MJFAS MALAYSIAN JOURNAL OF FUNDAMENTAL AND APPLIED SCIENCES
PRINT ISSN: 2289-5981 | ONLINE ISSN: 2289-599X

RESEARCH ARTICLE

Development and performance of BAC-ZS bacterial consortium as biofilm onto macrocomposites for raw textile wastewater treatment

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Article history
Received 16 December 2018
Revised 4 February 2018
Accepted 20 February 2018
Published Online 4 June 2018

Abstract

One of the most abundant dyes that are used extensively in the textile manufacturing are azo dyes, which may endanger water bodies since incomplete breakdown of dyes may cause mutagenic and carcinogenic compounds to persist. In this study, BAC-ZS, bacterial mixed culture consisting of three acclimatised decolourising bacteria were grown as biofilm onto macrocomposites. Different time duration between 3 to 14 days of biofilm development was studied to determine the density of biofilm attached onto macrocomposites. Sequencing batch reactors (SBRs) were set up for raw textile wastewater treatment to investigate the effectiveness of the treatment with and without the presence of biofilm (control). The treatment was performed under facultative anaerobic-aerobic condition for 20 days continuously with 48-hour of hydraulic retention time (HRT) cycle (consisting both conditions). Colour and chemical oxygen demand (COD) were monitored throughout the treatment process. Results showed that the colour and COD removal by the developed biofilm were 78.6 ± 1.4% and 76.4 ± 1.12% from initial values of 1400 ADMI and 660 mg/L, respectively while only 47.9 ± 0.9% colour and 38.0 ± 1.5% COD removal for the control. In conclusion, the biofilm of BAC-ZS mixed culture coated onto macrocomposites showed potential applications in the treatment of raw textile wastewater.

Keywords: Azo dye, biofilm, macrocomposites, sequential batch reactor, textile wastewater

INTRODUCTION

Wastewaters produced from textile industries have became an emerging global threat to the environment. In recent years, researchers have focused on manipulating bacteria for biodegradation of textile dyestuffs [1, 2, 3]. Chen and Chang [4] had reported that use of mixed bacterial cultures have greater advantages compared to pure cultures in treating real industrial wastewaters. This is because mixed bacterial cultures usually are more adaptable towards changes in pH, temperature, and feed composition as compared to pure cultures [5]. Azo dyes, which account for the majority of synthetic dyes used in textile factories, were often used as model dyes in biodegradation studies [6, 7, 8]. Unlike synthetic dyes solution, real textile wastewater is complex and generally consists of a mixture of dyes, organic contaminants such as ammonia, organic nitrogen, nitrate, phosphate, sulphate and heavy metals such as copper, chromium, cadmium, zinc and lead. Although various types of bacteria had been proven to decolourise and degrade azo dyes efficiently [9, 10, 11, 12, 13, 14] their abilities to decolourise and degrade real textile wastewater remains questionable.

At present, the sequential anaerobic-aerobic treatment process is most commonly used in treating textile wastewater [15, 16, 17]. This treatment method has been found to be the most successful in decolourising the dye contents in textile wastewaters due to the reduction of azo linkages in the anaerobic process before entering aerobic process which later on degraded the products produced from the anaerobic process [18, 19]. The reason is the biotransformation products generated cannot be further degraded via anaerobic treatment, but with the involvement of post-treatment of aerobic condition, the products formed can be completely mineralised [20]. Nowadays, biological treatment for textile wastewater using microorganisms has been widely studied compared to the physical and chemical treatment due to the eco-friendly towards environment, reproducible efficiency treatment and economical implementation [21]. Biofilm is best described as communities or clusters of microorganisms attached onto surfaces [22, 23]. Biofilm may be developed by a single or multispecies of microorganisms that have the ability to form at living and non-living surfaces [22]. Growth of microorganisms in the form of biofilm protect them from the adverse condition of the surrounding environment and act as a protector or barrier from the environmental stress, enable communication and exchange genetic material and, nutrient availability and persistence in different metabolic states [24, 25]. Biofilm can have very long biomass residence times when treatment requires slow growing organisms with poor biomass yield or when the concentration of wastewater is too low to sustain growth of activated sludge flocs [26, 27].

Decolourisation and biodegradation of textile wastewater using bacterial cultures is more preferable compared to fungi due to long growth cycle and some fungi especially white-rot fungi are not naturally found in wastewater in order to produce enzyme which

responsible for wastewater treatment such as laccase [28]. Moreover, long hydraulic retention time needed for complete decolourisation which also limits the application of fungal decolourisation system [29].

BAC-ZS is a bacterial consortium which is isolated from sludge of textile wastewater and palm oil mill effluent. BAC-ZS bacterial consortium consisted of three bacterial strains (*Brevibacillus panacihumi* ZB1, *Lysinibacillus fusiformis* ZB2 and *Enterococcus faecalis* ZL with accession number JF742761, JF742762 and JF742763, respectively), already proven in laboratory studies which could decolourised and degraded several azo dyes. Hence, the successful of those studies were further experimented using raw textile wastewater in order to see its performance.

In this study, a mixed bacterial culture known as BAC-ZS was grown as biofilm onto the macrocomposites as support material to degrade real textile wastewater in a sequential anaerobic-aerobic bioreactor. The bacterial consortium was previously shown to rapidly decolourise the model azo dye, Acid Orange 7 [30]. To the best of our knowledge, treatment of textile wastewater using decolurising bacteria grown as biofilm onto macrocomposites has very limited been reported before. Along with composition of macrocomposites that consists of several components such as zeolites and activated carbon that has been reported as application in wastewater treatment, thus, this study would be a beneficial in enhancing the prospect of treatment process.

EXPERIMENTAL

Sampling of wastewater

The raw textile wastewater was collected from a textile processing plant located in Johor, Malaysia. Table 1 shows the characteristics of the raw wastewater that were analysed according to the APHA methods [31]. The raw wastewater was then sterilised at 121°C, 1.5 kPa for 15 minutes and cooled to ambient temperature prior to use.

Table 1 The characteristics of raw textile wastewater used in this study.

Parameters	Value
Colour (ADMI)	1400 – 2100
COD (mg/L)	600 – 900
pH	7 – 9

Macrocomposites development

The development of macrocomposites was refered to article reported by Lim *et al*. [32].

Batch adsorption experiment of macrocomposites towards real textile wastewater

The equilibrium adsorption of real textile wastewater towards macrocomposites was performed by exposing 20.5 g of macrocomposites with varying studies of contact time and wastewater pH level on incubator shaker with 150 rpm at 25°C temperature. The amount of adsorbed (mg/g) was calculated using the formula reported by Vanderborght and Van Griekenm [33]:

$$Q = \frac{(C_i - C_f)v}{m} \quad -(1)$$

where;

Q = the amount of solute adsorbed from the solution (mg/g)
C_i = the concentration of adsorbate before adsorption (mg/L)
C_f = the concentration of adsorbate after adsorption (mg/L)
v = volume of the adsorbate (L)
m = weight of adsorbent (g)

The data then was fitted with Langmuir and Freundlich isoterms according to Igwe and Abia [34].

Preparation of bacterial inoculum

The bacterial consortium, BAC-ZS [30] at concentration of 10% (v/v) was added into a conical flask containing sterilised textile wastewater enriched with 10% (v/v) nutrient broth. The mixed bacterial culture was grown overnight at 150 rpm, 37°C in an incubator shaker.

Development of biofilm

The mix ratio design of the macrocomposites used in this study was developed and optimised using zeolites, activated carbon and cement [35]. Following that, the bacterial inoculum was added into a sterile 1 L container containing the macrocomposites. The development of biofilm was monitored within the duration time of 3 – 14 days. During the development process of biofilm, aeration was supplied using the air pump.

Determination of total biofilm dry cell weight

The macrocomposites with biofilm were dried in an oven to a constant weight at 105°C for 24 hours. Following that, the biofilm was removed from the surface of macrocomposites using the sodium hypochlorite according to procedure described by [36]. The calculation of total biofilm dry cell weight was determined using the equation:

Total biofilm dry cell weight (g) = A – B

A: dried weight of macrocomposites with biofilm (g)
B: dried weight of macrocomposites without biofilm (g)

Biofilm examination using field emission scanning electron microscopy (FESEM)

After 14 days of development duration, the biofilm coated onto macrocomposites was oven-dried at 105°C for 24 hours after which the samples were coated with gold particles and viewed using FESEM (FESEM, Supra 35 VP, Carl Zeiss).

Colony forming unit (CFU) analysis

The biofilm developed at different interval times were removed from the macrocomposites and vortexed with 10 ml of 0.9% saline solution. The homogenised biofilm was then centrifuged for 15 minutes at 4000 rpm and 4°C. Following that, the bacteria pellet was serially diluted before spread plating onto the nutrient agar. The experiment was done in triplicates.

Operation of wastewater treatment using the sequential batch reactor system

The macrocomposites with biofilm were placed into the reactor as illustrated in Fig. 1. The reactor was designed for a working volume of 4 L with total height of 16 cm and internal diameter of 20 cm. Only sterilised raw textile wastewater was fed into the reactor at the top of the tank using a peristaltic pump without the supplement of co-substrate. Air was supplied into the reactor by a fine air bubble diffuser from the bottom of the tank. The decanting of the wastewater took place using an outlet port located at 6.5 cm height from the bottom of the tank. The peristaltic pump and air supplied were controlled by an automatic timer.

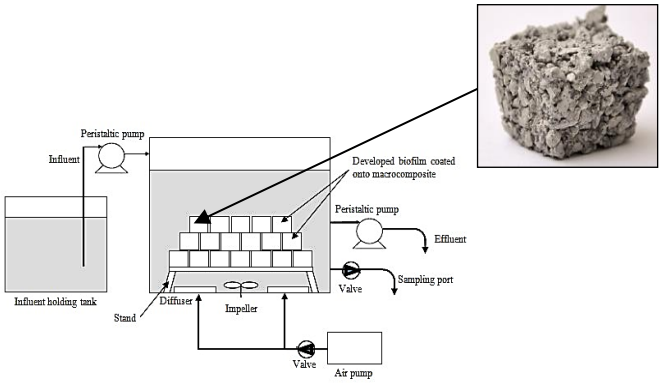

Fig. 1 Schematic representation of the SBR reactor system.

The reactor was run in anaerobic-aerobic sequencing mode of 48-hour that consists of four different phases in each cycle; feeding,

reacting, settling and decanting. The operating procedure and the time allocation for each cycle were described in Table 2. A control study using the macrocomposites without biofilm was performed.

Table 2 Operating procedure for the treatment process of each cycle.

Parameter	SBR treatment system
Working volume (L)	4
Feeding (minutes)	10
Mixing (minutes)	2
Reaction phase	
Facultative anaerobic (minutes)	1419
Aerobic (minutes)	1419
Settling (minutes)	10
Decanting (minutes)	10
Idle (minutes)	10
Temperature (°C)	25 ± 2

Determination of colour and chemical oxygen demand (COD)

The samples were taken and centrifuged at 4000 rpm at 4°C for 15 minutes. Only the supernatant was taken for the colour and COD measurement. Colour and COD reading were measured according to HACH Method via HACH DR5000 spectrophotometer (HACH Company, USA). All experiments were performed in triplicates and mean values were used for further calculations.

RESULTS AND DISCUSSION

Langmuir Adsorption Isotherm

The Langmuir theory is valid for the adsorption that takes place at a surface with a finite number of identical sites. The Langmuir model assumes that once a dyestuff molecule occupies a site, no further adsorption can take place at the same point. The model is based on adsorption homogeneity including equally available adsorption sites, monolayer surface coverage, and no interaction existing between the adsorbed molecules [37]. The equation for Langmuir isotherm is expressed as:

$$\frac{C_e}{q_e} = \frac{1}{k_l q_m} + \frac{C_e}{q_m} \quad -(2)$$

where;

C_e= the equilibrium concentration of the adsorbate (mg/L)
q_e = the amount adsorbed (mg/g)
k_l = the Langmuir constant related to the rate of adsorption (L/mg)
q_m = the maximum adsorption capacity (mg/g)

Table 3 Adsorption parameters of Langmuir and Freundlich isotherms.

Equilibrium model	Parameter	Value
Langmuir isotherm	q_m (mg/g)	0.5825
	k_l (L/mg)	0.0646
	R^2	0.9821
Freundlich isotherm	k_f (mg/g)	0.9982
	n	1.2009
	R^2	0.9176

Wang *et al*. [38] stated that a dimensionless constant separation factor or equilibrium parameter, R_L, is defined using the following equation:

$$R_L = \frac{1}{1 + k_l C_0} \quad -(3)$$

where C_0 is the initial concentration of wastewater (mg/L). Here, assumption have to be made as 1 ADMI unit equal to 1 mg/L due to complexity of wastewater. The R_L value indicates that the adsorption process is favourable when RL is between 0 and 1, and unfavourable when R_L is greater than 1 [38].

From this study, the maximum adsorption capacity (q_m) from Langmuir isoterm model was determined to be 0.5825 mg/g, k_l is 0.0646 L/mg, R_L is 0.011, which indicates that the equilibrium adsorption was favourable and the R^2 value is 0.9821 proving that the adsorption data fitted well to Langmuir isotherm model. The summary of the results were tabulated in Table 3 and showed in Fig. 2a.

Freundlich adsorption isotherm

The Freundlich equation represents the empirical relationship whereby it suggested that the adsorption energy of the adsorbate binding to a site on an adsorbent depends on whether or not the adjacent sites are already occupied. The equation assumes that the dye concentration on the adsorbent will increase when the dye concentration in the liquid increases too. The Freundlich isotherm is commonly designed for heterogeneous adsorption [39]. The equation for Freundlich isotherm is shown below:

$$\log q_e = \log k_f + \frac{1}{n} \log C_e \quad -(4)$$

where k_f and n are the physical constants of the Freundlich adsorption isotherm. The k_f and n are indicators of the adsorption capacity and adsorption intensity, respectively. The value of k_f and 1/n can be obtained from the intercept and slope respectively of the linear plot of experimental data of log q_e against log C_e.

From this study, the value of 1/n is 0.8327 while n is 1.2009, proving that the adsorption is favourable and the R^2 value is 0.9176. The summary of the results were tabulated in Table 3 and showed in Fig. 2b.

Adsorption kinetics

Xue *et al*. [40] reported that adsorption kinetic models are used to predict the adsorption characteristics and mechanisms, by which it is fundamentally important to be able to know the rate at which the adsorbate is removed/adsorbed by adsorbent in order to establish an adsorption treatment system. The kinetic of adsorption is important as it monitors the efficiency of the treatment process. Pseudo-first order and pseudo-second order are the kinetic models were studied for this framework. The linear form of the pseudo-first-order equation is given as [40]:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad -(5)$$

where q_e and q_t are the amount of textile wastewater adsorbed at equilibrium and at time t (mg/g), respectively and k_1 (h^{-1}) is the rate constant of adsorption. In the case of pseudo-first-order model, the R^2 value was found to be low as seen in Table 4, suggesting that the sorption kinetic does not follow pseudo-first order kinetic.

The experimental data was then examined by the pseudo-second-order kinetic model based on the linear form expressed as [41]:

$$\frac{t}{q} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad -(6)$$

where, k_2 (g/mg h) is the rate constant of pseudo-order adsorption. As shown in Table 4, the R^2 obtained from pseudo-second order model was found to be higher than pseudo-first order model. In order to further confirm the applicability of both kinetic models through the sum of error squares, SSE (%) was calculated as [42]:

$$\text{SSE (\%)} = \frac{\sqrt{\sum(q_e \exp - q_e \text{ cal})^2}}{N} \quad -(7)$$

where;

$q_e$exp = adsorption capacity at equilibrium experimental (mg/g)
$q_e$cal = adsorption capacity at equilibrium calculated (mg/g)
N = number of data point

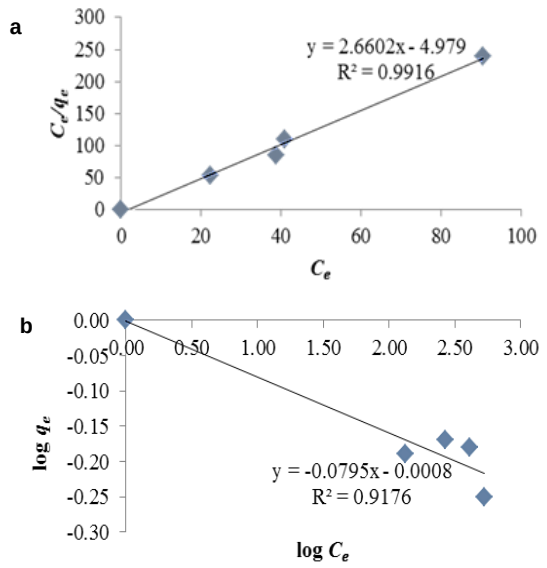

Fig. 2 Adsorption isotherm plotting graph (a) Langmuir model; (b) Freundlich model.

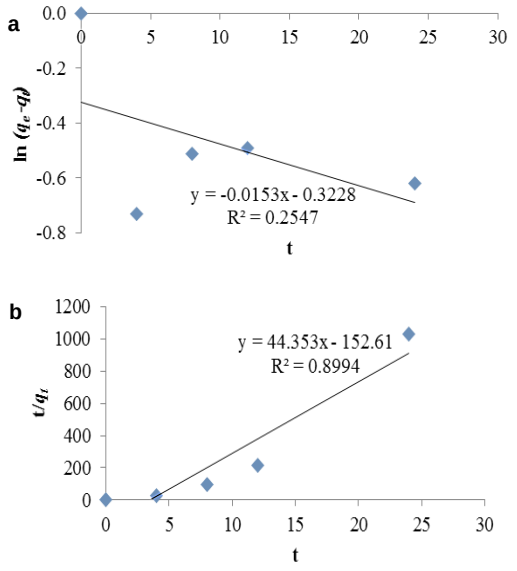

Fig. 3 Adsorption kinetics for textile wastewater removal by maccrocomposites (a) pseudo-first order; (b)pseudo-second order.

The corresponding graph for each adsortion kinetics are shown in Fig. 3. The result shows that the pseudo-second-order model has a lower SSE value when compared to pseudo-first-order model as shown in Table 4. The higher the value of R^2 and the lower the values of SSE (%), the better will be the goodness of fit. Thus, the sorption process can be approximated more accurately by the pseudo-second-order kinetic model than the pseudo-first-order kinetic.

Table 4 Kinetic parameters for the textile wastewater removal by macrocomposites.

Kinetic model	Parameter	Value
Pseudo-first order	k_1 (g/mg.h)	0.0153
	q_e exp (mg/g)	0.3753
	q_e cal (mg/g)	1.3810
	R^2	0.2547
	SSE (%)	0.2011
Pseudo-second order	k_2 (mg/g)	12.8903
	q_e exp (mg/g)	0.0200
	q_e cal (mg/g)	0.0225
	R^2	0.8994
	SSE (%)	0.0005

Development of biofilm

The rough surface of the macrocomposites as illustrated in Fig. 4a could provide a large surface area for bacteria attachment. This was witnessed with the formation of biofilm attached firmly on the macrocomposites after 14 days of development (Fig. 4b). According to [43], the substratum with rough surfaces provides large pores that could enhance the bacteria attachment.

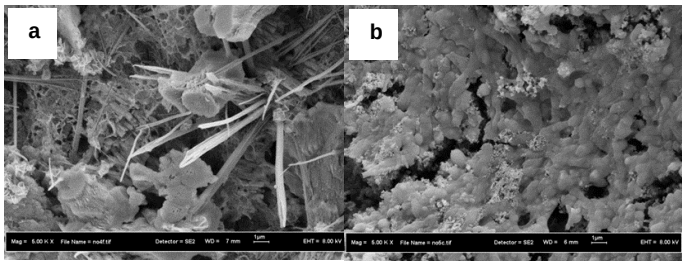

Fig. 4 FESEM observation on (a) macrocomposites structure only; (b) biofilm attached onto macrocomposites after 14 days of development. Resolutions of FESEM were taken at magnification of 5000 K.

Within the 14 days of biofilm development, the total biofilm dry weight showed a steady increase as seen in Table 5. In addition, the stable increase of numbers in viable total colony counts (Table 6) had reflected that the BAC-ZS had developed well living in textile wastewater condition and the bacteria were successfully adhered onto macrocomposites during the biofilm development process. Among the different development times, the biofilm developed on 14 days showed highest total dry weight of 0.54 ± 0.01 g (Table 5) and recorded highest total colony count of 6500.00 ± 608.00 x 10^6 CFU/mL (Table 6) as compared to biofilm developed on 3,7 and 10 days. According to Characklis [44], a common conceptual biofilm development model could be divided into three main phase: (i) a lag phase corresponding to the attachment of the microbial cells to the substratum, (ii) biofilm establishment phase which corresponded to the build up of the biofilm growth and (iii) the maturation phase with a constant number of cells. From the results obtained based on the total dry weight and total colony count of developed biofilm, it shows that the biofilm that formed is yet reaching its maturation phase due to the the number of cells appeared within it still increased. Thus, we chose the biofilm that developed for 14 days as our model onwards to be used in the treatment system.

Table 5 Total dry weight of developed biofilm.

Biofilm development (days)	Total biofilm dry weight (g)
3	0.08 ± 0.01
7	0.12 ± 0.01
10	0.28 ± 0.01
14	0.54 ± 0.01

Table 6 Total viable count of developed biofilm.

Biofilm development (days)	Colony count (x 10^6 CFU/mL)
3	0.35 ± 0.05
7	72.00 ± 4.00
10	350.00 ± 26.50
14	6500.00 ± 608.00

Treatment of textile wastewater in sequential anaerobic-aerobic batch reactor

The decolourisation of textile wastewater using BAC-ZS as biofilm achieved 78.6 ± 1.4 % of decolourisation (Fig. 5). Previously, Bay *et al.* [30] demonstrated BAC-ZS mixed bacterial culture required presence of yeast extract and glucose to fast decolourise Acid Orange 7 and no decolourisation was observed in the control without co-substrate. Interestingly in this study, the BAC-ZS in the form of biofilm could promote decolourisation without presence of co-substrate. Although several studies have been reported on decolourisation of real textile wastewater using mixed bacterial culture [45, 46], yet, the addition of co-substrate was required for the catabolic activities of

biofilm. Therefore, the success of BAC-ZS biofilm to decolourise textile wastewater without co-substrate had proven the ability of the mixed culture to use the complex textile wastewater as their sole carbon and nitrogen sources.

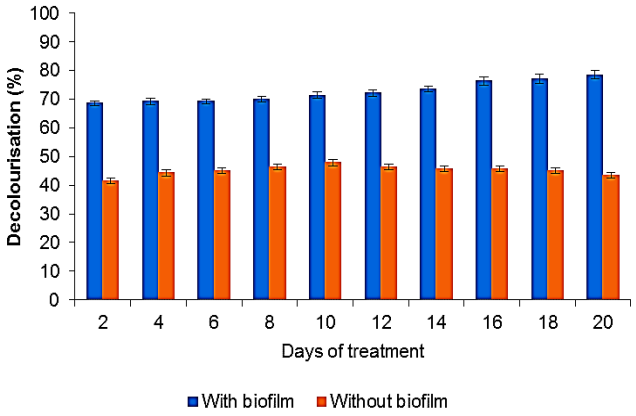

Fig. 5 Decolourisation of textile wastewater using SBR for 20 days of treatment.

In the control, 47.9 ± 0.9 % of decolourisation was recorded. The presence of zeolite and activated carbon as the main components of the macrocomposites assisted in absorption of colour [35, 47]. Activated carbon and zeolite are widely used in environmental application, especially in wastewater treatment [48, 49]. The characteristics of activated carbon such as high porosity make it an efficient adsorbent to trap metal ions, dyes, and other organic compounds through adsorption process. In addition, presence of zeolite allowed efficient ion exchange that would decrease the colour intensity of textile wastewater.

Pearce *et al*. [50] explained decolourisation of azo dyes is due to reduction of azo bond by electron donors; NADH, NADPH or FADH. However, presence of oxygen as stronger oxidant would have completed with the azo dyes as the terminal electron acceptors. This explained higher decolourisation during facultative anaerobic process compared to aerobic process as seen in Table 7. The finding in this study was aligned with the observation reported by Golob and Ojstrsek [51].

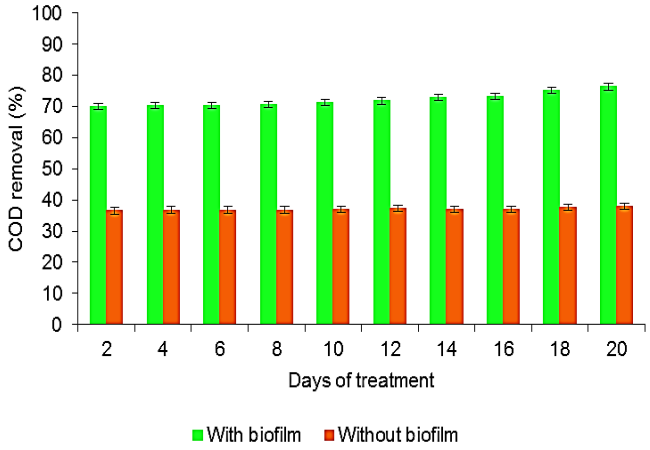

Fig. 6 COD removal of textile wastewater using SBR for 20 days of treatment.

Table 7 Decolourisation percentage in each process of treatment.

Treatment (Day)	Decolourisation using developed biofilm (%)		Decolourisation without using developed biofilm (%)	
	Facultative anaerobic process	Aerobic process	Facultative anaerobic process	Aerobic process
2	43.6 ± 1.15	25.0 ± 1.32	27.9 ± 0.80	13.6 ± 1.20
4	45.7 ± 1.57	23.6 ± 1.15	29.3 ± 0.50	15.0 ± 0.90
6	45.7 ± 0.92	23.6 ± 1.22	29.3 ± 0.60	15.7 ± 0.96
8	45.7 ± 0.79	24.3 ± 1.35	31.4 ± 0.88	15.0 ± 0.50
10	45.0 ± 0.87	26.4 ± 1.25	30.7 ± 0.96	17.2 ± 0.90
12	46.4 ± 0.85	25.7 ± 1.21	30.0 ± 0.50	16.4 ± 0.96
14	47.9 ± 0.79	25.7 ± 1.21	30.0 ± 0.50	15.7 ± 0.50
16	50.0 ± 0.87	26.4 ± 1.15	29.3 ± 1.00	16.4 ± 1.20
18	50.7 ± 1.47	26.4 ± 1.15	29.3 ± 0.90	15.7 ± 0.90
20	54.3 ± 1.03	24.3 ± 1.21	28.6 ± 0.96	15.0 ± 0.90

Table 8 Percentage of COD reduction in each process of treatment.

Treatment (Day)	COD reduction using developed biofilm (%)		COD reduction without using developed biofilm (%)	
	Facultative anaerobic process	Aerobic process	Facultative anaerobic process	Aerobic process
2	24.6 ± 0.79	45.5 ± 1.32	12.0 ± 1.02	24.6 ± 1.14
4	24.9 ± 0.26	45.5 ± 0.87	12.0 ± 1.14	24.9 ± 1.14
6	25.9 ± 0.66	44.4 ± 0.60	12.0 ± 1.00	24.9 ± 1.00
8	25.9 ± 0.78	44.7 ± 0.75	12.3 ± 0.94	24.6 ± 1.00
10	26.8 ± 0.92	44.5 ± 0.87	13.6 ± 0.90	23.5 ± 1.20
12	27.3 ± 0.61	44.6 ± 0.79	13.2 ± 0.90	24.1 ± 1.14
14	27.3 ± 0.44	45.6 ± 0.69	12.3 ± 0.94	24.9 ± 1.20
16	27.9 ± 1.05	45.5 ± 1.32	12.9 ± 0.90	24.7 ± 1.00
18	28.5 ± 0.87	46.7 ± 0.79	12.7 ± 1.20	24.9 ± 1.00
20	29.2 ± 0.69	47.1 ± 0.79	12.9 ± 1.00	25.2 ± 1.14

According to Fig. 6, maximum percentage of COD removal of 76.4 ± 1.12% was achieved in the reactor with biofilm. Higher COD removal in reactor with biofilm could reflect that BAC-ZS had degraded the textile wastes into simpler metabolites that useable for its cellular growth and cell maintenance. In the control, the macrocomposites without bioflim showed ability to remove 38 ± 1.5% of COD. Presence of activated carbon and zeolite in the macrocomposites could have acted as dye absorbent [52, 53, 54].

The drastic COD removal was occurred in post aerobic phase (Table 8). This result was agreeable with result obtained by Kapdan *et al*. [55], which also showed that the colour removal occurred mainly under facultative anaerobic phase while COD removal appeared to happen under aerobic condition of a sequential facultative anaerobic-aerobic treatment system. Besides, Pandey *et al*. [20] also reported that aerobic condition is required for complete mineralisation of dyes as the aromatic amines formed during reduction of textile dyes in the

anaerobic phase will only be mineralised via hydroxylation and ring-opening in the presence of oxygen.

CONCLUSION

As a conclusion:

a. Macrocomposites have the potential use as adsorbent for textile wastewater removal based on adsorption isotherm which fit Langmuir and Freundlich model. The adsorption kinetic studies show that the kinetic can be represented by a pseudo-second order model.
b. Biofilm was successfully developed onto macrocomposites using a novel bacterial consortium, BAS-ZS mixed culture obtained previously. The results showed that the 14-day of developed biofilm was the highest biofilm density compared to others between different time duration studies from 3 to 14 days.
c. Biofilm using BAC-ZS bacterial consortium was observed to grow and adapt very well with textile wastewater dyes without any of co-substrate added during the phase of wastewater treatment using sequential anaerobic-aerobic batch reactor.
d. Colour and COD analysis of sterilised textile wastewater were successfully obtained with comparison between with and without using the developed biofilm. The colour and COD removal using developed biofilm achieved maximum reading of 78.6 ± 1.4% and 76.4 ± 1.12% respectively after 20 days of treatment while only 47.9 ± 0.9% colour and 38.0 ± 1.5% COD removal achieved without using the developed biofilm that act as control study.

ACKNOWLEDGEMENT

We wish to thank to UTM PhD Zamalah scholarship for the financial aid and Ramatex Textile Industries (M) Sdn. Bhd. for providing access to their sites for wastewater sampling. We are also expressing our gratitude to UTM for providing equipment and facilities in order to perform this research.

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