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BIODECOLOURISATION OF METHYLENE BLUE IN WATER USING RECYCLED PAPER MILL ACTIVATED SLUDGE CULTURE IN A SEQUENCING BATCH REACTOR

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Abstract

In this research, an exploration of biodegradation of the methylene blue dye (MB) by mixed culture obtained from an activated sludge tank of a recycled paper mill was conducted. Three sequencing batch reactors (SBRs), each with a working of 5 L, the first reactor (R1) containing dye only (25 mg/L), the second reactor (R2) containing biomass mixed with dye (25 mg/L) (R2), and the third reactor (R3) containing only biomass were used in this study. For the first two weeks, the biomass was acclimatized with a ratio of COD (100):N (5):P (1) at a hydraulic retention time of 24 h in R2 and R3 reactors until both attained MLSS of 1400 mg/L. Then, the biomass in R2 was subjected to a treatment of 25 mg/L of synthetic methylene blue dye mixture for two weeks. Biodecolourisation of 15 and 85% was achieved within 14 days of treatment for R1 and R2, respectively. Fourier Transform Infra-Red Spectroscopy (FTIR) of the resultant effluent in the two reactors affirmed the biotransformation of the dye into different substances. The results demonstrated that biomass can provide an efficient platform for decolourisation of methylene blue dye and has improved the water quality for a better sustainable ecosystem.

INTRODUCTION

Textile dyeing wastewater is not only highly carcinogenic but also mutagenic, which has adverse effect not only to human health but also to aquatic environment [1, 2]. It has been reported that over 280,000 metric tons of dye effluents are produced each year from textile industries and believe it or not that the effluents are released into the environment especially water bodies with minimum or no treatment [3, 4]. Life below water facing dangerous situation whereby the light and oxygen transfer will be inhibited while adsorption of dyes into mammalian's skin will affected liver enzyme [5]. To maintain the quality of water in natural ecosystem, suitable treatment to treat contaminated medium is of high concern [6]. Physical and chemical treatment technologies such as coagulation-flocculation, adsorption, oxidation and filtration were usually utilised to treat dye contaminated effluents [7, 8].

However these technologies consume high energy, required the addition of chemical and it generates hazardous sludge contributing to the secondary pollution problem [9,10]. The best colour removal efficiency has been achieved by using RO member and nanofiltration which reduced pollution load as well as effluent colour to suitable levels that can be recycled and reused. Nanofiltration is limited, high in cost of equipment and membranes, and difficult in maintenance [11]. In contrast, biological treatment technology is an alternative treatment technology having an approach towards environment friendly, cost economical, and does not produce harmful sludge [12]. Biological treatment technology includes remediation by plants [2, 4] and microbes [13] which involve transformation and

degradation of dyes by their enzymatic production. Sequencing batch reactor (SBR) which utilises activated sludge containing bacteria has been frequently selected to treat dye contaminated wastewater due to its simple operation and degradation performance [14, 15].

The high concentration of colour, COD, suspended solids and other pollutants in textile effluent, it continues as one of the most complex wastewater to treat [16]. Therefore, in this study, activated sludge from a recycled paper mill was utilised in SBR to assess the decolourisation performance by bacteria in minimizing methylene blue (MB) in dye containing wastewater. MB was selected since it was listed as one of the most common dye used by industries [16]. The outputs from this recent study will throw light on decolourisation treatment technology not only for textile industry but for paper, leather, cosmetics and pharmaceuticals industries where application of dyes is directly related to these industries [17-18].

MATERIALS AND METHODS

Source of Biomass

Activated sludge from a recycled paper mill in Selangor, Malaysia was used as a source of biomass. The biomass taken from the activated sludge process had mixed liquor suspended solids (MLSS) of 2,400 mg/L prior to the experimental studies. The biomass was utilised as the inoculum for the sequencing batch bioreactors (SBRs).

Lab-scale Bioreactor Setup

The experiment was conducted using three laboratory scale sequencing batch reactor (SBRs). The 5 L cylindrical Plexiglas reactors acted as batch reactors in this experiment. The first reactor (R1) containing dye only, the second reactor (R2) containing biomass mixed with dye, and the third reactor (R3) containing only biomass were used in this study as illustrated in **Figure 1**. The biomass was inoculated into the R2 and R3 at a ratio of 3:10 (v/v), reducing the initial MLSS concentration to 1,400 mg/L (initially at 2,400 mg/L). Each reactor was provided with aeration at above 3 mg/L to ensure sufficient dissolved oxygen (DO) supply for aerobic conditions. The bioreactors were run for 14 days.

An amount of 3 L of recycled paper effluent was filled in each R2 and R3 for the first two weeks and was operated with one-day hydraulic retention time (HRT) for the purpose of biomass acclimatization. It was based on the optimal HRT obtained from a study conducted by Muhamad et al. [19]. In addition, nutrient was added to support microbial growth since the levels for nitrogen (N) and phosphorus (P) in the activated sludge were low. Therefore, urea (PETRONAS Fertilizer Sdn. Bhd., Malaysia) and trisodium phosphate (R & M Chemicals, Malaysia) were added in the reactors at a ratio of 100 (COD):5 (N):1 (P) [20].

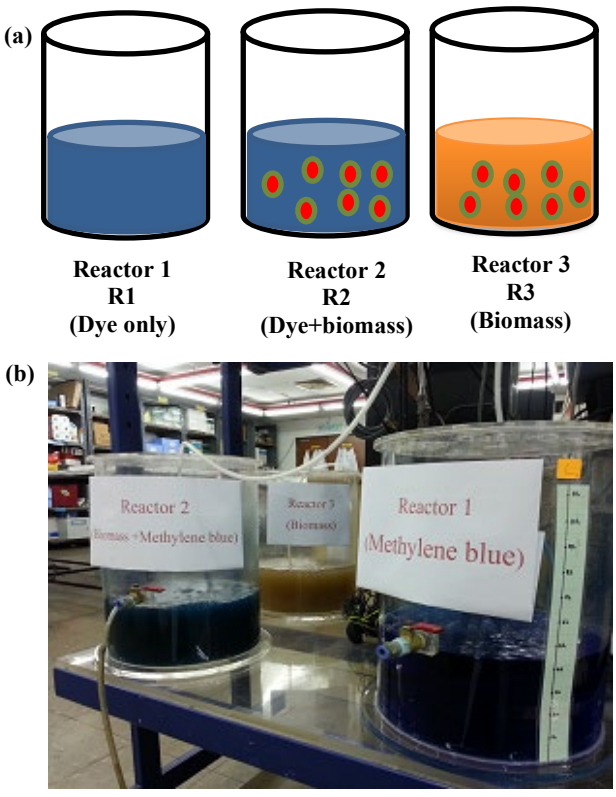


Figure 1. (a) Schematic diagram of sequencing batch reactors (SBRS) and (b) laboratory scale of SBRS applied through 14 days of treatment period.

Physicochemical Analysis of the Wastewater

Water quality parameters, including temperature (T), pH, Redox potential (ORP), chemical oxygen demand (COD), dissolved oxygen concentration (DO), and the suspended biomass concentration, MLSS were recorded at every sampling day. Each parameter were recorded using instruments listed in **Table 1**.

Table 1. Instruments involved for physicochemical analysis of the wastewater.

Parameter	Instrument	Brand, Model	Manufacturer	Method
T, pH, ORP	Multi-meter	Metrohm, 827	USA	-
DO	DO meter	YSI, 550A	USA	HACH Method 8000
COD	Spectrophotometer	HACH, DR3900	USA	
MLSS	-	-	-	APHA standard methods [21]

Biodecolourisation Experimental Runs

Biodecolourisation experiments were carried out using activated sludge of a recycled paper mill with initial MLSS of 1400 mg/L. After two weeks of acclimatized period, three sequencing batch reactors (SBRs) were used in this study. Each reactor with a working of 5 L, the first reactor (R1) containing dye only, the second reactor (R2) containing biomass mixed with dye (R2), and the third reactor (R3) containing only biomass. Methylene blue dyes with a concentration of 25 mg/L was added in R1 and R2. Every day, samples from each reactor were taken and centrifuged at 10,000 g for 10 min. After all particulate matter was removed, it was analysed using the UV–visible detector (Agilent 1100 Series, USA) at 665 nm. The biodecolourisation efficiency was calculated according to Equation 1 [22]:

$$\text{Biodecolourisation (\%)} = \frac{(A_i - A_f)}{A_i} \quad (1)$$

with, A_i constitutes the initial absorbance of the sample and A_f constitutes the absorbance of sample at the end of exposure period.

Analytical Procedures for the Detection of Biotransformation Products

Biotransformation was monitored using The Fourier Transform Infrared (FTIR) techniques (NICOLET 6,700 Spectrophotometer, USA). The FTIR spectra of Methylene blue dye with or without biomass treatment were analysed after 14 days of dye decolourisation experiments. This was done to confirm the biodegradation of the dye containing wastewater into different substances within the range of 400-4000 cm^{-1}. The samples were blended with KBr in the ratio of 5:95 [4] and were then analysed.

Statistical Analysis

IBM® SPSS® Statistics was utilised for statistical analysis of all experimental data obtained in this study where Statistical Package for Social Science (SPSS) program, version 21.0 (SPSS Inc., Chicago, IL) was used. Data was subjected to one-way analysis of variance (ANOVA) with Tukey–Kramer multiple comparison tests. Confidence limit used was 95% which indicated that any readings with $p<0.05$ represents the significant values [23, 24].

RESULTS AND DISCUSSION

Quality Characteristics of the Reactors

Generally, the mean values for temperature and pH ranged between 24-27 ˚C and 7.4-8.5 all through the 14 days respectively as shown in **Figure 2**. Decolourisation of dye by using mixed culture is influenced by temperature which enhances microbial growth and enzyme production within 25-35 ˚C [25]. Kishor et al. [26] found 99% decolourisation of MB dye within 6 h at optimum conditions of 30 ◦C temperature, 100 mg/L dye concentration, and pH 7 by *Bacillus albus* which was isolated from sludge of textile wastewater. From a review by Solís et al. [25], the best pH of treatment media is between pH 5-9 which is depend on the colour type. According to Kurade et al., [27] effluent with alkaline pH had faster decolourisation and reached the optimum level at pH 7. Cai et al. [28] proved that maximum methylene blue adsorption by sewage sludge was achieved at pH 9. Biological treatment with aeration system having DO concentration at a variance between 3-4.8 mg/L. It is worth noting that there is no significant difference between R1, R2, and R3 in DO concentrations ($p>0.05$) while there is a significant different for ORP conditions ($p<0.05$) among the reactors. ORP oscillated between -26 and -90 mV with R2 having the lowest ORP profile compared to other

reactors, giving evidence that R2 containing facultative culture that are able to survive in aerobic or anaerobic conditions [29]. The DO concentrations and ORP conditions are important for microbes to enhance degradation process [30]. For R2, the presence of oxygen had supported the mixed culture to degrade MB dyes compared with R1 (MB dyes only).

The biodecolourisation capability of methylene blue in improving water quality is studied. The Chemical Oxygen Demand (COD) and MLSS are among the analysis test used to examine the biomass ability to remove dye. The COD as well as MLSS test results are shown in **Figure 3**. There was statistically significant difference of COD removal values between the two treatments R1 and R2 (p<0.05). R2 was found to be most effective in treating dye contaminated influent by having the highest removal (62%) of COD due to the presence of biomass within 14 days comparing to R1 (MB only) with the removal was only 36% for COD. As shown in Figure 3, MLSS for R2 decreased when exposed to MB dye to 1,260 mg/L while R3 increased to 1,550 mg/L on day 5. It is noticeable that bacteria inside R2 when initially exposed to dye was loading shocked and required acclimatization process before regaining to grow. While bacteria in R3 which had no dye loading continued to grow.

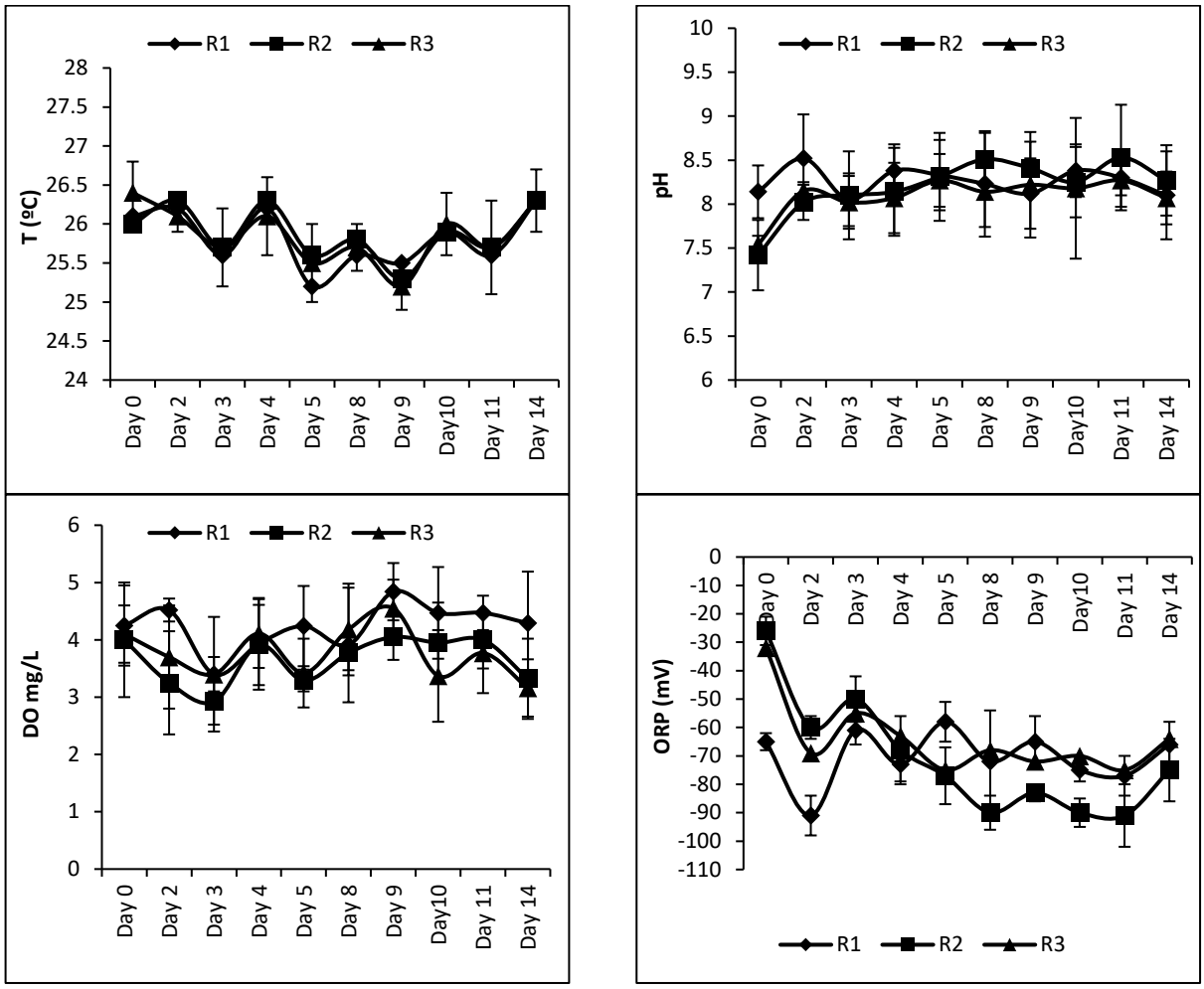


Figure 2. Physical parameters of dye contaminated water in each reactor (R1, R2 and R3) at each sampling day.

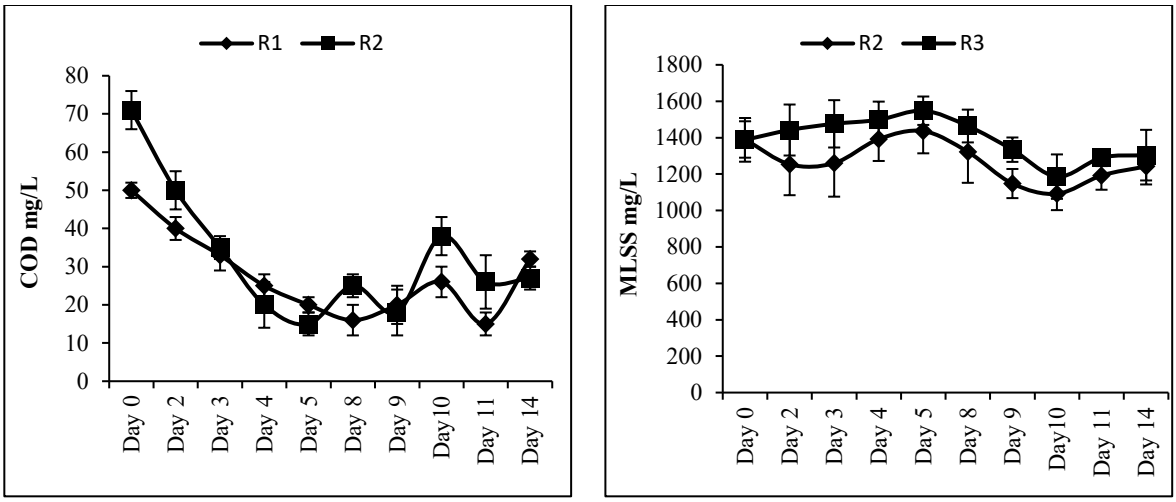


Figure 3 Variations of COD and MLSS for R1, R2, and R3.

Biodecolourisation and Biotransformation Analysis

UV–vis spectral analysis of the methylene blue dye represented the maximum absorbance at 665 nm during the initial exposure period and as time goes by, the absorbance was reduced due to the biodecolourisation [31]. Decolourised of dye solution in R1 was up to 15% within 14 day, whereas in R2 was 85% decolourisation of the dye within 14 day by biomass (**Figure 4**). It is clear that decolourisation was done first day and approximately fixed after day 3. Bacteria are the important factor in dye degradation mechanisms of adsorption and enzymatic degradation [26]. Du et al., [32] utilised *Aeromonas* sp. DH-6 for biodegradation of methyl orange (MO). After 12 h of exposure, almost 100% decolourisation of MO at initial concentration of 100 mg/L was achieved by *Aeromonas* sp. In different research, bacterial consortium was found to be acclimatized to the higher concentration of azo dyes which was utilized as nitrogen and carbon source in the synthetic wastewater [33, 34, 35].

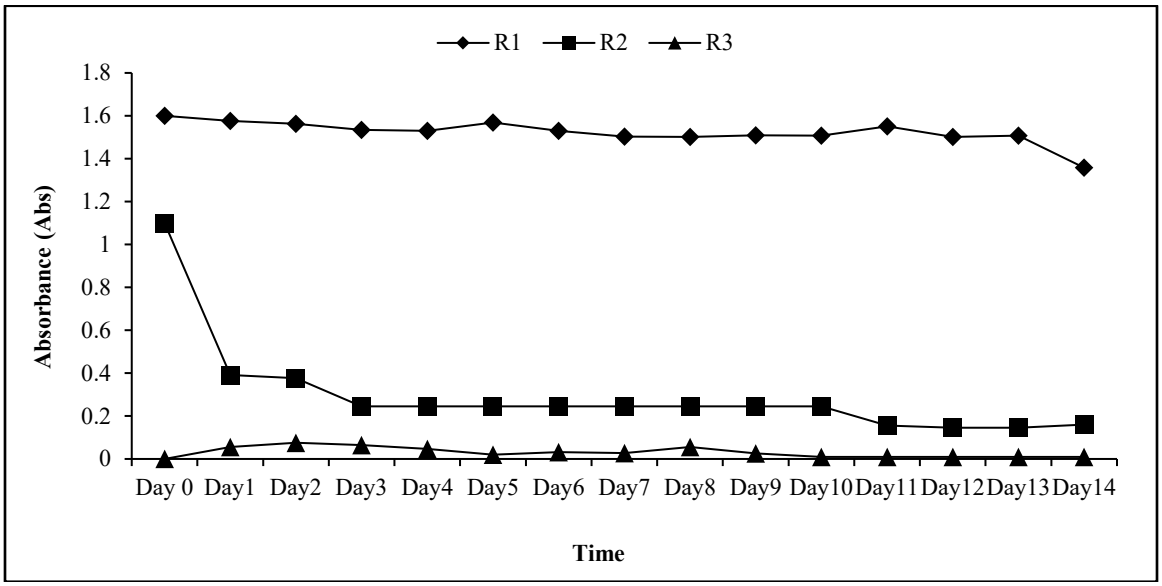


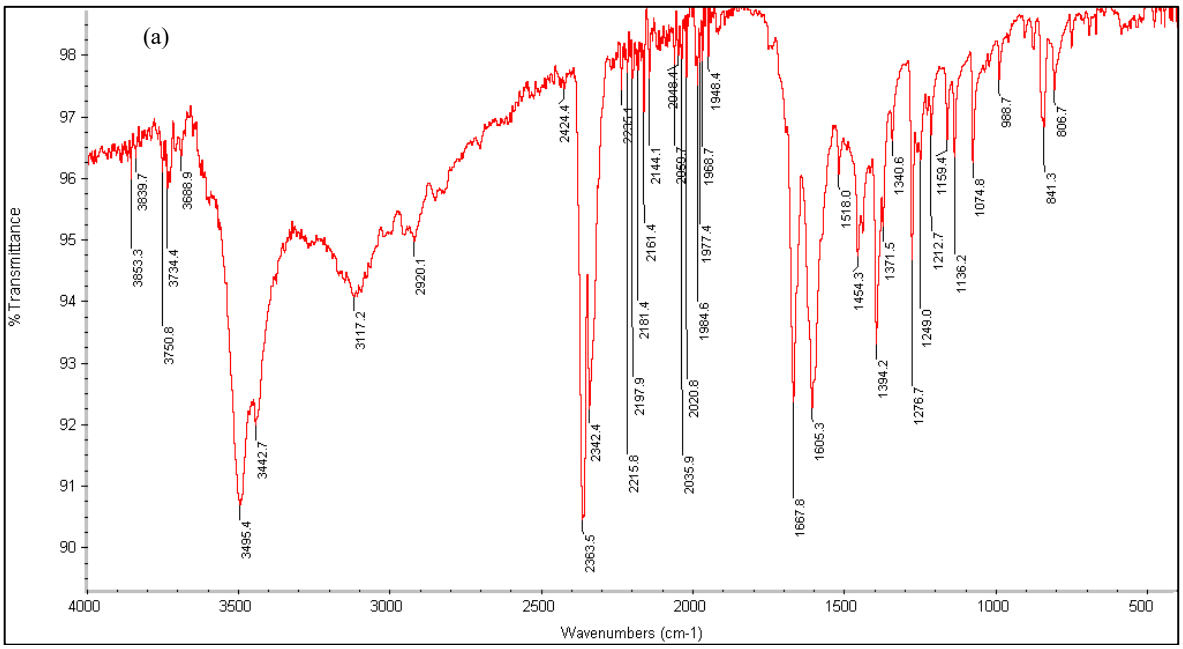
Figure 4. Absorbance of MB after treatment by consortium in activated sludge according to the three reactors (R1, R2 and R3).

From **Figure 5**, it was crystal clear that the decolourisation of MB by biomass in R2 occurred when compare to the reactor without biomass (R1). To further confirm that the biotransformation of the dye mixture in R2, the FTIR spectra of the control dye mixture obtained in R1 (**Figure 6(a)**) with spectra of the decolourised products of dye mixture by biomass in R2 (**Figure 6(b)**) were compared. From the figure, it was clearly seen that the peak between figures were significantly different. More peaks were observed in the dye mixture without the existence of biomass (control-R1).

The FTIR spectrum of R2 (dye + biomass) after 14 days of exposure represented significant difference in the peak position when compared with day 0. Lesser peak was observed in FTIR spectrum for R2. The final products showed peaks at 3,385.3 cm^{-1} for CH stretching (of alkynes), 1,641.6 cm^{-1} for –C=C– stretch (of alkenes), 1,413.4 cm^{-1} for C–C stretch (of aromatics), 1,049.9 cm^{-1} for C–N stretch (for aliphatic amines), 698.7 cm^{-1} for –C≡C–H : C–H bend (for alkynes). The results are in agreement with Merlin et al. [36], which found that 100 mg/L of mixed azo dye was degraded after five days. The medium intensity peak in 3335 cm^{-1} corresponds to N–H stretching vibration, indicating a primary aliphatic amine. The peak in 2116 cm^{-1} corresponding to C=C broadening vibration that indicates the occurrence of alkyne.

(a) (b)

Figure 5. The removal of MB dye by mixed culture at (a) Day 0 and (b) Day 14.



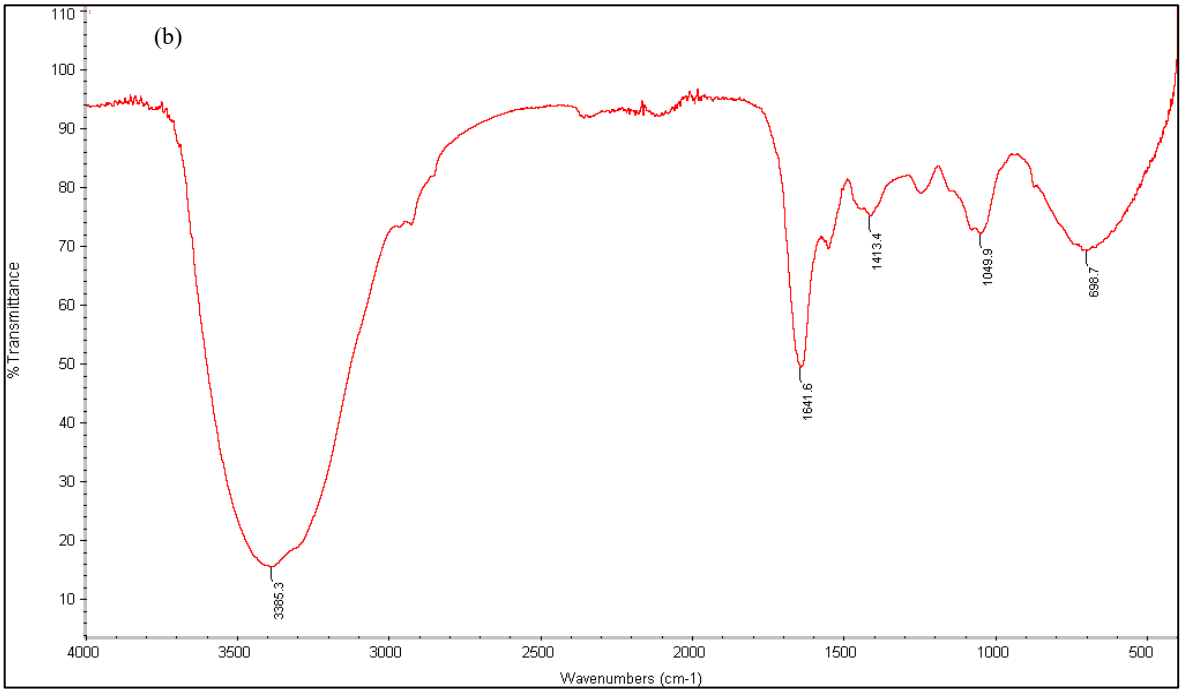


Figure 6. FTIR spectra of decolourisation of MB dye by biomass in R2: (a) Day 0 (b) Day 14.

CONCLUSION

Decolourisation of the dye by biomass reactor was observed and showed 85% MB dye removal after 24 h. The findings indicated that the biomass reactor had efficiently reduced colour from dye contaminated wastewater efficient over time. The activated sludge containing bacteria collected from a recycled paper mill revealed the outstanding ability of consortia system in decolourisation the MB dyes. The application of consortium reactor efficiently removed dye and COD from wastewater containing dye, thus increased the quality of textile wastewater before being discharged to the environment.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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