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RESEARCH ARTICLE

Biodecolourisation of acid red 27 Dye by *Citrobacter freundii* A1 and *Enterococcus casseliflavus* C1 bacterial consortium

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Abstract

The feasibility of *Citrobacter freundii* A1 and *Enterococcus casseliflavus* C1 bacterial consortium under sequential facultative anaerobic-aerobic treatment for complete dye degradation using AR-27 dyes shows 98% decolourisation and 100% COD removal after 72 hours treatment. Moreover, the UV-Vis spectroscopy and Fourier Transform Infrared (FTIR) Spectroscopic analysis confirmed that the azo linkage was cleaved after the decolourisation occurred. While, the cyclic voltammetry analysis also shows that the decolourisation of AR-27 by *C. Freundii* A1 and *E. Casseliflavus* C1 was an irreversible reaction and the detection of oxidation reaction under agitation proved the presences of AR-27 degradation process. Furthermore, the HPLC analysis has confirmed the AR-27 degradation through the decrease in catechol concentration.

Keywords: Bacteria consortia, Azo dye decolourisation, cyclic voltammetry, irreversible reaction, Catechol degradation

INTRODUCTION

Azo dyes are characterised as aromatic compound that consists of one or more azo linkage (-N=N-) which are commercially used in the textile, paper and cosmetic industries (Pandey *et al.*, 2007; Onn *et al.*, 2017). However, as these dye were released into the water body without proper treatment, it creates a serious water pollution. This is because dye containing wastewater is toxic and detrimental to the environment and human health when being discharged to the water body (Verma *et al.*, 2008). Thus, to avoid serious environmental pollution and human health hazards, several wastewater treatment approached has been implemented such as physical adsorption, chemical reduction and biological degradation to overcome such issues (Pandey *et al.*, 2007; Onn *et al.*, 2017). Recently, biological degradation has gather interests among researcher in treating azo dyes due to the versatility of the treatment to remove the pollutant, helped in azo dye degradation and mineralisation. Besides the nature of biological degradation involved the used of microorganisms such as bacteria, fungus and yeasts may produce a treatment that are more environmentally friendly and economical.

In addition, the application microrganisms as pure or consortia for azo dyes wastewater treatment has been proven to provide positive output especially in term of colour removal (Pearce *et al.*, 2003; Saratale *et al.*, 2011). However, the used of azo degrading bacteria in a form of consortia for biodecolourisation of azo dyes were described as an efficient practice to achieve complete degradation of azo dyes compares to the solely used of pure culture bacteria (Yoo *et al.*, 2001; Pandey *et al.*, 2007; Saratale *et al.*, 2011; Bay *et al.*, 2015). Therefore, in this study, two distinctive strains of azo degrading bacteria were tested in the form of consortia to determine the biodecolourisation using Acid Red 27 (AR-27) as a dye model. These locally isolated bacterial strains was identified as *C. freundii* A1 and *E. casseliflavus* C1 *via* 16rRNA gene sequence analysis.

Citrobacter sp. strain A1 was isolated from a sewage oxidation pond, which characterised as a Gram-negative enteric coccobacillus, facultative aerobe and mesophilic dye-degrading bacterium (Chan *et al.*, 2012). This organism degrades azo dyes efficiently *via* azo reduction and desulfonation, followed by the successive biotransformation of dye intermediates under an aerobic environment (Chan *et al.*, 2012). While, *Enterococcus* sp. strain C1 is a Gram-positive facultative anaerobe which was co-isolated with *Citrobacter* sp. strain A1 from a sewage oxidation pond (Chan *et al.*, 2012) and could degrade azo dyes very efficiently *via* azo reduction and desulfonation in a microaerophilic environment (Chan *et al.*, 2012).

Hence, the study of this chapter will focus on the decolourisation and degradation of Acid Red 27 (AR-27) by using *C. freundii* A1 and *E. casseliflavus* C1 baterial consortia based on colour removal efficiency (%), dry cell weight (mg/L), pH, DO (mg/L), COD (mg/L), DNS(mg/L),TPC(mg/L), cyclic voltammetry and High Performances Liquid Chromatography (HPLC) analysis.

EXPERIMENTAL

Bacteria culture and media

The two bacteria strains were obtained from the Microbiology Laboratory of the Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia.

Initially, the microorganisms were grown on nutrient agar (Merck, Germany) at 37 °C. Then, P5 medium containing K_2HPO_4 (35.3 g / L), KH_2PO_4 (20.9 g/ L), NH_4Cl (2 g/ L), glucose (10 g/ L), nutrient broth (20 g /L) and trace elements was prepared according to the procedures described by previous study (Chan *et al.*, 2012). For the starter culture, a single bacterial colony of *C. freundii* strain A1 and *E. casseliflavus* strain C1 was inoculated into a 250-mL conical flask containing 50 mL of P5 medium and incubated overnight at 37 °C with shaking at 200 rpm for 16 hours, respectively. Optical density (OD) of each bacterial culture was determined at 600 nm (GENESYS 10s UV–Vis Spectrophotometer). The inocula were ready to be used for both decolourisation efficiency and bioelectricity generation when the absorbance reading reached 1.0 ± 0.2 (Chan *et al.*, 2011; Chan *et al.*, 2012).

Decolourisation of azo dye

Acid Red 27 (AR-27) dye (Sigma Aldrich) (Fig. 1) was used for the determination of azo dye decolourisation using the *C. freundii* A1 and *E. casseliflavus* C1 bacteria consortia. The discoloration was measured using a UV-Vis spectrophotometer (GENESYS 10s UV-Vis). 50 ml of modified P5 medium (0.5 g/L glucose and 1.0 g/L nutrient broth) with Amaranth (0.1 g/L) was mixed with 10% v: v of pure and mixed culture of *C. freundii* A1 and *E. casseliflavus* C1 with 1:1 ratio in a facultative anaerobic condition. The samples were then incubated in an incubator shaker at 29±2ºC with 200 rpm for the aerobic treatment. Sampling was performed at a 10 minute interval. Each sample was centrifuged and the supernatant were analysed at 521 nm to determine the level of decolourisation. The decolourisation efficiency was determined according to equation (1):

$$\textit{Decolourisation efficiency (\%)} = \frac{(Ai - Af)}{Ai} \; x\; 100\% \qquad (1)$$

Where A_i refers to the initial absorbance of dye prior to operation, A_f refer to the final absorbance at any time. Then, Fourier Transform Infrared (FTIR) spectroscopy analysis (Thermo Scientific iD7 ATR) was performed to determine the azo bond spectrum before and after decolourisation.

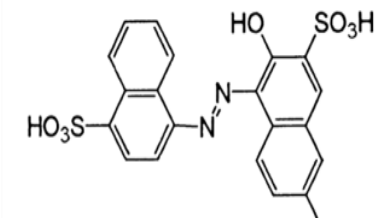

Fig. 1 Amaranth (AR 27) molecular structures.

Dry cell weight

Initially, the weight of filter paper is measured with aluminium foil and labelled before the housing filter was set up. The sample flask was stirred in order to suspend the culture evenly. Then, 1 ml of the sample was pipette out into a 2.0 mL micro-centrifuge tube and centrifuged at 10000×g for 3 minutes. The supernatant was discarded and the bacteria pallet was suspended with 10 mL sterile distilled water thrice to remove the excess component of P5 media to the filter paper by using the housing filter. The wet weight of the culture was immediately measured after the water has been pulled through as the first reading. Next, the cell paste was dried in a furnace at 100ºC for 24 h before the aluminium foil with the filter paper was weighted. The weight of the cell is calculated in mg/mL based on the equation (2).

$$\text{Dry cell weight} = [(\text{Cell dry weight} + \text{filter paper weight}) - (\text{filter paper weight})\text{mg}] / \text{sample volume (mL)} \qquad (2)$$

Determination of Redox Reaction by Cyclic Voltametric Analysis

Cyclic voltametric analysis was conducted to characterise the electron transfer synergy between microorganisms, microbial biofilm and microbial fuel cell anode (Fricke *et al*,. 2008). A three electrode system was implemented for the chroamperometry and cyclic voltametry analysis by using potentotiostat AUTOLAB PGSTST204 (Metrohm, Herisau, Switzerland). The analysis was performed by using glassy carbon electrode (Metrohm, Herisau, Switzerland), platinum sheet electrode (Metrohm, Herisau, Switzerland), and calomel electrode (HI55412, Hanna Instrument, Rhodes Island, USA) as the working electrode, counter electrode and a reference electrode. Cyclic voltametry (CV) analysis was conducted to observe the redox reaction during the AR-27 treatment The CV was conducted with a scan rate (10-100 mV/s) with a voltage range -0.6 to 0.8 V. The results were analysed using NOVA 1.1 AUTOLAB software (Metrohm, Herisau, Switzerland).

High Performances Liquid Chromatography Analysis

The HPLC analysis was conducted based from Anjaneya *et al.* (2011) and Bay *et al.*, (2013) with some modifications. Initially, the supernatant of AR-27 (90 mL) was collected during facultative anaerobic treatment (2 Hours) and aerobic treatment (24 Hours, 48 Hours and 72 Hours). The supernatant was transferred to a separating funnel and extracted with an equal volume of ethyl acetate. The top layer solution was collected and evaporated to dryness using a rotary evaporator. The residue was dissolved in 2 mL methanol. All samples were filtered using 0.2 µm nylon filters before eluting isocratically at a flow rate of 0.3 mL/min using LiChroCart, purospher STAR RP – 18, C18 column (5 µM, 4.6 mm × 250 mm). Mixture of methanol and nano pure water in the ration of 75:25 (v/v) was used for mobile phase. The analysis was conducted using the High Performance Liquid Chromatography (HPLC) with Photodiode Array and Fluorescence Detector (Agilent Technologies) at wavelength 280 nm.

RESULTS AND DISCUSSION

Azo dye decolourisation by *C. freundii* A1 and *E. casseliflavus* C1 bacteria consortium

The performances by *C. freundii* A1 and *E. Casseliflavus* C1 consortium for the AR-27 treatment was subjected to sequential facultative anaerobic-aerobic using glucose (0.5 g/L) and nutrient broth (1.0 g/L) as illustrated in Fig. 2. Based on the figure, 98% decolourisation was achieved within 2 h incubation under facultative anaerobic condition. The decolourisation of azo dye occurred due to the presences of azo reductase enzyme in *C. freundii* A1 and *E. casseliflavus* C1 that helped in cleaving the azo bond (-N=N-) which contributes to the dye colour (Lee, 2001; Chan *et al.*, 2012). Initially, slow growth of bacteria was recorded during the early phase of facultative anaerobic with initial biomass 0.12 ± 0.02 mg/mL. Then, the biomass increased to 0.46 ± 0.02 mg/mL after decolourisation was achieved after 2 h incubation. As decolourisation achieved, the decolourised solution was introduced into aerobic treatments. After 24 h, there was an increased in colour intensity of the decolourised solution, resulting in the decrease of the decolourisation percentage to 96%, while the bacteria biomass increased to 0.81 ± 0.07 mg/mL. Fig. 2 (a) also shows that the bacterial biomass continue to increase to 0.92 ± 0.01 mg/mL (72 h) before decreasing to 0.65 ± 0.02 mg/ml (120 h) during the aerobic treatment. Hence, it can be assumed that the decreased of bacteria biomass after 72 h incubation was cause by the nutrient deficiency and eventually leads the bacteria to enter their death phase.

Prior to the addition of the bacteria culture, the initial Chemical Oxygen Demand (COD) recorded in AR-27 solution was 2300 mg/L. However, the COD showed a slight decrease to 1900 mg/L (Fig. 2b) after decolourisation occurred. After 24 h of agitation, the COD reading continue to decrease to 900 mg/L (61 %) and there was a formation of the dark coloured observed. These dark coloured formation solution were suspected to be the results of auto-oxidation reaction occurred to the aromatic amine generated. Then, the COD

level of the decolourised AR-27 continued to drop to 100 g/L (95%) after 72 h of treatment.

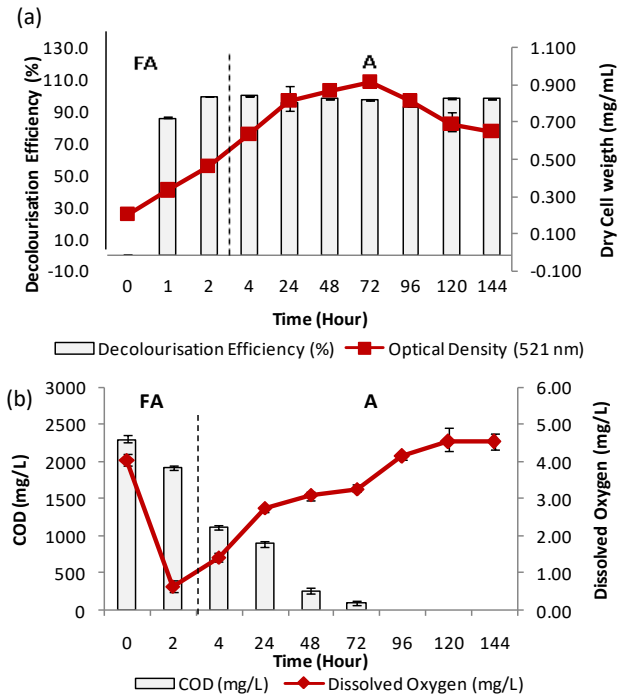

Fig. 2 Azo dye decolourisation of *C. freundii* A1 and *E. casseliflavus* C1 bacteria consortium. Decolourisation percentages and dry cell weight (a). COD removal and dissolved oxygen (b).

Besides COD removal, Fig. 2(b) also shows that the dissolved oxygen (DO) drastically decreased from 4.0 ± 0.2 mg/L to 0.63 ± 0.02 mg/L after decolourisation occurred. However, the concentration of dissolved of oxygen starts to increase to 1.43 ± 0.1 mg/L after 24 h aerobic treatments. The dissolved oxygen concentrations continue to increase alongside the incubation time under aerobic condition as shown in Fig. 2 (b). This was suported by previous literature which stated that under low oxygen concentrations, the biocatalyst metabolic activities might have initiated towards anaerobic reduction mechanism whereby the azo dye were considered as a competent electron acceptor along with the oxygen presence inside the bacteria cell (Mohan *et al.*, 2013). Moreover, according to Bay *et al.*, (2015), as oxygen is introduced after decolourisation occurred, the microorganisms will able to further degrade the aromatic amine produce during the facultative anaerobic treatment. This aromatic amine degradation was called mineralisation process which occurred under aerobic condition (Mohan *et al.*, 2013).

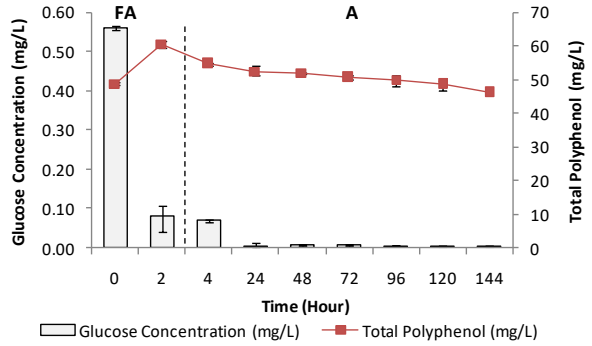

Fig. 3 Reducing sugar and total polyphenol analysis for AR-27 decolourisation.

During the AR-27 treatment, the DNS and total polyphenol analysis were conducted to determine the reducing sugar and total polyphenol concentration as shown in Fig. 3. In this study, the initial concentration of glucose supplied for the treatment was 570 ± 10 mg/L. However, the concentration of glucose decreased to 80 ± 4 mg/L as decolourisation was achieved after 2 h under anaerobic condition and continue to decrease drastically (4 ± 2 mg/L) after 24 h aerobic treatment. During decolourisation phase, glucose was utilised by bacteria cell metabolisms to generate adenosine triphosphate (ATP), hydrogen ion (H^+), and electron through glycolysis reaction. Due to the electron deficient properties of AR-27, the electron was transferred to the also linkage with the help of the electron carrier (NADH) and azo-reductase for reduction of the azo dye (Saratale *et al.*, 2011). While the bacteria completely utilised the remaining glucose for development of bacterial biomass after decolourisation was achieved.

According to previous literature, the degradation of azo dye usually will generate phenolic compound (Chengalroyen and Dabbs, 2013) such as aromatic amines and TPP was one of the analyses used to quantitatively determine the phenolic compound concentration (Dudonné *et al.*, 2009). Based on Fig. 3**,** during anaerobic treatment of AR-27, there is an increased of polyphenol recorded from 49 ± 1 mg/L during 0 h treatment to 61± 1 mg/L after 2 h. As decolourisation took place, the azo dye reduction by azoreductase enzyme will produce aromatic amines and contribute to the increased in the polyphenol concentration. However, as the decolourised AR-27 undergoes aerobic treatment, there was a slight decrease in polyphenol concentration recorded after 24 h (53 ±2 mg/L) and continue to decrease to 46 ±2 mg/L after 144 h incubation. The reduction of polyphenolic compound concentration during aerobic treatment might be caused by the mineralization of azo dye whereby the phenolic compound undergoes aromatic amine ring cleavage through the hydroxylation reaction in the aerobic degradation pathway (Hinteregger *et al.*, 1992).

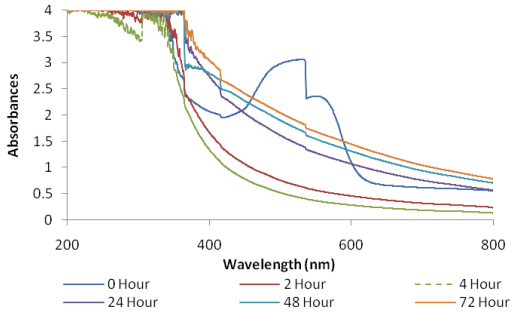

Fig. 4 Spectrum analysis for AR-27 decolourisation.

The degradation of AR-27 was foremost observed using UV-Vis spectrophotometry and the AR-27 solution showed the maximum absorption peak at 521 nm as seen in Fig. 4.. Once the decolourisation occurred, the major peak in the visible part of the spectrum had diminished. The significant reduction in the absorption peaks at 2h (facultative anaerobic condition); 24 h (aerobic condition) and 48h (aerobic condition) were compared to 0 h to confirm the degradation of AR-27. Although complete decolourisation was achieved, there was an increased of absorbances for the decolourised AR-27 during aerobic which causing the formation of a yellowish colour solution. This yellowish solution was suspected to be the excess colour of nutrient broth and product of oxidation reaction during mineralisation of decolourised AR-27 under aerobic condition.

Fourier Transform Infrared (FTIR) spectroscopy was used to determine the spectrum of the AR-27 dye before and after decolourisation. The FTIR analysis performed for the control and the decolourised sample was shown in Fig. 5. Based on Fig. 5, the control AR-27 dye displayed a peak at 3,311 cm^{-1} which corresponds to the intermolecular hydrogen bonding aromatic –OH and O–H stretching; a peak at 2,125.33 cm^{-1} for –H stretching of amines; a peak at 1,507.cm^{-1} for N=N stretching of azo linkage respectively.

Then, the decolourised AR-27 solution showed a peak at 3,343cm^{-1} for the intermolecular hydrogen bonding aromatic –OH and O-H stretching; a peak at 2,127 cm^{-1} for N-H stretching of amines. However, the peak located at 1,507 cm^{-1} for N=N stretching of azo group disappeared after 2 hours treatment. Thus, indicates that the azo linkage was cleaved biologically (Khouloud *et al.*, 2012; Singh *et al.*,

2015) by *C. freundii* A1 and *E. casseliflavus* C1 consortium after the decolourisation occurred.

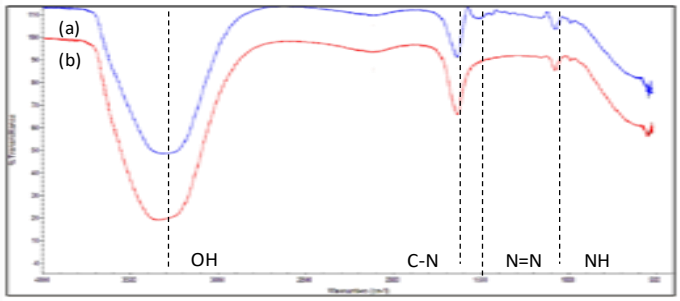

Fig. 5 FTIR spectra of azo bond reduction before (a) and after decolourisation (b).

Electrochemical analysis for AR-27 decolourisation by *C. freundii* A1 and *E. casseliflavus* C1 bacteria consortium

Cyclic-voltammetry (CV) analysis was used to measure the redox properties during the facultative anaerobic and aerobic treatment of AR-27 solution as shown in Fig. 6. Furthermore, CV analysis was also conducted to determine of mineralisation of the decolourised AR-27 solution. The presences of oxidation and reduction peak during aerobic treatment may indicate the mineralisation of the treated AR-27 solution.

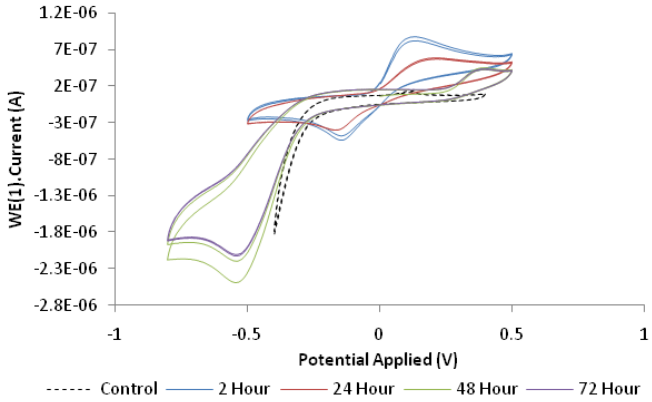

Fig. 6 Cyclic voltamograms analysis on AR-27 treatment in sequential facultative anaerobic (2 hour) and aerobic (24 hour, 48 hour and 72 hour) treatment.

Initially, inocula free AR-27 solution was used as a control and the result in Fig 6 shows the absence of any oxidation and reduction reaction. However, as *C. freundii* A1 and *E. casseliflavus* C1 consortia was introduced and undergoes facultative anaerobic decolourisation, after 2 h there is the presences of high oxidation potential (144 mV) and reduction potential (-146 mV). Thus, indicates that a biological oxidation and reduction was occurred due to the breaking the azo linkage (-N=N-) by *C. freundii* A1 and *E. casseliflavus* C1 which resulted the decolourisation of AR-27. Moreover, the separation between the oxidation and reduction peak was 290 mV indicates the decolourisation reaction was irreversible during 2 h facultative anaerobic treatment. As oxygen was introduced during aerobic treatment of AR-27, the oxidation and reduction peak start to decreased and the result was illustrated in Fig. 6.

After 24 hour aerobic treatment, the oxidation and reduction peak recorded were 224 mV and -165 mV respectively. The difference between the oxidation and reduction peak was 389 mV during 24 h aerobic treatment, thus shows that the reaction was irreversible under the presences of oxygen. It is expected that the detection of oxidation peak was due to oxidative degradation that occurred during the aerobic treatment. This oxidative degradation indicates that the presences of aromatic amine degradation during the aerobic treatment of the decolourised AR-27 solution. Furthermore, Fig. 6 also shows weak oxidation peak and strong reduction peak during 48 h and 72 h aerobic treatment of AR-27. After 48 h aerobic treatment, the oxidation and reduction peak recorded was 380 mV and -551 mV respectively. While the oxidation peak (380 mV) and reduction peak (546 mV) was recorded after 72 h aerobic treatment. Hence, the different between the oxidation and reduction peak for 48 h and 72 h was 931 mV and 926 mV respectively. Thus, shows that the reaction was irreversible for the AR-27 treatment during 48 h and 72 h aerobic treatment. Previous study by Steter *et al.*, (2014) also reported similar findings whereby the peak observe during decolourisation and degradation indicates the occurrence of polymerisation of azo compound. Hence, proves the abilities of *C. freundii* A1 and *E. casseliflavus* C1 in decolourisation and degradation of AR-27.

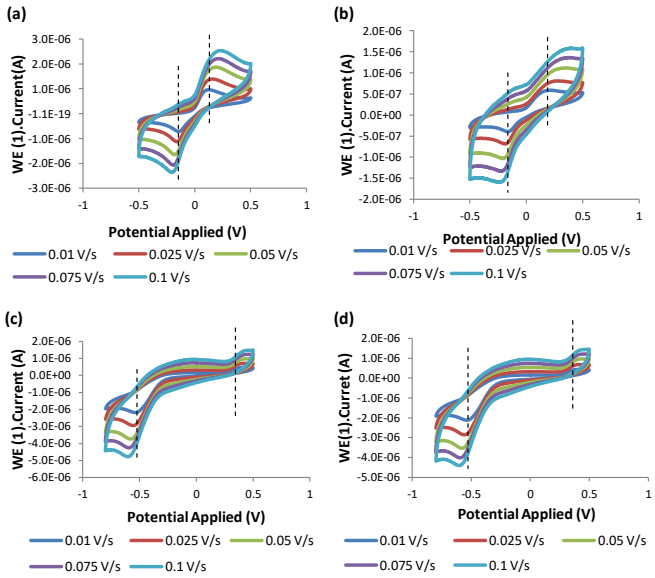

Fig. 7 Cyclic voltammogram analysis for AR-27 treatment in sequential facultative anaerobic-aerobic with differences scan rates (a) 2 h, (b) 24 h, (c) 48 h and (d) 72 h.

Further analysis was conducted to further prove the redox reaction observed during the AR-27 treatment in facultative anaerobic-aerobic condition was irreversible type was shown in Fig. 7. According to *Brownson and Banks* (2014), by applying various scan rates, the diffusion layer thickness was dramatically changed by reflecting the competition between electrode kinetic and mass transport. Thus, differences scan rates starting from 0.01 V/s – 0.1 V/s was applied during the AR-27 treatment for the irreversible type reaction determination. Based on the Fig. 7, there was a shift of individual redox peak magnitude and wide separation between the peaks during AR-27 treatment as higher scans rates was applied during the CV analysis. The irreversible reaction was observed during the AR-27 decolourisation by CV analysis was due to the electron transfer rates were smaller compared to the mass transport (*Brownson and Banks*, 2014). This was vice verse to a reversible type reaction in which the electron transfer rates and the peak potential were independent of the applied voltametric scan rates *(Brownson and Banks*, 2014*)*. In conclusion, the CV analysis of the AR-27 treatment using *C. freundii* A1 and *E. casseliflavus* C1 under sequential facultative anaerobic-aerobic condition was an irreversible reaction.

Determination of catechols degradation by *C. freundii* A1 and *E. casseliflavus* C1 bacteria consortium.

According to Pandey *et al.*, (2007), aromatic amines formed during azo dye reduction, have been reported to be more easily degraded under aerobic conditions. Furthermore, the presences of phenolic group in these aromatic amines can cause the decolourised azo dyes to have high toxicity properties. Hence, in this study, catechol was selected as the phenolic compound model for degradation as previous studies by Chan *et al.*, (2012) has detected the presences of this compound after decolourised AR-27 undergoes aerobic treatment through LC-MS analysis. Thus, the determination of

phenolic compound catechol by *C. freundii* A1 and *E. casseliflavus* C1 bacteria consortium was conducted using HPLC analysis.

In this study, HPLC analysis was conducted for the sample at the end of facultative anaerobe treatment (2 h) and throughout the aerobic treatment (24 h, 48 h, and 72 h) and the concentration of catechol detected during the AR-27 treatment by *C. freundii* A1 and *E. casseliflavus* C1 wasas shown in Fig 8. The retention time for the detection of catechol was estimated at ~4.02 minutes based on standard (Fig. 8a).

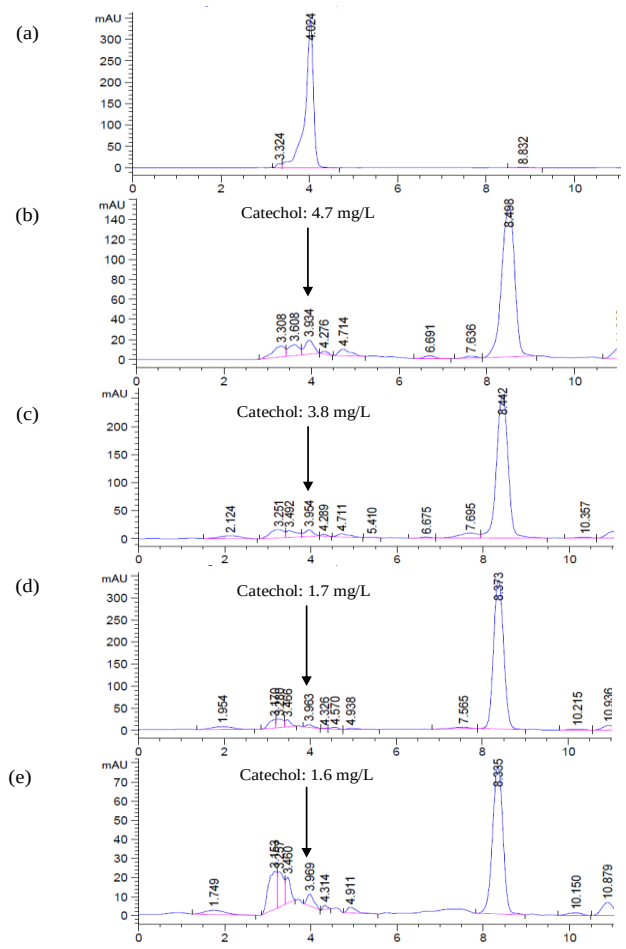

Fig. 8 HPLC analysis for AR-27 treatment based on catechol degradation (a) Standard catechol, (b) 2 h of facultative anaerobic treatment, (c) 24 h aerobic treatment, (d) 48 h aerobic treatment and (e) 72 h aerobic treatment. Catechol was detected with the approximate retention time of ~4.02 minutes.

Based on Fig. 8 (b), 4.7 mg/L of catechol was detected after 2 h facultative anaerobic treatment. However, after 24 h agitation, the aerobic treatment has reduced the catechol to 3.8 mg/L (Fig. 8c). The catechol concentration continues to decrease to 1.7 mg/L and 1.6 mg/L after 48 h (Fig. 8d) and 72 h (Fig. 8e) aerobic treatment, respectively. Hence, shows 66% reduction of catechol concentration was achieved at the end of AR-27 treatment. This may indicates the potential phenolic degradation by *C. freundii* A1 and *E. casseliflavus* C1.

Lastly, Fig. 9 shows the proposed pathway for AR-27 and catechol degradation that was constructed based on Chan *et al.*, (2012). According to previous studies, the decolourisation of AR-27 generates sulfonated aromatic amines, namely 1-aminonaphthalene-4-sulfonic acid and 1-aminonaphthalene-2-hydroxy-3,6-disulfonic acid (Zhee van deer *et al.*, 2005; Chan *et al.*, 2012; Hadibarata *et al.*, 2014). Initially, AR-27 undergoes a partial cleavage of the azo linkage (-N=N-) to produce hydrazo intermediates. This hydrazo intermediates were eventually transformed to aromatic amines after undergoes symmetric reductive cleavage reaction (Chan *et al.*, 2012; Hadibarata and Nor, 2014) in which was the first step of AR-27 degradation. Furthermore, the *C. freundii* A1 and *E. casseliflavus* C1 consortia used in the AR-27 decolourisation study was previously proven to be azo degrading microorganisms. This was supported by Lee (2003) who discovered the presences of azo reductase activity in the presence of NADH and riboflavin on the crude cell-free extract of *E. casseliflavus* C1. While Wahab (2007) proven that *C. freundii* A1has the ability to expressed azo reductase enzyme called flavin reductase for reduction of azo dye

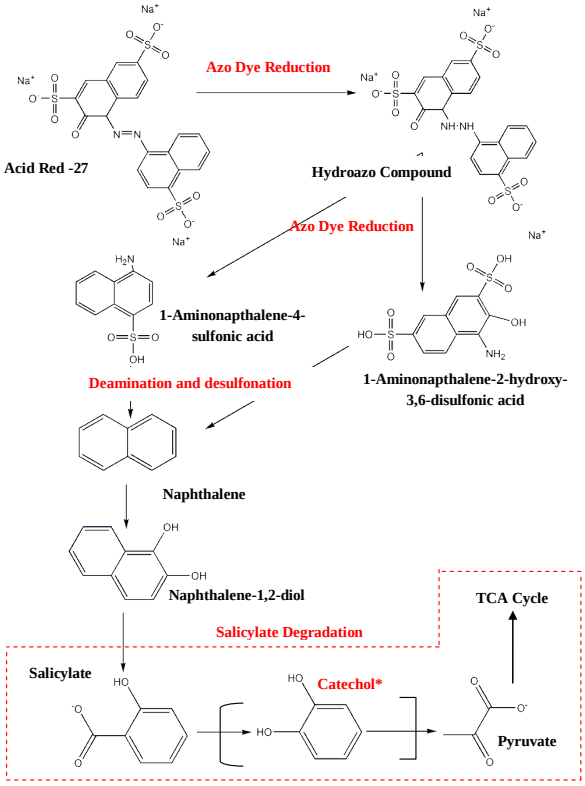

Fig. 9 Proposed AR-27 and Catechol* degradation pathway by *C. freundii* A1 and *E. casseliflavus* C1 bacteria consortium (Chan *et al.*, 2012)

The aromatic amine 1-aminonaphthalene-4-sulfonic acid and 1-aminonaphthalene-2-hydroxy-3,6-disulfonic acid may undergo rapid biodegradation to produce naphthalene *via* deamination and desulfonation reaction (Chan *et al.*, 2012). Under aerobic condition, naphthalene will undergo the naphthalene degradation pathway to generates naphthalene-1,2-diol before eventually converted to salicylate. Then, salicylate undergoes salicylates degradation pathway to generates catechol in which eventually mineralised to pyruvate. Pyruvate will entered the TCA cycles to generates ATP for the bacteria metabolisms. Hence, proving that *C. freundii* A1 and *E. casseliflavus* C1 consortia has the ability for AR-27 degradation under sequential aerobic-anaerobic treatment.

CONCLUSION

This study demonstrates the potential of the *C. freundii* A1 and *E. Casseliflavus* C1 bacterial consortia in Acid Red 27 (AR-27) dye decolourisation and removal. The feasibility of sequential facultative anaerobic-aerobic treatment for complete dye removal and degradation using AR-27 dyes resulted 98% decolourisation removal under static condition at 29 ± 2ºC Furthermore, after 72 hours aerobic treatment the bacteria consortia able to achieved 100% COD removal and 5% total polyphenol content removal. Plus, the cyclic voltammetry analysis demonstrates that the decolourisation of AR-27 by *C. freundii* A1 and *E. Casseliflavus* C1 was an irreversible reaction and the detection of oxidation reaction under agitation proved the presences of decolourised AR-27 mineralisation process. For the AR-27 degradation determination, it was confirmed by the decrease in

catechol concentrations through the High Performance Liquid Chromatography (HPLC) peaks detection.

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