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Algal diversity and distribution in Waste Stabilization Ponds treating faecal sludge leachate from drying vegetated beds

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ABSTRACT

Waste Stabilization Ponds (WSP) were tested at pilot scale for the polishing of faecal sludge leachate from planted drying beds in Yaoundé, Cameroon. Water was sampled at three different depths (10, 30, 45 cm) and three different hydraulic retention times (HRT) (4, 7 and 10 days) in two maturation basins in series for physico-chemical and biological analyses. As a removal mechanism, algae diversity, density and biomass were assessed and correlated to the physical parameters within the ponds. Results showed the presence of nine algal species belonging to three divisions, four classes, six orders, eight families and eight genera. Among these species found in WSPs, *Chlamydomonas globosa, Monoraphidium convolutum and pseudanabaena catena* were the most abundant whatever the basin, the hydraulic retention time (HRT) and depth. PO_4P, NH_4N and the total chlorophyll showed strong correlation with the algal biomass (0.582, 0.731 and 0.895 respectively) at the surface (0-15 cm) followed by TSS, temperature and COD (0.556, 0.509 and 0.533 respectively) at HRT 4 days. These correlations were not observed at HRT 7 and 10 days.

Keywords: Algal dynamic, Waste Stabilization Pond, faecal sludge leachate, depth, hydraulic retention time.

INTRODUCTION

Waste Stabilization Ponds (WSP) have beingused worldwide for many decades for the treatment of wastewater from different origins in small municipalities. The reasons for this choice are simplicity for construction, very low operational cost as well as good nutrient and pathogen removal (Koné, 2002; Mara, 2003). This technology is suitable and recommended for Sub-Saharan countries because of the high insolation and availability of land. Among the many processes responsible for the removal of pollutants in WSP are microalgae through uptake of

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nutrient and sedimentation. This availability of nutrients in turn affects the growth of algae which at a certain extent becomes a new source of pollution. As demonstrated by Mbwele et al. (2003); Powell et al. (2008), algal concentration within the WSP depends on nutrient loading, temperature and sunlight. The hydraulic retention time also affects the algal biomass which in turn as first order removal process affects the performance (Heaven et al., 2012).

Despite this algal effect on WSP performances, few studies have investigated the algal dynamic and diversity within WSP treating wastewater and are mainly in temperate regions (Short et al., 2007; Bulent et al., 2013). Data for tropical areas are still on collection, especially algal dynamics in WSP treating faecal sludge leachate at different hydraulic retention times. This paper reports on algal dynamics within microcosm treatment ponds treating faecal sludge leachate in Yaoundé, a tropical region.

MATERIALS AND METHODS

Site of study

This study was conducted at pilot-scale at the University of Yaoundé I, in Cameroon. The field site located 760 m above sea level at 3°45 N and 11°32 E has a typical equatorial Guinean climate characterized by two rainy seasons (September to mid-November and mid-March to June) and two dry seasons (mid-November to mid-March and July to August). The annual average rainfall is 1600 mm, and the daily temperature ranges from 22 °C to 35 °C.

Experimental layout and operation

The experimental layout consisted of two leachate collection tanks (LCT) of $1m^3$ each, connected in series to a leachate application tank (LAT) of the same capacity using a PVC pipe provided with a balloon tap system. The balloon floating system enabled the regulation of the hydrostatic pressure in the second tank connected to microcosm WSPs in order to ensure a constant flow pressure. The WSP microcosms consisted of 6 PVC maturation ponds (M1) of 31 cm x 25 cm x 50 cm in size (L x l x H) connected in parallel to the LAT via 15 mm diameter distribution pipes controlled each at the inlet of each pond with a screw tap. These M1 ponds were connected in series each to a maturation pond (M2) of the same size using a short PVC pipe (Figure 1). The functional depth for both M1 and M2 was 45 cm.

System operation

To the M1 ponds, leachate was applied in continuous flow for a HRT of 4, 7 and 10 days with one replication. Screw taps were placed at the inlet of ponds to regulate the flow adjusted after a tracer test using NaCl solution of conductivity 3mS/cm. Wood boxes were made to receive the ponds in order to reduce the effect of temperature on the sides of the ponds and a shade frame was constructed and covered with transparent plastic paper to prevent rainfall in the ponds.

Water sampling and analysis

Water was sampled at the inlet and outlet of each pond. Within each pond, the sampling was done at three different depths (10, 20 and 40 cm) for physico-chemical as well as biological analyses. An adapted sampling apparatus made up of an 80 mL polyethylene syringe prolonged at its extremity with a 50 cm long and 1 cm diameter polyethylene pipe and fixed on a graduated ruler served for the sampling. Physical parameters were assessed *in-situ* using portable Hach pH-meter model HQ11d for pH and Eh, the WTW Oxi Cal 325 for Dissolved Oxygen (DO) and the conductivity meter model WTW 3310 for electrical conductivity. Suspended Solids, DBO_5, NH_4N, PO_3P were analysed in the laboratory according to the standard methods (APHA, 2005).

The biological analysis of water samples consisted in the determination of the algae biomass and density as described by Ütermöhl (1958) respectively. Algae observations and counting were performed using a light microscope, model Olympus CH-2. The determination of species was done following identification keys published in several reference books (Bourelly, 1984; Compère, 1976; Couté and Rousselin, 1975; Gasse, 1986; Krammer and Lange-Bertalot, 1986-1991).

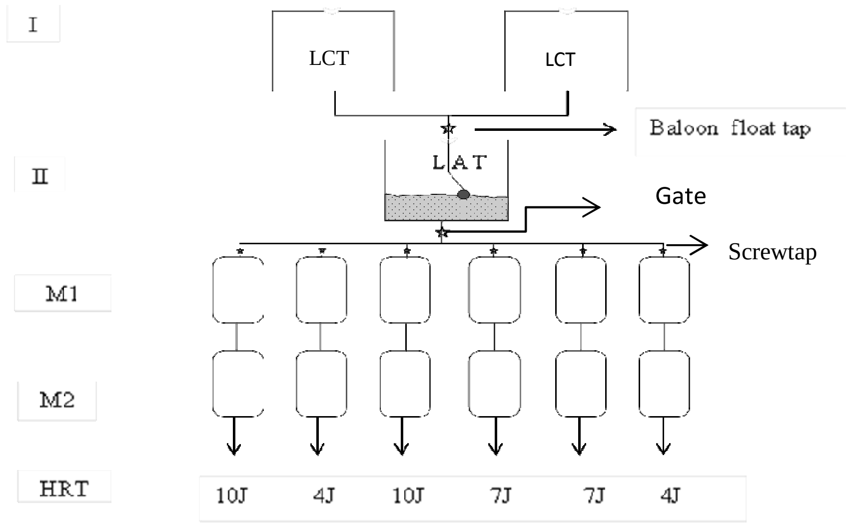

Figure1: Schematic representation of the experimental layout for the investigation of hydraulic retention time (HRT) effect on pilot waste stabilization ponds (WSP), algal diversity and dynamic. LCT: Leachate Collection Tank; LAT: Leachate Application Tank; M1: Maturation pond 1; M2: Maturation pond 2; HRT: Hydraulic Retention Time.

Statistical analysis

Data obtained were evaluated using ANOVA for comparison and Pearson's correlation test at 95% confident level.

RESULTS

Water characteristics in WSP ponds at the different HRT

Table 1 summarizes the main physico-chemical characteristics of water inside the different maturation ponds (M1 and M2) at the three HRT (4, 7, 10 days) and depths (surface, middle and bottom that correspond respectively to 10, 20 and 40 cm).

This table showed a significant decrease in concentrations as the retention time increased for almost all the parameters except for physical parameters (EC, pH, SS) and DO. No significant difference was observed between these different physical parameters with respect to the depth, the maturation ponds and HRT (P>0.05). on the contrary, the nutrient (NH_4-N, PO_4P) and organic content (COD, DBO_5.) analysed only per maturation ponds at the surface (10 cm) and not following the depth gradient showed a significant difference between the ponds and between the different HRT ($p < 0.05$).

Algal diversity and distribution

Microscopic observations enabled the identification of nine (09) algae species belonging to 03 divisions, 04 classes, 06 orders, 08 families and 08 genera (Table 2).

Figure 2 presents the distribution of algae species as a function of the HRT and depth. The number of species remains in all cases between 3 and 6. It appears that the species distribution is neither affected by the HRT nor by depth. This was confirmed by the Soerensen index of similarity which showed that at 4and 7 days the algal diversity was the same (S= 1). This similarity is not exactly the same between 4 and 10 days (0.6) and 7 and 10 days (0.6), but considerable. No difference was observed between the maturation ponds M1 and M2 in terms of algal distribution. Among the species found in WSPs, *Chlamydomonas globosa*, *Monoraphidium convolutum* and *Pseudanabaena catena* were the most abundant whatever the HRT and the depth (Figure 3).

Algal biomass

The algal biomassas function of the HRT and depth is presented in the Figure 4. During the experiment, the algal biomass varied from 0.19 to 0.4µg/l. The averages of the algal biomass in each hydraulic retention time did not show any significant difference, although 10 days retention time presented the relatively highest algal biomass. The depth also did not significantly affect the algal biomass ($p>0.05$) whatever the maturation pond. However, a decreasing trend of algal biomass values is observed from the surface to the bottom of the either use 'for each retention time' (changes with respect to HRT) or 'during each retention time period' (which indicates changes in the course of each HRT period).

Algal density

Regarding the algal density, no significant difference was observed between the different hydraulic loads and sampling depths, although 10 days HRT and surface water presented higher values. Mean values obtained were 8.74 x 10^7; 10.78 x 10^7; 13.93 x 10^7 cells/mL and 7.4 x 10^7; 6.92 x 10^7; 7.94 x 10^7 cell/mL for HRT 4, 7 and 10 days, respectively for maturation pond 1 and maturation pond 2.

Correlations between water physico-chemical and biological parameters

At the HRT 4 days, strong correlations were observed between the algal biomass and PO_4-P, NH_4-N and the total chlorophyll with correlation coefficients of 0.582, 0.731 and 0.895 respectively. The algal biomass was affected but slightly by the temperature, the suspended solids (SS) and the COD as shown by lower correlation coefficients of 0.509, 0.556 and 0.533 respectively. On the contrary, there was no significant correlation between the biomass production and physico-chemical parameters at the HRT of 7 days. At HRT of 10 days, a strong correlation (0.93) was observed only between the biomass production and the total chlorophyll content in water.

Table 1: Physical and chemical parameters of water within the M1 and M2 basins at the different depths and different HRT.

Parameters	**Variables**				
n = 10	**Ponds**	**Position**	**HRT 4 das**	**HRT 7 days**	**HRT 10 days**
T °C	M1	S	24.8 ±2.1a	24.4±1.74a	24.89± 1.17a
		M	23.7 ±0.8a	23.7±1.12a	24.43±1.109a
		B	23.4 ±0.5a	23.5±1.25a	24.15± 0.84a
	M2	S	24.3±1.64a	23.8±1.01a	25± 1.13a
		M	23.6±0.53a	23.6± 1.06a	24.33±0.71a
		B	23.8±1.35a	23.5± 1.04a	24.06±0.49a
EC (µS/cm)	M1	S	1607±902a	1631±132a	1679±1023a
		M	1597±893a	1634±117a	1686±102a
		P	1600±896a	1634±105a	1687±1027a
	M2	S	1570±868a	1580±1,09a	1628±963a
		M	1573±868a	1551±1,21a	1631±965,5a
		B	1557±857a	1553±1,16a	1633±968,3a
pH	M1	S	7,56±1,31a	7,3±1,32a	7,34±0,99a
		M	7,52±1,2a	7,44±1,17a	7,33±1,02a
		B	7,49±1,21a	7,44±1,05a	7,28±1,077a
	M2	S	7,54±1,25a	7,47±1,09a	7,42±0,99a
		M	7,47±1,19a	7,49±1,21a	7,38±0,92a
		B	7,47±1,12a	7,18±1,16a	7,28±0,95a
		S	17,15±66,75a	19,5±71,5a	19,58±20,2a

Eh (mV)	M1	M	14,88±63,6a	19,3±57a	19,3±18,1a
		B	15,3±65,3a	30,98±45a	30,98±16,5a
	M2	S	16,15±66a	30,32±45a	29,46±27,2a
		M	15,48±65,6a	22.7±55.8a	31.93±19a
		B	6.31±1.66a	20.4±60.2a	32.98±18a
SS (mg/L)	M1	S	24.3±94,04a	23.8±101a	25±74.7a
		M	23.6±87.4a	23.6±90.1a	24.33±69.5a
		B	23.8±88.8a	23.5±91.4a	24.06±67.8a
	M2	S	40 ±95.4a	38±89.05a	56±62.26a
		M	32±84.4a	31±88.4a	32±56.06a
		B	41±80.7a	24±87.5a	24±61.67a
DO (mg/L)	M1	S	12.08 ± 1.14a	11 ± 1.76a	16.7 ± 2.1a
		M	4.1 ± 0.7b	8.23 ± 1.91a	11.5 ± 2.33a
		B	3.32 ± 0.53b	6.6 ± 1.49b	8.94 ± 1.460a
	M2	S	6.8 ±1.30a	14.2 ± 2.66a	16.3 ± 1.9a
		M	5.2 ± 1.19a	10.8 ± 2.18a	12 ± 2.5a
		B	3.9 ± 0.81a	10.1 ± 2.79a	9.56 ± 1.92a
PO_3P^-(mg/L)	M1	S	31.78 ± 25.73a	14.93 ± 12.26b	6.47 ± 4.60b
	M2	S	18.23 ± 16.25a	8.28 ± 8.74b	2.03 ± 1.06c
NH_4N (mg/L)	M1	S	17.58 ± 15.33a	8.30 ± 6.09b	4.98 ± 3.71b
	M2	S	10.96 ± 9.18b	4.99 ± 4.16b	2.15 ± 1.32b
DCO (mg/L)	M1	S	197 ± 121.77a	133.5 ± 84.95a	86.5 ± 65.25a
	M2	S	128.58 ± 95.44a	85.0 ± 62.52b	44.14 ± 38.26b
DBO_5 (mg/L)	M1	S	47.17 ± 27.11a	30.84 ± 21.42a	23.25 ± 16.93a
	M2	S	29.92 ± 15.05a	14.17 ± 6.78b	12.42 ± 8.31b

For each parameter, numbers with the same letter are not significantly different at p = 0.05 following the Newman and Keuls test of comparaison. M1: Maturation pond 1; M2: maturation pond 2; S: surface; M: Middle; B: bottom.

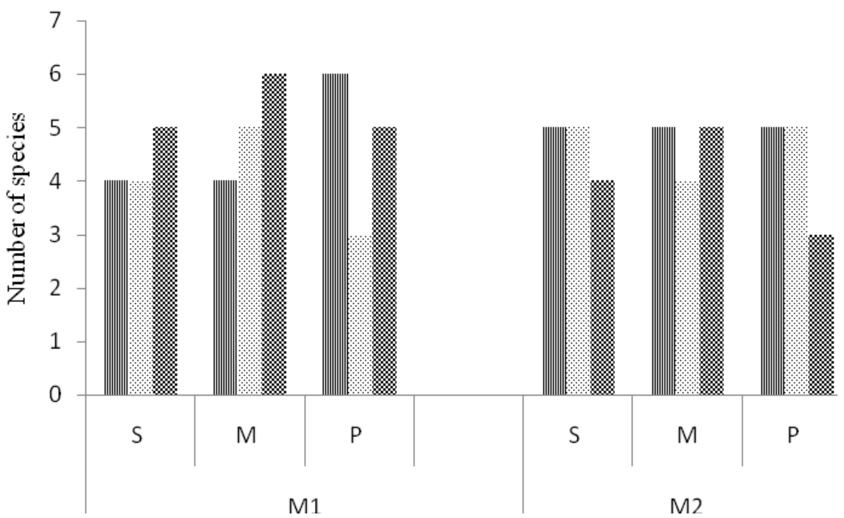

Figure 2: Number of species found at different depths (Surface, Middle and Bottom corresponding respectively to 10, 20 and 40 cm) as function of the hydraulic retention time (HRT) per maturation ponds M1 and M2.

Table 2: Classification of algae species found within the maturation ponds.

Divisions	Classes	Orders	Families	Genera	Species
Cyanophyta	Cyanophyceae	Chroococcales	Cyanobacteriaceae	*Aphanothece*	*Aphanothece elabens*
					Aphanothece nidulans
			Microcystaceae	*Microcystis*	*Microcystis densa*
		Pseudana baenales	Pseudanabaenaceae	*Pseudanabaena*	*Pseudanabaena catenata*
Chlorophyta	Chlorophyceae	Chlamydomonadales	Chlamydomonadaceae	*Chlamydomonas*	*Chlamydomonas globosa*
		Sphaeropleales	Scenedesmaceae	*Coelastrum*	*Coelastrum cambricum*
			Selenastraceae	*Monoraphidium*	*Monoraphidium convolutum*
Chrysophyta	Chryso-phyceae	Nitzchiales	Nitzschiaceae	*Nitzschia*	*Nitzschia* sp.
	Bacillario-phyceae	Achnanthales	Achnanthaceae	*Achnanthes*	*Achnanthes exigua*

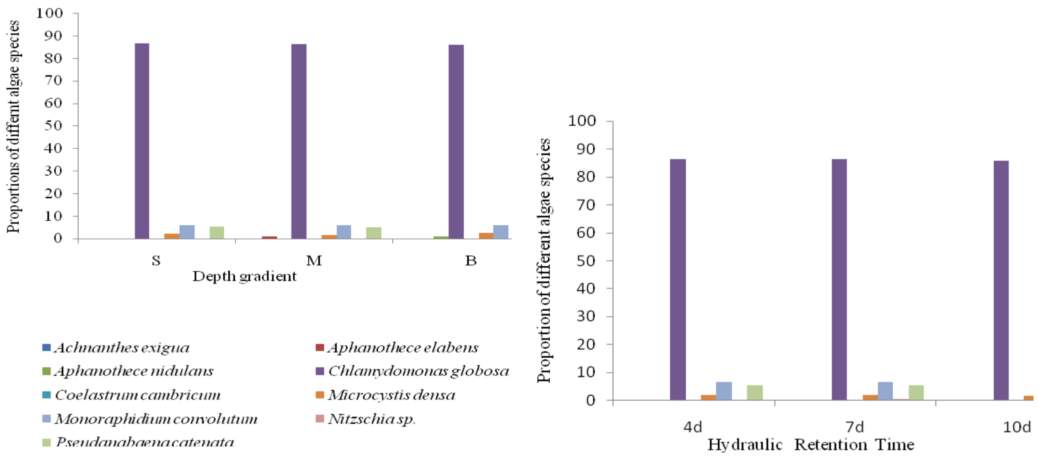

Figure3: Distribution of the different algae species according to the depth gradient and Hydraulic retention time.

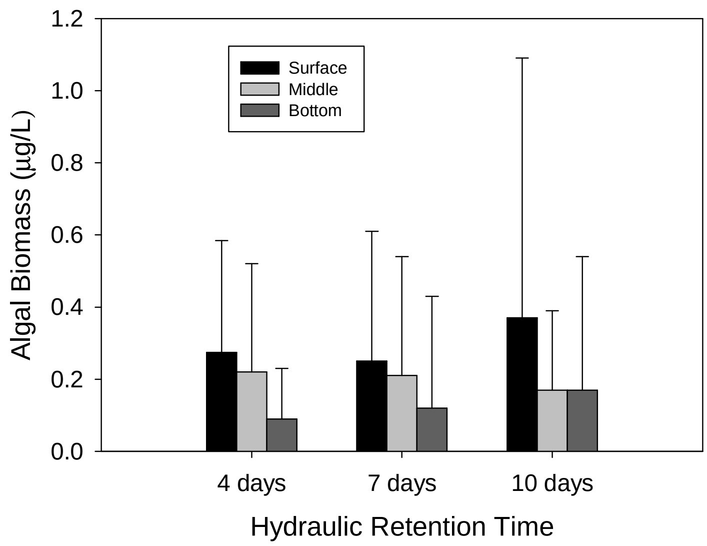

Figure 4: Variation of algal biomass per hydraulic retention time (HRT) and depth.

DISCUSSION

Water characteristics

The effluent physical parameters did not significantly differ between the two maturation ponds or the different sampling depths. This can be due to the fact that these ponds are of a small depth (45 cm) and therefore, climatic factors (insolation and wind) that influence most water characteristics such as temperature, pH and redox potential, acted almost similarly in both basins. This hypothesis can be completely verified given the fact that nutrient inputs is the same at the beginning of the experience and ponds acted as a complete-mixed reactor as shown by tracer study. This is in good agreement with Sweeney et al. (2007) who showed in investigating temporal and spatial variations of physical, biological and chemical parameters in a large WSP that temperature is the most determinant factor affecting these parameters. Nevertheless, the evolution of nutrient and organic matter content differed significantly from one pond to another and between the three HRT. The quality of effluents from the second maturation ponds M2 was better than that of maturation pond M1 whatever the HRT. This is due to the fact that pollutants were firstly reduced in the maturation ponds M1 before arriving M2 as they were in series. Concerning the different HRT, the long duration of the retention time improved substantially the quality of the effluent. This can be explained by the prolonged contact time with microorganisms for organic matter degradation, as well as a prolonged exposure to sunlight and other removal mechanisms to act (Kadlec and Wallace, 2009; Short et al., 2007).

Except for the SS, all parameters at the exit of the WSP (M2) were within the Cameroon guidelines for discharge or re-use of effluent from waste treatment plant (MINEP, 2008). This suggests that WSP are effective in polishing faecal sludge leachate.

Algal diversity and distribution

Nine algae species were found within the ponds. The fact that algal diversity was similar whatever the HRT as shown by the Sorensen similarity index [S (4d, 7d) = 1; S (7d, 10d) = 0.6 and S (4d, 10d)= 0.6)] can be explained primarily by the similarity of climatic factors (mainly temperature) at the different HRT during the experiment. Also, the operating depth of the ponds (45 cm) was not enough to create any significant drop of temperature or light active radiation to significantly affect biological processes (photosynthesis and respiration). Despite the nutrient content decreases from M1 to M2, and from HRT 4 days to HRT 10 days, the algae species number is not affected according to the values of Sorensen Index. A simpler hypothesis is that nutrients present in these basins did not affect algal growth and diversity. But this hypothesis is less likely because it has been demonstrated that great amount of nutrient and organic components are important for the growth and development of algae (Bulent et al., 2013). We can thus consider that although organic matter and nutrient decreased from one basin to another, the threshold level was not reached at the end of the experiment to impact the algae diversity. Thus nutrient and organic content of water remained sufficient for algal growth throughout the experiment. Similar conclusions were reached at by Mbwele et al. (2003) and Bulent et al. (2013) in their respective investigations on WSP.

The more dominant species found are in order of importance: *Chlamydomonas globosa*, *Monoraphidium convolutum* and *Pseudanabaena catena* for all HRT and depths. These species belong to the Chorophyta and Cyanophyta divisions, therefore confirming the observations of Short et al. (2007) who presented blue-green algae as the most commonly taxa dominating WSP phytoplankton communities. However, the species richness is inferior to that found by Nya (2001) in Biyem-Assi (Cameroon) microphyte lagoon domestic wastewater treatment plant. This difference observed can be due to the longer retention time and the bigger size of ponds as also indicated by Mara (2003) who showed that WSP pond ecology and performance depend on the pond geometry and size which impacts on the hydraulic. Also, the WSP species richness was lower than that obtained by Nguetsop et al. (2009) who found for two natural oligotrophic wetlands of the West Cameroon Highlands an average of 20 species. This can be due to the fact that algal diversity in wetlands reduces with the increase of pollution up to a certain level as the hyper-eutrophication gradually reduces algae population in favour of macrophytes and land instalment (Short et al., 2007).

Algal biomass and density

The algal biomass and cell density did not show any significant change whatever the HRT or the Depth. This agrees well with the variations of physical parameters which are only slight and did not show any significant difference from M1 to M2 and from one retention time to another. The high cell density throughout the experiment can be explained by the fact that the influent quality applied here provided enough nutrients for algal growth. These conditions are also favoured by the available oxygen shown by the permanent positive redox potential indicating an oxidative milieu at any depth and HRT whatever the maturation pond. However, the algal biomass and density were higher at the surface although not significantly different to those obtained at the middle and bottom of the pond, irrespective of the pond (M1 or M2), probably because of the light intensity which, due to high suspended solids, was slightly reduced at the bottom, but was still at an intensity active for photosynthesis. This statement was confirmed by the strong negative correlation of the SS and Biomass ($r^2 = -0.7154$). Powell et al. (2008) formerly noticed this downwards decreasing biomass and attributed it to light penetration impaired by SS and their effect on non-motile algae sedimentation. The algal density was higher than that obtained by Short et al. (2007) in Australian WSPs which averaged 2 x 10^4 cells/mL despite the high strength of influent (500 kg/ha/d).

Conclusion

WSP are effective in polishing faecal sludge leachate. During this process, the high microalgae biomass and density observed pointed algal uptake as contributing in the removal of nutrients in wastewater. However, for shallow ponds (<45 cm), the depth effect is non-significant on the algal dynamic, the temperature and dissolved oxygen remaining almost the same. Short HRT (≤ 10 days) did not significantly affect algae dynamics. The algae diversity (9 species), although stimulated by the presence of nutrients in wastewater, is negatively correlated to high SS content due to excess nutrient in wastewater. Green algae and blue algae were confirmed as dominant taxa in WSP.

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