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Effect of Nitrogen Sources on Actual Textile Wastewater Decolorization using Bioaugmentation in a Sequential Facultative Anaerobic-Aerobic Bioreactor

Zulkefli Daud[1]*, Zaharah Ibrahim[2], Adibah Yahya[2], Azmi Aris[3] and Zainab Ari[2]

[1]*Department of Science and Mathematics, Institute of Teacher Education Malaysia, Tun Hussein Onn Campus, 83009 Batu Pahat, Johor, Malaysia.*
[2]*Faculty of Biosciences and Medical Engineering, University of Technology Malaysia, 81310 Skudai, Johor, Malaysia.*
[3]*Centre for Environmental Sustainability and Water Security, University of Technology Malaysia, 81310 Skudai, Johor,Malaysia*

Bioaugmentation is one of the preferred bioremediation technologies for textile wastewater, but its efficacy may decrease due to the lack of nutrients. Thus, the study aims to investigate the effects of nitrogen sources on actual textile wastewater decolorization using bioaugmentation in sequential facultative anaerobic-aerobic bioreactor. Treatment I (absence of nitrogen sources), Treatment II (urea addition) and Treatment III (yeast extract (YE) addition) were conducted within 190 days of monitoring. Its bioaugmentation was dominated by *Enterococcus* sp 33C, *Enterococcus faecalis* FUA 3334 and *Bacillus cereus* strain M212. The treatment system was operated under a natural environment without any adjustments except hydraulic retention time (HRT) which was set around 72 hrs. The result found that all treatments obtained the highest color and chemical oxygen demand (COD) removal of more than 80%. However, the highest average color removal (75±9%) was obtained with YE, while the absence of nitrogen sources and urea addition caused the average color removal of about 73±9% and 57±13%, respectively. The same results were found for COD removal, where the highest average was obtained with YE (80±6%), followed by the absence of nitrogen sources (78±5%) and urea (66±12%). YE addition also achieved the lowest color intensity (120 ADMI) and COD concentration (66 mg/L) compared to other treatments. Meanwhile, the addition of urea and YE showed a decrease in biokinetic coefficients including maximum specific biomass growth rate (μ_m), specific biomass growth rate (μ), endogenous decay rate (k_d), half-saturation coefficient (K_s), and biomass yield (Y). Treatment with YE addition shown the best effect in the treatment of actual textile wastewater. The results of this study showed that the addition of nitrogen sources can increase nutrient for bioaugmentation growth.

Keywords: Bioaugmentation, actual textile wastewater, decolorization, nitrogen sources, anaerobic-aerobic bioeactor

I. INTRODUCTION

Anaerobic and aerobic processes are found to be successful in treating textile wastewater. Even today, the sequential anaerobic–aerobic process is one of the most accepted technologies for wastewater bioremediation containing azo dyes (Assadi *et al.*, 2017). Bioremediation efficiency can be enhanced by the use of bioaugmentation with specific microbial. Since textile wastewater

*Corresponding author's e-mail: zulmydinamik@yahoo.com

contains a large amount of colors, highly toxic and non-biodegradable pollutants (Jilani, 2015; Ibrahim *et al.*, 2015), compounds from pollutants may be inefficient for bio-metabolic enzymes. This causes ineffective bioremediation through resist biodegradation or degradation occurs slowly (Jilani, 2015). In addition, inadequate nutrients in the treatment system also affect dye degradation activity (Ramachandran *et al.*, 2013). One of the essential nutrients for microbes is nitrogen (N). Nutrients are required by bioaugmentation to transform textile effluent into degradable compounds (Khan *et al.,* 2016). However, nitrogen often does not exist in sufficient quantities in textile wastewater (Jilani, 2013). Microbes will break down dyes to get nitrogen (Siuli, 2017), however some strains cannot utilize dyeing wastewater because it contains toxic compounds (Stolz, 2001). Therefore, enrichment technique through the control of nitrogen sources in a wastewater stream or the addition of certain nitrogen sources can help stabilize bioaugmentation population to enable the effectiveness of residual targets metabolism (Fan *et al.*, 2009).

According to Wang *et al.* (2017) this technique may be able to achieve consistent results in dye decolorization.

Shinkafi *et al.* (2016) revealed that yeast extract (YE) was the best nitrogen source for dyes decolorization by a bacterial consortium. Bayoumi *et al.* (2010) found that peptone was the best nitrogen source for dye decolorization by different bacterial strains. While, Joe *et al.* (2011) found that peptone and YE were highly effective for decolorization (97%) of RBB dye by *Pseudomonas aeruginosa* CR 25. Likewise, Gomaa (2016) found that peptone or YE was the best inducers for decolorization of black B and congo red dyes by 4 bacterial strains including *Bacillus* sp. and *Pseudomonas* sp., but the addition of urea resulted in the reduction of decolorization. Shah *et al.* (2013) and Saratale *et al.* (2008) found that urea caused less in decolorizing ramazol black B by *Bacillus* sp.. On the other hand, Pant *et al.* (2008) found that the addition of urea shown a significant increase in decolorization of synthetic dyes by fungi. Besides color and COD removal, the addition of nitrogen sources can also affect on the determination of biokinetic coefficients (Mardani *et al.*, 2011). Jawad *et al.* (2015) found that mean values of K, K_s, k_d, Y and μ_m during operating the UASB reactor for textile effluent were 0.31/d, 77.5mg/L, 0.32/d, 0.83 mg/mg and 0.26/d, respectively. However, Naghizadeh *et al.* (2008) determined biokinetic parameters in municipal wastewater treatment with a submerged membrane reactor and found that the coefficients of K_s, k_d, Y and μ_m were 65.5 mg/L, 0.5/d, 0.67 mgVSS/mgCOD and 1.86/d, respectively. Based on both studies, the biokinetics coefficients were vary depending on the treatment system and the characteristics of wastewater.

This study aims to investigate the effect of actual textile wastewater decolorization by adding urea and YE in sequential facultative anaerobic-aerobic bioreactor using bioaugmentation. Apparently no major studies have been found to clarify the effect of urea and YE on actual textile wastewater. The confirmation of the effectiveness of this nitrogen sources would open-up the possibility of using the waste products industries such as urea and residual fermentation industries like yeasts in the future.

II. MATERIALS AND METHODS

Textile wastewater was obtained from a textile factory located in Batu Pahat, Johor. Some of the important characteristics were pH (7.7-10), temperature (29-42°C), biological oxygen demand (BOD) (58-177 mg/L), color (317-1670 ADMI) and COD (367-912 mg/L. The BOD/COD ratio was between 0.09-0.25, indicating that the actual textile wastewater was in low biodegradability. Treatment was carried out in a bioreactor containing facultative anaerobic reactor (5 L), aerobic reactor (2.5 L) and reservoir tank. A schematic diagram of bioreactor is shown in Figure 1. Bioaugmentation was dominated by *Enterococcus* sp 33C, *Enterococcus faecalis* FUA 3334 and *Bacillus cereus* strain M212. Operating parameters were not adjusted to represent the real conditions except total HRT which was continuously operated around 72 hrs. The study parameters for all treatments in general, were almost identical. Table 1 shows the parameters of the study. Three treatments were conducted within 190 days of monitoring ie; I (absence of nitrogen sources), II (urea addition) and III (YE addition). The addition of urea and YE concentrations were 2.5 g/L. Color, COD, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), biomass of biofilm, pH, temperature, dissolve oxygen (DO), BOD, total nitrogen (TN) and total phosphorus (TP) were measured according to APHA (American Public Health Association) standard methods. The color and COD removal, color intensity and COD concentration were evaluated to test the significant differences between the different nitrogen sources using Analysis of Variance (ANOVA) and Least Significant Difference (LSD). Statistical significance was accepted at $p<0.05$. Biokinetics coefficients estimation including maximum specific biomass growth rate (μ_m), specific biomass growth rate (μ), endogenous decay rate (k_d), half-saturation coefficient (K_s), and biomass yield (Y) were determined based on Monod model.

III. RESULTS AND DISCUSSIONS

A. Color and COD removal

Color and COD removal for textile wastewater were measured by analysing color intensity and COD concentration before and after treatment using sequential facultative anaerobic-aerobic bioreactor. Through this treatment system, dye decolorization occurred under anaerobic condition and removal of COD occurred under aerobic condition. The result is consistent with Supaka *et al.* (2004) which found that the treatment of dye wastewater at the combination of anaerobic and aerobic conditions showed that the majority of colors were removed by the anaerobic process, whereas the COD was removed by aerobic process.

Overall, the results found that all treatments (Treatment I, II and III) obtained the highest color removal of more than 80%. The highest average color removal of 75±9% was obtained with the addition of YE, while the lowest average color removal about 57±13% was obtained by the addition of urea. The absence of nitrogen sources caused 73±9% in the average color removal. For the lowest color intensity, YE addition gained about 120 ADMI, followed by the absence of nitrogen sources (190 ADMI) and urea addition (300 ADMI). The range and average color removal and intensity are shown in Table 2, while Figure 2 and Figure 3 show the color removal and color intensity. In this study, YE addition has shown the best average color removal compared to other treatments. This result is consistent with other studies such as by Rajeswari *et al.* (2011) which found 98% decolorization of mixed dye, meanwhile Shinkafi *et al.* (2016) found 81.94% decolorization of textile wastewater, but the color intensity was not mentioned. Highest average color removal and lowest color intensity by YE addition may be due to nicotinamide adenine dinucleotide hydride (NADH) regeneration as an electron donor for dye reduction (Hu, 1994) while lowest average color removal and highest color intensity by urea addition may be due to increase acidity of pH when urea dissolved in liquid culture (Shah *et al.*, 2013). The average color removal with absence of nitrogen sources (73±9%) was still high compared to YE addition (75±9%), while the average color intensity (239±55 ADMI) was lower than YE addition (280±85 ADMI). In fact, maximum color removal by absence of nitrogen sources (88%) was also higher than YE addition (85%). This may be due to the fact that some microbes have a high biomass growth rates. This is one of the reasons why bacteria in treatment without nitrogen sources or nutrient deficiency can survive better than bacteria in treatment with the addition of nitrogen sources. Thus, the color removal and color intensity were greatly affected by the type of microbial consortia, in addition to the dye chemical structure (Nosheen *et al.*, 2010). By adding YE, a non-specific microbes may grow better in the treatment system and therefore get the highest average color removal and lowest color intensity. This is in line with the significant difference in color removal and color intensity in all treatments (ANOVA), except the treatment between absence of nitrogen sources and YE addition (LSD) (Table 2). Accordingly, the use of YE was proven effective in color removal and confirmed that YE was the best nitrogen source for efficient decolorization as also mentioned by Baêta *et al.* (2016) for various textile dyes.

As in color removal, all treatments obtained the highest COD removal of more than 80%. The highest average COD removal was obtained by YE addition (80±6%), followed by absence of nitrogen sources (78±5%) and urea addition (66±12%). The lowest concentration of

COD by YE addition was 66 mg/L, while absence of nitrogen sources and urea addition had the lowest COD concentration of about 70 mg/L and 85 mg/L, respectively. The range and average of COD removal and concentration are shown in Table 3. Figure 4 and Figure 5 show COD concentration and COD removal.

All treatments had significant differences in COD removal and COD concentration (ANOVA), but there was no significant difference between treatment with the absence of nitrogen sources and YE addition (LSD) (Table 3). However, treatment by YE addition was still the best in COD removal and COD concentration compared to other treatments based on the addition of YE showed the highest average COD removal (80±6%) and lowest COD concentration (66 mg/L). COD removal in the range of 65-89% by addition of YE in this study is in line with Patel *et al.* (2015) which found that COD removal was between 60-70%, but the maximum COD removal around 89% was almost equal to the removal of 90% COD by Gebregiorgis *et al.* (2018). On the other hand, removal of COD (83%) and average COD concentration (196±60 mg/L) using treatment with urea addition were different compared to COD removal (95%) and average COD concentration (113 mg/L) through research by Alparsalan (2003). To date, no record has been obtained from other studies for COD removal and concentration by the addition of YE using similar or almost identical treatment systems. Different results in this study compared to other studies may be due to different treatment systems or different operating parameters and most studies also add other substances such as glucose, methanol and basal medium. NADH regeneration as an electron donor for dye reduction (Hu, 1994) and increased pH acidity (Shah *et al.*, 2013) may also affect the highest COD removal by YE addition and the lowest COD removal by urea addition as described in color removal.

B. Biokinetic coefficients estimation

Analysis of biokinetic coefficients based on Monod model, generally found that treatment with nitrogen sources addition has decreased μ_m, μ, K_s, k_d and Y under facultative anaerobic and aerobic conditions. This showed clearly that the types of nitrogen sources (substrate) and microbes (MLSS) can have a significant impact on the determination of biokinetic coefficients. This result is in line with Mardani *et al.* (2011). Table 4 shows biokinetic coefficients of this study and other treatments.

The addition of urea and YE have decreased μ_m and μ. This is consistent with Anderson *et al.* (1996) which stated that the use of non-biodegradable substrate will decrease μ_m. However, μ_m was found to be higher in the absence of nitrogen sources compared to the addition of urea and YE. This may be due to the growing population of organisms as mentioned earlier in color and COD removal. In this study, μ_m for treatment with YE addition under anaerobic condition was 1.7010/d while under aerobic was 0.1418/d. This result was different compared to Jawad *et al.* (2015) which found μ_m during operating the up flow anaerobic sludge blanket (UASB) reactor for textile effluent was about 0.26/d. Similarly, Gnanapragasam *et al.* (2011) and Naghizadeh *et al.* (2008) found that μ_m were 0.037–0.094/d and 1.86/d, respectively (Table 4). μ_m was different compared to other treatments may due to different nitrogen sources, reactor configurations, wastewater characteristics and microorganisms. Besides that, μ, K_s, k_d and Y were also different from other treatments that may be due to the same cause.

The addition of urea and YE also resulted in K_s reduction. This results was in line with Tuzun (2009) which reported that low K_s indicated a higher affinity between COD and microbes. Further analysis found that K_s was higher than μ_m in all treatments indicating that low biomass growth rate (Grady *et al.*, 1999) This may be caused to unstable MLSS, organic loading rate (OLR) or the practical identifiability limitations of Monod model (Chandran & Smets, 2005). Although K_s decreased in this study, it was still higher in all treatments indicating that the accumulation of amine or residual CH_4-COD resulted in inhibition (Isik & Sponza, 2004). This accumulation indicates that the dye was not utilized and the dye azo bond was not cleaved because the electrons releasing from urea or YE and NADH utilization were stopped (Kudlich *et al.*, 1997). YE addition has shown better than urea addition in reducing K_s under aerobic conditions. For k_d, it represented the rate of biomass lost due to the endogenous respiration. In this study, all treatments showed that k_d was lower than μ_m. This result may indicates that the amount of sludge at higher MLSS was not reduced. k_d in this study is less than Orhon *et al.* (1999) findings (0.16/d) for treatment of textile wastewater. Hence, lower mortality rates maybe due to the long HRT and sludge retention time (SRT) having a positive effect on microbial growth (Selvabharathi *et al.*, 2016).

Further analysis found that Y decreased by adding urea and YE. However, Y was higher than k_d in all treatments. According to Grady *et al.* (1999) this result indicates that the treatment requires low oxygen concentration because more electrons are in the substrate and will be stored in the biomass. Therefore, long HRT and low oxygen concentration may still be needed in the treatment system. Y has also decreased with increased MLSS with the addition of different nitrogen sources, which may be due to the amount of biomass produced by the current growth substrate. Based on biokinetic coefficients in this study, the use of nitrogen sources mainly YE has a good potential to improve the performance of actual textile wastewater treatment. Biokinetic coefficients have shown important implications for biological reactions during treatment.

Table 1. The parameters of the study

Stage	Parameters	I (absence of N sources)	II (Urea)	III (Yeast Extract)
Facultative anaerobic	DO, mg/L	0.4-1.9	0.3-1.9	0.5-1.5
	pH	7.8-8.9	7.9-9.7	7.8-8.6
	Temp, ^{o}C	25.1-27.8	24.7-28.7	23.8-27.5

	Init Col, ADMI	660-1620	760-1580	690-1770
	Init COD, mg/L	342-545	490-635	390-704
	MLSS, mg/L	80-120	90-160	130-210
	MLVSS, mg/L	60-100	70-110	110-180
	OLR, kgCOD/m^3.d	0.22	0.29	0.26
Aerobic	DO, mg/L	3.1-6.6	2.1-6.2	2.3-4.3
	pH	8.4-9.3	6.2-9.0	7.7-8.7
	Temp, ^{o}C	24-26.3	23.2-27.6	21-26
	Init Col, ADMI	170-410	110-630	556±95
	Init COD, mg/L	149-288	148-444	199-618
	MLSS, mg/L	50-120	85-150	40-300
	MLVSS, mg/L	40-80	60-100	25-200
	OLR, kgCOD/m^3.d	0.46	0.64	0.78

Table 2. The range and average color removal and intensity

Reaction	I (absence of N source)		II (urea)		III (yeast extract)	
	Range	Average	Range	Average	Range	Average
Initial colour, ADMI	660-1620	966±384	760-1580	1244±238	690-1770	1143±251
*Color after treatment, ADMI	190-410	239±55**	300-670	507±95	120-560	280±85**
*Colour removal, %	64-88	73±9	22-81	57±13	47-85	75±9

*ANOVA: (F(2,282)=198, p<0.05), (F(2,282)=66.88, p<0.05), **LSD: no significant difference

Table 3. The range and average COD removal and concentration

Reaction	I (absence N source)		II (urea)		III (yeast extract)	
	Range	Average	Range	Average	Range	Average
Initial COD, mg/L	342-545	458±400	490-635	577±53	390-704	535±94
*COD after treatment, mg/L	70-128	98±18**	85-331	196±60	66-154	105±20**
*COD removal, %	70-86	78±5	41-83	66±12	65-89	80±6

*ANOVA: (F(2,282)=155.5, p<0.05), (F(2,282)=74.789, p<0.05), **LSD: no significant difference

Table 4. Biokinetic coefficients of this study and other treatments

Treatment/ wastewater (ww) type	Nitrogen sources	MLSS mg/L	μ_m/d	μ /d	K_d/d	K_s mg COD/L	Ymg MLSS/mg COD	Ref
Facultative anaerobic (actual textile ww)	absence of N source	476	5.2165	0.5322	0.0095	2719.6	1.404	This study
	urea	572	0.2360	0.0918	0.0011	331.8	0.299	
	YE	575	1.7010	0.1581	0.0056	2070.6	0.832	
Aerobic (actual textile ww)	absence of N source	617	7.5350	0.7044	0.1593	978.4	0.721	
	urea	730	0.7364	0.1316	0.0077	492.5	0.847	
	YE	823	1.1294	0.1418	0.0395	661.8	0.509	
Anaerobic (Synthetic textile dye)	Starch	-	0.037 – 0.094	-	-	213.4 – 985.6	-	Gnanapragasam *et al.* (2011)
UASB reactor (textile effluent)	-	-	0.26	-	0.32	77.5	0.83	Jawad *et al.* (2015)
Submerged membrane reactor (municipal ww)	-	-	1.86	-	0.5	65.5	0.67	Naghizadeh *et al.* (2008)

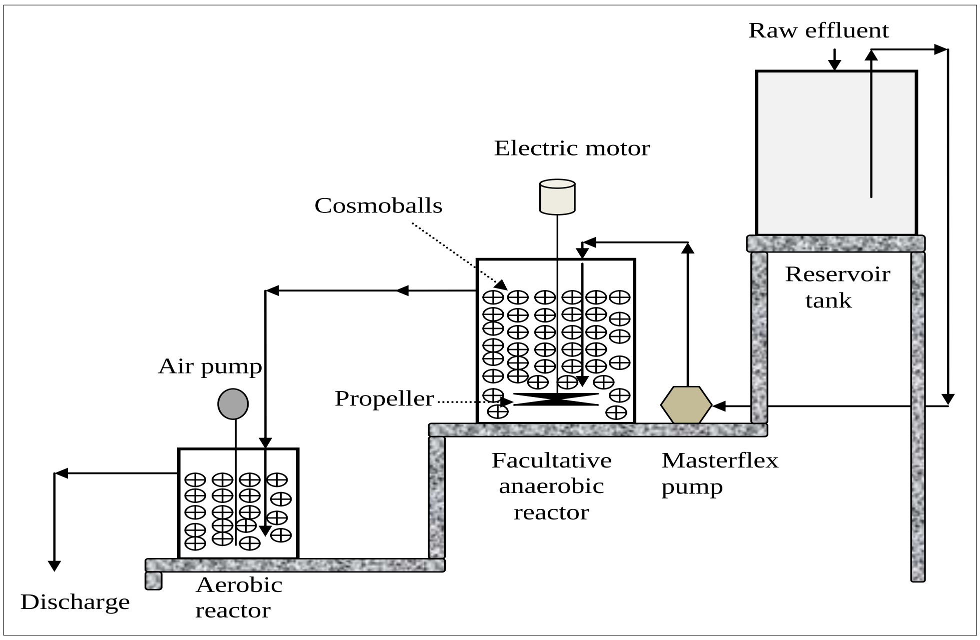

Figure 1. Schematic diagram of bioreactor

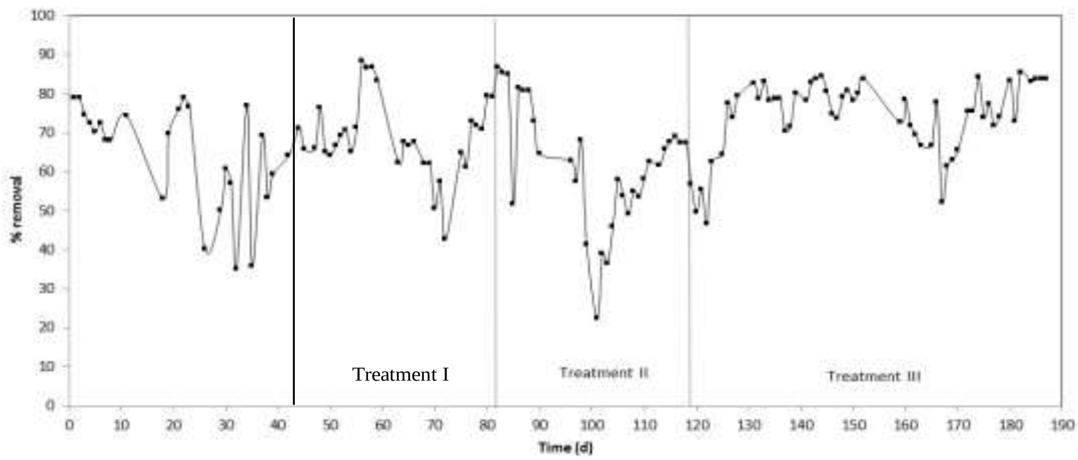

Figure 2. Total color removal after complete treatment for treatment I, treatment II and treatment III

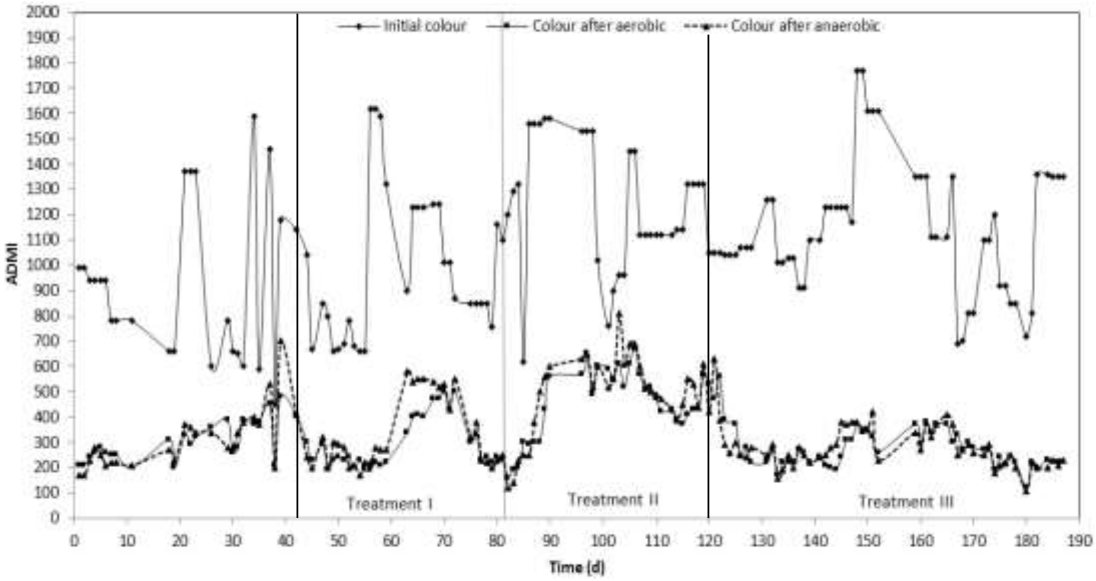

Figure 3. Color intensity for initial color, color after anaerobic and color after aerobic

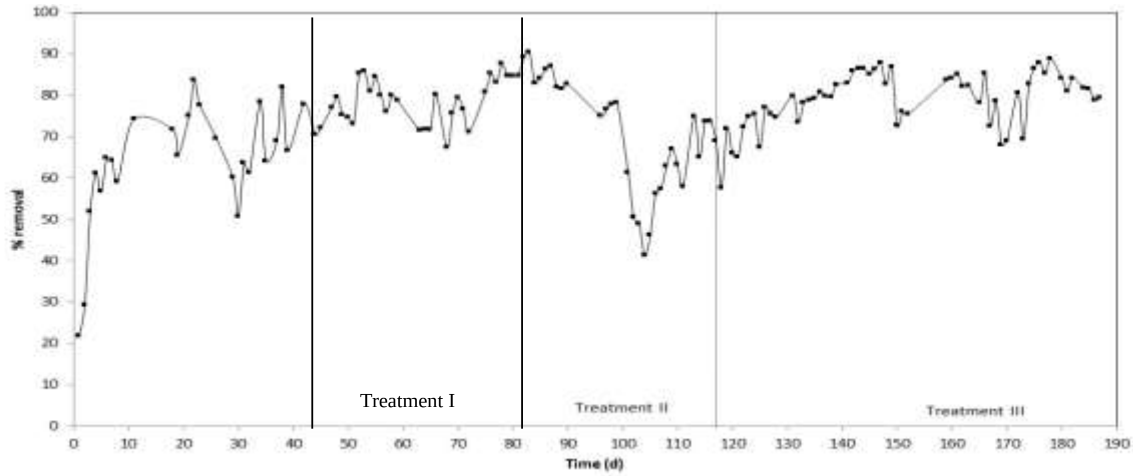

Figure 4. TotalCOD removal after complete treatment for treatment I, treatment II and treatment III

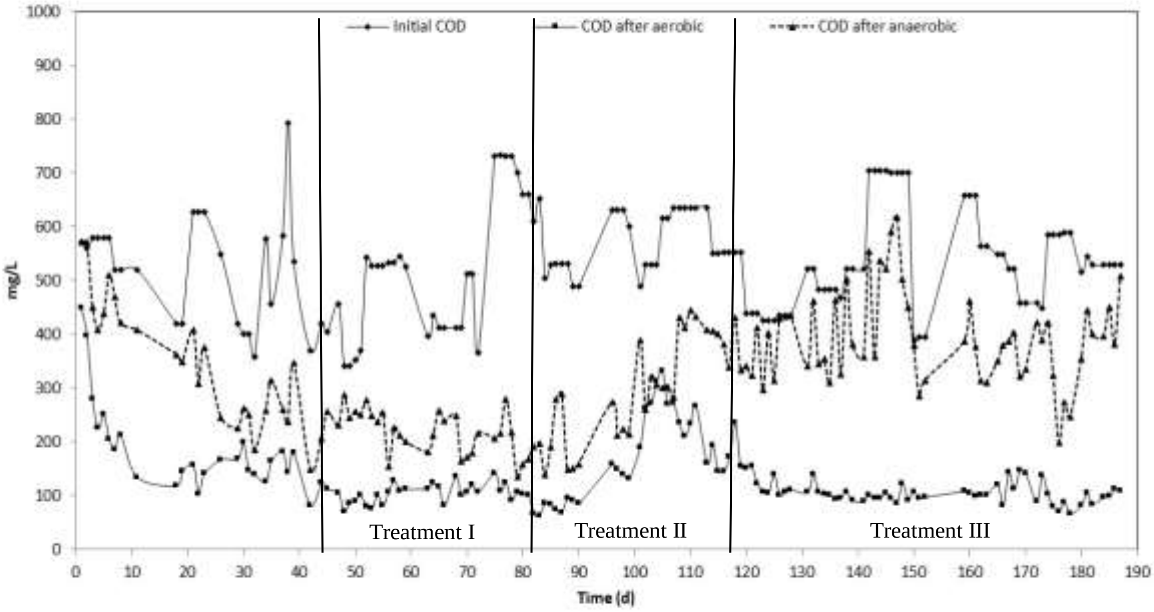

Figure 5. COD concentration for initial COD, COD after anaerobic and COD after aerobic

IV. CONCLUSION

Effect of actual textile wastewater decolorization by adding urea and YE in sequential facultative anaerobic-aerobic bioreactor using bioaugmentation can be studied in two aspects; effect on color and COD removal and effect on biokinetic coefficients. The addition of YE in this study has enhanced the color and COD removal of actual textile wastewater better than the addition of urea and absence of nitrogen sources. The highest average color and COD removal obtained with the addition of YE were (75±9%) and (80%±6%), respectively. The addition of YE also achieved the lowest color intensity (120 ADMI) and COD concentration (66 mg/L). For biokinetic coefficiants, the values of μ_m, μ, k_d, K_s, and Y have been decreased by the addition of urea and YE. Different characteristics of textile wastewater and different type of nitrogen sources may have resulted in different values of biokinetic coefficients. Overall, the results of the study showed that the use of urea and YE have the potential to enhance the decolorization of actual textile wastewater from the industry. However, urea and YE from market are relatively expensive and can result in higher treatment costs. Consequently, the use of urea from the waste products industry and residual fermentation industries such as yeast in the textile wastewater treatment system can be studied in the future.

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