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Articles

Removal of organic matter present in wastewater of a pharmaceutical plant using a RBC (rotating biological contactor)

Remoción de materia orgánica presente en aguas residuales de una planta farmacéutica mediante un RBC (contactor biológico rotatorio)

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Abstract

A RBC (Rotating Biological Contactor) system is a technology using a biological process by which microorganisms present in wastewater are fixed to partially-submerged parallel rotating discs forming a biofilm through bioaugmentation processes. This technology allows for the removal of contaminating organic matter from the water improving treatment processes. In this research work, a laboratory-scale RBC composed of four discs submerged approximately 40 % in a volume of 1 l (0.001 m^3) was used for the purpose of reducing high levels of COD and BOD contained in the wastewater of a pharmaceutical plant with the aid of two different microbial consortiums. An initial characterization of COD and BOD of the wastewater sample was carried out in order to compare with six samples obtained from the reactor in operation. A maximum COD removal of 2 560 mg O_2/l was obtained which, although very high, is beyond of the permissible limits allowed by Colombian law (ordinance 0631 of 2015) but is significant because it represents an efficient removal percentage of 99.67 % with respect to the values obtained at the initial characterization.

Keywords: Bioaugmentation, biofilm, COD removal, RBC.

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Resumen

Un sistema RBC (Rotating Biological Contactor) es una tecnología de tipo BIO mediante la cual los microorganismos presentes en las aguas residuales se fijan a discos giratorios paralelos parcialmente sumergidos formando una biopelícula mediante procesos de bioaumentación. Esta tecnología permite eliminar la materia orgánica contaminante del agua mejorando los procesos de tratamiento. En este trabajo de investigación se utilizó un RBC a escala de laboratorio compuesto por cuatro discos sumergidos aproximadamente al 40 % en un volumen de 1 l (0.001 m^3), con el propósito de reducir los altos niveles de DQO y DBO contenidos en las aguas residuales de una planta farmacéutica con la ayuda de dos consorcios microbianos diferentes. Se realizó una caracterización inicial de DQO y DBO de la muestra de aguas residuales para comparar con seis muestras obtenidas del reactor en operación. Se obtuvo una remoción de DQO máxima de 2 560 mg O_2/l, la cual, aunque muy alta, está más allá de los límites permisibles permitidos por la ley colombiana (ordenanza 0631 de 2015), pero es significativa, porque representa un porcentaje de remoción eficiente del 99.67 % con respecto a los valores obtenidos en la caracterización inicial.

Palabras clave: bioaumentación, biopelícula, remoción de DQO, RBC.

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Introduction

Emerging contaminants are compounds of different chemical origin and nature whose presence in the environment is not significant from the distribution and/or concentration standpoint, but which have potential to generate ecological impacts as well as harmful effects on health. Such compounds enter the environment through different means including nondomestic wastewater from pharmaceutical industries (Barceló & López, 2007; Murray, Thomas, & Bodour, 2010; Gil, Soto, Usma, & Gutierrez, 2012; Stuart, Lapworth, Crane, & Hart, 2012; Herrero *et al*., 2012). This wastewater is not properly treated, and its final destination is the sewerage network without fulfilling the Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), fat and oil requirements of Bogota Capital District ordinances 3956 and 3957 from 2009 and the "manufacture of pharmaceutical products, medicinal chemical substances, and botanical products for pharmaceutical use" section of Colombian ordinance 0631 on wastewater disposal of 2015.

This is the case of a pharmaceutical plant in the city of Bogota that has its own treatment facility for nondomestic wastewater which does not fulfill the current guidelines for COD and BOD generating excessive fats and oils. Emerging contaminants are also present in the wastewater given that these compounds are common residues in such industries. These are not treated properly and when they enter the environment, they generate a significant ecological impact and harmful effects on health, reasons that make necessary to redesign the fat trap using an RBC system to improve the current wastewater treatment.

RBC systems are a BIO-type fixed-film technology for wastewater treatment consisting of parallel spaced rotating discs which are partly submerged in a tank through which wastewater is directed (Nozaic & Freese, 2009) (Wang, Wu, & Shammas, 2009). The treatment process hinges on bioaugmentation of complex microbial communities which form a biofilm adhered to the surface of the disc (Martin-Cereceda, Serrano, & Guinea, 2001).

According to Schmitt, García-Cundinach and Dalmau-Soley (2005) the advantages of using biodiscs include: (1) flexibility to operate at lower flows than designed for, thereby increasing treated water quality; (2) easy and fast assembly, low operating costs – these are all-plastic compact reactor-decanter units which are entirely protected from the elements and require minimal civil engineering work; (3) minimal energy consumption by the biodiscs compared to associated technologies: activaded sludge with fine-bubble diffusors or superficial turbines,

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biological filters, oxidation channels, etc.; (4) reduced space and volume requirements, and (5) reduced atmospheric contamination. Because there is no dispersion of the water into the air, aerosol formation and associated atmospheric contamination problems are not present.

Rajani-Rani, Sreekanth and Himabindu (2011) devised a biological treatment including a three-stage laboratory-scale RBC for pharmaceutical wastewater with high COD and BOD levels. The RBC consisted of 18 parallel discs that rotated in a three-stage deposit, with 6 discs per stage. Reactor performance was assessed at three different organic loading rates and at three different RPMs. The study established that the optimal COD was 4 500 mg/l with an elimination efficiency of 95.3 % at 5 rpm. When the rotational speed was increased from 5 to 15 rpm, the COD removal efficiency was decreased from 95.3 to 70 %, respectively.

In 2015, in an article published by Su *et al*. (2015), an RBC was used in the treatment of wastewater from the workshop at a local pharmaceutical plant in Harbin, China. Results obtained by the RBC were not optimal, because its removal percentage was 40 % having initially achieved removal percentages in the neighbourhood of 80 %. This reduction in removal was explained through several factors including the toxicity of the type of wastewater which could have affected the capacity of the microorganism population growing in the biofilm, the concentration of the influent, and the ambient temperature.

Tecnología y ciencias del agua, ISSN 2007-2422, 14(4), 305-348. DOI: 10.24850/j-tyca-14-04-07

In this paper we propose the implementation of an RBC system to be used in the wastewater treatment facility of a pharmaceutical plant in the city of Bogota, to improve its current treatment process. Our objective is to evaluate the behavior of an RBC reactor in the treatment of effluent from this pharmaceutical plant, using a laboratory-scale reactor with 4 discs submerged at 40 %. RBC treatment efficiency is evaluated measuring COD and BOD values to determine the system's removal percentages.

Materials and methods

Fat traps are small floatation tanks in which fat rises to the surface and is retained in a chamber while cleared water is released downward to another chamber. Generally, this process does not involve mechanical parts (Unión Temporal Acuambiental, 2008). In our case, the pharmaceutical plant's fat trap consists of four stages, each located in one of four rectangular chambers through which wastewater flows. Wastewater arrives directly to chamber 1 from the pharmaceutical plant, following on to chamber 2 with a 30 min to 1 h hydraulic retention time where there is high sedimentation rate as well as mixing by which all the organic load is dissolved. After chamber 2 the process continues in the following chambers.

Tecnología y ciencias del agua, ISSN 2007-2422, 14(4), 305-348. DOI: 10.24850/j-tyca-14-04-07

Initial assessment of COD and BOD

In order to make an initial characterization of the wastewater, a first sampling of the fat trap at the pharmaceutical plant was carried out, specifically sampling chamber 1, the access, and chamber 2. On the day of the sampling, the industry was producing creams and lotions which was evidenced by a large amount of fats and oils exiting from the tubing in the fat trap. For the initial characterization, COD was assessed by closed reflux and volume method using IDEAM protocol TP0086 in which a 2.5 ml sample from chamber 1 and a 0.15 ml sample from chamber with a dilution factor of 1 000 were used. BOD_5 was determined using IDEAM protocol TPOO87.

Evaluation of consumer consortiums of pharmaceutical organic matter

Since the critical COD and BOD results occur in chamber 2, this is the chamber that was selected for future reengineering with the RBC system.

Therefore, subsequent assessments for COD and BOD were performed on this chamber to evaluate its behavior in the proposed experimental scenarios.

In this phase, two microbial consortia were composed to evaluate the degree of removal when acting on samples from chamber 2. In an earlier stage of this project approximately 300 bacterial strains were obtained by bio prospecting wastewater; from these, 16 were selected and evaluated in three different time frames. The bacterial strains were organized in 2 consortia and inoculated during 24, 48, and 72 h to evaluate their efficiency in COD and BOD removal. The first consortium consisted of morphotypes with hydrolytic activities (amylases, proteases, and lipases) which were able to grow in 50 mg/l of acetaminophen and ibuprofen. The second consortium consisted of morphotypes with the same hydrolytic activities which were able to grow in 50 mg/l of acetaminophen, ibuprofen, and meloxicam. The last test for these 8 bacteria was to grow in 100 mg/l of acetaminophen. The bacteria were morphologically different; however, in this last consortium 2 morphotypes have features of Gram-negative bacilli with exopolysaccharide production typical of *Pseudomonas*, a microorganism that has been widely studied in removal processes for xenobiotic and recalcitrant contaminants.

Approximately 5 l of wastewater were collected. At the time of collection, the pharmaceutical plant was producing shampoo and the chambers had been cleaned five days before, so the wastewater was less turbid than in the initial sampling.

COD assessment for consortium 1 was performed by closed reflux and volume method using IDEAM protocol TP0086 (IDEAM, 2007b) with a 0.05 ml sample at a dilution factor of 1000. BOD_5 was determined using IDEAM protocol TP0087 (IDEAM, 2007a). COD assessment for consortium 2 was performed by closed reflux and volume method using IDEAM protocol TP0086 with a 0.00125 ml sample a high dilution factor of 10 000. BOD5 was determined using IDEAM protocol TP0087.

Prior to COD and BOD assessment of consortia 1 and 2, samples were centrifuged in 14 ml Falcon tubes at 4 000 RPM for 20 min. COD value was obtained from the centrifuged consortium 1 sample by closed reflux and volume method using IDEAM protocol TP0086 with a 0.25 ml sample at 1 000 dilution factor and the BOD_5 value using IDEAM protocol TP0087. In the same fashion, COD value was obtained from the centrifuged consortium 2 sample by closed reflux and volume method using IDEAM protocol TP0086 with a 0.5 ml sample at 100 dilution factor and the BOD_5 using IDEAM protocol TP0087.

McFarland assay

The preparation of the McFarland assay was following the pattern specified in Table 1.

Table 1. McFarland assay.

N°	$BaCl_2$ 0.048 M ml	H_2SO_4 0.36 M ml	V_f ml	N° cells
0.5	0.05	9.95	10	$1.5x10^8$
1	0.1	9.9	10	$3x10^8$
2	0.2	9.8	10	$6x10^8$
3	0.3	9.7	10	$9x10^8$
4	0.4	9.6	10	$12x10^8$
5	0.5	9.5	10	$15x10^8$
6	0.6	9.4	10	$18x10^8$
7	0.7	9.3	10	$21x10^8$
8	0.8	9.2	10	$24x10^8$
9	0.9	9.1	10	$27x10^8$
10	1	9	10	$30x10^8$

V_f: final volume.

Once all tubes were prepared, 3 ml were taken from each and placed in the cell for absorbance reading in the spectrophotometer. The absorbance of the tubes containing strains was measured in the same

fashion to allow for comparison and determination of the absorbance value of the sample.

Fixation solution as substratum

Using a 250 cm^3 beaker, 2.5 g of sodium alginate were dissolved in 100 cm^3 of ultra-pure water. The solution was hot plated at 365 °C for 15 min while homogenizing until no particulate matter remained. The solution was allowed to cool for 5 min and then a compact disc (CD) was immersed in it to allow it to impregnate its surfaces.

Similarly, 3.5 g of pure $CaCl_2$ were dissolved in 100 cm^3 of ultra-pure water until achieving a homogeneous mix in which the CD was placed in for 10 minutes.

Fixation matrix

Once the disc was impregnated by the mix, it was placed in a 1 500 ml beaker, submerged in 50 ml of chamber 2 sample, and allowed to sit for approximately 27 h. The intent was to see what would be the behavior of

Tecnología y ciencias del agua, ISSN 2007-2422, 14(4), 305-348. DOI: 10.24850/j-tyca-14-04-07

the biofilm, *i.e.,* if it would detach from the disc surface during this prolonged contact with water of if it would remain attached to the surfaces of the disc.

Scanning electron microscopy

A small 1 x 1 cm segment of the disc used for the *fixation matrix* was removed for observation in the electron microscope to assess the behavior of the biofilm.

Fixation solution and strains

The sodium alginate solution described in the *fixation solution* section was prepared again but in this instance the strains from consortium 2 were added to it. This mix was applied with a brush on the surfaces of the reactor's four discs to create a biofilm which was then fixed on to the surfaces by spraying them with the $CaCl_2$ solution so the biofilm would not detach from the discs' surfaces.

Tecnología y ciencias del agua, ISSN 2007-2422, 14(4), 305-348. DOI: 10.24850/j-tyca-14-04-07

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Running the laboratory-scale RBC

The sample was treated in an RBC with discs submerged approximately 40 % and rotating at 50 rpm. Four discs were used. These consisted of plastic polycarbonate with a reflecting aluminum layer and some of them had labels on one of their surfaces. These discs are commonly known as mini CDs or pocket CDs, have an 8 cm diameter, and were spaced 3 cm apart on a steel axis which was 13.2 cm long and 0.5 cm in diameter. Taking into account that the disk contains an internal hole, the total area of each disk is 0.0048 m^2. The reactor had an actionable volume of 1 l (0.001 m^3) and its tank was cubical and made in glass. A switch-operated electric motor coupled to a set of pulleys was used to rotate the discs. A diagram of the RBC used for testing is presented in Figure 1.

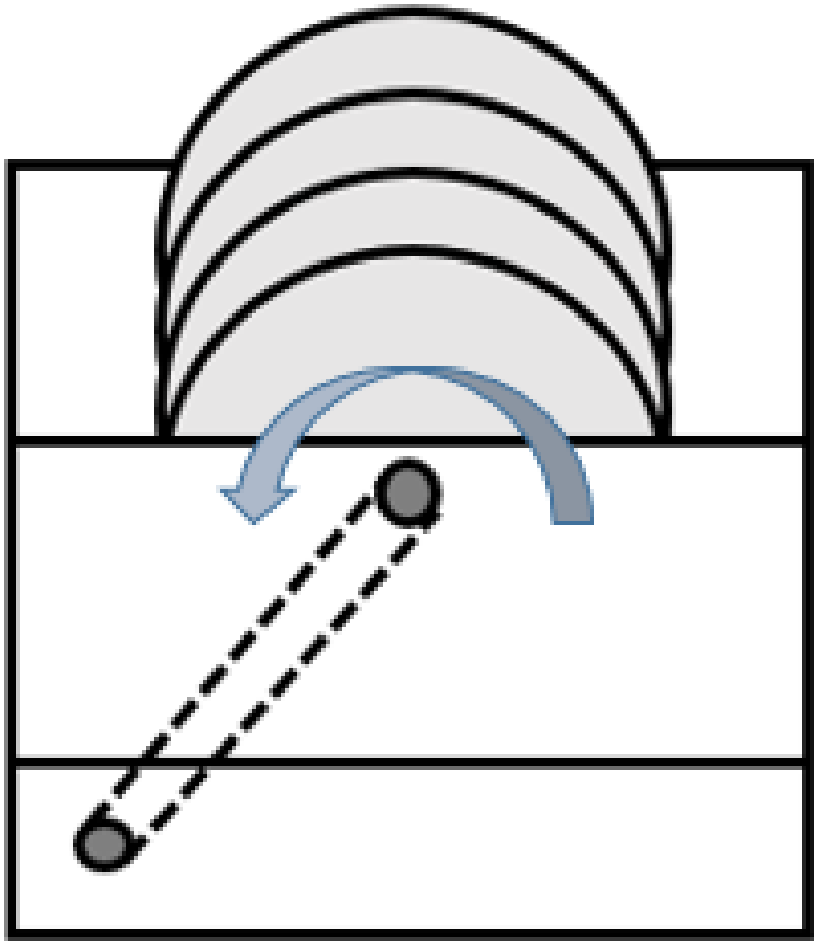

Figure 1. Diagram of the RBC used for testing.

The behavior of the reactor was monitored for 3 hours, and 50 ml samples were taken every 30 minutes from the start to be later assessed in the laboratory for COD and BOD with a total of 6 samples collected.

Tecnología y ciencias del agua, ISSN 2007-2422, 14(4), 305-348. DOI: 10.24850/j-tyca-14-04-07

Results and discussion

Initial assessment of COD and BOD

Table 2 shows the critical value of COD present in chamber 2 which is 768 000 mg O_2/l. This value is much higher in comparison with chamber 1. Therefore, 768 000 mg O_2/l is the reference value which should be reduced with the use of the consortia and in subsequent assessments for COD and BOD in order to determine which microbial consortium has the best removal activity on the samples. This allows then for the development the biofilm that should be adhered to the biodiscs of the RBC. Column 3 results from the application of Equation (1) where *sample ml* is a constant value of 2.5 ml:

$$COD = \frac{(target\ mL\ consumed - sample\ mL\ consumed) * N\ FAS * 8 * 1000}{sample\ mL} \quad (1)$$

N FAS: normality of ferrous ammonium sulfate

Table 2. Initial assessment of COD from chambers 1 and 2.

Sample	ml consumed	COD (mg O_2/l)
Chamber 1	3.6	435.2
Chamber 1	4	384
Chamber 2	1	768 000
Chamber 2	1	768 000
Standard	7	0

In Table 3 can be seen the BOD value obtained by applying Equation (2):

$$BOD_5 = \frac{(OD\ consumed - OD\ consumed\ by\ strain)}{Vs} * V \qquad (2)$$

V: volume contained within the Winkler flasks.

Vs: volume of the sample within the Winkler flasks.

It can be seen that the BOD_5 values from chamber 1 and chamber 2 are very different, those from chamber 1 being very low compared to chamber 2.

Tecnología y ciencias del agua, ISSN 2007-2422, 14(4), 305-348. DOI: 10.24850/j-tyca-14-04-07

Table 3. Initial assessment of BOD_5 from chambers 1 and 2.

Sample	**BOD_5 (mg O_2/l)**
Chamber 1	198
Chamber 2	2 051
Standard	23.6
Standard	6.4

DO: dissolved oxygen.

Vs: sample volume.

The initial characterization of the effluent from the pharmaceutical plant had higher levels of COD and BOD than those permitted for discharge into superficial bodies of water and public sewerage systems in ordinance 631 of 2015 (Ministerio de Ambiente y Desarrollo Sostenible, 2015). This can be seen in Table 4, where the COD value in chamber 2 exceeds the permitted value in 1 920 times and the BOD exceeds the permitted value in 12.67 times.

Table 4. Comparison of the results from chamber 2 with Ordinance 631 of 2015.

	Chamber 2	**Permissible value Ord. 631 of 2015**
COD (mg O_2/l)	768 000	400
BOD (mg O_2/l)	2 051	150

In the paper by Su *et al*. (2015), experiments were run on pharmaceutical wastewater which contained fluid residue from the manufacturing of antibiotics in a workshop from a local pharmaceutical plant in Harbin, China. This fluid residue contained branched ferments, residual penicillin, and various substances including acetone, amyl butyric ester, formaldehyde and sulfate radical. Pharmaceutical wastewater in the tests was a mixture of antibiotic production residual liquid (APRL) and either discharging sewage (DS) from the pharmaceutical plant or a mixture of RWW and natural water (NW) from a creek. In initial assessments to establish the state of the sample, COD values of 400 000 mg O_2/l were found for APRL, 350 mg O_2/l for DS and 20 mg O_2/l for NW water. The COD removal efficiencies were within 45-50 % when organic input concentration was below 100 mg/l. With an increase of organic input concentration from 400 to 800 mg/l, the COD removal increased slightly.

Initial BOD values obtained in the pharmaceutical plant in Harbin are less than those obtained in the pharmaceutical plant subject of the

present article. Although this value is less, it still is a very high BOD level which indicates that pharmaceutical industries release a great organic load into bodies of water which, if not reduced, causes major contamination problems.

Assessment of the consortia consuming organic matter as BOD and COD

The results of the assessments for COD and BOD on samples at 24, 48, and 72 h are presented, each performed on 2 repeats, repeat 1 = R1 and repeat 2 = R2, for more certainty. With these results we attempt to establish which consortium is best to constitute the biofilm, by comparing the percentages of removal.

Tecnología y ciencias del agua, ISSN 2007-2422, 14(4), 305-348. DOI: 10.24850/j-tyca-14-04-07

Consortium 1 – COD

According to Table 5 removal percentages are high, surpassing 50 %, meaning that consortium 1 has an important removal rate in the sample at the different time points and supported by repeat testing.

Table 5. COD values from consortium 1.

Sample	ml consumed	COD (mg O_2/l)	% of removal
24h R1	4.3	51 200	93.33
24h R2	4.3	51 200	93.33
48h R1	4.2	64 000	91.67
48h R2	4.4	38 400	95.00
72h R1	4.3	51 200	93.33
72h R2	4.1	76 800	90.00
Standard	4.7	0	

BOD

Table 6 was obtained in the same manner as Table 3, with the difference that in the present Table chambers 1 and 2 are not analyzed. Only chamber 2 is assessed at the 3 proposed time points with repeat testing for more data certainty. BOD values obtained at the different time points and respective repeats do not have major variations between them, hovering in the range of 1 840 to 2 070 mg O_2/l.

Table 6. BOD values from consortium 1.

Sample	BOD_5 (mg O_2/l)
24h R1	1 842
24h R2	2 069
48h R1	1 944
48h R2	1 980
72h R1	2 052
72h R2	2 035
B	37
B	56

Consortium 2 – COD

In Table 7 it is evidenced that consortium 2 has the worst removal data as it did not have much incidence on the sample and even further, in the 72 h hour R2, it surpassed the threshold of 768 000 mg O2/l yielding a negative removal of 50 %.

Table 7. COD values from consortium 2.

Sample	ml consumed	COD (mg O_2/l)	% of removal
24h R1	4.2	512 000	33.33
24h R2	4.1	640 000	16.67
48h R1	4	768 000	0.00
48h R2	4	768 000	0.00
72h R1	4	768 000	0.00
Standard	4.6	0	

Tecnología y ciencias del agua, ISSN 2007-2422, 14(4), 305-348. DOI: 10.24850/j-tyca-14-04-07

BOD

In Table 8 the BOD values are elevated and have similar values to those presented in Table 6, which also shows that these values do not show major variation at the different time points shown in column 1.

Table 8. BOD values from consortium 2.

Sample	BOD_5 (mg O_2/l)
24h R1	1 820
24h R2	1 739
48h R1	1 634
48h R2	1 953
72h R1	1 939
72h R2	1 885
Standard	27.9
Standard	23.7

With the above results from Table 5, Table 6, Table 7 and Table 8 it can be seen that the COD and BOD_5 values from consortia 1 and 2 are

very elevated given that the microorganisms in the consortia contributed a high amount of organic matter load. Because of this, every sample with consortia was centrifuged and reassessed for COD and BOD obtaining the values below.

Centrifuged consortium 1

COD

In Table 9 an inverse effect on the percentages of removal is evident. Given that a greater removal was expected after centrifuging in comparison with the results from the non-centrifuged consortium 1 in Table 4, the considerable drop in values demonstrated that consortium 1 was not suitable to constitute the biofilm for the RBC.

Tecnología y ciencias del agua, ISSN 2007-2422, 14(4), 305-348. DOI: 10.24850/j-tyca-14-04-07

Table 9. COD values for centrifuged consortium 1.

Sample	ml consumed	COD (mg O_2/l)	% of removal
24h R1	1.2	332 800	56.67
24h R2	0.8	384 000	50.00
48h R1	0.7	396 800	48.33
48h R2	1.2	332 800	56.67
72h R1	0.9	371 200	51.67
72h R2	0.8	384 000	50.00
Standard	3.8	0	

BOD

On centrifuging the sample and comparing Table 10 with Table 6, it can be seen that even after centrifuging BOD_5 values show no significant change as the values on both tables are very similar.

Table 10. BOD values for centrifuged consortium 1.

Sample	BOD_5 ($mg\ O_2/l$)
24h R1	1 445
24h R2	1 812
48h R1	2 006
48h R2	1 598
72h R1	1 953
72h R2	1 832
Standard	18.7
Standard	14.2

Centrifuged consortium 2

COD

Removal results in Table 11 are the highest reaching almost 100 % at all hours and repeats, demonstrating that centrifuged consortium 2 is

suitable to be used in the RBC in comparison with the results of the other consortium which showed removal percentages lower than those in Table 11.

Table 11. COD values for centrifuged consortium 2.

Sample	ml consumed	COD (mg O_2/l)	% of removal
24h R1	2	6 400	99.17
24h R2	1.8	8 960	98.83
48h R1	1.5	12 800	98.33
48h R2	2	6 400	99.17
72h R1	1.5	12 800	98.33
72h R2	1.6	11 520	98.50
Standard	2.5	0	

BOD

Comparing Table 6, Table 8, Table 10 and Table 12 it can be surmised that BOD values do not show major variation even after centrifuging.

Values do not fluctuate in the same degree as the COD values for each of the consortia, but these are lower than the COD values obtained in the different scenarios.

Table 12. BOD values for centrifuged consortium 2.

Sample	BOD_5 (mg O_2/l)
24h R1	1 844
24h R2	1 788
48h R1	2 013
48h R2	1 990
72h R1	1 936
72h R2	1 926
Standard	18.7
Standard	10.6

Figure 2 is based on the final COD data from the consortia at the different time points and allows for a visual comparison of the data with the COD line of reference of 768 000 mg O_2/l obtained initially from chamber 2. In Figure 2, centrifuged consortium 2 has the lowest COD values, which are represented in Table 11 with its high percentages of

removal and graphically indicating that consortium 2 is the most suitable to develop the biofilm for the RBC.

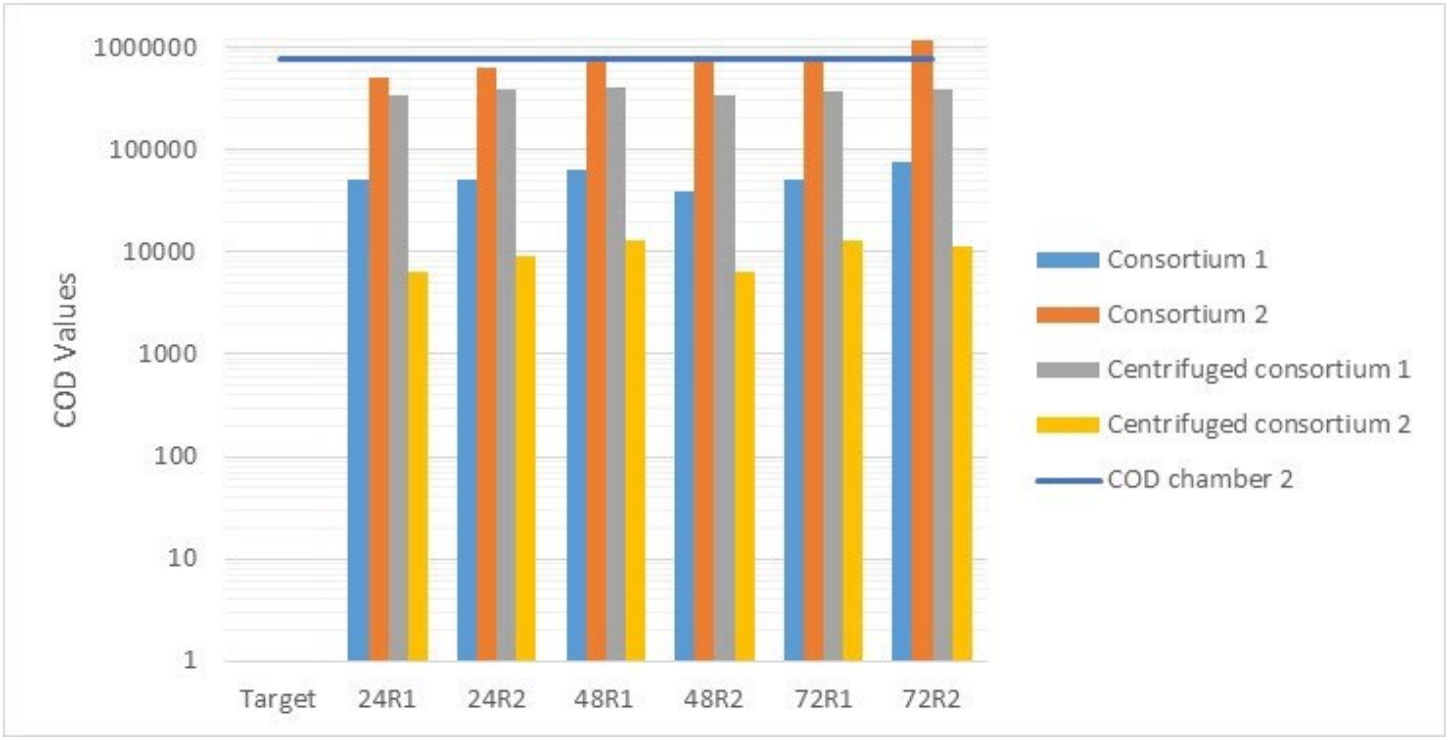

Figure 2. Comparison of COD values against the consortia.

Tecnología y ciencias del agua, ISSN 2007-2422, 14(4), 305-348. DOI: 10.24850/j-tyca-14-04-07

McFarland assay

Comparing the strains in the tubes with the patterns prepared in the McFarland assay it was determined that the strains had a turbidity similar to standard No. 7 which is equivalent to 21 x 10^8, hence every ml of our strains had a microorganism concentration of 21 x 10^8.

Fixation matrix

After approximately 27 h the disc was inspected to assess its behavior after prolonged contact with the water. The biofilm had remained adhered to the disc all day, as it can be seen in Figure 3, indicating that the fixation solution was suitable for the reactor.

Tecnología y ciencias del agua, ISSN 2007-2422, 14(4), 305-348. DOI: 10.24850/j-tyca-14-04-07

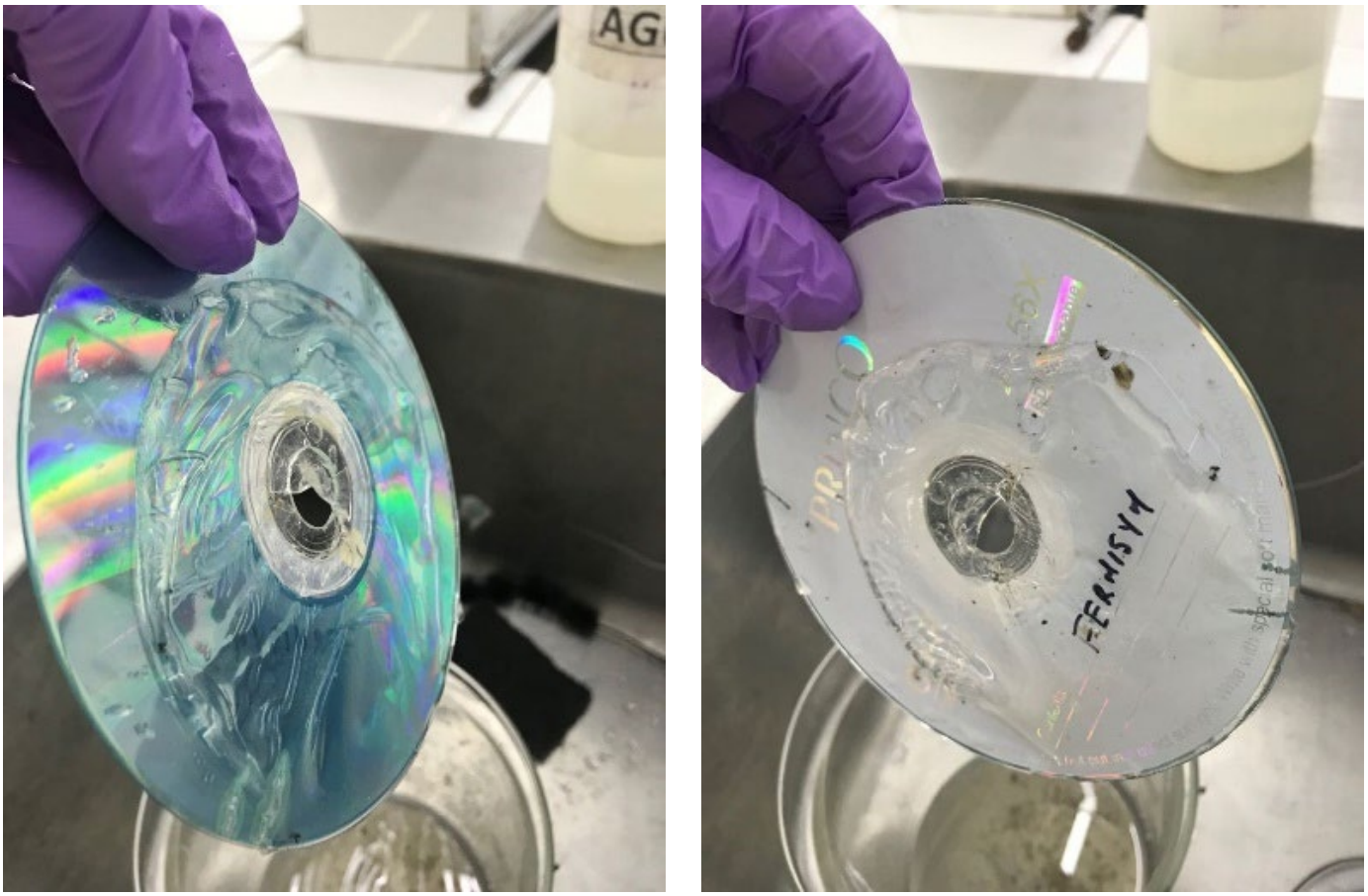

Figure 3. Fixation test after 27 h.

The disc had remained static within the beaker during the fixation assessment, whereas discs within the reactor are in continuous movement raising the possibility of biomaterial loss. Due to the latter, the discs in the reactor received three layers of alginate with the strains. The initial layer was present at the beginning of the reactor's operation and

Tecnología y ciencias del agua, ISSN 2007-2422, 14(4), 305-348. DOI: 10.24850/j-tyca-14-04-07

the other 2 reinforcement layers were applied at 30 min and at 90 min of operation.

Scanning electron microscopy (SEM)

Figure 4A corresponds to the disc's surface at 3 mm without biofilm and shows its composition and matrices. Figure 4B represents the same disc surface but at 200 µm and it shows the disc materials and pores on its surface. The biofilm is already adhered to the disc in Figure 4C and corresponds to the clear material against the alginate's gray background. Figure 4D shows the same as in the previous figure but at 20 µm to better appreciate the layer of alginate. A prism-shaped figure present in Figure 4E is a focused view of the alginate at 5 µm and the small adhered structure corresponds to bacterial colonies resting on it. Figure 4F is a 200 µm view of the polymer without the disc. In Figure 4G, at 100 µm, the porosity of the biofilm is evident and a clear space in it represents a fissure of the biofilm; the image also shows some dune-like formations which correspond to the way the alginate layer was mixed and applied. Figure 4H is the same shown previously but with more contrast. Figure 4I, at 100 µm, shows fissures in the biofilm. Finally, Figure 4J, at 30 µm, shows the pores that harbor microorganisms and fissures in the membrane.

Tecnología y ciencias del agua, ISSN 2007-2422, 14(4), 305-348. DOI: 10.24850/j-tyca-14-04-07

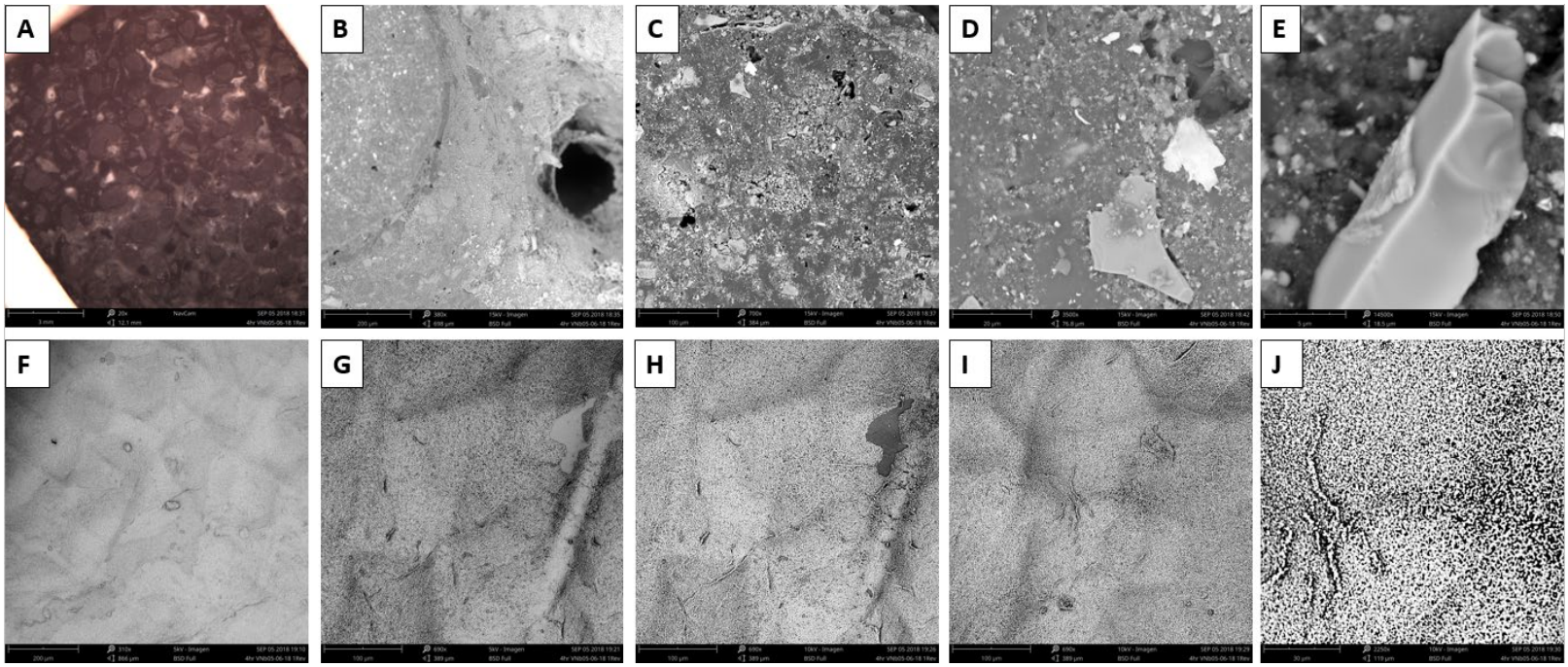

Figure 4. SEM results.

Assessment of RBC behavior with regards to BOD and COD

Figure 5A shows the discs during the experiments and Figure 5B shows the RBC in operation.

Tecnología y Ciencias del Agua
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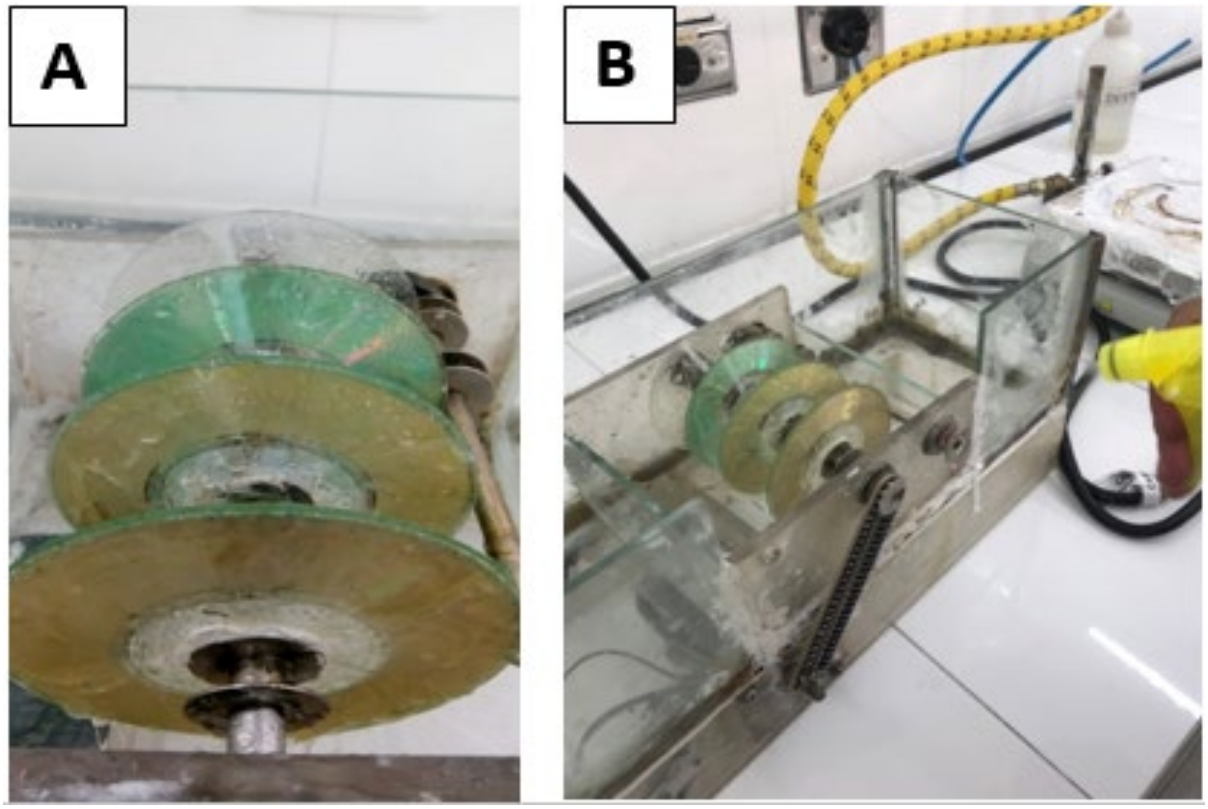

Figure 5. A: Discs with biofilm. B: Reactor used in the experiment.

COD

In Table 13 removal percentages exceed 99 % which means that the system has good performance in wastewater treatment. Comparing with Table 5, Table 7, Table 9, and Table 11, it can be seen that the reactor

Tecnología y ciencias del agua, ISSN 2007-2422, 14(4), 305-348. DOI: 10.24850/j-tyca-14-04-07

obtained the best results in removing organic load, although if it is compared with column 3 of Table 4 it is clear that even using the RBC system on the wastewater from the pharmaceutical plant, the object of our study, the permissible limits according to ordinance 0631 of 2015 are not met.

Table 13. COD values to assess the performance of the operating RBC-

Sample	ml consumed	COD* (mg O_2/l)	% of removal
7:00 R1	9	2 560	99.67
7:00 R2	8.8	5 120	99.33
7:30 R1	8.7	6 400	99.17
7:30 R2	8.7	6 400	99.17
8:00 R1	8.9	3 840	99.50
8:00 R2	8.9	3 840	99.50
8:30 R1	8.8	5 120	99.33
8:30 R2	8.6	7 680	99.00
9:00 R1	8.9	3 840	99.50
9:00 R2	8.9	3 840	99.50
9:30 R1	8.9	3 840	99.50
9:30 R2	8.7	6 400	99.17
Standard	9.2	0	

In this case the COD indicates that after 3 hours of the experiment the water remains with a high oxygen demand to be able to decompose the organic matter contained within it.

We cannot state that there was significant variation of COD removal every 30 min in the sample within the reactor. In other words, no matter what time passed, the sample didn't show lower COD readings that in the 30 minutes before.

BOD

Comparison of the BOD values in Table 14 obtained from assessment of the RBC with those obtained from the consortia in Table 6, Table 8, Table 10, and Table 12 demonstrates that the RBC was able to remove part of the degradable organic matter, which was difficult to accomplish with the isolated consortia. These values diminished but, similar to those of COD, are still above the permissible 150 mg O_2/l required in ordinance 631 of 2015.

Tecnología y ciencias del agua, ISSN 2007-2422, 14(4), 305-348. DOI: 10.24850/j-tyca-14-04-07

Table 14. BOD values to assess the performance of the operating RBC.

Sample	DO (mg/l)	DO_i (mg/l)	DO_f (mg/l)	V (ml)	DO consumed (mg/l)	DO consumed by strain	V_m (ml)	BOD_5 (mg O2/l)
7:00 R1	0.68	6.61	4.73	305	1.88	0	1	573
7:00 R2	0.68	6.61	4.74	315	1.87	0	1	589
7:30 R1	0.68	6.57	4.58	305	1.99	0	1	607
7:30 R2	0.68	6.57	3.92	305	2.65	0	1	808
8:00 R1	0.68	6.57	4.53	311	2.04	0	1	634
8:00 R2	0.68	6.57	3.78	323	2.79	0	1	901
8:30 R1	0.68	6.52	4.1	305	2.42	0	1	738
8:30 R2	0.68	6.52	4.07	313	2.45	0	1	767
9:00 R1	0.68	6.54	6.39	335	0.15	0	1	50
9:00 R2	0.68	6.54	7.62	317	-1.08	0	1	-342
9:30 R1	0.68	6.5	6.74	305	-0.24	0	1	-73
9:30 R2	0.68	6.5	6.5	303	0	0	1	0
Standard	0.68	6.77	5.46	308	1.31	0	10	40.4
Standard	0.68	6.77	5.57	308	1.2	0	10	36.9

Rajani-Rani *et al*. (2011) reported biological treatment of wastewater from a pharmaceutical plant with high COD and BOD values using a scale-size three-stage RBC. They constructed an RBC with 18 parallel discs and assessed the reactor's performance at 3 different (5,

10, and 15) rpms. These authors found that the optimal COD load was 4500 mg O_2/l with a removal efficiency of 95.3 % at 5 rpm. On increasing rotation velocity from 5 to 15 rpm, their efficacy of COD removal went from 95.3 to 70 %. Comparing with the present study, where 4 parallel discs were assessed in one stage at 50 rpm and the lowest COD load obtained was 2 560 mg O_2/l for a removal efficiency of 99.67 %, favorable results were also accomplished even with fewer discs at a higher rotation velocity.

In the paper by Su *et al*. (2015), removal of COD diminished from 80 to 40 % during nine days of testing because the quantities and types of bacteria, fungi, and heterotrophs in the RBC's biofilms varied and began to acclimatize to new wastewaters or reproduced variations suitable for the new wastewater. This was due to the increasing quantity of biorecalcitrant substances that could not be degraded and to an increase in the toxicity of the wastewater, generating a scenario where toxicity was more intense towards bacteria and fungi than their treatment and resistance capabilities. Also, the low temperatures (10 to 16 °C) during the acclimatizing period generated a decrease in COD elimination. This acclimatizing effect did not affect the elimination process in this paper because the system did not receive new wastewaters that could propitiate the entry of new substances that could contain bio recalcitrant elements. Temperatures in the laboratory in which testing was carried out fluctuated between 25 and 28 °C so temperature did not have negative impact on COD removal, as shown by the good results in Table 13.

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Conclusions

Evaluation and comparison between the different consortia allowed the identification of consortium 2 as suitable to form the biofilm given its high removal percentages in the scenario where centrifugation applied. The system was efficient in removing the organic matter present, with the best results —COD removal of 99.67 %— within the first 30 minutes of operation of the RBC. SEM allowed study of the biofilm morphology and understanding of how the biofilm and the microorganisms interact and function as a unit. The experiment reveals the need for a settler at the outflow of the reactor in order to reduce the amount of solids contained within the system, as a considerable amount of solids was visible suspended in the system while assessing the performance of the RBC. Also, for the system to perform within the guidelines of ordinance 631 of 2015 it is necessary to supplement it with a complementary process either before or after the reactor. The very high relationship between COD and BOD shows that in the studied wastewater of pharmaceutical origin there is a larger amount of chemically-degradable products rather than biological, which explains why COD was used to assess the removal efficiency of the system.

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