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Implementation of the sludge biotic index in a petrochemical WWTP in Brazil: improving operational control with traditional methods

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Abstract Microbiological analysis of activated sludge is an important tool for monitoring wastewater treatment plants (WWTP). The utilization of the sludge biotic index (SBI) provides helpful information in examining the quality of biological treatment process and has been tested for several different systems. Although its utilization has been increasing, it is still not widespread, especially in Brazil. Also, its applicability has been considered limited for some particular systems. Thus, it becomes important to evaluate the relations among operational and biological parameters of each WWTP in order to characterize the system and its variations. In this work, microscopic analysis were performed once a week for 1 year ($n = 54$) and the results were compared to the physicochemical, operational parameters and efficiency of the plant along the period. The four seasons were comprised and analyzed, as we cannot neglect the influence of environmental changes in this subtropical region. Not only had we found a strong influence of the evaluated parameters on the structure of the biological community but there is also a good correspondence of SBI with the performance of the WWTP. More importantly, including microscopic analysis in the operational routine made it possible to notice even the slightest changes in the biological community that were not enough to diminish the SBI classification of the sludge, but were satisfactorily informative to show in advance to operators when to take corrective actions about an increase of COD and BOD in the influent and when it was necessary to discard the exceeding sludge.

Keywords Activated sludge microbiology · Microbial ecology · Microfauna · Wastewater treatment · Process performance

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Introduction

The microorganism's ability to purify sewage has been explored in wastewater treatment plants (WWTP) for almost a century and biological WWTPs are considered the most common and one of the most important applications of biotechnology in the world [7, 20]. Contradictory to its extensive use and recognized benefits taken from microbial activities, the deeply complex ecological communities in WWTP are barely understood [7]. Among other industrial applications, activated sludge is one of the important classes of continuous culture—a technique whose importance has been recently revisited [5]. Wastewater treatment has been proposed to be a subdiscipline and a model for the study of microbial ecology, as the system is a suitable and amenable ground for testing fundamental ecological questions [7]. Besides, it is considered that the concepts of microbial ecology are the foundation for managing processes in environmental biotechnology, and the microbial communities should be properly managed in order to provide us with services such as detoxification and treatment of water and wastewater [17]. Despite the emergence of alternative methods of biological treatment in the last years, the conventional activated sludge (CAS) bioreactors are still the most frequently applied secondary treatment methodology [20]. In this system, microbial communities consisting mainly of bacteria, protozoan, fungi, and small metazoans are kept in suspension under aeration to permit the organic matter to be consumed [11]. Bacteria are central in this process, and their communities have been proven to be influenced by the influent quality as well as changes in these communities significantly affect the effluent characteristics [23]. Nonetheless, it is believed that protozoans and metazoans also have an important role in the maintenance of the activity of the microbial communities by selecting species, as they release growth-stimulating compounds, excrete mineral nutrients, and keep the metabolic rates elevated [6]. Similar function of metazoans was recently described for gravity-driven membrane (GDM) filtration systems, where these organisms were shown to prevent high biofilm accumulation, enhancing the permeate flux [8]. In addition, these groups are also considered bioindicators, and the use of indexes based on microscopic analyses to evaluate the quality of treatment systems has increasingly been applied [3]. This is possible because the community structure of the activated sludge presents quite a fast response to operational changes [11, 15]. Biotic indexes are common in hydrobiology since the estimate of biotic integrity may be the best tool to assess the effect of multiple stressors in aquatic environments [18]. In 1994, Madoni created the sludge biotic index (SBI), and since then it has gradually been increasingly used by researchers and WWTP workers as an additional parameter to control the quality of activated sludge systems [3, 14]. This index correlates presence and abundance of protozoans, amoebas, and metazoans with quality of the waste and the biological process and has been shown to be efficient to evaluate the depurative conditions of activated sludge plants, despite being limited in some aspects [3]. Although the value of these analyses has been recognized, their utilization is not widespread in Brazil and their results are frequently underutilized, possibly because engineers and other WWTP operators are not trained in biology, and these two sciences have not been powerfully integrated, as discussed elsewhere [7]. The microscopic analysis of the sludge is swift and inexpensive. Even though morphological identification is often argued not to be accurate, more sophisticated methods such as molecular techniques are not available for routine in most of the municipal and state WWTPs in Brazil, and it makes Madoni's index based on identification of key protozoan groups particularly useful. In this context, the aim of this work is to implement the microscopic evaluation of the activated sludge in a WWTP located in the third Brazilian Petrochemical Plant (Triunfo City, Rio Grande do Sul, Brazil) applying Madoni's SBI index, and correlate it with physicochemical analysis, operational parameters, and performance of the system. We want to assess whether the microorganisms are or are not reflecting the quality of the treatment in this plant. We also examined the dynamics and structure of microbial communities in order to investigate the effect of the seasons and the physicochemical variables on the efficiency of treatment.

Materials and methods

Wastewater treatment plant

This study was performed in a WWTP dedicated to the treatment of waste from Brazilian Third Petrochemical Plant, City of Triunfo, Rio Grande do Sul, Brazil (29° 51′35.02″S, 51°20′50.17″W) (Fig. 1). This WWTP belongs to the State Sanitation Company, *Companhia Riograndense de Saneamento (CORSAN)* and is called SITEL (Integrated System for Treatment of Wastewater). It has been operating since 1982, with two bioreactors of conventional activated sludge (CAS) with volume of 13,000 m^3 each, with interchanged operation, from which all the samples were obtained.

Sampling

Sampling was realized on a weekly basis in 1 year (from July 2011 to August 2012) totalizing 54 samples. For microscopic analyses, 50–200-ml samples were collected into new clean plastic bottles with a 1-l capacity, allowing the presence of air and analyzed immediately. For physicochemical analyses, samples were collected in dark 5-l gallons and preserved according to standard methods until the analyses were performed [1].

Microbiological analysis

The analyses were performed in our biology laboratory (SEBIO/SITEL), using an optic microscope (Zeiss AXIO LabA1), ×400 magnification. After homogenization, 50 μl of sludge were applied in a microscope glass and all the organisms found were counted. The taxonomic groups were identified morphologically and the sludge biotic index (SBI) was calculated according to Madoni (1994).

Operational parameters and physicochemical analysis

Operational parameters were monitored by our operational department (SEOPER/SITEL). Dissolved oxygen (DO)

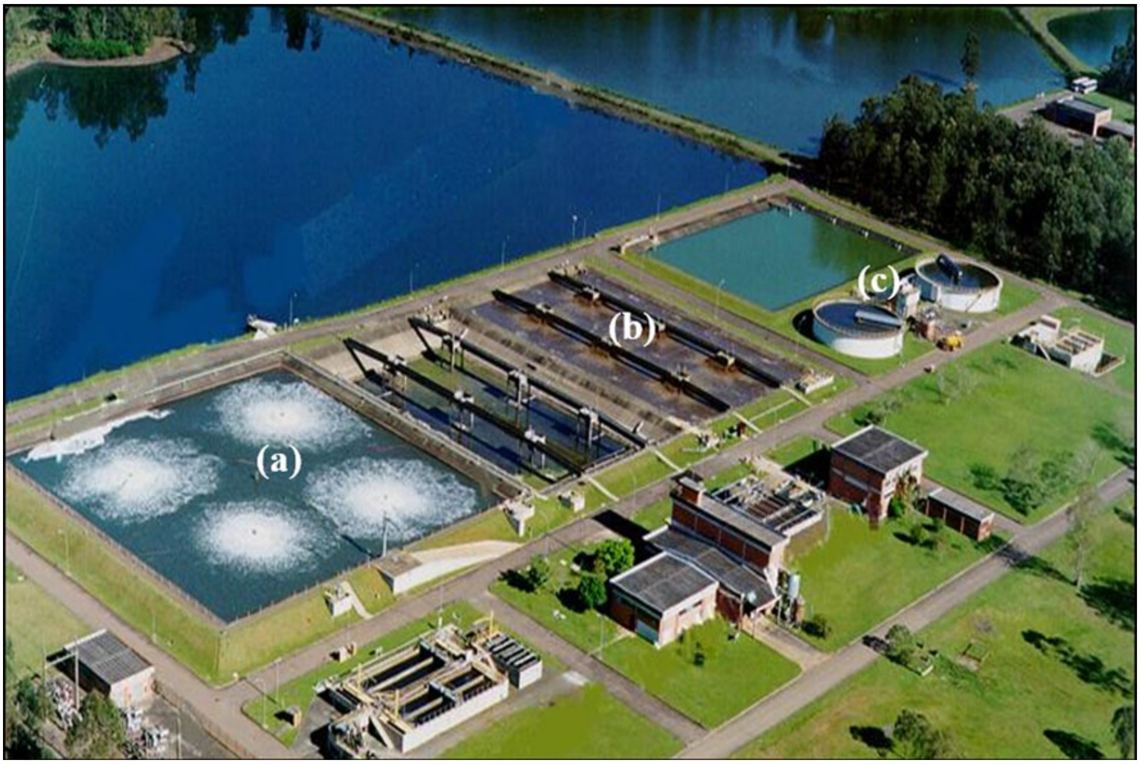

Fig. 1 Aerial view of the WWTP housing the equalization tank (**a**), the aeration tank (**b**), and the settling tanks (**c**)

and pH are monitored by fixed electrodes (Metler M300) installed 80 cm from the liquid surface next to the exit of the bioreactor. Treatment flow (TF), sludge recycling flow (SRF), and sludge discard (SD) are controlled by Parshall flumes and monitored online (Nivetec Echotrek ST-300), with the sizes of the flumes being 18″, 18″, and 6″, respectively. Oxygen uptake rate (OUR), temperature (T), and food: microorganism ratio (F/M) were also monitored. Other analysis as chemical oxygen demand (COD), biological oxygen demand (BOD), mixed liquor volatile suspended solids (MLVSS), and mixed liquor suspended solids (MLSS) were performed in our physicochemical laboratory (SEFIQ/SITEL) using standard methods [1]. The efficiency of the secondary treatment was estimated by the reduction (%) of COD and BOD (respectively, daily and weekly analyzed, in samples from organic influent and exit of secondary treatment). A tertiary treatment is performed in the WWTP by passage of effluent by eight stabilization ponds. However, the final effluent was not considered in this study.

Data analysis

Richness and abundance of organisms from microbial community were represented by the total number of taxonomic units and by the number of individuals collected throughout the year, respectively. Variation of richness and abundance of organisms through the year were evaluated by analysis of variance (ANOVA). Tukey tests were applied a posteriori to estimate individual differences. Variation in the organisms composition was described through principal components analysis (PCA). A permutational multivariate analysis of variance (PERMANOVA) was performed in order to check the significance of differences in the species compositions between seasons [2]. A correlation matrix among physicochemical parameters was established in order to choose only not strongly correlated variables to be used in further analyses. All results with r^2 higher than 0.55 were considered significant (Online Resource 2). Canonical correspondence analysis (CCA) was performed to verify the relation between the environmental variables with the composition of the community in the different seasons. Likewise, similar analysis was performed considering the variation of physicochemical and operational parameters related to the seasons. All statistical analyses were performed using PAST 2.17b software [12].

Results

Environmental and operational features

The most carefully controlled variables were pH (adjusted in an equalization tank prior to the entrance of influent in the aeration tank to be kept around 7.0) and DO, which was

Table 1 Variation of physicochemical and operational parameters along the period studied

	Mean	Minimum	Maximum	Range
pH	7.0	6.6	7.6	1
DO (mg/l)	3.7	0.7	6.9	6.2
T (°C)	21.6	15.5	27	11.5
COD (mg/l)	335.9	193	758	565
MLSS (mg/l)	8,806	7,300	11,750	4,450
MLVSS (mg/l)	6,418	5,097	8,529	3,432
TF (m^3/h)	530	200	740	540
SRF (m^3/h)	313	160	430	270
SD (m^3/week)	174	0	1,250	1,250
F/M	0.05	0.0083	0.0901	0.0818
OUR (mgO_2/l/h)	7.9	2.4	29	26.6

pH potential of hydrogen, *DO* dissolved oxygen, *T* temperature, *COD* chemical oxygen demand, *MLSS* mixed liquor suspended solids, *MLVSS* mixed liquor volatile suspended solids, *TF* treatment flow, *SRF* sludge recirculation flow, *SD* sludge discard, *F/M* food: microorganisms ratio, *OUR* oxygen uptake rate

kept higher than 2.0 mg/l, except for one shock load situation where it fell as low as 0.7 mg/l. The mean and range of all monitored physicochemical and operational factors are illustrated in Table 1.

It is worth noticing one isolated situation where operational problems had led to a shock load. In this sample, the COD raised to 758 mg/l, more than 200 % above the mean. In this event, the DO fell as low as 0.7 mg/l and the OUR reached 29 mgO_2/l/h.

The first three axes from CCA analysis (Fig. 2) have explained 96 % of the variation of physicochemical and operational parameters in seasons studied. According to the relations between the physicochemical variables and the ordination axes, the samples from winter were related to the first axis and encompassed the highest SD values. During autumn, the highest TF were found. For pH, COD, T, and DO, the highest values occurred during summer. The lowest DO values were registered in spring.

Biological description

The biological investigation revealed an average of 4,544 organisms/ml, and the group of crawling ciliates were predominant (43.1 %), followed by testate amoeba (23.5 %). Testate amoeba from genus *Arcella*, *Euglypha* and other not identified, together with small flagellates were the only groups found in all of the samples. Crawling ciliates from genus *Aspidisca*, were present in 53 out of 54 samples, showing the ability of these groups to survive through the system variations. Despite their resilience, the abundance of these groups has varied considerably among the samples. In addition, it was observed that among the 19 taxonomic groups identified, 14 were present in more than 50 % ($n \geq 27$) of the samples. Tardigrades were the least common group, being found in only four samples (7 %). The frequency and abundance of organisms is shown in Table 2.

In the single event of shock load mentioned above, microscopic analysis revealed a rapid response from organisms. Several dead rotifers were seen, as well as stalked ciliates found free instead of attached to the flocs, and weaker dispersed flocs.

Microbial community structure

In our study, the mean richness varied from 12 taxonomic units (spring) to 15 (winter), which was not significantly different among the seasons of the year ($F_{3.48} = 6.421$, $p = 0.593$). On the other hand, the total abundance varied from 1,580 organisms/ml to 8,720 organisms/ml individuals and the mean abundance has varied significantly among

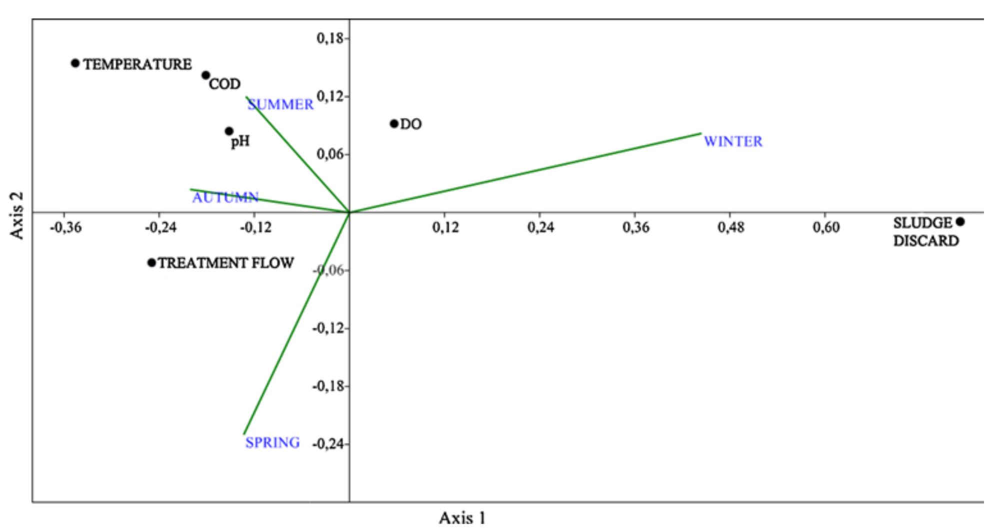

Fig. 2 Canonical correspondence analysis (CCA) of the relationships between seasons and physicochemical/operational parameters; *pH* potential of hydrogen, *DO* dissolved oxygen, *COD* chemical oxygen demand

Table 2 Frequency and abundance of the main identified groups

Key groups	Taxonomic units	Frequency	Abundance (org/ml)		
		Number[a] (%)	Min	Max	Range
Crawling ciliates	*Aspidisca* spp.	53 (98)	0	4,980	4,980
	Euplotes spp.	34 (63)	0	500	500
	Crawling ciliates not identified	54 (100)	160	3,440	3,280
Free-swimming ciliates	*Paramecium* spp.	30 (56)	0	760	760
	Free-swimming ciliates not identified	12 (22)	0	300	300
Attached ciliates	Attached ciliates	52 (96)	0	1,000	1,000
Carnivorous ciliates	*Amphileptus* spp.	29 (54)	0	320	320
	Trachelophylum spp.	47 (87)	0	820	820
Testate amoebae	*Arcella* spp.	54 (100)	40	600	560
	Euglypha spp.	54 (100)	180	1,200	1,020
	Testate amoebae not identified	54 (100)	40	1,080	1,040
Small metazoan	Rotifers	36 (67)	0	240	240
	Nematodes	17 (31)	0	60	60
	Tardigrades	4 (7)	0	40	40
	Aeolosoma spp.	24 (44)	0	80	80
	Chaetenotus spp.	21 (39)	0	220	220
	Catenula spp.	28 (52)	0	140	140
Flagellates	*Euglena* spp.	43 (80)	0	1,860	1,860
	Small flagellates not identified	54 (100)	140	5,040	4,900

[a] Number of observations out of 54 samples

Min minimum of organisms/ml, *Max* maximum of organisms/ml

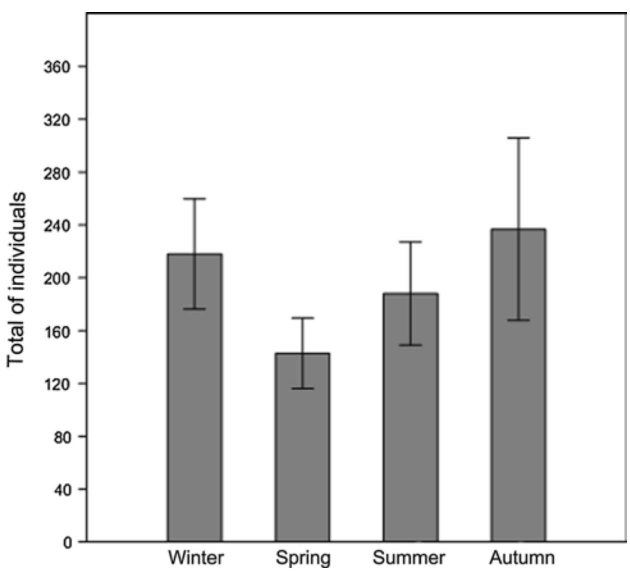

Fig. 3 Variation of abundance between the seasons ($F_{3.48} = 2.87$, $p = 0.04978$): the total of individuals refers to the mean of individuals counted in 0.05-ml samples

the seasons ($F_{3.48} = 2.87$, $p = 0.04978$) (Fig. 3). The mean abundance was higher during autumn when compared to all the other seasons (Tukey, $p = 0.044$).

The PERMANOVA test has shown that the composition of the microbial community was significantly different among the seasons ($R = 1.656$, $p < 0.05$). Pairwise comparisons have shown that these differences are mainly between the composition from autumn against the ones from winter ($p = 0.0219$) and from spring ($p = 0.0229$). The three first axes of PCA analysis explained 66 % of organisms variation, showing that community composition and parameters values are more similar during summer and autumn than the ones from winter and spring (Fig. 4).

The first three axes from CCA analysis have explained 80.7 % of the variation in the organisms composition within the time extent the study was conducted. According to the relations between the environmental variables and the ordination axes, the amounts of COD, pH (related to the first axis), treatment and sludge recycling flows (related to the second axis), and disposal of exceeding sludge, temperature and DO (related to the third axis), all influenced significantly the organisms composition throughout the time (Online Resource 3, Fig. 5).

Ciliates from the genus *Aspidisca*, the Gastrotricha *Chaetenotus* and Tardigrades were more abundant in the samples obtained during the summer, especially when the values of pH, COD, and sludge discard were higher, together with the lowest temperatures observed in that season, sludge recycling and treatment flow. Besides, other crawling ciliates, attached ciliates, *Arcella,* rotifers, and nematodes were more abundant in samples obtained when the values from these variables were lower. These organisms appear to be associated with high rates of DO.

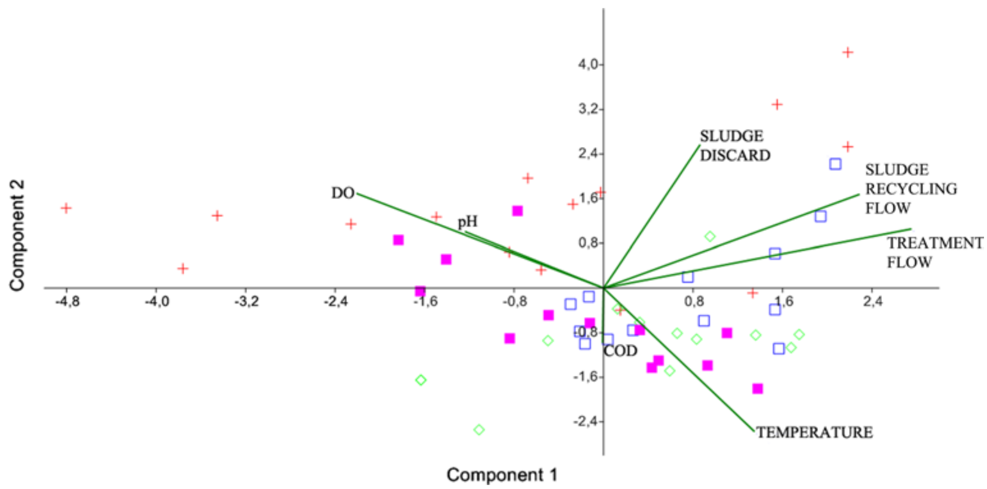

Fig. 4 Principal component analysis (PCA) showing the relations between samples and the physicochemical and operational parameters; samples are targeted as follows: *plus* winter samples; *unfilled diamond* summer samples; *unfilled square* spring samples and; *filled square* autumn samples. *pH* potential of hydrogen, *DO* dissolved oxygen, *COD* chemical oxygen demand

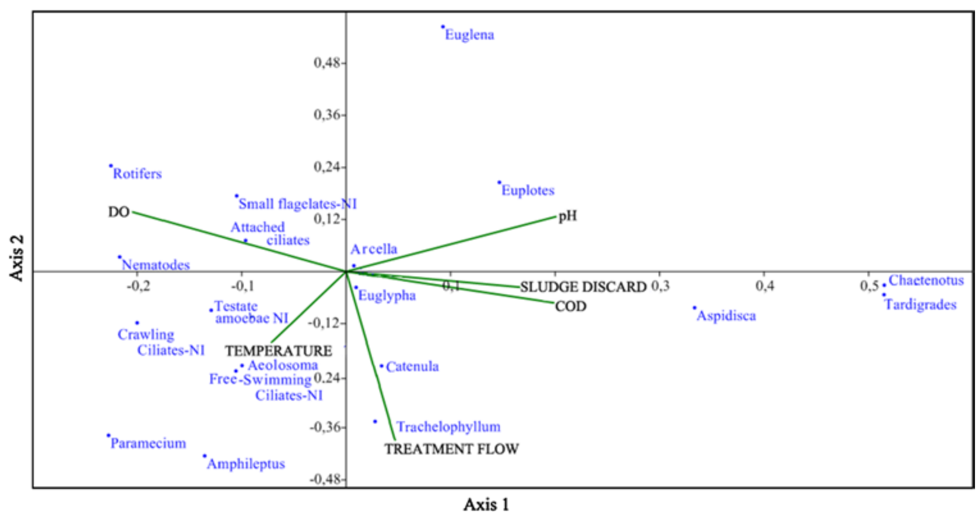

Fig. 5 Canonical correspondence analysis (CCA) of the relationships between analysis showing physicochemical/operational parameters and abundance of taxonomic units found in the study; *pH* potential of hydrogen, *DO* dissolved oxygen, *COD* chemical oxygen demand

Flagellate algae from the genus *Euglena* had their abundance increased when temperature, treatment, and recirculation flows were lower. On the other hand, Annelids from the genus *Aeolosoma*, ciliates from the genus *Trachelophyllum*, and platyhelminthes from the genus *Catenula* were more abundant when these variables were higher. These organisms had little correlation with other variables.

Ciliates from the genus *Amphileptus*, *Paramecium*, and other free-swimming ciliates were more abundant when temperature, DO, treatment, and recirculation flows were higher, associated to low values of pH, COD, and sludge discard.

Aspidisca, *Amphileptus*, *Euglypha*, testate amoeba, rotifers, nematodes, tardigrades, *Chatenotus*, and *Euglena* were more abundant in spring and summer, while the other groups were associated to autumn and winter.

Another CCA analysis was performed in order to evaluate the relationships among functional groups and physicochemical and operational parameters. As we have seen, a relatively large variation in composition and abundance of taxonomic units along the time but not in the richness, the goal of this analysis was to verify if the same pattern would be observed when turning over taxonomic groups by the key groups used in the establishment of SBI. For this analysis, composition and abundance of key groups were not found to respond to the influence of the same variables (Online Resource 1).

Performance and IBL

The performance of the WWTP is shown here by its efficiency in removing COD and BOD, as it is shown in Fig. 6. The mean COD removal was 75 %, varying from 68 % (in September/2011) to 80 % (January, March and April/2012). Also, the mean BOD removal was 97 %, varying from 95 % (June/2011) to 99 % (December/2011, January and April/2012). This efficiency was confirmed by an almost constant value of SBI, which was stable at class I (valued 10 in the first 52 samples and 9 in the last 2).

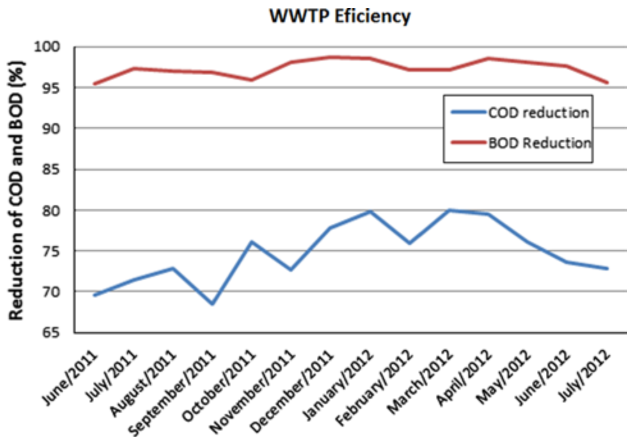

Fig. 6 Efficiency of the secondary treatment from the WWTP measured by removal of BOD and COD; *COD* chemical oxygen demand, *BOD* biological oxygen demand

Discussion

Several studies have reported that the community structure responds to operational variations [4, 13, 16, 19, 23] although even systems which were started with identical operational conditions can present distinct communities for some groups of organisms [21]. Our results not only corroborate this, but also have shown the applicability of SBI for monitoring petrochemical waste treatment by CAS bioreactors, as an almost constant SBI was coherent with an almost constant BOD removal efficiency.

Biological description

The group of metazoans with longer life cycles had their occurrence increased whenever the sludge got older. On the other hand, after the disposal of exceeding sludge, flagellates and small ciliates were in higher number, in agreement with previous community succession studies [6, 14].

The effect of shock load events has been studied for bacterial communities, highlighting the functional resistance presented by bacterial groups in the system [19]. In our microfauna observation, the event of a shock load was recognized immediately in the biological analysis as stated in the "Results" section. In the week following this event, there was a complete absence of organisms from the small metazoan group in the sample (data not shown). Based on these findings, we consider that the biological analysis was satisfactory in predicting an increase of COD and BOD, whose results are obtained with a delay of 1 and 5 days, respectively, helping the agility of the corrective actions.

Another important remark concerning operation was that we could safely keep the sludge in the system longer than it was stated in the original project. SITEL is a WWTP that works with extended aeration and long residence time of the sludge in the system (not kept constant, but always longer than 60 days), so the microfauna is diverse and equilibrated, as expected [14]. However, after several weeks without disposal of exceeding sludge (or with only a few cubic meters disposed), the number of small metazoans would increase, nematodes were found, and the flocs would be more open and weak, with a boost in dispersed bacteria. These observations were consistent with an increase of the sludge volume index (SVI) and, eventually, beginning of flotation in the secondary settling tank (data not shown). Whenever this happened, a discard of exceeding sludge was followed by immediate recovery of the system.

Environmental interactions and microbial community structure

The microbial communities found in activated sludge are complex assemblages of microorganisms that maintain dynamic equilibrium by responding to changes in environmental conditions by shifts in the structure of the community (richness, abundance, and composition) [10]. In our study, we observed differences in community abundance and composition between seasons combined with no significant changes in richness. Furthermore, temporal variations on community structure in activated sludge are strongly influenced by operational and/or environmental parameters [9, 13]. Thus, knowledge of the ecology of microbial communities is imperative in understanding the factors that may influence the efficiency and stability of a WWTP and mainly outline strategies for process improvement.

Our findings indicate a temporal variation of abundance and composition, exhibiting a fluctuating pattern for the variation of organisms among seasons, along with parameters such as temperature and others (Fig. 4). However, the key groups showed a relative constant pattern (Online Resource 1), which can be explained by the stability of the system, as our WWTP receives a quite similar influent all through the time (always from the same industries in the petrochemical plant) and it has maintained a constant performance (efficiency of BOD removal). In a recent study, seasonal changes in microbial community structure monitored by phospholipid fatty acid profiles were also found not to affect the BOD removal. The authors considered that, even with the change, the new dominant species were also able to utilize the organic compounds present in the influent [23]. Moreover, inside our bioreactors, the variables that compose the ecosystem for the microbial community tend to be less variable than the influent itself, once the disturbances are minimized as the influent does not enter directly into the aeration tank. Instead, it is driven first to an equalization tank, where it is homogenized, temperature-stabilized, and, if needed, the pH is adjusted before it enters gradually into the tank (mean 530 m^3/h in a 13,000 m^3 tank). On the other hand, throughout the year, weather variations can influence parameters such as temperature and consequently, pH, DO, and COD, which are essential for the presence and function of the community inside the reactor [13, 22]. This was also demonstrated here by both the variation of physicochemical results shown by PCA analysis (Fig. 4) and by the differences in composition of organisms throughout seasons (PERMANOVA and PCA analyses).

Performance, monitoring, and SBI

According to Madoni (1994), a class I SBI (values from 8 to 10) indicates very well colonized and stable sludge, with excellent biological activity and performance. By implementing this new routine analysis, we become familiar with the communities of our CAS bioreactor so that changes observed in microscopic analyses could be used for predicting a rise in COD and BOD values. For example, when the shock load occurred, the changes were noticed straightforwardly, allowing immediate corrective actions to be taken. These actions were the reason why the disturbance did not last long enough to diminish the SBI-based class of the sludge.

Additionally, exceeding sludge disposal is often a problem in terms of area, costs, and/or pollution. Applying the results of this work, we were able to reduce the discard of sludge by keeping it longer in the system than it was fixed in the original project of the WWTP, and it was safe for the system because we closely followed the response of the sludge organisms.

For these reasons, we consider that monitoring WWTP by using this simple and inexpensive analysis should be encouraged, especially in developing countries.

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Ethical standards The authors declare that the experiments comply with the current Brazilian laws.

Conflict of interest The authors declare that they have no conflicts of interest.

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