the acclimatization phase, by determining COD. When a stable COD removal was verified, the bacterial population was considered acclimated, and short- and medium-duration tests began.

The controlled variables were: Hydraulic Retention Time (20 hours); Organic Load (1 and 2 times the concentration obtained from synthetic wastewater); Temperature (controlled at 30 °C using a water bath) and Support Mass/Residual Water Volume Ratio (approximately 1 : 10 volume ratio).

The measured parameters (for both inflow and outflow) were: Chemical Oxygen Demand (COD); Biochemical Oxygen Demand (BOD); Nitrogen; Phosphorus, and Suspended solids.

Each experimental condition was intended to last at least 30 days. However, shorter periods were used due to time constraints and because the bacterial inoculum from the treatment plant was highly active.

2.4 Mathematical Modeling of Dynamic Treatment Processes

The generated data was statistically analyzed to determine significant differences in process efficiency based on the variables proposed in Stage 3. Mathematical modeling theories were applied to the data generated from the operation of the bioreactors, and calculations were performed using the Microsoft Excel data analysis package.

3 Results and Discussion

3.1 Water Quality of the Samalá River

Through Table 1 (Point A) and Table 2 (Point B), the physicochemical parameters of the points established in the research are presented. Additionally, the mean and standard deviation are provided, showing an average chemical oxygen demand (COD) of 96.38 with a standard deviation of 63.08.

Table 1 Point A. Samalá River

Date	COD (mg.L^{-1})	BOD (mg.L^{-1})	N_{tot} (mg.L^{-1})	P_{tot} (mg.L^{-1}))	Settleable solids (mL.L^{-1})	Color (UPtCo)	pH	Turbidity (NTU)	Nitrite (mg.L^{-1})	Nitrate (mg.L^{-1})	Total suspended solids (g.L^{-1})
13/08/2021	175	105	1.0	4.30	2.5	4,180	7.57	488	0.06	3.0	0.928
19/08/2021	173	103	1.4	8.95	4.5	5,290	7.68	594	0.105	2.0	1.096
26/08/2021	50	40	2.0	1.88	0.5	528	7.66	96.8	0.086	0.0	0.146
9/09/2021	40	36	2.4	1.70	1.0	485	7.54	101	0.0064	3.8	0.271
20/09/2021	135	80	0.8	4.50	0.5	171	7.47	14.3	0.0039	0.0	0.040
24/09/2021	50	40	1.0	2.55	0.4	293	7.63	27.8	0.051	1.1	0.080
1/10/2021	20	20	2.1	1	0.7	295	7.56	60.4	0.124	1.1	0.119
29/03/2021	128	123	6.6	4.15	–	–	–	–	–	–	0.076
Average	96.38	68.38	2.16	3.55	1.44	1,606.00	7.59	197.47	0.06	1.57	0.38
Std.Dev.	63.08	39.02	1.89	2.72	1.53	2,164.79	0.07	238.82	0.05	1.45	0.44

Table 2 Point B. Xequijel River

Date	COD (mg.L^{-1})	BOD (mg.L^{-1})	N_{tot} (mg.L^{-1})	P_{tot} (mg.L^{-1})	Settleable solids (mL.L^{-1})	Color (UPtCo)	pH	Turbidity (NTU)	Nitrite (mg.L^{-1})	Nitrate (mg.L^{-1})	Total suspended solids (g.L^{-1})
9/09/2021	185	80	13.6	9.6	2	1,069	6.14	171	0.0082	2.6	0.928
20/09/2021	40	20	15.9	16.95	0.5	685	6.78	80.7	0.004	3.6	0.133
24/09/2021	581	250	21.3	11.25	0.7	650	7.13	63.3	0.01	1.2	0.111
1/10/2021	294	100	20.4	8.3	1	806	6.89	80.9	0.007	3.6	0.232
29/03/2022	285	197	15.8	9.46	–	–	–	–	–	–	0.253
Average	277.00	129.40	17.40	11.11	1.05	802.50	6.74	98.98	0.01	2.75	0.33
Std.Dev.	198.37	92.72	3.30	3.43	0.67	189.82	0.42	48.72	0.00	1.14	0.34

3.2 Batch-type Runs

In Fig. 1, the following results were observed: the sample started with a COD of 1208. 350 mL of the stock solution was placed in six flasks to be treated differently. The blue bar represents the initial parameter of the stock sample. Literal I underwent an aerobic treatment without a wood-based substrate, resulting in a COD of 279. Literal II received an anaerobic treatment without a substrate, reducing COD to 482. OA and OB were treated with *Opuntia* spp. substrate under aerobic and anaerobic conditions, increasing COD to 2,173 and 1,957, respectively. TA and TB were treated with *Stenocereus*

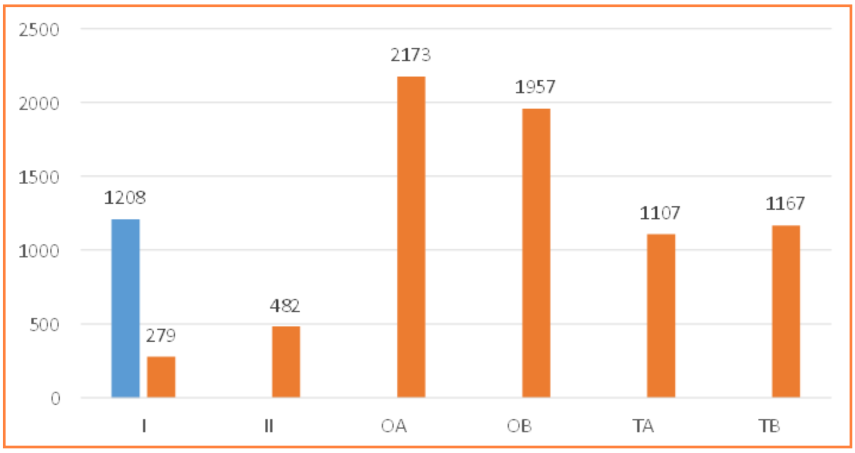

Figure 1 Comparison of COD behaviour in the different treatments. Run 1

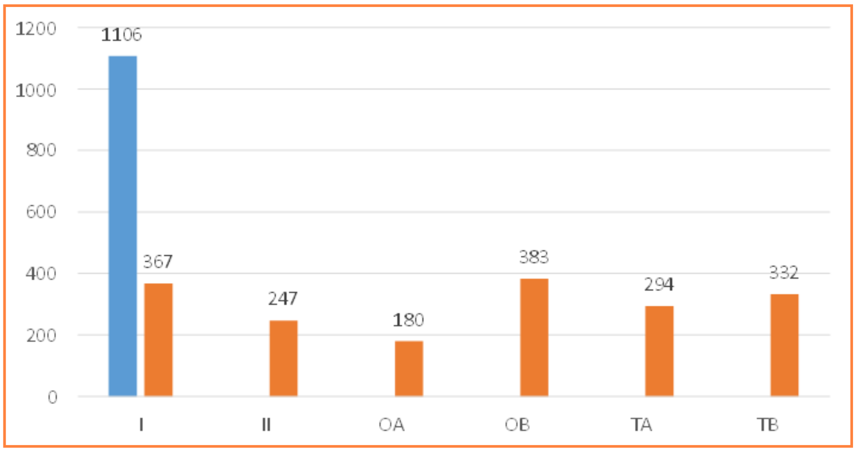

Figure 2 Comparison of COD behaviour in the different treatments. Run 2

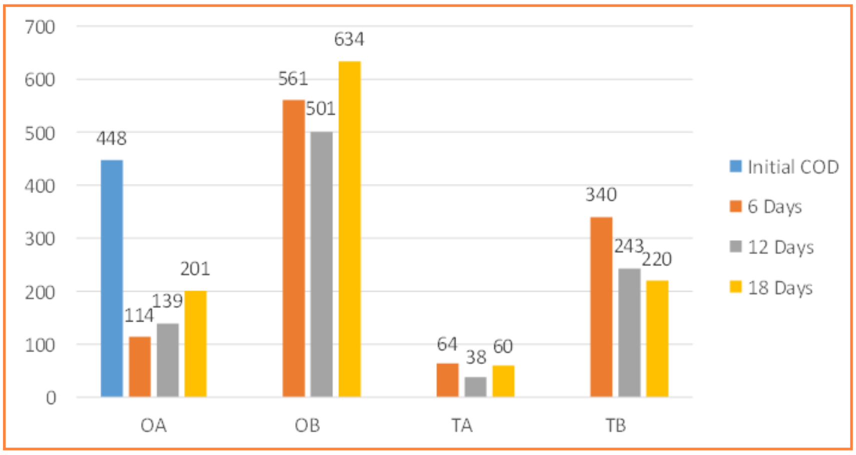

Figure 3 Comparison of COD behaviour in the different treatments
Runs from 26/11/21 to 14/12/22

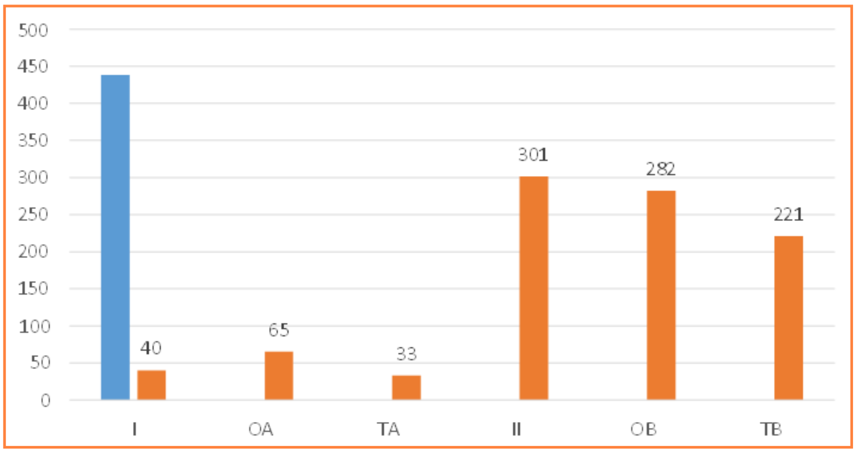

Figure 4 Comparison of COD behaviour in the different treatments
Run for March 4th

spp. substrate under aerobic and anaerobic conditions, and after one week of treatment, COD was reduced to 1,107 and 1,167, respectively. In the samples where substrate was used, its addition contributed resin to the system, which influenced the COD results. In subsequent tests, the substrate was washed to avoid the presence of resin in the system.

Fig. 2, shows the following results; the sample started with a COD of 1,106. 350 mL of the stock solution of synthetic wastewater was placed in six flasks to be treated differently, following the same treatment methods as in the previous run. The treatment that showed the highest COD reduction was the aerobic treatment, both with *Opuntia* spp. and *Stenocereus* spp. substrates. When comparing substrates, *Opuntia* spp. showed better performance in COD reduction under aerobic treatment, while *Stenocereus* spp. performed better under anaerobic conditions.

In Fig. 3, the following results were noticed: when conducting a longer-duration test under the same conditions, it was observed that the substrate with the best performance in COD reduction was *Stenocereus* spp., both in aerobic and anaerobic conditions. However, a phenomenon occurred at 18 days where the COD increased again. In the samples, particles detached from the wood used as a substrate were observed, especially in those where *Opuntia* spp. was used. Over time, this substrate begins to release wood particles, which could be the reason for the increase in COD after 18 days. Similar results were observed in Fig. 4.

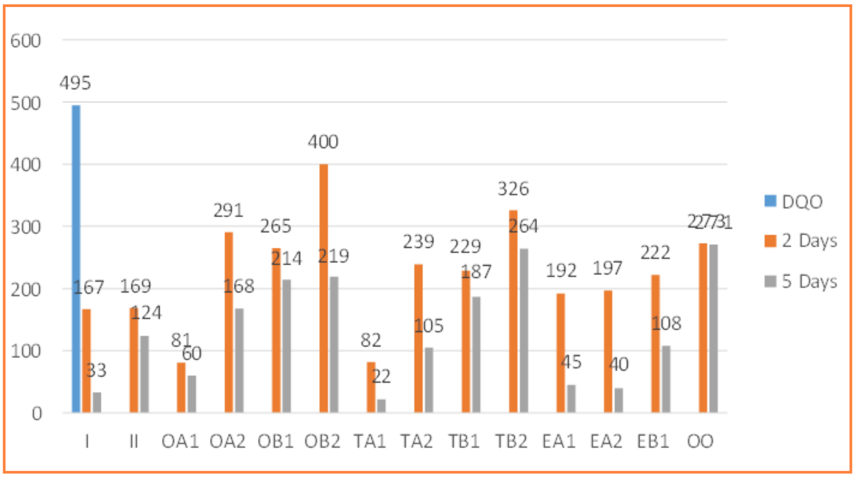

Figure 5 Comparison of COD behavior in the different treatments
Runs for 10/03/22 to 15/03/22

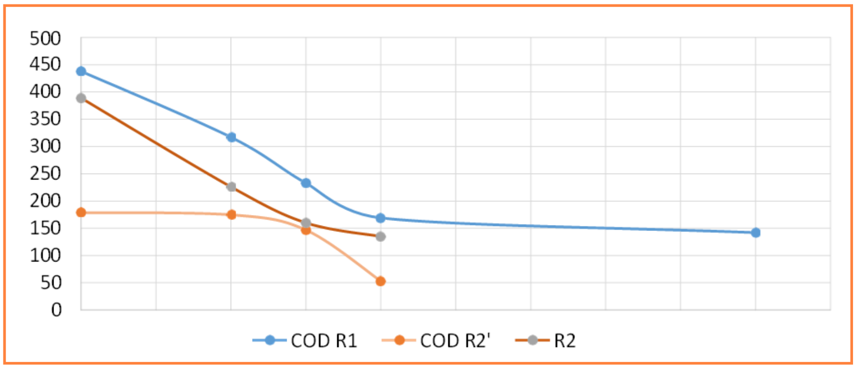

Figure 6 COD concentration
% COD removal in 4 days; total = 63.4%; dissolved = 70.4%

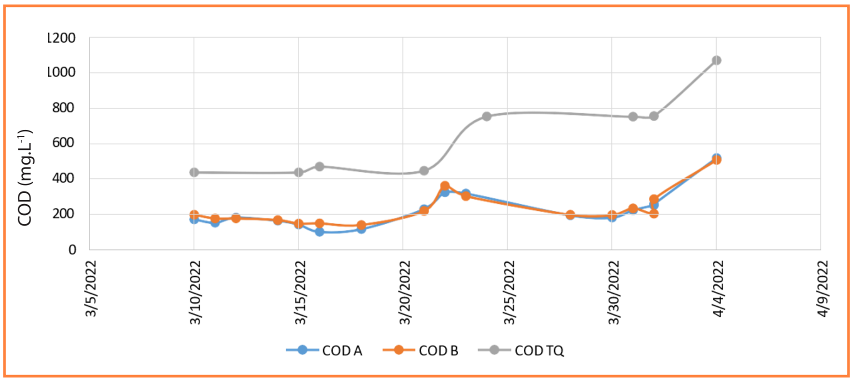

Figure 7 Organic material removal

Lastly, in Fig. 5 where the experiment was repeated once more, but in duplicate; the results were similar, and the aerobic *Stenocereus* spp. exhibited a greater reduction than the rest. However, the samples in which the substrate had been used multiple times showed a greater COD reduction after treatment. Based on these results, it can be concluded that *Stenocereus* spp. under anaerobic conditions was the most efficient treatment and that the efficiency of the treatment was affected by repeated use of the substrate, possibly due to bacterial growth on its walls.

3.3 Long-term Reactors

After performing the batch tests, two types of reactors in anaerobic and aerobic conditions (UASB and FBR) were assembled in a laboratory scale to perform a longer duration. Tests included two parallel runs, including one reactor of each type without any packing (identified as A) and another that used the prepared cacti as packing for biofilm growth (identified as B). Figs 6 and 7 show the removal of organic material, COD and BOD, respectively, for reactors in UASB conditions, an experiment that ran for 28 days. Similar performance was observed in removal of COD, which was of about 62% and 60%, for A and B reactors, respectively. Removal of BOD, is higher in A, corresponding to approximately 57% and B of around 50%. This could be due to the release of some material still in the packaging, as it is a biodegradable material.

Figs 8 and 9 show the concentration of total phosphorus and total nitrogen, as nutrients, in both the inlet and outlets of both reactors. In summary, little to no removal was determined to happen. This is expected and due to the necessity of a combination of processes to achieve the removal of either of these nutrients. For these systems, a combination

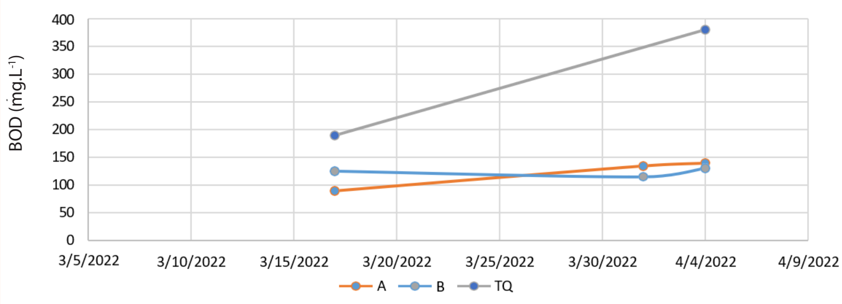

Figure 8 Total phosphorus concentration
removal of N and P nutrients

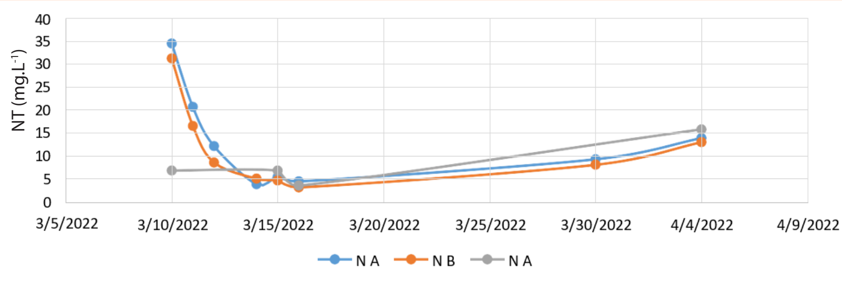

Figure 9 Total nitrogen concentration
low removal of N and P

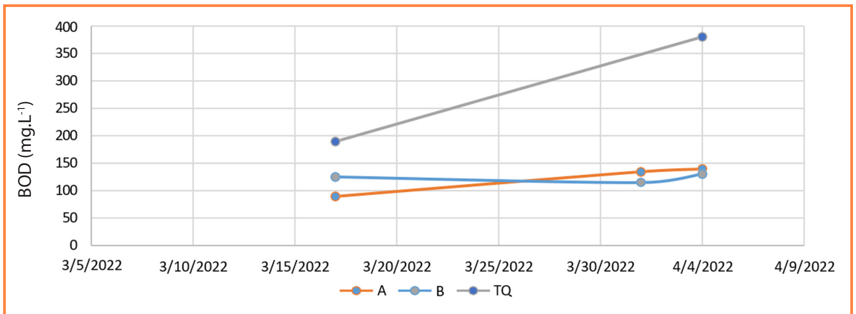

Figure 10 BOD concentration
removal of N and P nutrients

of aerobic, anaerobic, and anoxic environments is required. Possibly, only a percentage of the particulate nutrients are removed. The initial peak in both graphs may be due to the adjustment phase and the release of dead biofilm.

Fig. 10 shows the removal of COD by the aerobic reactor, which in general achieved a 63.4% for total COD and 70.4% for dissolved COD. The experiment had to be stopped at this point due to technical constraints, but it was possible to determine the reaction rate of this reactor, which is $K = 1.75 \cdot 10^{-4} \pm 0.15$ for a zero-order kinetic model.

The results obtained show consistency when compared to behaviours described in the literature, particularly the findings of Ehlinger et al. (1987), in which biofilm reactors were found to be highly stable but required a long maturation process. This can be corroborated in the various graphs, where the initial runs did not show significant reductions and, in some cases, even increases in certain parameters due to the presence of resin from the wood used. Comparing the performance of fluidized bed reactors and fixed bed reactors, the behaviour described by Ehlinger et al. (1987) and Lindgren (1983) is observed. The fluidized bed reactors reached maturity faster than the fixed bed reactors and were also more efficient in contaminant reduction. Overall, the results presented indicate that *Stenocereus* spp. is the most efficient substrate for treating synthetic wastewater, particularly in reducing COD, as well as nitrogen and phosphorus. This may be due to *Stenocereus* spp. having a less porous structure compared to *Opuntia* spp., which could facilitate bacterial growth while preventing particle detachment that could affect measurements, as observed during the experiment. It is important to mention that a potential source of error in the experiment was the presence of organic matter on the surface of the treated water, which could cause contamination during measurement. Filtration is recommended to avoid contamination during sample collection. The research objective was achieved, demonstrating that it may be possible to use biofilms attached to cacti for municipal wastewater treatment.

4 Conclusions

It was determined that, at a laboratory scale, the removal of COD from synthetic wastewater using fluidized bioreactors with biofilms attached to cactus-based supports ranged from 50% to 63%, suggesting their potential viability as an alternative treatment method.

The water from the Samalá and Xequijel Rivers was characterized, yielding average values of 96.38 and 277 with a standard deviation of 63.08 and 198.27 for COD, 68.38 and 129.40 with a standard deviation of 39.02 and 92.72 for BOD, 2.16 and 17.40 with a standard deviation of 1.89 and 3.30 for nitrogen, and 3.55 and 11.11 with a standard deviation of 2.72 and 3.43 for phosphorus, as key research values. The most efficient natural support and method for wastewater treatment was aerated *Stenocereus* spp., achieving an 86.75% COD reduction, followed by aerated *Opuntia* spp. at 77.29%, anaerobic *Stenocereus* spp. at 51.36%, and anaerobic *Opuntia* spp. at 18.21%. The efficiency of wastewater treatment using fluidized bioreactors with natural supports ranged from 50% to 63%. The studied reaction followed a zero-order kinetic model, with an equation of $K = -1.75 \times 10^{-4} \pm 0.15$.

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