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Biomonitoring of wastewaters in treatment plants using ciliates

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The aim of this project has been to study and compare the ciliate populations present in rotating biological reactors treating three different wastewaters. Wastewaters chosen were a maize mill (nejayote), a sugarcane/ethyl alcohol plant (vinasses) and a recycled paper mill (whitewaters). The initial dissolved organic contents, measured as soluble chemical oxygen demand (COD) and biochemical oxygen demand in five days (BOD_5), were 2040 ± 150 mg COD L^{-1} and 585 ± 5 mg BOD_5 L^{-1} for nejayote; 2000 ± 20 mg COD L^{-1} and 640 ± 5 mg BOD_5 L^{-1} for vinasses and 960 ± 200 mg COD L^{-1} and 120 ± 10 mg BOD_5 L^{-1} for whitewaters. Results obtained indicate that ciliated protozoa proliferated in the different chambers of each rotating biological reactor (RBR). Saprobity indices, as a quantitative evaluation parameter, indicate that there are no universal species of ciliates associated with specific BOD_5 concentrations. Therefore, the number of species of ciliates present in the effluent indicate qualitatively the efficiency of removal of pollution from the wastewaters during treatment in the rotating biological reactors.

Keywords: biomonitoring; wastewaters; treatment plants; ciliates; water quality bioindicators

Introduction

Industrial wastewater treatment, within the framework of environmental health issues, has become a vital topic for multidisciplinary research. In this paper, research is focused on the study of the effect of environmental parameters on the density and relative composition of ciliate communities thriving in aerobic biological wastewater treatment systems.

A practical application of ciliate community composition and density is the characterization of these communities and their association with saprobity (concentration of biodegradable organic material dissolved) at a given pH, dissolved oxygen tension and temperature of treatment [2,11,12]. If these correlations are statistically reproducible, it is possible that a continuous treatment plant could be monitored using microscopic observations. Ciliates, due to their size, wide distribution and other characteristics, have been used often as saprobity indicators in natural water sources [5,11] and domestic wastewater treatment systems [4,6–8,21], but not in industrial wastewater treatment systems with similar concentrations of biodegradable dissolved organic material. Typical concentrations of domestic wastewater, measured as Biochemical Oxygen Demand (BOD_5), fluctuate between 110 and 400 mg L^{-1} [22]. Figure 1 shows the different sections of a saprobic system, each level is associated with a particular BOD_5 and a group of ciliate species denominated indicator [4,6,25].

Biological monitoring may allow the systems to operate with fewer chemical analyses. Such analyses may be costly and generate polluted liquid effluents and fumes. Indeed, direct observation of statistically significant samples represents a complete view, not only of the ecosystems present in the wastewater system being treated, but also of the effect of the abiotic environmental parameters on the biota.

Therefore, biological monitoring may also direct proper operating conditions and rectify disturbances due to ecological imbalances in the treatment system, as well as detecting pathogens [5,18]. The objective of this work was the identification of ciliated protozoa as saprobity indicators in RBRs working with different industrial wastewaters. Information about their potential as indicators as well as description of the saprobic system and scale of saprobity are presented elsewhere [3,26,27]. These abundant nonpathogenic micororganisms are ubiquitous, easy to detect, to culture, and identify to genus. They represent a high permanence in the environment and with a narrow association with the parameter to be used [8,18,19,24].

Materials and methods

Wastewaters

For this research, three types of industrial wastewaters were chosen. The main consideration was that organic materials in the wastewaters are biodegradable. Sources chosen were the paper industry (wastewaters known as whitewaters), the food industry (maize mills wastewaters, known as nejayote), and the sugarcane mills/distilleries (distillery liquid wastes known as vinasses). Description of the processes where these wastewaters were produced are presented elsewhere [10,23,28]. Their original compositions and abiotic characteristics are presented in Table 1. A constant initial concentration with the lowest amount of dissolved organics, measured as chemical and biochemical oxygen demand, was chosen. After analytical monitoring, whitewaters showed an average content of 2000 mg COD L^{-1}. This was the limit used for nejayote and vinasses. Tap water (non-chlorinated) was added to the samples of nejayote and vinasses to reduce their initial concentration. However, the whitewaters collected had a lower concen-

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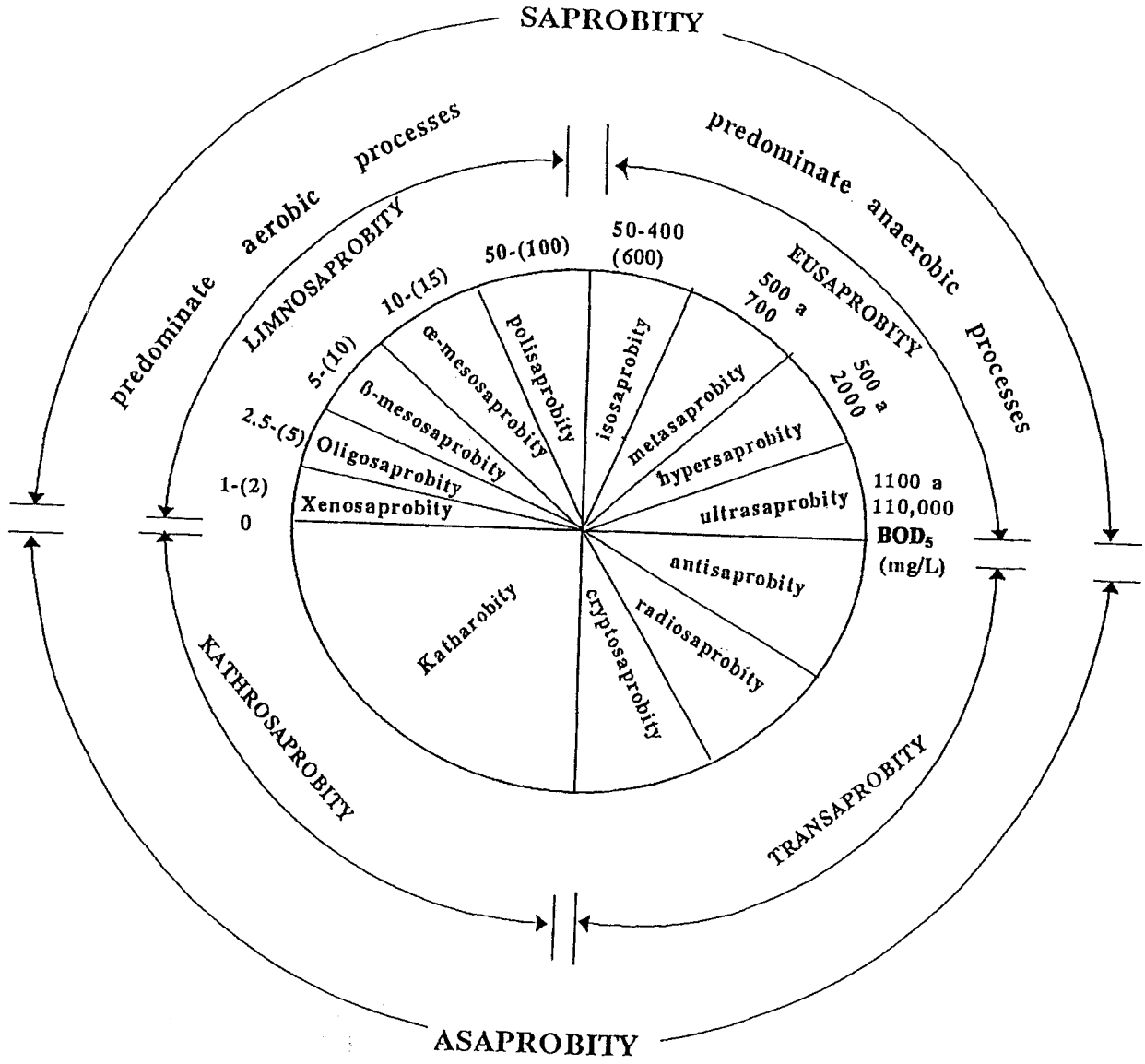

Figure 1 Saprobity system [20,25]. Upper half circle: from Greek *Sapros* = rotten, *bios* = life; organisms that live on dead or decaying organic matter; *Limne* = pool, pond, lake; superficial fresh waters, aerobic water sources; *Eu* = good, true saprobity; anaerobic processes in water sources. Lower half circle: from Greek *Trans* = beyond, no life beyond these limits; *Krypton* = hidden, no apparent life; *Kathairein* = clean, pure; clean water, no life in it; all these are included in the asaprobity region, with the Greek privative *a* before saprobity meaning the opposite of saprobity.

Table 1 Characteristics of wastewaters studied[a]

Parameter	Nejayote[b]	Vinasses[b]	Whitewaters
Chemical oxygen demand (mg COD L^{-1})	20 000–30 000	69 000–128 000	1500–2500
Five-day biochemical oxygen demand (mg BOD L^{-1})	8000	31 500	470–700
pH	5–13	4.5–5.5	6.3–7.2
Temperature (°C)	15–30	15–50	15–30
Dissolved oxygen (mg DO L^{-1})	0.0	0.0	0.0
C : N : P average ratio	10 : 1.5 : 0.9	100 : 2.3 : 0	100 : 1.7 : 0.1

[a]Values for nejayote are from [23], for vinasses from [10] and for whitewaters from [28].
[b]Diluted to 2000 mg COD L^{-1} with tap water.

tration and were fed into the reactor. Wastewater characteristics were determined using Standard Methods [1].

Experimental conditions

Experiments were carried out at 20 ± 0.5°C in rotating biological reactors (RBRs) under similar abiotic conditions changing only the type of substrate (wastewater). Each RBR has a working volume of 20 L, divided into 10 chambers or stages, with 20 discs of 30 cm diameter, rotating at 20 rpm (Figure 2). Hydraulic residence time was 1 day. Temperature of the influent treating wastewater fluctuated between 16 and 21°C, and pH varied between 5.2 and 6.6. Dissolved organic concentration, measured as chemical oxygen demand (COD) [1], and 5-day biochemical oxygen demand (BOD_5) [1], were adjusted by dilution with tap water to similar levels (2000 mg COD L^{-1}, and 600 mg BOD_5 L^{-1}) with the exception of whitewaters that had

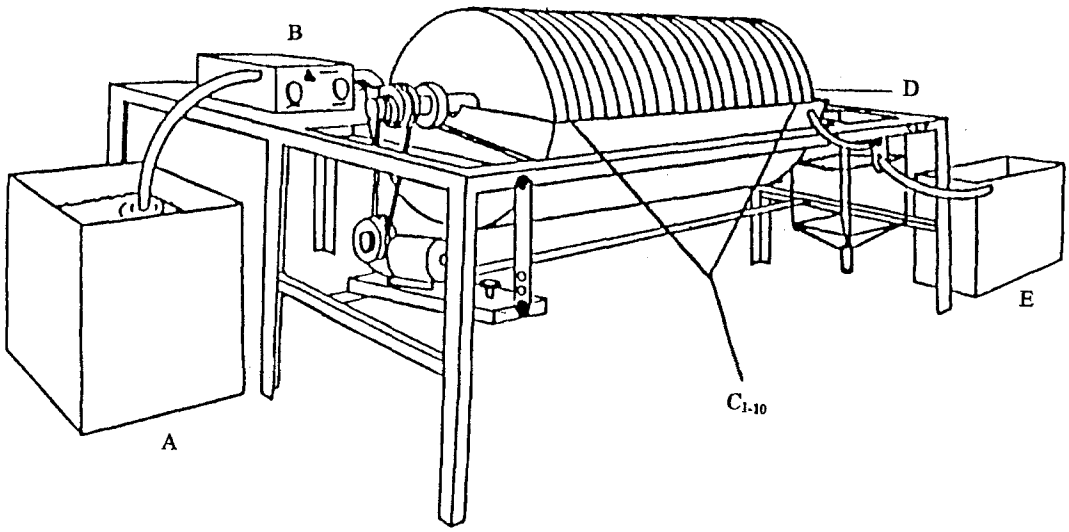

Figure 2 Experimental Rotating Biological Reactor (RBR). A = influent (wastewater raw), B = peristaltic pump, C_{1-10} = reactor chambers or stages (1–10), D = discs, E = effluent.

960 mg COD L^{-1} and 120 mg BOD_5 L^{-1} (The paper industry at that time was using a higher proportion water : pulp, giving a more dilute wastewater.) Dissolved oxygen was monitored to corroborate microaerophilic or aerophilic conditions [1].

To determine the diversity of ciliates, for each wastewater, a 'natural' sample from soil was collected and stagnant water samples from the three different wastewaters were cultivated in Petri dishes following techniques already reported [20]. The three RBRs were inoculated with the same volume of all cultures derived from soil and stagnant water in order to obtain similar initial communities of ciliates. The cultures in Petri dishes permitted a taxonomic determination of potential ciliates present in the RBRs. This information made in the recognition and enumeration of ciliates in the samples from RBRs during microscopic analysis easier.

Microscopic observations

Microscopic analysis was directed to identify ciliate species, as these microorganisms have proved to be good saprobity indicators [4,13–16,21]. To determine diversity, abundance and distribution of ciliates in the different wastewater samples, microscopic observations were performed in fresh preparations (using vital dyes and phase contrast), and in permanent preparations (silver staining). From both observations, morphological data were collected, such as total organism length, width, somatic and bucal ciliature, and the position and form of the macronucleus.

An Olympus microscope BH-2, coupled with phase contrast and a photographic camera, was used to observe the ciliates. Ciliate identification was based on specialized literature [13–15]. Ciliates counting was performed once a pseudo steady-state was reached (when variations in daily COD determinations were ±10%), using a modification of the Lackey technique [1,20,21]. Total ciliates were determined in a 0.1-ml sample from each chamber.

Results and discussion

Observations from Petri dish cultivations and RBR's mixed liquor, showed the presence of twenty-two species of ciliates (Table 2). In both cases, the diversity of ciliate in vinasses was slightly lower than in the other two wastewaters.

Microscopic analysis from Petri dishes demonstrated that five species were common to all three wastewaters. Four species were found exclusively in whitewaters, whereas five were present only in nejayote, and one in vinasses. Nejayote and whitewaters from the RBRs had 11 different species of ciliates, whereas the reactor with vinasses had only nine distinct species (Table 2).

Nineteen out of 22 ciliate species found in the Petri dishes were observed as well in the mixed liquor of RBRs. *Podophrya fixa, Paramecium aurelia*, and *Cyclidium glaucoma* were present in all three reactors, both in Petri dishes and in RBRs. Some species were found exclusively in RBRs; three from whitewaters, six from nejayote and one from vinasses.

Tables 3 to 5 show the ciliates' distribution and abundance in the RBRs fed with the different wastewaters, as well as some environmental parameters monitored during the experiments. All ciliates found are euritolerant to temperature, pH, and dissolved oxygen as compared to the tolerance of the content of organic biodegradable dissolved matter (measured as BOD_5) in the different wastewaters. Dissolved oxygen, pH, and temperature in the three systems were not limiting abiotic factors for ciliate proliferation [4,13–15,25]. As seen in the tables, some species are more tolerant than others to dissolved organic matter.

The number of ciliate species (specific richness) was higher in the central stages of the three reactors where the dissolved organic concentrations were at an intermediate concentration.

According to the initial ratio, BOD_5/COD for the three wastewaters, vinasses (0.32) appear to be more bidegrad-

Table 2 Registered ciliates in the three studied wastewaters in Petri dishes and mixed liquor from RBRs

Ciliates	Presence (+ or absence (–)[a] in:					
	Petri dishes prepared from			RBRs containing		
	N	V	W	N	V	W
(1) *Aspidisca cicada* [13]	+	–	+	–	–	+
(2) *Blepharisma americanum* v *dawsoni* [17]	–	–	+	–	–	–
(3) *Colpidium colpidium* [9]	+	–	–	+	–	–
(4) *Colpidium colpoda* [15]	–	+	–	–	+	–
(5) *Cyclidium glaucoma* [15]	+	+	+	+	+	+
(6) *Chilodonella uncinata* [13]	–	–	+	–	–	–
(7) *Dexiostoma campylum* [15]	+	–	–	+	–	–
(8) *Dexiotricha plagia* [29]	–	–	+	–	–	+
(9) *Epistylis plicatilis* [14]	+	+	–	+	+	–
(10) *Glaucoma scintillans* [15]	+	–	–	+	–	–
(11) *Litonotus lamella* [18]	+	+	+	–	+	+
(12) *Opercularia coarctata* [14]	+	+	–	+	–	–
(13) *Opercularia microdiscum* [7]	+	–	+	+	–	+
(14) *Oxytricha fallax* [13]	+	+	+	–	+	+
(15) *Paramecium aurelia* Complex [15][c]	+	+	+	+	+	+
(16) *Paramecium caudatum* [15]	–	+	+	–	+	+
(17) *Podophrya fixa* [18]	+	+	+	+	+	+
(18) *Tetrahymena pyriformis* Complex [15]	+	–	–	+	–	–
(19) *Uronema nigricans* [15]	–	+	+	–	–	–
(20) *Vorticella campanula* [14]	+	–	–	+	–	–
(21) *Vorticella convallaria* Complex [14]	–	–	+	–	–	+
(22) *Vorticella octava* Complex [14]	–	+	+	–	+	+
Total	14	11	14	11	9	11

[a]+ Present in some samples; – total absence.
[b]N = Nejayote; V = Vinasses and W = Whitewaters.
[c]The term Complex includes many species which were morphologically indistinguishable.

able than nejayote (0.286) and whitewaters (1.025). The ciliate counts in each reactor during their relatively constant operating conditions were: 120 250 ciliates ml^{-1} for vinasses; 62 690 ciliates ml^{-1} for nejayote and 11 380 ciliates ml^{-1} for whitewaters.

The greatest diversity or heterogeneity (H), in Shannon–Wiener index terms, was found first in the RBR with whitewaters (H = 3.0602), second using nejayote (H = 2.1722) and finally vinasses (H = 1.8267). In these three kinds of industrial wastewater, ciliate diversity decreased as the biodegradability of wastewater increased. The description and analysis of water quality obtained in RBRs and its association with ciliated community structure and the calculation of Shannon–Wiener index are reported in an internal publication [20].

On the other hand, considering the BOD_5 concentration measured, seven stenosaprobic species of ciliates were found in the RBRs. *Colpidium colpidium* and *Dexiostoma campylum* were observed between 255 and 535 mg BOD_5 L^{-1} (iso-metasaprobic level) from RBR with nejayote. In vinasses, *Colpidium colpoda* and *Podophrya fixa* were found at 50–120 mg BOD_5 L^{-1} (polisaprobic level). From whitewaters, *Litonotus lamella, Vorticella convallaria* and *Vorticella octava*, were present between 30 and 75 mg BOD_5 L^{-1} (polisaprobic level). These ciliates may be considered as saprobity indicators for each reactor. From the seven species listed only one, *Colpidium colpidium*, was not found in the technical literature among the saprobity indicators [4,6,13,15,21,25]. In general terms, the seven ciliates were found in a higher level of concentration of BOD_5 than reports published previously.

This behavior, according to the saprobity 'circle' (Figure 1), shows that saprobity characteristics correspond to higher concentrations of biodegradable compounds. The fact that the reactors have 'artificial aeration' derived from disc rotation, and hence, better conditions for degradation

Table 3 Ciliate distribution and concentration (organisms ml^{-1}) in the RBR fed with nejayote. Environmental parameters are included

Ciliate/RBR chambers	1	2	3	4	5	6	7	8	9	10
Colpidium colpidium	1760	870	440	0	0	0	0	0	0	0
Cyclidium glaucoma	10390	12150	5550	850		800	250	360	340	0
Dexiostoma campylum	870	340	160	0	0	0	0	0	0	0
Glaucoma scintillans	20	50	10	0	0	0	0	0	0	0
Paramecium aurelia	30	230	150	40	10	0	100	40	160	0
Tetrahymena pyriformis	350	1970	120	0	20	10	10	0	0	0
Epistylis plicatilis	10	350	110	100	0	610	1480	150	90	0
Opercularia coarctata	0	30	120	160	130	110	510	950	470	350
Opercularia microdiscum	0	230	140	50	70	0	370	100	310	220
Vorticella campanula	0	20	7970	450	880	300	1890	440	840	290
Peritricus, mobile forms	10	10	0	0	0	0	0	30	50	0
Podophrya fixa	0	30	70	60	60	290	120	70	0	0
Dissolved oxygen (mg L^{-1})	0.35 ± 0.29	1.36 ± 0.49	2.48 ± 0.24	3.17 ± 0.28	5.08 ± 0.51	5.06 ± 0.66	5.51 ± 0.39	5.70 ± 0.40	5.41 ± 0.18	6.00 ± 0.16
Biochemical oxygen demand (mg (BOD_5^{20}) L^{-1})	535 ± 10	–	255 ± 20	–	140 ± 10	–	110 ± 5	–	–	95 ± 5
Chemical oxygen demand (mg COD L^{-1})	1760 ± 130	1600 ± 100	1470 ± 90	1420 ± 90	1290 ± 50	1250 ± 30	1200 ± 20	1160 ± 40	1080 ± 60	1040 ± 100

Numbers 1–10 represent bioreactor chambers (1 = entry chamber; 10 = final chamber or effluent).
Total number of ciliates = 62 690 ml^{-1}.
Average temperature of liquid, 19.6 ± 1.2°C.
Average pH value of liquid, 7.92 ± 0.23 units.

Table 4 Ciliate distribution and concentration (organisms ml^{-1}) in the RBR fed with vinasses. Environmental parameters are included

Ciliate/RBR chambers	1	2	3	4	5	6	7	8	9	10
Colpidium colpoda	0	10	0	0	0	0	0	0	10	120
Cyclidium glaucoma	0	10	210	270	50	680	550	530	390	50
Litonotus lamella	30	380	3280	1550	2290	3630	4200	5720	8780	5010
Paramecium aurelia	0	10	2820	110	20	0	100	40	160	0
Paramecium caudatum	20	70	60	150	200	320	110	30	70	260
Oxytricha fallax	0	60	390	50	0	180	0	0	10	0
Epistylis plicatilis	0	210	0	280	0	80	10	80	60	220
Vorticella octava	710	2880	12170	6790	11090	8210	5530	5680	4020	4520
Peritricus, mobile forms	60	2540	5170	950	2490	3120	1520	2240	1320	740
Podophrya fixa	0	0	0	0	90	0	0	10	10	170
Dissolved oxygen ($mg\ L^{-1}$)	0.35 ± 0.29	1.16 ± 0.49	2.48 ± 0.24	3.17 ± 0.28	5.08 ± 0.51	5.06 ± 0.66	5.51 ± 0.39	5.70 ± 0.40	5.41 ± 0.18	6.00 ± 0.16
Biochemical oxygen demand ($mg\ (BOD_5^{20})\ L^{-1}$)	400 ± 40	–	320 ± 30	–	120 ± 10	–	80 ± 10	–	–	50 ± 5
Chemical oxygen demand ($mg\ COD\ L^{-1}$)	1790 ± 30	1580 ± 20	1310 ± 10	1230 ± 40	1000 ± 10	790 ± 20	610 ± 20	400 ± 20	400 ± 10	400 ± 10

Numbers 1 to 10 represent bioreactor chambers (1 = entry chamber; 10 = final chamber or effluent).
Total number of ciliates = 120 250 ml^{-1}.
Average temperature of liquid, 20.1 ± 0.17°C.
Average pH value of liquid, 7.74 ± 0.51 units.

Table 5 Ciliate distribution and concentration (organisms ml^{-1}) in the RBR fed with whitewaters. Environmental parameters are included

Ciliate/RBR chambers	1	2	3	4	5	6	7	8	9	10
Cyclidium glaucoma	90	0	50	10	210	0	60	1140	40	140
Dexiotricha plagia	40	60	80	20	0	20	10	100	130	0
Litonotus lamella	0	0	0	0	0	0	0	30	0	40
Paramecium aurelia	160	40	320	30	130	130	40	150	0	100
Paramecium caudatum	100	0	280	0	30	10	30	30	10	110
Aspidisca cicada	0	0	140	10	60	10	20	27	60	0
Oxytricha fallax	30	10	60	0	40	0	50	0	0	0
Opercularia microdiscum	0	70	0	0	0	0	0	0	30	0
Vorticella convallaria	0	0	0	6	200	280	410	370	90	10
Vorticella octava	0	0	0	6	200	280	410	370	90	10
Peritricus, mobile forms	0	350	380	600	1250	570	340	240	350	0
Podophrya fixa	0	10	60	0	30	70	50	10	20	40
Dissolved oxygen ($mg\ L^{-1}$)	2.90 ± 0.30	4.35 ± 0.40	4.80 ± 0.50	5.20 ± 0.50	5.20 ± 0.50	5.10 ± 0.50	4.95 ± 0.50	5.20 ± 0.50	5.55 ± 0.55	5.90 ± 0.60
Biochemical oxygen demand ($mg\ (BOD_5^{20})\ L^{-1}$)	90 ± 10	–	65 ± 10	–	50 ± 10	–	45 ± 10	–	–	30 ± 5
Chemical oxygen demand ($mg\ COD\ L^{-1}$)	730 ± 50	650 ± 70	600 ± 60	540 ± 30	500 ± 30	450 ± 30	430 ± 10	400 ± 20	410 ± 20	390 ± 20

Numbers 1 to 10 represent bioreactor chambers (1 = entry chamber; 10 = final chamber or effluent).
Total number of ciliates = 11 380 ml^{-1}.
Average temperature of liquid, 20.0 ± 0.07°C.
Average pH value of liquid, 7.83 ± 0.12 units.

of these compounds, may account for these differences from natural environments. The higher concentration of dissolved oxygen and higher availability of bacteria as food for the ciliates, may provide more opportunities for survival in environments with higher amounts of dissolved biodegradable organic material.

Colpidium colpidium and *Dexiostoma campylum* were found in the nejayote reactor as stenosaprobic ciliates, however, only *D. campylum* has been catalogued in the saprobic system as ciliate poly-isosaprobic [15]. According to the BOD_5 values found in this study (255–535 mg $BOD_5\ L^{-1}$), *D. campylum* may be classified as an isometasaprobic organism. For vinasses, the two stenosaprobic species (*Colpidium colpoda* and *Podophrya fixa*) have been catalogued, one as poly-isosaprobic [15] and the other as alpha-saprobic, respectively [16]. However, according to the levels of organic matter in the RBRs, measured as BOD_5 (between 50 and 120 mg L^{-1}), they could be classified as polysaprobes [15,16]. As before, this may be because in the reactors, conditions are more favorable for these ciliates than in natural environments.

Finally, the three species of ciliates from whitewaters are considered stenosprobic; two, *litonotus lamella* and *Vorticella convallaria*, as α-mesosaprobe [16,14] and the third, *Vorticella octava*, as β-α-mesosaprobe [14]. However, in this study, they were present in the RBRs within the interval of 30–75 mg $BOD_5\ L^{-1}$ that would classify them as polysaprobes, corresponding to a more polluted environment (one level) than those reported in the specialized literature.

Therefore, it may be inferred that, as a consequence of oxygen availability due to rotation of the discs, ciliates found in the three different wastewater treatment reactors correspond to those present in polluted natural sources of water with lower concentrations of dissolved organic biodegradable matter. It should be mentioned that previous information comes from direct observations in polluted rivers that perhaps do not have enough turbulence and oxygenation to reach equivalent aeration conditions as these RBRs. Finally, some amoeba species were found, but have not yet been identified.

According to these results, future research is going to be focused on the study of the possible presence of pathogens such as *Entamoeba histolytica, Naegleria* spp and *Acanthamoeba* spp.

Also, complementary studies on the chemical aspects of the metabolites present in each type of wastewater have to be conducted in order to determine their effect on the microbiological diversity and abundance of the whole ecosystem. The technological approach to this research should contemplate the modification of abiotic parameters to favor the presence of non-pathogens and waste-removing organisms over the opposite microbial communities.

Conclusions

The microbial cultures in dishes gave a similar number of ciliate species to RBRs. This makes recognition and subsequent numeration of ciliates in samples from rotating biological reactors easier.

The number of ciliate species was higher in the RBR's central stages from the three wastewaters studied. The ciliate community was more diverse in RBRs with whitewaters (less biodegradable wastewater) than in the other two. However, the ciliate concentration was greater in the more biodegradable wastewater (vinasses).

From the total number of ciliates found in the three wastewaters used, *Cyclidium glaucoma, Paramecium aurelia* complex and *Podophrya fixa* were common in both Petri dishes and RBRs. *Glaucoma scintillans, Tetrahymena pyriformis* complex, *Dexiostoma campylum, Colpidium colpidium, Opercularia coarctata* and *Vorticella campanula* were exclusive to nejayote, whereas *Colpidium colpoda* was found only in vinasses, and *Dexiotricha plagia* and *Vorticella convallaria* were found only in whitewaters. The majority of the ciliates present in the experiments were eurisaprobic, and their diversity (richness and abundance) varied as a function of the type of substrate fed to the reactor.

Thus, in a municipal or industrial wastewater treatment where these types of effluents are purified, with simple microscopic observations by a trained technician, it is possible to determine, through the community structure of ciliates, the quality of water during its biological treatment, and therefore indirectly, the physicochemical behavior of the RBRs.

Saprobic systems are not directly applicable to evaluate water quality in RBRs with industrial wastewater. However, it is possible to establish for the RBRs, a qualitative correlation between the ciliates and the biodegradable dissolved contaminant concentration, measured as mg $BOD_5\ L^{-1}$, when and only when, the samples to be evaluated come from the same treatment system at controlled operating conditions.

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