

# Consortia of microalgae and bacteria in the performance of a stabilization pond system treating landfill leachate

R. H. R. Costa, C. L. Martins, H. Fernandes and V. F. Velho

## ABSTRACT

This study treated sanitary landfill leachate and was conducted in a pilot-scale system composed of three serial ponds (P1, P2 and P3), followed by a rock filter, in order to evaluate the microbial consortium influence on system performance and to investigate microorganism dynamics in the process. The system was broken into three stages, with a continuous flow rate ( $Q = 200 \text{ L d}^{-1}$ ) for 43 weeks. The stages were as follows: conventional operation (stage I), 12 h aeration in P2 (stage II), and 18 h aeration in P2 (stage III). The results showed the possibilities for treating landfill leachate, presenting an average efficiency of 75% for both filtered biochemical oxygen demand and ammonium. At the end of stage III, the ammonium concentration was  $6 \text{ mg L}^{-1}$ , which is lower than that established by Brazilian regulations for wastewater discharge (CONAMA 430/2011). The aeration applied in P2 led to a change in the microbial consortia during the second and third stage, which influenced the quality of the final effluent. The best performance was seen in stage III, where the system showed high microbial diversity, including the presence of nitrifying bacteria.

**Key words** | landfill leachate, microbial consortia, sanitary landfill, stabilization ponds

R. H. R. Costa (corresponding author)

C. L. Martins

H. Fernandes

V. F. Velho

Department of Sanitary and Environmental

Engineering,

Campus Universitário,

Trindade, CEP 88010 970, Florianópolis,

SC,

Brazil

E-mail: [rejane@ens.ufsc.br](mailto:rejane@ens.ufsc.br)

## INTRODUCTION

The degradation of municipal solid waste in landfills produces large amounts of leachate, which contains a variety of contaminants, such as hydrocarbons and their derivations, heavy metals, ammonium, etc. If not properly treated, leachate can pollute surface and ground waters (Jiang *et al.* 2009).

Activated sludge and its variations are largely utilized to treat sanitary landfill leachate. The most common and effective landfill leachate treatment method is on-site activated sludge coupled with the necessary pre-treatment (Wisniewski *et al.* 2006). However, stabilization ponds are a simple way to treat effluents, and their use has been shown to be a promising treatment for landfill leachate (Renou *et al.* 2008; Leite *et al.* 2011). Practical application and simplicity in both design and operation are important advantages of the pond system treatment.

Studies have reported that conventional pond system use in the treatment of leachate still results in high final concentrations of COD and ammonia, especially recalcitrant compounds, which are common in this type of effluent (Thörneby *et al.* 2006). Given their complexity, the use of aerated ponds has proven to be a very auspicious technology

in order to obtain final effluent with a significant reduction of carbonaceous and nitrogenous fractions (Renou *et al.* 2008; Mehmood *et al.* 2009).

The pond system operates through chemical, physical and biological processes. The presence of microorganisms is essential for all wastewater treatment stages and in the degradation of organic matter, there is a natural succession of these microorganisms. The symbiotic interactions of microalgae and bacteria form the basis of the biological oxygen demand (BOD) in the wastewater treatment ponds. A consortium of microalgae and bacteria can be more efficient in detoxification of organic and inorganic pollutants, as well as removal of nutrients from wastewaters, compared to individual microorganisms (Subashchandrabose *et al.* 2011), which further enhances their growth. This group culture concept has proven itself to be promising in the improvement of wastewater treatment, encompassing not only nutrient removal, but also biomass harvesting that was observed in research with high rate algal ponds (HRAPs), as described by Park & Craggs (2010).

A thorough understanding of the microbiology and ecology of the microorganisms involved in the leachate

degradation processes are extremely important in procedural control and treatment efficiency. What sets the stabilization pond system apart from all other treatment technologies is the involvement of the micro-algae process. In this respect, the microbiology of these ponds more closely mimics that of a polluted lake system than do other treatment technologies (Pearson 2005). The higher treatment efficiency is achieved because of the relationship between these microorganisms, the physical-chemical and seasonal variations in the environment, and operational conditions (Kargi & Pamukoglu 2003).

Studies investigating the structures of microbial communities and their performances in different treatment systems have reported a link between these community structures and the efficiency with which nutrients are removed (Xie *et al.* 2010; Araujo *et al.* 2010). The microbial population of a bioreactor is responsible for the decomposition of leachate pollutants. To gain biological insights into this process, microflora have been investigated using different methods (microscopy, fluorescence *in situ* hybridization (FISH), polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE)). However, few studies have been conducted on stabilization ponds that connect the role of microorganisms and the subsequent treatment performance of landfill leachate.

Therefore, this study aimed to evaluate the landfill leachate treatment system using three ponds, in series (P1-anaerobic, P2-facultative and P3-maturation), followed by a rock filter (RF), with an emphasis on the consortium of microalgae and bacteria for three different operational

conditions. For this purpose, the specific objective of this work was to take a taxonomic approach to the microorganisms found in different stages of treatment, in order to determine the relationship between the biological profile and physico-chemical parameters.

## MATERIAL AND METHODS

### Leachate production

The leachate used in the experiment was obtained from a sanitary landfill in the city of Biguaçu, Santa Catarina, Brazil (27°21'42 S 48°38'24 W). The landfill has been operated since 1990, receiving domestic and hospital wastes from 22 cities, which requires an average production of 800 to 1,000 ton d<sup>-1</sup>. The leachate was transported to the laboratory by a tank truck and was stored in a closed equalization tank with a capacity of 5 m<sup>3</sup>, which was refilled monthly. From the equalization tank, the leachate was pumped into the treatment system.

### Pilot-scale treatment system

The experiment was conducted in a pilot-scale stabilization pond system, composed of three ponds in series, anaerobic (P1), facultative (P2) and maturation (P3), followed by an RF (Figure 1). This was performed at the Federal University

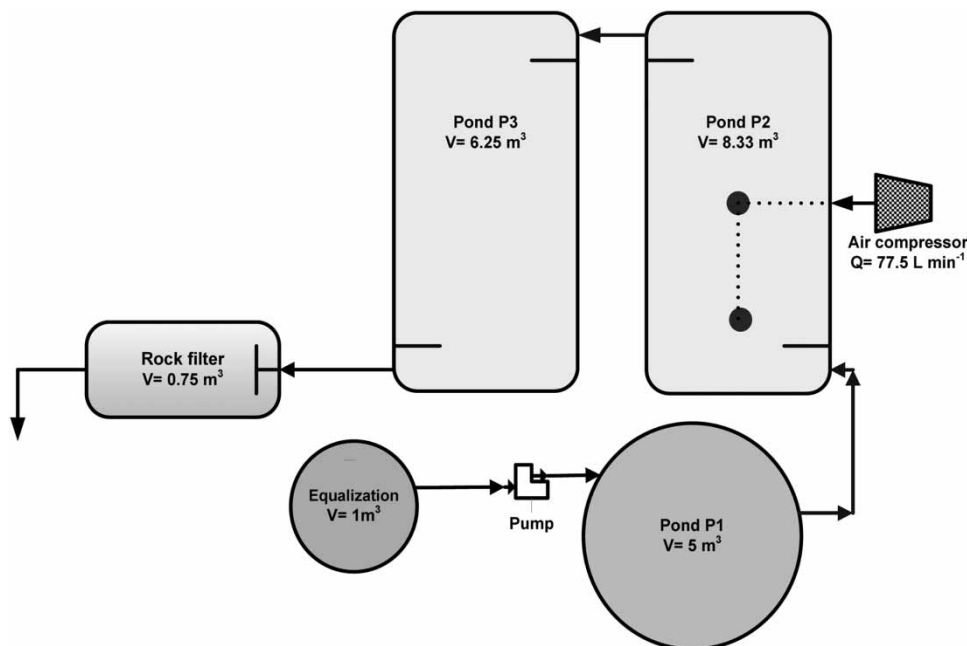


Figure 1 | Treatment system scheme.

**Table 1** | Physical and operational characteristics of the treatment system

Characteristics	P1	P2	P3	RF
Length (m)	–	4.36	4.36	3
Width (m)	–	2.4	2.4	0.5
Diameter (m)	1.85	–	–	–
Depth (m)	1.85	0.8	0.6	0.5
Volume (m <sup>3</sup> )	5	8.33	6.25	0.75
Hydraulic retention time – HRT (day)	25	42	31	4
Flow rate (L d <sup>-1</sup> )	200	200	200	200

of Santa Catarina, in Florianópolis, Brazil. The main physical and operational conditions of the treatment system are presented in Table 1. P1 had a cylindrical shape, while P2 and P3 were rectangular. Acrylic plates were installed in both the inlets and outlets of P2 and P3 to ensure the flow direction and to avoid short-circuiting. The RF used for effluent polishing was filled with gravel stones (commercial no. 4: 38–76 mm diameter) and fed with a hydraulic flow rate of 0.25 m<sup>3</sup> m<sup>-3</sup> d<sup>-1</sup>.

The depths of the facultative and maturation ponds were based on the literature (Shilton 2005; von Sperling 2007) and previous work developed by the research group (Gehring *et al.* 2010; Leite *et al.* 2011).

## Operation

Three operational conditions were studied, corresponding to different mechanical aeration exposures (with or without), in P2. The aeration was introduced with the aim of improving the removal of ammonia; aeration times were studied in order to produce residual dissolved oxygen (DO) of greater than 3 mg L<sup>-1</sup>. For the provided air (stages II and III), two ceramic diffusers were used, located at the pond bottom at a distance of 1/3 to 2/3 from the effluent inlet, with an action radius up to 5 m each. The air was supplied by an air compressor (Schulz MSI 2.6 ML – 12 HP – 60 Hz) with an aspiration capacity of 77.5 L min<sup>-1</sup> (33.5 Kg O<sub>2</sub> d<sup>-1</sup>), which guaranteed the necessary oxygen for aerobic organic matter stabilization (oxygen requirement: 0.05 Kg O<sub>2</sub> d<sup>-1</sup>).

The total monitoring period was 43 weeks, comprising three stages.

**Stage I Conventional operation:** the system was operated normally, without mechanical aeration in P2. The ponds were subjected to different loads and/or environmental variations (daily and seasonally). This condition was maintained for 15 weeks.

**Stage II 12 h of aeration:** P2 received mechanical aeration for a period of 12 h (9:00 pm – 9:00 am). This condition was maintained for a total of 13 weeks.

**Stage III 18 h of aeration:** P2 received mechanical aeration for a period of 18 h (3:00 pm – 9:00 am). This condition was maintained for 15 weeks.

## Samples and monitoring

The samples were collected weekly, at 10:00 am, from different points of the system: pond inlets and outlets, and the RF. The analysed parameters were soluble chemical oxygen demand (SCOD), filtered biochemical oxygen demand (FBOD), total Kjeldahl nitrogen (TKN), ammonium (NH<sub>4</sub>-N), turbidity, and total suspended solids (TSS). For measures *in situ* (pH, DO and temperature) a probe (YSI 6600 V2; YSI Inc., OH, USA) was installed in a central location in the ponds, at 0.10 m depth; all physical and chemical analyses were performed according to *Standard Methods* (APHA AWWA WEF 2005). The results were explored using Statistica 6.0 (STATSOFT). For the chlorophyll *a* and plankton analysis, samples were collected from the central point of the ponds (P1: 0.10 m depth; P2: 0.1, 0.4 and 0.7 m depths; P3: 0.1, 0.3, and 0.5 m depths). The samples were preserved with an acetic lugol solution (1:100) and stored at –4 °C until analysis. For the second and third stages, the sample point was maintained in P1. However, for P2 and P3, the monitoring occurred at three different times: 10:00 am, 2:00 pm and 6:00 pm at a central point, at three different depths (0.1, 0.4, and 0.7 m).

## Microbiological identification

The genera of the planktonic microorganisms were identified using an optical microscope (Olympus BX41) and an inverted microscope (Bioval XDS-1). Taxonomic identifications were determined as described by Canler *et al.* (1999) and Bellinger & Sigeo (2011). The quantification of dominant groups was performed with a Sedgewick–Rafter chamber, using an optical microscope with a 200-fold increase.

FISH analyses were performed, essentially as described by Amann *et al.* (1995). Samples from P2 and P3 were filtered before fixation, using filter paper with a porosity measurement of 14 µm. All samples were fixed in 4% paraformaldehyde–phosphate-buffered saline and placed on 0.6% gelatine and 0.06% KCr(SO<sub>4</sub>)<sub>2</sub> gelatine-coated glass slides. For bacteria identification, probes were used as presented

in Table 2. Total microbial cells were detected by staining with 1% 4',6-diamidino-2-phenylindole (DAPI). The slides were examined with an Olympus BX40 microscope. All samples were analysed against DAPI (considered 100%).

## RESULTS AND DISCUSSION

In all stages of the study, the three ponds presented homogenous temperatures, between 17 °C and 25 °C. The highest temperatures were registered during stage III (above 30 °C), coinciding with the summer season. The total accumulated precipitation amounts were 366 mm, 393 mm and 1,146 mm for stages I, II and III, respectively. The solar irradiance averages during each stage were

285 ± 97, 275 ± 132 and 355 ± 161 W m<sup>-2</sup>. The pH values ranged from 9.0 to 9.6. The main value of DO in the photosynthetic ponds (P2 and P3) in stage I was 1.0 mg L<sup>-1</sup>. The highest DO values were obtained near the pond surface, where there was the highest algal incidence. During stage II, the average was 3.4 mg L<sup>-1</sup> in P2 and 2.1 mg L<sup>-1</sup> in P3. During stage III, the average was 4.1 mg L<sup>-1</sup> in P2 and 3.1 mg L<sup>-1</sup> in P3. Table 3 shows the average values of the measured parameters and removal efficiency rates. In P2, the applied loads varied between 300–470 kg SCOD ha<sup>-1</sup> d<sup>-1</sup> and 150–220 kg NH<sub>4</sub>-N ha<sup>-1</sup> d<sup>-1</sup> and presented average removal rates of 35–45% and 65–95%, respectively.

The BOD/COD ratios were 0.44, 0.17 and 0.37 in stages I, II, and III, respectively. The obtained results indicated a low biodegradability of the leachate organic fraction, which points to the old age of the influent (Jiang et al. 2009). This can be confirmed by the sanitary landfill's length of operation (approximately 23 years). The pond system efficiently removed the FBOD, turbidity, NH<sub>4</sub>-N and TNK, with a focus on the third stage, which presented a good removal of NH<sub>4</sub>-N (average of 82% for all stage periods). According to Darota & Ewa (2008), the BOD/COD ratio reflects the biodegradability of leachate, and hence, indirectly reflects the degradation phase of the landfill refuse (an important quantitative indicator of the landfill degradation phase).

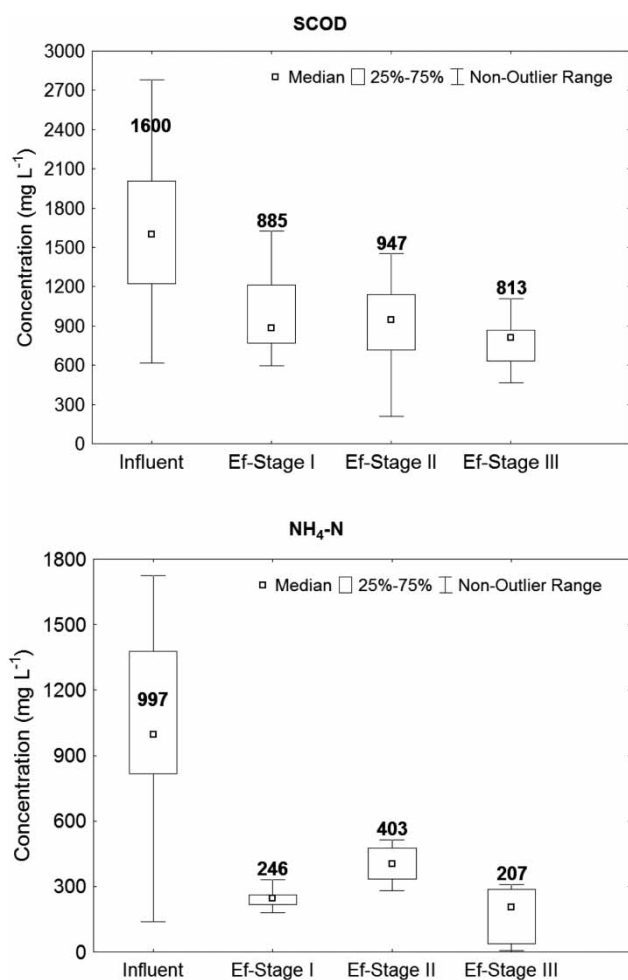
The final average concentrations of SCOD were very high (above 700 mg L<sup>-1</sup>). The removal rates varied between 42 and 48%. These values reflected the presence of recalcitrant compounds, such as humic compounds and chlorinated organic and inorganic salts (Thörneby et al. 2006). The determined results are consistent with others reported by Renou et al. (2008) for different pond systems treating landfill leachate. SCOD removal varied between 40 and 97%. Figure 2 shows that the average SCOD of the

**Table 2** | Probe, specificity and sequences used for *in situ* hybridization (references from Probase)

Probe name	Specificity	Sequence 5'-3'
EUB mix (I + II + III)	Most bacteria Planctomycetales Verrucomicrobiales	I- ctgcctcccgtagca II- cag ccaccctagggtg ctg III- ccaccctagggtg
NEU	<i>Nitrosomonas</i> sp.	cccctctgctgcaactactcta
NIT 3	<i>Nitrobacter</i> sp.	cctgtgctccatgctccg
AMX	Anammox	aaaaccctctactgt
Eury 499	<i>Methanosarcina</i> , <i>Methanosaeta</i> , Methanomicrobiales groups	cggtctgcccggccct
DSV 407	Some SRB of the <i>Desulfovibrionaceae</i>	ccgaaggccttctccct
Nso 190	<i>Betaproteobacteria</i>	cgatcccctgctttctcc
ARC 915	Archae	gtgctccccgccaatctct

**Table 3** | Physicochemical parameters and removal efficiency rates during the research stages. Data *n* = 15 (average ± standard deviation)

Sampling point	Stage	SCOD (mg L <sup>-1</sup> )	FBOD (mg L <sup>-1</sup> )	NH <sub>4</sub> -N (mg L <sup>-1</sup> )	TKN (mg L <sup>-1</sup> )	Turbidity (NTU)
Influent	I	1,789 ± 492	793 ± 554	1,108 ± 215	1,471 ± 238	147 ± 64
	II-12 h	1,770 ± 623	323 ± 171	1,342 ± 438	1,909 ± 426	96 ± 30
	III-18 h	1,313 ± 204	483 ± 179	845 ± 66	1,088 ± 125	84 ± 37
Effluent	I	1,009 ± 405	143 ± 26	240 ± 84	326 ± 110	42 ± 6
	II-12 h	920 ± 357	99 ± 60	397 ± 79	578 ± 91	31 ± 8
	III-18 h	761 ± 167	123 ± 97	149 ± 119	227 ± 188	23 ± 6
Removal (%)	I	44	82	78	78	71
	II-12 h	48	69	70	70	68
	III-18 h	42	75	82	79	73



**Figure 2** | Box-plot of the concentrations of SCOD and  $\text{NH}_4\text{-N}$  obtained during system monitoring (raw influent and RF effluent) for each operational phase.

effluent remained between 800 and 950  $\text{mg L}^{-1}$  during the monitored stages. Conversely, the values of  $\text{NH}_4\text{-N}$  were lower in stage III, and at the end of this stage, which lasted two weeks, the average concentration was 6  $\text{mg L}^{-1}$ . This is below the limit established by Brazilian legislation for surface water discharge.

Table 4 summarizes the main plankton groups and genera found in the pond system treatment. The presence of bacterioplankton was detected at P1, such as cocci, spirilla and bacilli, which function under anaerobic conditions, both covered and without light exposition. A great number of cylindrical or bacillus cells was verified, which occurred sometimes as pairs and occasionally as chains. The presence of spiral bacteria in the form of isolated cells often showed variations in the spiral, e.g., in length, number, and amplitude. Short organisms with uncompleted spirals (vibrios) were also found. Neither phytoplankton nor zooplankton

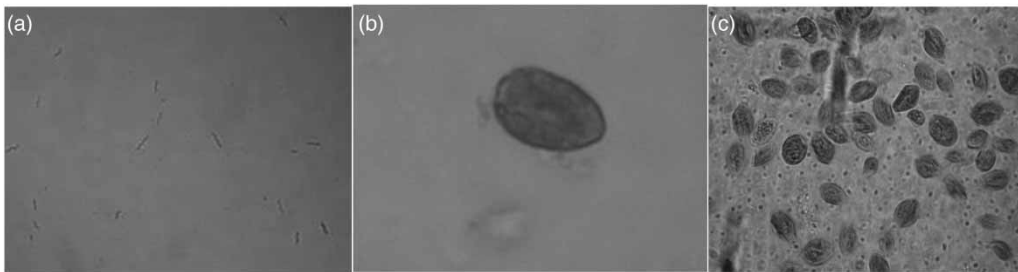
**Table 4** | The main plankton groups and genera found in the treatment ponds

Group/Pond	Stage I			Stage II-12 h			Stage III-18h		
	P1	P2	P3	P1	P2	P3	P1	P2	P3
Bacterioplankton (cocci, spirilla, bacilli)	X			X			X		
Phytoplankton ( <i>Chlamydomonas</i> , <i>Cryptomonas</i> , <i>Navícula</i> , <i>Nitzschia</i> and <i>Phacus</i> )		X	X	X	X		X	X	
Zooplankton (Ciliates)				X	X		X	X	

were observed in this pond. Del Nery et al. (2013), when studying a poultry slaughterhouse wastewater treatment plant in an aerated overload facultative pond (983  $\text{kg BOD (ha day)}^{-1}$ ), observed phylogenetic associations with dominant microbial groups responsible for organic matter decomposition. This was associated with bacteria from anaerobic and sulphur-rich habitats, faeces, soils and pathogenic organisms. Furthermore, Almasi & Pescod (1996) determined that sulphate-reducing bacteria were predominant in anoxic stabilization ponds used for wastewater treatment, rather than acidogenic bacteria. This caused sulphide and hydrogen sulphide build-up in the pond content.

Figures 3(a)–3(c) show microscope photos of organisms found in P1, P2 and P3, respectively.

In stage I, the photosynthetic ponds, P2 and P3, showed that the phytoplankton were mainly composed of the *Chlamydomonas* genera (Division *Chlorophyta*, Class *Chlorophyceae*, Order *Volvocales*, Family *Chlamydomonadaceae*), totalling over 50% of the total number samples, followed by the *Cryptomonas* genera. Both genera presented motility capacities ensuring the migration of these organisms along the water column during high solar radiation periods (Lee 1999). The genera found in photosynthetic ponds are characterized by their ability to resist high load conditions and to survive in contaminated environments. They can assimilate nitrogen as nitrate, nitrite, ammonium or other small molecules, such as urea and acetamide (Harris 2001). Almasi & Pescod (1996) reported that motile flagellate algae (*Euglena* and *Chlamydomonas*) exist under anoxic conditions for ponds with a volumetric organic load between 10 and 30  $\text{g BOD}_5 (\text{m}^3 \text{d})^{-1}$ . *Euglena* spp. are more sensitive to sulphide toxicity than *Chlamydomonas*, which are four times more tolerant than *Euglena*. Athayde (2001), studying algal dynamics in waste stabilization ponds in north-east Brazil, identified that the *Chlamydomonas*, *Pyrobotrys*, *Phacus* and *Euglena* genera were the most resistant algae to high organic loadings ( $770 \text{ k BODs (ha day)}^{-1}$ ).



**Figure 3** | (a) P1: *Spirilla* (1,000-fold); (b) P2: *Cryptomonas* sp. (1,000-fold); (c) P3: *Chlamydomonas* sp. (400-fold).

It was observed that the phytoplankton groups were distributed mainly on the surface throughout all periods of the day in P2, while in P3, they were more evenly distributed along the water column. Other microorganisms, such as *Navicula*, *Nitzschia* and *Phacus*, were also found, especially at the beginning of this stage, which tended to gradually disappear with the predominance of the *Chlamydomonas* genera. In studies with leachate in shallow ponds, using five serial ponds, these algae genera were also present, and were able to support the high concentrations of ammonium, commonly found in this type of effluent (Frascari *et al.* 2004; Leite *et al.* 2011).

Figure 4 shows the relationship between concentrations of Chlorophyll *a* and TSS in P2 and P3. In P2 these variables were functions of the operational conditions. In accordance with previous work (Fernandes *et al.* 2013) a stratification in the P2 water column was observed during stage I. During stages II and III the pond presented complete mixing with average of 250 mg TSS L<sup>-1</sup>. The operational conditions, despite climatic conditions being decisive to P2, which can also be explained by solar irradiation. These values were similar during stages I and II (285 ± 97 and 275 ± 132 W m<sup>-2</sup>, respectively) but with large differences to chlorophyll *a*.

During stage I, the average concentration of chlorophyll *a* was 617 µg L<sup>-1</sup>, providing a green colour in the pond. In stage II, with 12 h aeration, the settled sludge in P2 was resuspended, increasing the TSS concentration. A change of colour was observed as well as a reduction of chlorophyll *a* in approximately 60% of samples (values <10 µg L<sup>-1</sup>). In stage III, the solar irradiation increased to 355 ± 161 W m<sup>-2</sup> but the chlorophyll *a* remained lower (390 µg L<sup>-1</sup>) than stage I.

P3 operated as a maturation pond during the monitored stages. The average values of chlorophyll *a* were 360, 449 and 661 µg L<sup>-1</sup> for stages I, II and III, respectively, and it was not affected by the artificial aeration.

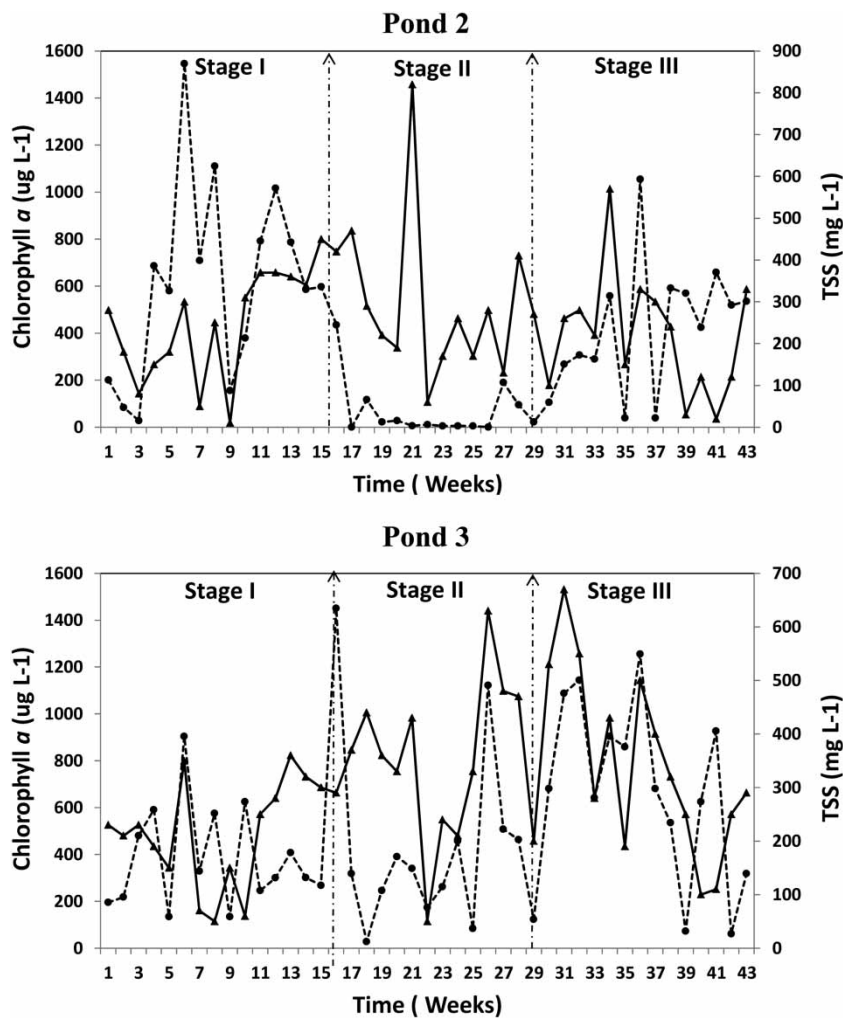
In stage II, with a 12 h aeration, a change was observed in microorganisms, with considerably decreased numbers of

phytoplankton in P2 and P3, and an increased incidence of crawling ciliate organisms. According to Samaras *et al.* (2009), ciliates, amoebae, and rotifers play an important role in this type of ecosystem, since they feed on bacteria, in addition to organic substances and other small organisms. They maintain and rejuvenate the bacterial population through this predation, but also contribute to the flocculation process by improving effluent quality, making them suitable indicators of good quality. For SCOD, the increase in oxygen during the artificial aeration period (averages of 3.4 ± 1.7 and 2.1 ± 1.3 mg L<sup>-1</sup> at P2 and P3, respectively) had little influence on the pond's removal efficiency, with an average of 48% (average concentration of 920 mg L<sup>-1</sup>). Concerning the NH<sub>4</sub>-N, decreased removal was observed (Table 2), with an average efficiency of 70% (increasing the average concentration of NH<sub>4</sub>-N to 400 mg L<sup>-1</sup> in the effluent).

In stage III, the arrival of other microorganism genera, such as *Navicula*, *Cryptomonas* and *Chlamydomonas*, as well as free crawling ciliate organisms, were observed, which established a natural consortium with microalgae. According to Mohammadi *et al.* (2011), the death of smaller species, such as bacteria, because of predation by larger species, such as protozoa, may reduce the population of the smaller species, like algae. Furthermore, this generally results in a reduction of the total microorganism population and, consequently, excess sludge, reducing the TSS values. This was observed at the end of stage III (Figure 4).

It is important to note that this stage occurred between spring and summer, when temperatures were between 24 °C and 25 °C. The average concentrations of chlorophyll *a* were 400 and 660 µg L<sup>-1</sup>, in P2 and P3, respectively (Figure 4), which showed higher values than those found in previous stages, where the average temperature was lower (ranging from 17 °C to 21 °C). In P3, the behaviour of TSS was similar to that observed for chlorophyll *a*.

There was also a considerable increase of phytoplankton species in P2 and P3 at stage III, with algal bloom



**Figure 4** | Relationship between Chlorophyll *a* (●) and TSS (▲) concentrations during stages I, II, and III in P2 and P3.

events during the summer. According to Lee (1999), algae blooms are related to high temperatures. Indeed, they were more common at the end of the summer when the temperatures were sufficiently high. At the end of this stage, the treatment system produced an effluent with an FBOD concentration of  $114 \text{ mg L}^{-1}$  (82% removal efficiency) and  $6 \text{ mg L}^{-1}$  for  $\text{NH}_4\text{-N}$  (99% removal efficiency). The obtained values complied with the limits established by Brazilian legislation for surface water (BOD and ammonium concentrations should be lower than  $120 \text{ mg L}^{-1}$  and  $20 \text{ mg L}^{-1}$ , respectively).

A parallel study concerning nitrogen transformation in the same treatment system (Martins et al. 2013) showed that the main form of nitrogen transformation/removal was by dead/inert algae settling (64–79%), followed by volatilization (12–27%) and algae assimilation (1–6%). Nitrification/denitrification did not occur in stages I, II or III.

FISH analysis was also carried out in a parallel study (Fernandes et al. 2013). In stage I, P1 samples showed the presence of high numbers of bacteria (75% of DAPI). Nitrifying bacteria (*Nitrosomonas*, *Nitrobacter*, and beta-proteobacteria) were found in small percentages (10% of DAPI), probably because of the low DO (near or equal to  $0 \text{ mg L}^{-1}$ ) and high pH (9.2). Archaea and methanogenic Archaea groups (*Methanosarcina*, *Methanosaeta*, and other Methanomicrobiales groups) were also found (25% of DAPI). Sulphate-reducing bacteria (SRB) groups from the *Desulfovibrionaceae* genera were also found (25% of DAPI). The samples of P2 showed the presence of ammonium oxidizing bacteria (20 to 40% of DAPI), nitrifying bacteria (*Nitrosomonas* – 10 to 25% of DAPI) and *Nitrobacter* (15% of DAPI). In the second and third stages, P1 and P2 samples were analysed and presented a slight increase of organisms in the presence of ammonium oxidizing bacteria (30 to 40% of DAPI), nitrifying bacteria *Nitrosomonas* and

*Nitrobacter* (10 to 25% of DAPI) and Eubacteria (80% of DAPI). The presence of those microorganisms is in accordance with evidence from the literature on anaerobic and facultative ponds (Pearson 2005). No Anammox (anaerobic ammonium oxidation) microorganisms were detected in this study. This result is in agreement with those obtained by Araujo et al. (2010), studying wastewater treatment with three shallow polishing ponds in series. The FISH analysis did not indicate the presence of Anammox bacteria nor ammonia oxidizing bacteria. However, some nitrifying bacteria were observed in pond water columns, like *Nitrosomonas* and *Nitrobacter*. Similar results were obtained by Sawaitayothin & Polprasert (2007) for constructed wetland treatment of landfill leachate, in which neither Anammox bacteria nor nitrifying bacteria (genera *Nitrospira*, *Nitrospira moscoviensis*) were found. These authors still showed the presence of about 49% to EUB 338 mix probes, which included heterotrophic and autotrophic bacteria, being the remaining 51% of inactivated bacterial cells and Archaea.

The P3 samples were not evaluated because of the interference of high algal concentrations in the unit.

## CONCLUSIONS

The stabilization pond system was able to treat landfill leachate, reducing the effluent carbonaceous (69–82% of FBOD) and nitrogen concentrations (18–99% of  $\text{NH}_4^+\text{-N}$ ). It was observed that the treatment was effected in P2 by various microbial consortia, which varied according to the operating conditions. The plankton and microbial changes that occurred in the three stages greatly influenced system performance, interfering in the final effluent quality. The best pond system performance occurred in stage III (18 h aeration), where a balance of the microbial diversity in the ponds and restoration signs were observed in the system, due to the presence of phytoplankton genera, as well as crawling ciliate organisms and nitrifying bacteria. Under these conditions, the concentration of  $\text{NH}_4^+\text{-N}$  in the effluent was significantly below the limit established by Brazilian legislation for surface water discharge.

## ACKNOWLEDGEMENTS

The authors would like to thank the Financing Fund for Studies of Projects and Programs/Research Program Basic Sanitation (FINEP/PROSAB) and the Brazilian National

Research and Development Council (CNPq) for their financial support.

## REFERENCES

- Almasi, A. & Pescod, M. B. 1996 *Wastewater treatment mechanism in anoxic stabilization ponds*. *Water Science and Technology* **33** (7), 125–132.
- Amann, R. 1995 *In situ* identification of microorganism by whole cell hybridization with rRNA-targeted nucleic acid probes. In: *Molecular Microbial Ecology Manual* (A. D. L. Akkerman, J. D. van Elsland & F. J. de Bruijn, eds). pp. 1–15.
- APHA AWWA WEF 2005 *Standard Methods for the Examination of Water and Wastewater*. 21st edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.
- Araujo, J. C., Correa, M. M. S., Silva, E. C., Campos, A. P., Godinho, V. M., Von Sperling, M. & Chernicharo, C. A. L. 2010 *Investigation of aerobic and anaerobic ammonium-oxidising bacteria presence in a small full-scale wastewater treatment system and three polishing ponds*. *Water Science and Technology* **61** (3), 737–743.
- Athayde, S. T. S. 2001 *Algal and Bacterial Dynamics in Waste Stabilization Ponds and Wastewater Storage and Treatment Reservoirs*. PhD Thesis, University of Liverpool, Liverpool, UK.
- Bellinger, E. G. & Sigeo, D. C. 2011 *Freshwater Algae: Identification and use as Bioindicators*. 2nd edn, Wiley-Blackwell, Oxford, UK, 284 pp.
- Canler, J. P., Perret, J. M., Duchène, P. & Cotteux, E. 1999 *Aide au Diagnostic des Stations d'épuration par l'observation Microscopique des Boues Activées* (The diagnosis of wastewater treatment plants in activated sludge by microscopic observation). Copyright Cemagref Éditions (1999), Tec et Doc Lavoisier, Cachan, France.
- Darota, K. & Ewa, K. 2008 *The effect of landfill age on municipal leachate composition*. *Bioresource Technology* **99**, 5981–5985.
- Del Nery, V., Damianovic, M. H. Z., Pozzi, E., Nardi, I. R., Caldas, V. E. A. & Pires, E. C. 2013 *Long-term performance and operational strategies of a poultry slaughterhouse waste stabilization pond system in a tropical climate*. *Resources, Conservation and Recycling* **71**, 7–14.
- Fernandes, H., Viancelli, A., Martins, C. L., Antonio, R. V. & Costa, R. H. R. 2013 *Microbial and chemical profile of a ponds system for the treatment of landfill leachate*. *Waste Management* **24**, 183–194.
- Frasconi, D., Bronzini, F., Giordano, G., Tedioli, G. & Nocentini, M. 2004 *Long-term characterization, lagoon treatment and migration potential of landfill leachate: a case study in an active Italian landfill*. Elsevier, *Chemosphere* **54**, 335–343.
- Gehring, T., Silva, J. D., Kehl, O., Castilhos, A. B., Costa, R. H. R., Uhlenhut, F., Alex, J., Horn, H. & Wichern, M. 2010 *Modelling waste stabilisation ponds with an extended version of ASM3*. *Water Science and Technology* **61** (3), 713–720.



- Harris, E. H. 2001 *Chlamydomonas* as a model organism. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**, 363–406.
- Jiang, J., Zhang, C., Li, C. & Huang, Y. 2009 A new method applied for the evaluation of municipal solid waste landfill stabilization. *Environmental Engineering Science* **26** (6), 1123–1130.
- Kargi, F. & Pamukoglu, M. Y. 2003 Aerobic biological treatment of pre-treated landfill leachate by fed-batch operation. *Enzyme Microbiology Technology* **33**, 588–595.
- Lee, R. E. 1999 *Phycology*. 3rd edn, Cambridge University, Cambridge, UK.
- Leite, V. D., Pearson, H. W., Sousa, J. T., Lopes, W. S. & Luna, M. L. D. 2011 The removal of ammonia from sanitary landfill leachate using a series of shallow waste stabilization ponds. *Water Science and Technology* **63** (4), 666–670.
- Martins, C. L., Fernandes, H. & Costa, R. H. R. 2013 Landfill leachate treatment as measured by nitrogen transformations in stabilization ponds. *Bioresource Technology* **147**, 562–568.
- Mehmood, M. K., Adetutu, E., Nedwell, D. B. & Ball, A. S. 2009 In situ microbial treatment of landfill leachate using aerated lagoons. *Bioresource Technology* **100**, 2741–2744.
- Mohammadi, A. R., Mehrdadi, N., Bidhendi, G. N. & Torabian, A. 2011 Excess sludge reduction using ultrasonic waves in biological wastewater treatment. *Desalination* **275**, 67–73.
- Park, J. B. K. & Craggs, R. J. 2010 Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. *Water Science and Technology* **61** (3), 633–639.
- Pearson, H. 2005 Microbiology of waste stabilization ponds. In: *Pond Treatment Technology* (A. Shilton, ed.), IWA Publishing, London, UK.
- Renou, S., Givaudan, J. G., Poulain, S., Dirassouvan, F. & Moulin, P. 2008 Landfill leachate treatment: review and opportunity. *Journal of Hazardous Materials* **150**, 468–493.
- Samaras, P., Papadimitrio, C. A., Vavoulidou, D., Yiangou, M. & Sakellaropoulos, G. P. 2009 Effect of hexavalent chromium on the activated sludge process and on the sludge protozoan community. *Bioresource Technology* **100**, 38–43.
- Sawaitayothin, V. & Polprasert, C. 2007 Nitrogen mass balance and microbial analysis of constructed wetlands treating municipal landfill leachate. *Bioresource Technology* **98**, 565–570.
- Shilton, A. 2005 *Pond Treatment Technology*. IWA Publishing, London, UK.
- Subashchandrabose, S. R., Ramakrishnan, B., Megharaj, M., Venkateswarlu, K. & Naidu, R. 2011 Consortia of cyanobacteria/microalgae and bacteria: biotechnological potential. *Biotechnology Advances* **29**, 896–907.
- Thörneby, L., Mathiasson, L., Martensson, L. & Hogland, W. 2006 The performance of a natural treatment system for landfill leachate with special emphasis on the fate of organic pollutants. *Waste Management and Research* **24**, 183–194.
- von Sperling, M. 2007 Waste Stabilisation Ponds. In: *Biological Wastewater Treatment Series*. IWA Publishing, London, UK, v 3.
- Wiszniewski, J., Robert, D., Surmacz-Gorska, J., Miksch, K. & Weber, J. V. 2006 Landfill leachate treatment methods: a review. *Environment Chemical Letters* **4**, 51–61.
- Xie, B., Lv, B. Y., Hu, C., Liang, S. B., Tang, Y. & Lou, J. 2010 Landfill leachate pollutant removal performance of novel biofilter packed with mixture medium. *Bioresource Technology* **101**, 7754–7760.

First received 26 November 2013; accepted in revised form 19 May 2014. Available online 30 May 2014