The Distribution of Microalgae in a Stabilization Pond System of a Domestic Wastewater Treatment Plant in a Tropical Environment (Case Study: Bojongsoang Wastewater Treatment Plant)

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Abstract. The Bojongsoang Wastewater Treatment Plant (WWTP) serves to treat domestic wastewater originating from Bandung City, West Java, Indonesia. An abundant amount of nutrients as a result of waste decomposition increases the number of microalgae populations present in the pond of the wastewater treatment plant, thereby causing a population explosion of microalgae, also called algal blooming. In a stabilization pond system, the presence of algal blooming is not desirable because it can decrease wastewater treatment performance. More knowledge about the relationship between the nutrients concentration and algae blooming conditions, such as microalgae diversity, is needed to control and maintain the performance of the wastewater treatment plant. Therefore this study was conducted, in order to reveal the diversity of microalgae in the stabilization pond system and its relationship with the water characteristics of the comprising ponds. The results showed that the water quality in the stabilization pond system of Bojongsoang WWTP supported rapid growth of microalgae, where most rapid microbial growth occurred in the anaerobic pond. The microalgae diversity in the stabilization ponds was very high, with various morphologies, probably affiliated with blue-green algae, green algae, cryptophytes, dinoflagellates and diatoms. This study has successfully produced information on microalgae diversity and abundance profiles in a stabilization pond system.

Keywords: *Bojongsoang WWTP; domestic wastewater; microalgae diversity and abundance; nutrients; stabilization pond.*

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1 Introduction

The Bojongsoang Wastewater Treatment Plant (WWTP) is a domestic wastewater treatment facility managed by the Regional Water Company (PDAM) of Bandung city, located in the southern part of Bandung, Indonesia. This plant currently deals with almost 60% of domestic wastewater originated from Bandung city, which has a total population of approximately 3 million people. The treatment processes that occur in Bojongsoang WWTP comprise of physical or mechanical processes and 3 stages of biological processes in the stabilization pond system, consisting of an anaerobic, a facultative and a maturation pond. The wastewater treated by the Bojongsoang WWTP comes from various sources of domestic waste. The decomposition products of these compounds in the stabilization pond system become nutrient sources for microorganisms such as microalgae. The abundant amount of nutrients will increase the number of existing microalgae populations in the stabilization ponds and initiate a population explosion of microalgae, also known as algal blooming.

In a stabilization pond system, algal blooming can reduce the efficiency of the wastewater treatment. On the other hand, if we conduct simple modification techniques, such as inactivation and immobilization, the microalgae consortium biomass can be utilized as a promising and environmentally friendly biomaterial for absorbing heavy metals. Heavy metal biosorption technology can potentially be applied in Indonesia because of the availability of various local types of biomass that are cost-competitive and easy to develop. Investigation of the sorption capacity of the microalgae consortium from Bojongsoang WWTP has been accomplished in previous studies and we obtained interesting results on the competitive sorption character of the microalgae biomass in algal blooming condition [1]. However, the huge potential of microalgae consortium utilization is still constrained by the unavailability of information on its microalgae diversity and abundance profiles.

Therefore, in the current study we produced information on the distribution profile of growing microalgae abundance and diversity by integrating conventional microbiology techniques and characterization of the physico-chemical properties of water as limiting factor for microalgae growth. These approaches have the advantage of enabling investigation in detail of a single microbial entity of microalgae, its viability and its relationship with the abiotic environment.

2 Materials and Methods

Sampling points were located at Bojongsoang WWTP's stabilization pond set 1B, which consists of anaerobic pond 1B (AN 1B), facultative pond 1B (F 1B), and maturation pond 1B (M 1B), as shown in Figure 1. The depths of the anaerobic, facultative and maturation ponds were 4, 2 and 1.5 m, respectively.

The influent of this plant has an average BOD value of 360 mg/L with a flow discharge of 80,835 m^3/day. The effluent BOD of the maturation pond was observed to be 30 mg/L on average. The average annual air temperature and humidity in this area are approximately 25 °C and 80%, respectively.

(a) (b)

Figure 1 Study area of Bojongsoang WWTP, Bandung, Indonesia. (a) Plant layout, (b) flow diagram of biological process.

2.1 Sampling and *In Situ* Water Quality Measurement

Water quality measurements were carried out either *in situ* or in the laboratory. Sampling was performed using a LaMotte water sampler. Three sampling points were established in this study, i.e. the inlet (In), the middle (M) and the outlet (Out), with composited vertical variation for water quality analysis and without composited vertical variation for microalgae observation. Water quality parameters that were measured, the location of the measurement and the methods used are shown in Table 1.

Table 1 Water quality parameters, measurement location and methods used.

Water Quality Parameter	Measurement Location	Equipment/Method
Water transparency	*In situ*	*Secchie* disc
pH	*In situ*	pH meter
Dissolved Oxygen (DO)	*In situ*	DO meter
Conductivity	*In situ*	Conductivitymeter
Ammonia	Laboratory	Spectrophotometry [2]
Nitrite	Laboratory	Spectrophotometry [2]
Nitrate	Laboratory	Spectrophotometry [2]
Total Phosphate (Total P)	Laboratory	Spectrophotometry [2]

2.2 Morphological Analysis of Microalgae Abundance and Diversity

Samples were precipitated using Lugol with a 10:1 ratio. The precipitated samples were then identified using a binocular microscope and identified according to microbial identification manual [3]. The abundance of microalgae was counted using an improved Neubauer haemocytometer counting chamber [4]. Subsequently, the critical value, diversity index (H') [5] and similarity index (S) using the Sorensen Index [6] were determined.

3 Results and Discussion

3.1 *In Situ* Water Quality Analysis

Water transparency values in all ponds were very low, ranging from 2.38 ± 0.43 cm to 2.88 ± 1.03 cm (Table 2). According to Arthington, *et al.* [7], turbid water has a water transparency value of 0.25 to 1 m. The AN 1B pond had the highest turbidity level. Turbidity occurred because the microalgae biomass increased rapidly as long as growth factors were available, such as nutrients derived from the treated wastewater and sufficient sunlight. The temperature in all ponds ranged from 28.13 ± 1.22 °C to 30.80 ± 2.06 °C (Table 2), depending on the weather conditions and the location of the ponds. Overall, these values were in the normal temperature range for microalgae growth. According to Reynolds (1982) in Kawaroe, *et al.* [8], the optimal temperature range for microalgae growth is 25-40 °C.

Based on the results of pH measurements, it was found that the AN 1B pond had a lower pH value than the F 1B and M 1B ponds, i.e. 5.64 ± 0.42 (Table 2) while F 1B and M 1B had pH values of 6.96 ± 0.16 and 7.95 ± 0.08, respectively. Based on the statistical analysis using T-test, there was no significant difference in pH value between F 1B and M 1B (p value > 0.05), but both F 1B and M 1B had a pH higher than the N 1B pond (p value < 0.05). The anaerobic pond seemed to have no obvious methanogenic phase, where the dissolved organic material is degraded into organic acids, alcohols, ammonia, sulfide, hydrogen, CO_2 and water by acid-forming bacteria that reduce the pH value. Meanwhile, in F 1B and M 1B, pH values increased due to the process of microalgae photosynthesis that absorbs dissolved CO_2 in water. A decrease of the CO_2 concentration increases the pH value of the water [9].

The concentration of dissolved oxygen (DO) ranged from 3.59 ± 1.53 mg/l in AN 1B to 8.99 ± 2.12 mg/l in F 1B (Table 2). The DO levels in F 1B and M 1B were relatively higher than the DO level in the AN 1B pond, due to the process of photosynthesis by photosynthetic organisms, which produced oxygen and reduced organic loading, which requires oxygen for its biochemical reaction, in both ponds. Based on the statistical analysis using T-test, the DO levels in F 1B and M 1B were not significantly different from each other (p value > 0.05), but both ponds had higher levels than AN 1B (p value < 0.05).

The conductivity value shows the ability of water to transport an electrical current as a result of dissolved minerals in the ionized water. Based on the statistical analysis using T-test, the conductivity was significantly different between all ponds (p value < 0.05), where AN 1B had the maximum conductivity (511.42 ± 7.99 µS/cm) and the minimum conductivity was shown in M 1B (349.25 ± 3.65 µS/cm) (Table 2).

Table 2 Physico-chemical characteristics of pond water.

Water Quality Parameter	Pond		
	AN 1B	F 1B	M 1B
Water transparency (cm)	2.38 ± 0.43	2.69 ± 0.85	2.88 ± 1.03
Temperature (°C)	28.13 ± 1.22	29.88 ± 1.75	30.80 ± 2.06
pH	5.64 ± 0.42	6.96 ± 0.16	7.95 ± 0.08
Dissolved Oxygen (mg/L)	3.59 ± 1.53	8.99 ± 2.12	9.71 ± 1.14
Conductivity (µS/cm)	511.42 ± 7.99	429.47 ± 5.68	349.25 ± 3.65

3.2 Nutrient Load Factor Analysis

N-organic compounds in domestic waste are decomposed by microorganisms to form ammonia compounds (NH_3). At low pH (acid condition) ammonia compounds turn into ammonium (NH_4^+). Therefore, as shown in Figure 2, the concentration of ammonia compounds in F 1B and M 1B were lower than that in AN 1B, because the majority of ammonia was turned into ammonium. Statistically, ammonia concentrations in all ponds were significantly different from each other (p value < 0.05). These results demonstrated that ammonia concentrations significantly declined from AN 1B to M 1B.

Nitrification, a process of oxidation of ammonia into nitrite and nitrate, is the most important process in the nitrogen cycle and takes place in aerobic conditions [10]. From Figure 2 it can be seen that nitrite compounds tended to increase starting from the M 1B inlet point towards the M 1B outlet point. Statistically it was shown that AN 1B and F 1B had similar nitrite concentrations (p value > 0.05), while M 1B had significantly different concentrations or a higher value when compared with AN 1B and F 1B (p value < 0.05). This phenomenon appears due to high DO values (aerobic condition) in M 1B, which makes the nitrification process become thoroughly established.

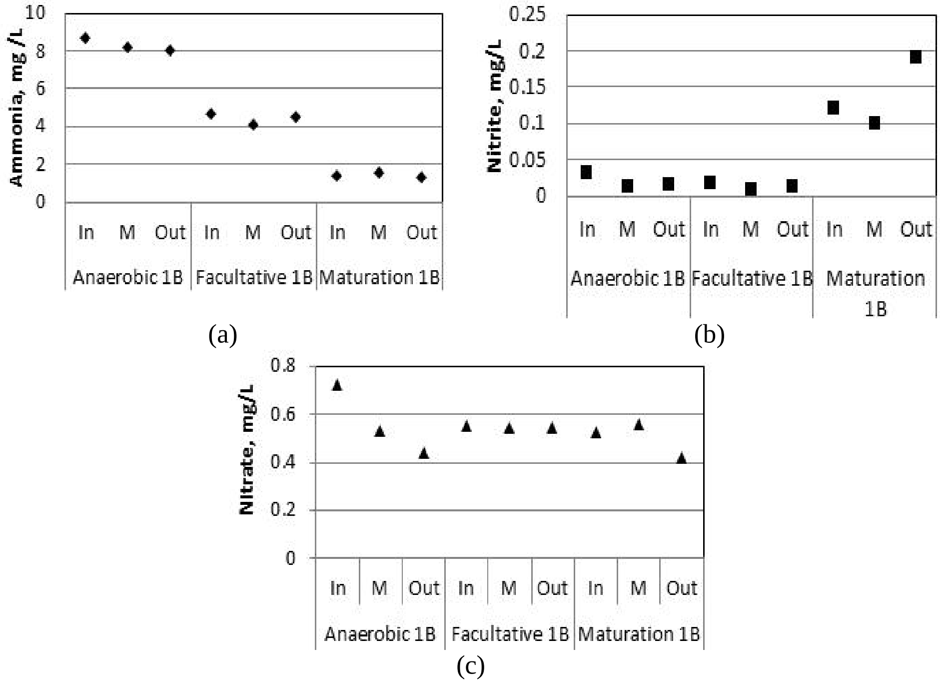

Figure 2 Profile of (a) ammonia, (b) nitrite and (c) nitrate concentrations of stabilization pond set 1B of Bojongsoang WWTP.

Nitrate is the main form of nitrogen in natural waters and a nutrient for aquatic plants and algae growth. Based on the measurement results, nitrate compounds tended to be stable and there was no impairment or significant improvement. As shown in Figure 3, the maximum total P concentration based on in situ measurement was in AN 1B (8.17 ± 0.08 mg/l) and the minimum value was in pond F 1B (0.49 ± 0.13 mg/l). Based on statistical analysis using T-test, total P concentrations in the AN 1B ponds were significantly different in the F 1B and M 1B ponds (p value < 0.05), which indicates a significant decrease. However, total P concentrations in F 1B pond were almost the same as those in M 1B (p value > 0.05). Absorption of ortho-phosphorous is also correlated directly with the presence of microalgae, the number of ions in the water and the existence of macronutrients and organic compounds as well (Wetzel, 2001 in Annisa [11]).

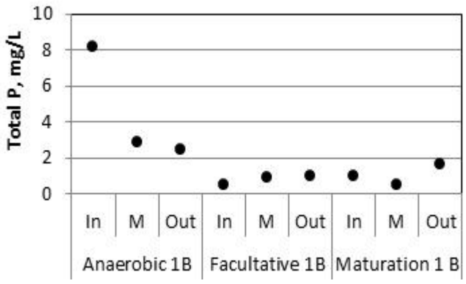

Figure 3 Profiles of total phosphate concentrations in stabilization pond set 1B of Bojongsoang WWTP.

3.3 Effect of Depth Variation on Physico-Chemical Factors of Water and Abundance of Microalgae

According to Effendi [10], the vertical stratification of the water column in standing waters depends on the intensity of the light entering the water. A decrease of the light intensity thus caused the water temperature to decline so that the dissolved oxygen and the pH value also decreased. Also, H_2S production by sulfate reducing bacteria created a low pH condition at the bottom of the pond. In contrast, the conductivity value, which indicates the concentration of dissolved particles in the water, increased due to dead cells of bacteria and algae and other solid particles settling on the bottom of the pond during the anaerobic decomposition process (Figure 4).

Figure 5 shows the microalgae abundance profile by depth variation of stabilization pond set 1B of Bojongsoang WWTP. As a result of vertical stratification and differences in water quality parameters, the abundance of microalgae tended to decrease along with depth increase.

The pH and conductivity had a positive correlation with the abundance of microalgae in each pond (Table 3). According to Senapati, *et al.* [12], the conductivity also has a positive correlation with the density of phytoplankton, especially during the wet season. Based on linear regression using the Pearson correlation statistical analysis, the variation of depth had no influence on water quality and the number of cells, indicated by negative values (–) in the test results. However, some water quality parameters had an influence on or a positive correlation with cell growth, indicated by positive values (+) in the test results. The correlation of each parameter was different for each pond. A matrix of linear regression results for each parameter relationship is shown in Table 3.

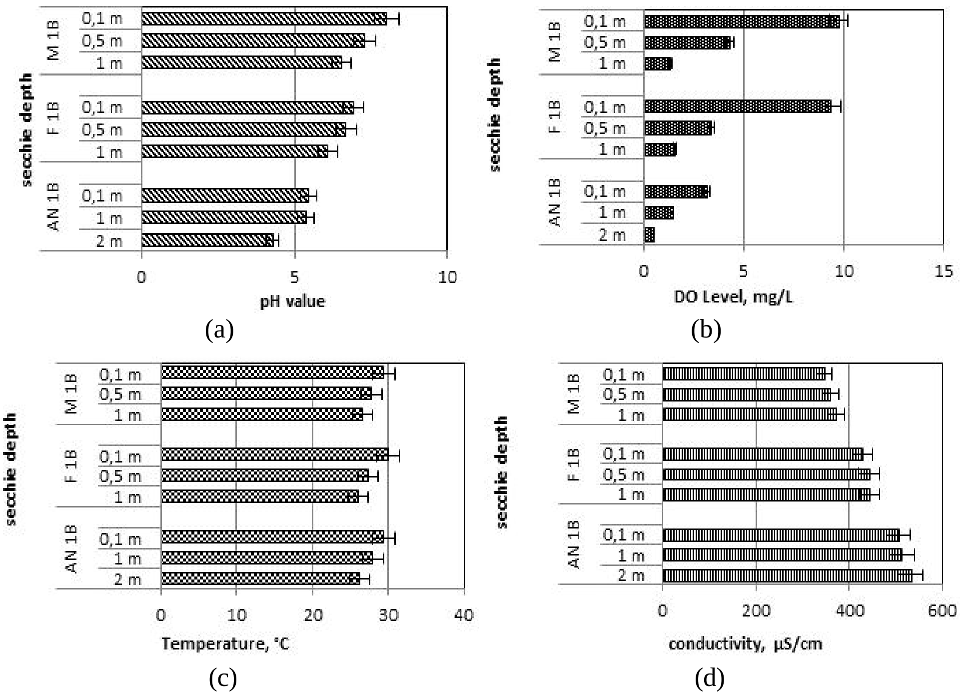

Figure 4 Profiles of water quality parameters. (a) pH, (b) dissolved oxygen, (c) temperature, and (d) conductivity by depth variation of stabilization pond set 1B of Bojongsoang WWTP.

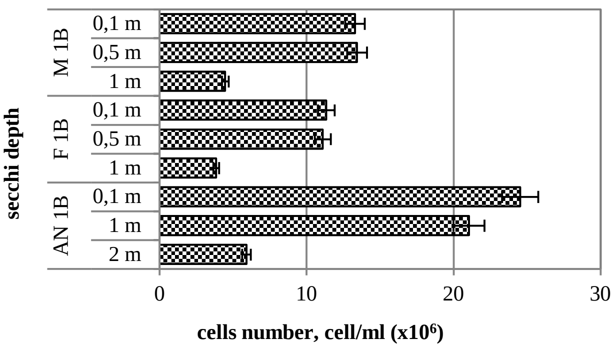

Figure 5 Microalgae abundance profile by depth variation of stabilization pond set 1B of Bojongsoang WWTP.

Table 3 Matrix of correlations among parameters based on linear regression test of pearson correlation.

Pond	Parameter	Total Cells	Depth	pH	DO	Temperature	Conductivity
Anaerobic 1B	**Total Cells**		-	+	-	+	+
	Depth	-		-	-	-	+
	pH	+	-		+	-	-
	DO	-	-	+		+	-
	Temperature	+	-	-	+		-
	Conductivity	+	+	-	-	-	
Facultative 1B	**Total Cells**		-	-			+
	Depth	-		-	-	-	+
	pH	+	-		+	+	-
	DO	+	-	+		+	-
	Temperature	+	-	+	+		+
	Conductivity	+	+	-	-	+	
Maturation 1B	**Total Cells**		-	+	+	-	+
	Depth	-		-	-	-	+
	pH	+	-		+	-	-
	DO	+	-	+		+	-
	Temperature	-	-	-	+		+
	Conductivity	+	+	-	-	+	

Notes:
(–) : No correlation
(+) : Positive correlation

3.4 Morphological Analysis of the Abundance and Diversity of Microalgae

Morphological microalgae identification was performed at the genus level. The diversity of microalgae in stabilization pond set 1B of Bojongsoang WWTP were dominated by the *Cyanobacteria* and *Chlorophyta* division (Figure 6). Both divisions were found in all ponds; its genera are quite common in some wastewater treatment plants [13]. The dominant algae species is influenced by the organic compounds loading, where the algae have capability to tolerate anaerobic conditions [9]. Therefore, the AN 1B pond had the highest microalgae abundance.

Based on the calculation of the diversity index (H') at all points, it was shown that the diversity index was in the range of 1 to 3, which means a moderate level of diversity. Based on the similarity index (S) test, the microalgae community in all ponds in this study (AN 1B, F 1B, and M 1B) had a similarity index above 50%, which indicates that the microalgae communities were similar. Critical value calculation results indicated that the stabilization pond set 1B was dominated by the genera of *Synechococcus, Chroococcus, Mycrocystis,* and

Chlorella, which were distributed across all three ponds. According to Mezrioui, *et al.* (1994) in Kantachote, *et al.* [13], some Cyanobacteria, such as *Synechococcus*, in wastewater treatment ponds are able to produce toxic compounds that inhibit *E. coli* and other bacteria growth. In addition, there were some genera that were only found in the F 1B and M 1B ponds, such as *Spirulina* (Figure 7).

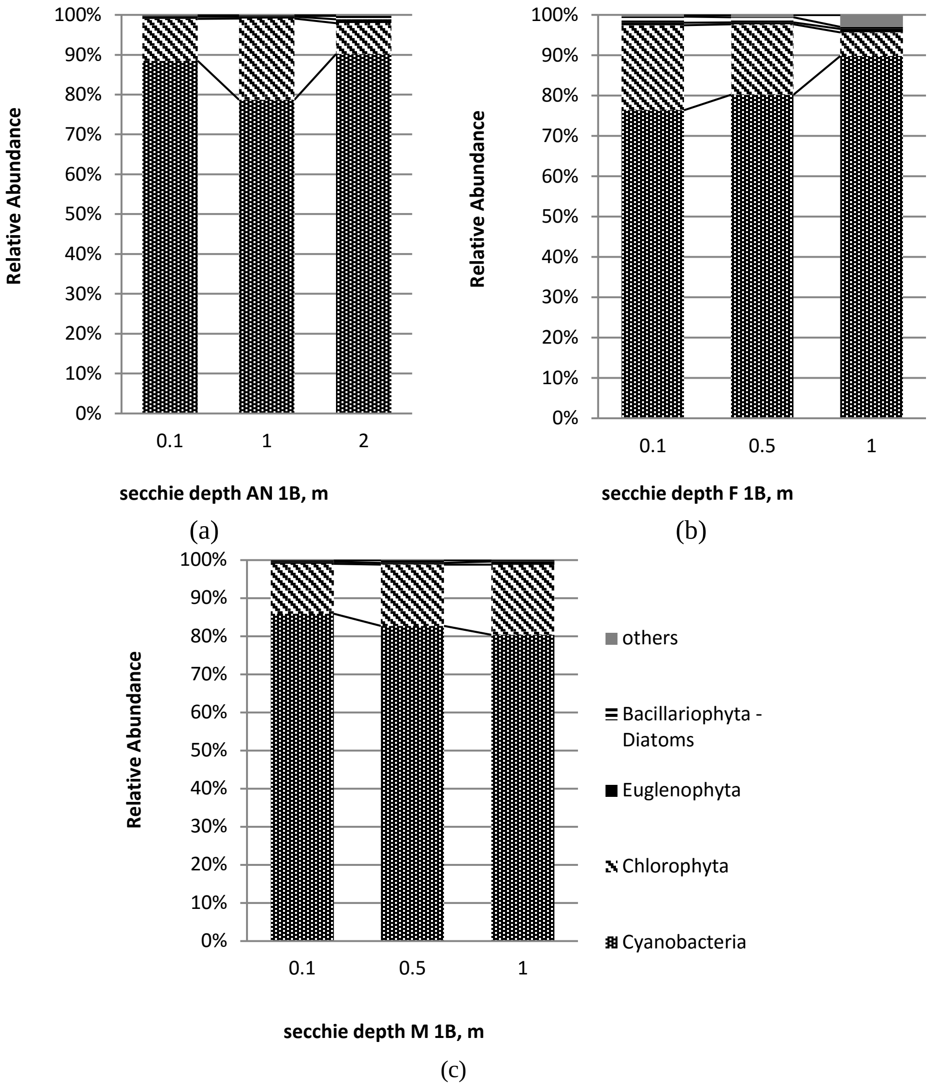

Figure 6 Microalgae diversity profiles in (a) anaerobic, (b) facultative, and (c) maturation pond of stabilization pond set 1B of Bojongsoang WWTP.

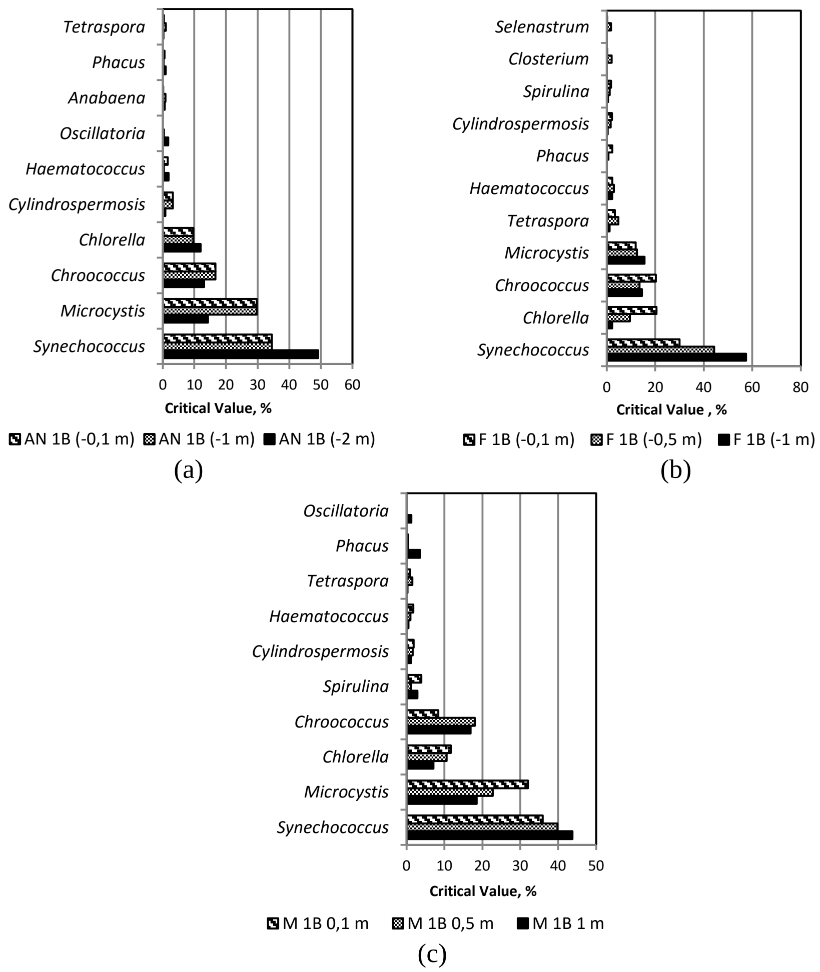

Figure 7 Critical values of microalgae genera diversity profiles in (a) anaerobic, (b) facultative, and (c) maturation pond of stabilization pond set 1B of Bojongsoang WWTP.

4 Conclusions

The anaerobic, facultative and maturation ponds in Bojongsoang WWTP were shown to have distinctive characteristics based on physical and chemical factors. The differences in the characteristics of the water in a vertical pattern affected the total abundance of microalgae but had little influence on its diversity due to the shallow water depth. Microalgae in stabilization pond set 1B of Bojongsoang WWTP were dominated by the genera of *Synechococcus*, *Chroococcus*, *Mycrocystis* and *Chlorella* with a fairly uniform distribution in each pond and with a moderate diversity index and similarity index. This variety of microalgae indicates that the future application of this consortium as biosorbents to overcome heavy metals pollution at an optimum absorption level is promising. By understanding the characteristics of each microalgae that has been identified, further attempts to cultivate an artificial environment at a large scale and use them as commercial biosorbents becomes more achievable.

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