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**Treatment of colour industry wastewater and concomitant biogenic electricity production using stacked mono-chamber type Microbial Fuel Cells**

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## Abstract

The scalability of any Microbial Fuel cell (MFC) based system is of vital importance if they are to be utilised for potential field applications. In this study, an MFC–aerobic integrated bioreactor system was investigated for its scalability for the purpose of treatment of simulated colour industry wastewater containing the commercial azo dye Acid Orange–7 (AO-7) and two other types of real colour industry wastewater. The colour industry wastewater originated from acid dyebaths for wool colouring and leather tanning. The influent containing real wastewater was fed to the reactor in continuous mode at ambient temperature. Three MFC units were integrated to act in unison as a single module for wastewater treatment and an aerobic bioreactor mimicking an activated sludge system operating downstream to the MFC module was installed in order to ensure more complete degradation of colouring agents found in the wastewater. Total colour removal in the final effluent exceeded 90% in all experiments where both synthetic (AO-7 containing) and real wastewater was used as the influent feed. The COD reduction also exceeded 80% in all experiments under the same conditions. The MFC modules connected in parallel configuration allowed obtaining higher current densities than that can be obtained from a single MFC unit. The maximum current density of the MFC stack reached  $1150\text{mA}\cdot\text{m}^{-2}$  when connected in parallel configuration. The outcome of this work implies that suitably up-scaled MFC–aerobic integrated bioprocesses could be used for colour industry wastewater treatment in industrially relevant conditions with possible prospects of bio-electricity generation.

**Key words:** Microbial Fuel cells, Azo dyes, Stacked MFCs, Bioremediation, Bioenergy

## 6.1. Introduction

MFCs used for the purpose of azo dye removal have been gaining interest in recent years. Many recent studies in this area have demonstrated the potential of these systems for environmental remediation and industrial waste material removal. Novel biological processes evaluated for xenobiotic removal from industrial wastewater must ultimately be capable of dealing with real industrial wastewaters operating in industrially relevant conditions. Furthermore, the reactor systems tested for such applications must ultimately be scalable systems from laboratory scale to pilot scale or fully fledged industrial scale reactors capable of effectively handling large industrial effluent volumes. Hence, it is imperative to assess the scalability of any laboratory scale MFC based bioreactors tested for the purpose of potential industrial waste remediation processes. Although the potential of MFC systems for the remediation of azo dye contaminated wastewater has been explored in an increasing number of studies in recent years, very few studies to-date have explored the potential of these systems to be scaled up. Hitherto, several studies have demonstrated that the MFC based systems to be promising platforms to effectively deal with simulated wastewaters contaminated with industrial azo dyes using small laboratory scale reactors [1, 2, 3, 4, 5, and 6]. All of these studies were conducted in MFCs operating under 500 mL limit of reactor working volume. However, only a very limited number of studies attempted to investigate the possibility of operating MFC reactors to be up-scaled in order to deal with azo dye containing real industrial wastewater. To-date, only a handful of studies was published that investigated any attempts to scale-up any bio-electrochemical system for treating azo dye contaminated wastewater [7, 8, and 9].

Furthermore, the industrial applicability of such MFC based processes for azo dye wastewater treatment largely depends on the ability of these systems to effectively deal with real industrial wastewater originating from the colour industry. The wastewater originating from the colour industry may possess many adverse traits such as toxic auxiliary compounds, extremes of pH, temperatures and salinities that may critically hinder the metabolism of exo-electrogenic microbes found in MFC anodes. Therefore, it is imperative that any suitable laboratory scale MFC based process designed for colour industry wastewater treatment undergoes thorough testing with real colour industry wastewater before undertaking any attempts to scale up the process. Many of the published work in this area only utilise model colour industry wastewater often containing single azo dyes and seldom contain many of the auxiliary compounds and salts routinely applied in the colour industry for process enhancement. Another major drawback of MFC based systems using model dye wastewaters is that almost all synthetic wastewaters used utilise a buffering system. This is highly disadvantageous in practical terms due to inhibitory reagent costs if buffer salts are to be introduced in large quantities into colour industry wastewater prior to treatment. Low ionic strengths and solution conductivities resulting from low salt contents of some of the real colour industry wastewater could be a problem as shown in many previous studies that MFC electrochemical performance and anode ionic strength are positively correlated [10, 11]. Only a few recent studies tested the feasibility of utilising MFC technology for real wastewater treatment [2, 8]. Hence, it is also important that real colour industry wastewater is utilised where possible in MFC based systems developed for potential industrial scale wastewater treatment applications. Although the use of buffer salts is highly disadvantageous for real wastewater treatment applications of MFCs, the use of colour industry wastewater

could be advantageous because colour industry wastewater is routinely found to be moderately or highly saline. Auxiliary salts such as NaCl and Na<sub>2</sub>SO<sub>4</sub> used in colour industry processes elevate the salinity of colour industry wastewater. Therefore, establishing microbial consortia that could tolerate such unbuffered but saline conditions in MFCs while effectively dealing with colour without much deterioration of MFC electrochemical performance would be highly beneficial as potential scale-up models.

Furthermore, it is ideal for a potential scale-up model for industrial wastewater treatment if the reactor system could operate in continuous mode. Batch systems are disadvantageous due to discontinuity and operational limitations such as the inability to deal with larger volumetric loading rates. The use of single chamber air – cathode type MFCs is best suited for continuous feeding of colour industry waste containing media. Unlike their aqueous cathode counterparts, they do not need active aeration of the cathode. Hence, in terms of operational energy expenditure, mono-chamber MFCs are more energy efficient compared to their two chamber aqueous cathode counterparts. Therefore, they are capable of registering higher power densities. Larger scale MFCs employed for potential industrial applications should also be capable of withstanding field conditions such as wide temperature fluctuations and operate in non-temperature controlled environments if they are to be more energy efficient.

In previous work, it has been demonstrated that the MFC electrochemical performance is not a linear function to its size. Most notably, a negative correlation was found between the anode surface area and the maximum power density obtainable from an MFC system [12]. Therefore, a modular scale up strategy where numerous smaller integrated MFC units functioning as a single unit would be more

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2  
3 advantageous compared to a linear MFC scale up strategy. Previously, Kim et al.,  
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5 2010 [13] demonstrated that tubular single chamber MFCs could be used for a  
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7 successful modular scale up to treat low-strength synthetic wastewater. However,  
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9 when electrically connecting several MFC units in series configuration, a serious  
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11 potential drop occurs [14]. A parallel MFC configuration of an MFC stack operating in  
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13 unison would be the most suitable strategy to circumvent this problem. In our  
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15 previous study, it was demonstrated that azo dyes can be successfully degraded into  
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17 non-toxic simpler compounds by using an integrated tubular MFC aerobic process  
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19 [15]. Hence, it would be advantageous to assess the scalability of the same or a  
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21 similar system that could effectively degrade azo dyes with concomitant bio-  
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23 electricity generation.  
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28 Therefore, the aim of this study was to attempt a modular scale – up of the  
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30 integrated two stage MFC-aerobic reactor system in order to remove colour and  
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32 remediate colour industry wastewater. Real industrial wastewater originating from  
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34 acid dyebaths for wool and leather was used in this study. This was because the use  
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36 of real wastewater rather than synthetic wastewater in any potential scale up model  
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38 is essential if the ultimate aim of the process development is field application. The  
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40 reactor operation in continuous flow mode in ambient laboratory temperature was  
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42 tested in order to investigate the operational capabilities and robustness of the  
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44 scaled up model in field conditions.  
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**Materials and Methods**

**2.4.1. Chemicals and real wastewater**

The azo dye AO-7 was procured from Sigma Aldrich (UK) and was used without further refinement. The mixed COD reagent Ficodox Plus <sup>TM</sup> was purchased from Fisher Scientific (UK).

The real industrial wastewater used in this study was obtained from a previous Europe-wide industrial study relating to colour industry wastewater treatment (Sophied project FP-6 <http://www.sophied.net/>). The industrial wastewater originated from two acid dyebaths in Europe used respectively for tanning of leather and colour fixation on wool fabrics. The exact dye content and other auxiliary components of the mixtures were unknown. Typical characteristics of the two industrial wastewaters are listed in Table – 1.

**Table - 1**

Conductivity and TDS measurements were taken using an Oakton PC-700 (Oakton Instruments, UK) conductivity probe. ORP measurements were made using a BASi Ag/AgCl reference electrode (BASi reference electrodes, USA). The Ag/AgCl reference electrode contained 3M NaCl electrolyte and was +196 V versus the standard hydrogen electrode (SHE) at 25°C.

**2.4.2. Modular scale-up of the integrated MFC-aerobic bioreactor system and operation**

Three MFC units were incorporated to produce a modular scale-up model for the initial MFC stage of the two-stage system. The system components and the



hydraulic flow of the up-scaled system are shown in Figure- 1. The MFCs were arranged in tandem but operated as a single unit in terms of hydraulic flow.

### Figure - 1

The working volume of a single MFC module was 400 mL and had a combined working volume of 1200 mL in the integrated reactor system. The MFC reactor construction was of polyvinyl chloride (PVC) tubes and had an internal diameter of 3.5 cm and a length of 60 cm. The anode and the cathode were made of carbon paper (PRF composite materials, Dorset, UK) and had surface areas of 192 cm<sup>2</sup> (projected) and 128 cm<sup>2</sup> (measured) respectively. The electrode spacing between the anode and the cathode was approximately 1.3 cm. The hollow concentric anode ran through the length of the PVC tube. Platinum powder was used as the oxygen reduction catalyst in the cathode and was coated on to the cathode membrane-facing side at 0.35 mgcm<sup>-2</sup>. The air facing side of the cathode contained a Polytetrafluoroethylene (PTFE) diffusion layer in order to minimise water loss through the cation exchange membrane. The Pt catalyst layer and the PTFE diffusion layer was applied as described earlier in section 2.3.2. Electrical connections on the electrodes were secured by soldering copper wire onto the electrodes and the exposed junctions were sealed with non-conductive epoxy. A cation exchange membrane (CMI-7000 – Membranes International, USA) separated the anode and the cathode in all three MFC modules. The spacing between the anode and the cathode was approximately 1.5 cm in each MFC module. Three MFC modules were connected in parallel to a 500 Ohm external resistor and the voltage across the resistance was monitored using a Picolog ADC-24 (Pico Technology, UK) data logging system at a data recording interval of 10 minutes. The external

electrical circuit in the system is indicated in Figure- 2. Real or model dye wastewater was continuously fed with an up-flow configuration to the MFC modules using a peristaltic pump. The effluent from the MFC stage was collectively fed into the second aerobic stage of the integrated bioreactor system (Figure - 1). The aerobic bioreactor stage was actively aerated continuously through an airstone sparger at an air flow rate of 400 mL air per minute. The aerobic reactor was continuously agitated using a magnetic mixer. The working volume of the aerobic stage was 2L. The Hydraulic Residence Time (HRTs) of the MFC stage and the subsequent aerobic stage respectively were 13.3 hours and 22.2 hours.

**Figure - 2**

**2.4.3. Inoculum source and growth medium**

For the initial study, model wastewater containing AO-7 ( $35 \text{ mgL}^{-1}$ ) was prepared as indicated in an earlier study [16]. For the latter part of the study involving real industrial wastewater, the colour industry effluent was modified by supplementing it with  $2 \text{ gL}^{-1}$  of molasses to act as a co-substrate during colour removal. The industrial wastewater influents from wool colouring and leather tanning indicated COD values of  $3950 \pm 40 \text{ mgL}^{-1}$  and  $4250 \pm 30 \text{ mgL}^{-1}$  following the supplementation of  $2 \text{ gL}^{-1}$  of molasses co-substrate. The MFC stage was inoculated with an azo dye adapted mixed microbial consortium from a continuously fed tubular MFC system treating AO-7 as described in an earlier study [16].

**2.4.4. Experimental design**

Experiments were designed and carried out with the aim of investigating the effect of scale-up of the integrated MFC-aerobic two-stage bioreactor system for azo dye

removal and to investigate the reactor performance when used for the treatment of model and real industrial wastewaters. A modular scale-up was used in which the MFC stage comprised of three 400 mL MFC units acted in unison to act as the initial stage of the two-stage system.

During the initial experiment where model wastewater containing AO-7 was used, the AO-7 loading rate was maintained at  $126 \text{ gm}^{-3}\text{day}^{-1}$  (COD loading of  $5.76 \text{ kg CODm}^{-3}\text{day}^{-1}$ ). In the experiment where real colour industry wastewater was used, COD loadings of  $7.11 \text{ kgCODm}^{-3}\text{day}^{-1}$  (dye wastewater from wool colouring) and  $7.65 \text{ kgCODm}^{-3}\text{day}^{-1}$  (dye wastewater from leather tanning) respectively were maintained. Colour and COD removal performance of the reactor system during the experimental runs were monitored.

## Analytical methods

### 2.4.5. Assessment of colour removal performance

Colour removal when using AO-7 as the model azo dye in the wastewater feed was carried out spectrophotometrically at the maximum absorbance ( $\lambda_{\text{max}}$ ) of AO-7 (484 nm) using a UV-Visible Spectrophotometer (Perkin-Elmer Lambda 35, USA). The AO-7 removal efficiency (RE) was expressed as a percentage, calculated according to the expression-1.

$$RE(\%) = \frac{C_{0,AO7} - C_{t,AO7}}{C_{0,AO7}} \times 100 \quad \text{----- 1}$$

Where,  $C_{0,AO7}$  and  $C_{t,AO7}$  are AO7 concentrations (mM) at the start and at each time point respectively.

In order to assess the colour removal in more complex real industrial wastewater, UV – visible scans of real wastewater and the decolourised samples were used (Perkin

– Elmer Lambda-35 UV-Visible spectrophotometer). The decolourisation efficiencies (DE) were established by calculating the peak area reduction within the wavelength region 400nm – 650nm of the UV-vis scans of influent and effluent wastewater samples, as described in expression - 2.

$$DE (\%) = \frac{A_0 - A_t}{A_0} \times 100 \text{ ----- 2}$$

Where,  $A_0$  and  $A_t$  are absorbance peak area values between the wavelength range 400 nm - 650 nm of UV-Visible spectra scans in the starting solution and at each time point respectively.

**2.4.6. Assessment of COD removal performance**

COD analysis of all samples was carried out in accordance with the closed reflux titrimetric method as described by the UK Environment Agency, APHA method 5220D, as described in an earlier study [15].

**2.4.7. HPLC analysis of the metabolites formed during decolourisation in the two-stage scaled-up process**

HPLC gradient elution of the metabolites contained in the samples was carried out in order to investigate the chemical changes that took place during the two-stage decolourisation process. The separation of metabolites using HPLC gradient elution was carried as described previously [16]. The Dionex GS50 HPLC system was equipped with a Phenomenex Gemini® reversed phase C18 column (5µm, 150 X 4.6mm). The detection of the metabolites were done at 248 nm using a photodiode array detector (Dionex PDA-100). The mobile phases were Acetonitrile (mobile phase-A) and HPLC grade water (mobile phase-B). Subsequent to a 1minute equilibration before injection, a linear gradient of 10% - 90% of mobile phase-A

(Acetonitrile) was used over 29 minutes for the elution of metabolites. The sample injection volume was 20 $\mu$ L and a flow rate of 1 mLmin<sup>-1</sup> was used.

#### 2.4.8. Electrochemical monitoring of the scaled-up MFC system during dye wastewater treatment.

The external circuits of the three integrated MFC modules were connected in parallel configuration to a 500 Ohm load as shown in figure- 2. The ADC-24 (Pico-Technology, UK) data logging system was connected in parallel across the 500 Ohm resistance. Polarisation curves of the collective three-module system were recorded by varying the external resistance from 1  $\Omega$  to 1 M $\Omega$  and recording the registered voltage across each external load. Current flowing across each resistance was calculated using the Ohm's law, as shown in expression – 3. The power produced was calculated as earlier described according to expression -4. Current and power values were normalised to the anode surface area (192 cm<sup>2</sup>) and were used to construct power-current plots.

$$I = E/R \text{ ----- 3}$$

Where, I is the current flowing through the load (mA), E is the potential across the resistor (mV) and R is the external resistance ( $\Omega$ )

$$P = EI \text{ ----- 4}$$

Where, P is Power produced (mW), E is the potential (mV) and I is the current (mA)

## 6.2. Results and discussion

### 6.2.1. Decolourisation and COD removal in AO-7 containing model wastewater in the scaled up MFC-aerobic reactor system

In a previous study, it was demonstrated that MFC- aerobic integrated reactor system was capable of fully degrading AO-7 containing simulated colour industry wastewater into non-toxic simpler metabolites [16]. The modular scale – up of the same two – stage system was tested initially in continuous mode in this study using the same synthetic medium containing AO-7 (35 mgL<sup>-1</sup>).

Figure - 3

The results indicate that the colour and COD removal reached above 90% and therefore, the performance of the scaled-up two-stage MFC-aerobic reactor system was comparable to the similar but smaller reactor system (200 mL working volume) utilised in a previous study [15] (Figure 3A and 3B). This suggests that the integrated MFC-aerobic two-stage reactor system is capable of operating without any deterioration of its colour and COD removal performances when it is scaled-up by a volume scale factor of 6 compared to a previous study [16] (1200 mL total working volume in the three integrated MFC modules) and operating on the same synthetic wastewater medium containing AO-7. Previously it was demonstrated in the same study that an MFC – aerobic two stage reactor set – up was the ideal configuration for more complete degradation and toxicity removal of azo dyes such as AO-7. Therefore, the same two – stage MFC aerobic reactor set up was used in the up-scaled model reactor.

### 6.2.2. Decolourisation of real colour industry wastewater in the scaled up system

For any potential wastewater treatment system tested for field application, it is essential to possess the capability to effectively deal with real wastewater types. This is especially relevant to biological wastewater treatment systems developed for the removal of xenobiotic compounds. Although the use of model wastewater often containing a single dye is ubiquitous in many studies done in this area, the use of real colour industry wastewater is relatively rare. It is not always possible and adequate to extrapolate the information obtained from studies utilising model wastewater into a scenario where real colour industry wastewater is encountered. Therefore, it is essential to conduct experiments utilising real colour industry wastewater when attempting to scale up prospective wastewater treatment systems. Therefore, in this study, two types of real colour industry wastewater were tested; namely, acid dyebath wastewater from wool colouring and acid dyebath wastewater from leather tanning.

When using colour industry wastewater from wool colouring, the effluent from the MFC stage and the aerobic stage indicated sequential colour reduction during the (Figure – 4). Subsequently however, the cumulative colour removal from both MFC and aerobic stages reached > 90% decolourisation (Figure 5A).

#### Figure - 4

Furthermore, COD reduction indicated a stepwise reduction compared to the influent COD, indicating a COD level below  $500 \text{ mgL}^{-1}$  for the final effluent (Figure 5B).

**Figure - 5**

Therefore, similar to the findings of a previous study that used an integrated MFC – aerobic process [16], it can be expected that due to the stepwise and sequential COD reduction, some of the decolourisation metabolites formed in the anaerobic MFC stage are further oxidised into simpler compounds during the aerobic stage.

**Figure - 6**

Similar to the previous observations with real colour industry wastewater from wool colouring, the intense colour of the influent was almost completely removed from wastewater originating from leather tanning when it was used as the influent feed for the scaled-up integrated MFC – aerobic reactor system (figure – 6). This clearly indicates that the scaled-up integrated MFC module coupled to the aerobic reactor system is capable of effectively removing colour from both types of colour industry wastewater tested in this study. The final colour removal efficiency of the combined MFC-aerobic stages of the reactor reached over 90% (Figure 7A) when leather tanning wastewater was used as the influent. This clearly demonstrates that the scaled-up integrated MFC – aerobic system is a versatile bioreactor system that can effectively deal with different types of real colour industry wastewater.

**Figure - 7**

Residual COD levels of the samples also indicated a stepwise reduction at the end of the MFC stage and the aerobic stage compared to the influent COD when leather tanning wastewater was used as the influent. This suggests that decolourisation metabolites were further oxidised into simpler compounds at the aerobic reactor



stage of the integrated system. The unmodified (without the addition of the co-substrate molasses) COD values for the two types of wastewaters were  $1000 \pm 60$   $\text{mgL}^{-1}$  and  $1280 \pm 40$   $\text{mgL}^{-1}$  respectively for wool colouring wastewater and leather tanning wastewater. The residual COD values of the final effluent discharged from the aerobic stage when both types of wastewater were used in the scaled – up reactor system were below  $500$   $\text{mgL}^{-1}$  (figures 5B and 7B). This clearly suggests that organic components contained in the two types of wastewater (including colouring agents) were oxidised in a stepwise manner during the process into simpler and colourless compounds. When leather tanning wastewater was used as the influent, colour removal at the end of the MFC stage remained at 80% or below, but the overall colour removal of the combined MFC + aerobic process was over 90%. This is in contrast to other work done using this two stage system. When azo dyes (i.e. AO-7) were used as model compounds in synthetic wastewater, most of the colour removal (> 90%) occurred at the anaerobic MFC stage rather than at the aerobic stage because most azo moieties ( $-\text{N}=\text{N}-$ ) undergo reductive degradation in the anaerobic MFC stage. However, contrary to the observations relating to azo dyes such as AO-7, when leather tanning wastewater was used as the influent, the aerobic stage accounted for more than 10% of the total decolourisation (Figure – 7A). This suggests that leather tanning wastewater used here may have contained other types of dyes belonging to dye classes such as anthraquinone or triphenylmethane dyes which could be amenable to degradation in the aerobic reactor stage.

**6.2.3. Electrochemical performance of the parallel connected MFC modules during real and simulated wastewater treatment**

Connecting stacked MFCs in series configuration could lead to undesirable energy losses due to effects such as voltage reversal and potential drop across each MFC unit. Voltage reversal is thought to occur due to sudden fuel starvation in the anode of one or more MFCs in an MFC stack and as a result, a sudden loss of bacterial catalytic activity transferring electrons to the anode [17, 18 and 15]. However, voltage reversal is mainly encountered during batch operation of MFC stacks and hence, operating MFCs in continuous mode and by ensuring good mixing, the undesirable energy losses of voltage reversal could be circumvented. The voltage drop across each MFC during a series connection of an MFC stack mainly occurs due to the internal resistance of MFCs [19]. MFC systems when used as a single unit, suffer from the intrinsic theoretical maximum voltage output upper limit of about 1.2 Volts. However, due to various internal energy losses as discussed by [20], the actual voltage obtained under field conditions is lower than 1.2 V. Therefore, when scaling up MFC systems, it is essential to connect multiple MFC units in either series or parallel configurations in order to obtain useful and high enough voltage or current outputs. Parallel connection of multiple MFCs is useful in terms of obtaining high current densities.

The current production of an MFC module during AO-7 containing simulated wastewater is depicted in figure

– 8A. Current densities across a  $R_{\text{ext}}$  of  $500 \Omega$  reached a maximum of approximately  $200 \text{ mA m}^{-2}$  and stayed relatively stable throughout continuous reactor operation.

### Figure - 8

Polarisation curves and power current plots of individual scaled-up MFC units indicated average maximum current and power densities of  $550 \text{ mA m}^{-2}$  and  $49 \text{ mW m}^{-2}$  respectively during colour industry wastewater treatment (Figure – 8B). The average open circuit potential (OCP) of an individual MFC module of the integrated MFC stage reached  $297 \pm 31 \text{ mV}$  during the same operational period.

When the MFC modules were connected in parallel configuration, maximum current densities as high as  $1150 \text{ mA m}^{-2}$  could be obtained while the maximum power density obtainable from the parallel connected MFC modules remained a modest  $54 \text{ mW m}^{-2}$  (Figure – 8C). However, the OCP remained at  $275 \text{ mV}$ . This indicates that the parallel connected MFC units allows drawing a larger maximum current while the maximum power density obtainable from such parallel connected MFC units is not notably different to the maximum power that can be drawn compared to a single MFC unit. The OCP of the parallel connected MFCs ( $275 \text{ mV}$ ) remained close to that of a single MFC unit ( $297 \text{ mV}$ ), indicating that no significant enhancement of voltage output is possible in parallel MFC configuration. These findings are in agreement with an earlier study conducted by Zhuang et al., 2012 [19] in which they used parallel and series connected MFC stacks and found that current outputs can be enhanced when the MFC units are stacked in parallel configuration with no significant enhancement to the voltage output or power density.

**6.2.4. Degradation of colouring agents contained within colour industry wastewater in the MFC – aerobic two stage process**

Effective colour removal from both types of colour industry wastewater was achieved using the scaled – up MFC-aerobic bioreactor system as shown in figures 6.3 and 6.5. However, it is necessary to assess the degradation of various colouring components contained within colour industry wastewater during the two stage treatment process. Comparison of HPLC elution profiles of the influents and the effluents at various stages of the two stage process was carried out for this purpose.

**Figure - 9**

HPLC analysis of the samples obtained from the two-stage operation reactor when leather tanning wastewater was used as the reactor feed indicates that numerous metabolites were produced at the end of the aerobic stage of the treatment process (Figure – 11). The HPLC spectrum of the aerobic stage effluent (final effluent) was markedly different from the HPLC spectra of the blank medium, real wastewater influent feed and the MFC stage effluent.

HPLC chromatograms of the samples obtained from the integrated bioreactor system operating on wool colouring wastewater being used as the influent feed also indicated that many metabolites were produces at the end of the decolourisation process in the final effluent (aerobic stage effluent) (Figure 12). These chromatograms indicate that during the decolourisation process of both types of wastewater, a clear biotransformation of the colouring agents contained in the wastewater takes place. Furthermore, the number of metabolites produced at the

end of the aerobic stage is greater than the number of metabolites present at the end of anaerobic MFC stage in both instances where two different types of colour industry wastewater was used (Figures 11 and 12). This clearly indicates that decolourisation metabolites produced as a result of biotransformation of colouring agents at the MFC stage undergoes further catabolism into simpler compounds in the aerobic stage.

### 6.3. Conclusions

The findings of this study indicate that the MFC – aerobic integrated bioreactor system could be successfully scaled up by combined operation of numerous MFC modules in order to handle larger colour industry wastewater volumes. The system was capable of effectively handling wastewater originating from acid dyebaths for wool colouring and leather tanning in continuous flow mode at ambient temperature. The parallel configured MFC connection of numerous MFC modules allowed the current that can be drawn from the system to be enhanced. The findings of this study implies that suitably scaled – up MFC stacks operating in continuous flow mode could potentially be incorporated with aerobic processes such as activated sludge systems in order to treat complex colour industry wastewater. This brings about possibilities of effective colour industry wastewater treatment, good colour removal, and potential bio-energy recovery with the use of MFCs during wastewater treatment.

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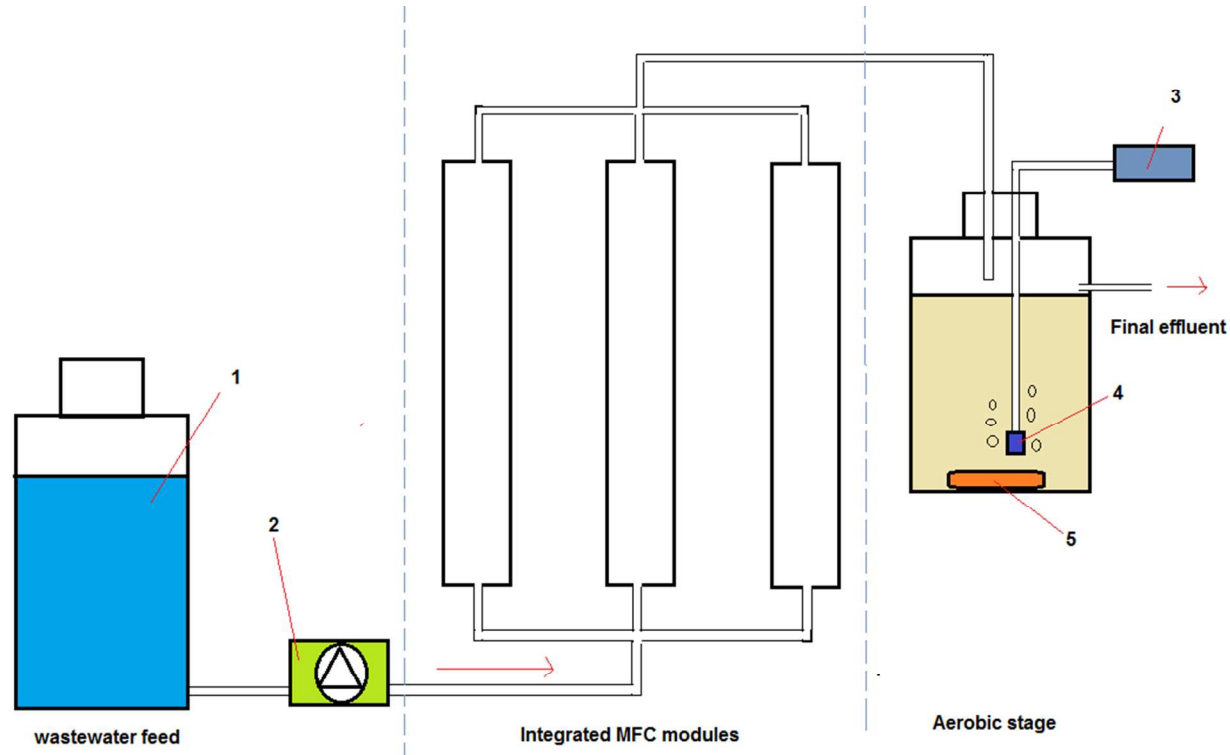
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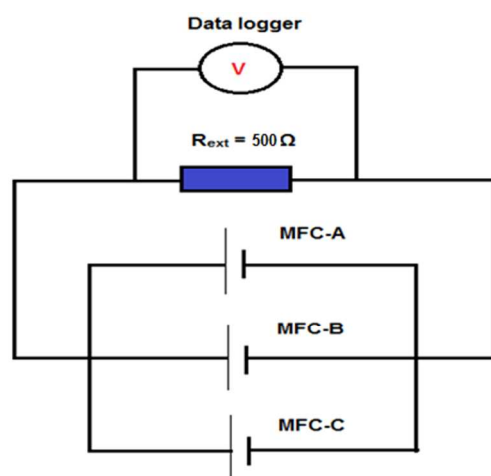
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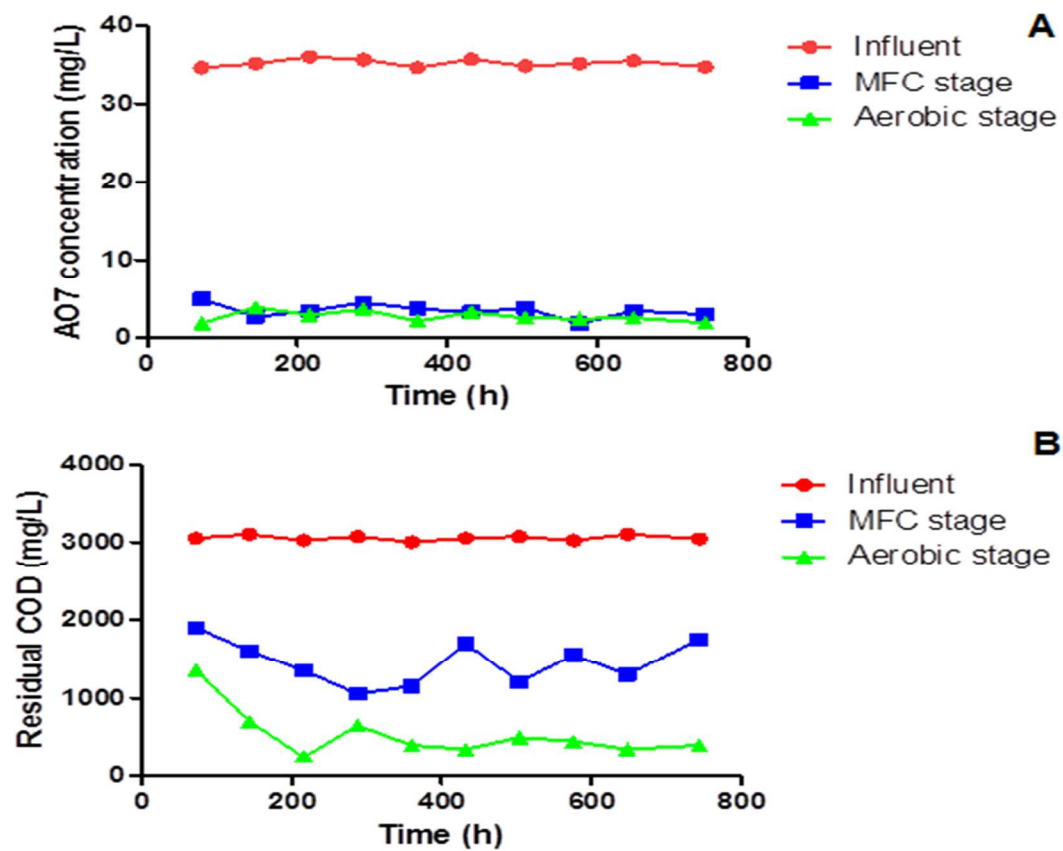


**Figure- 1:** Components and the hydraulic flow of the up-scaled two-stage integrated bioreactor system featuring integrated MFC modules. (1) Wastewater feed (2) peristaltic pump (3) air pump (4) air stone sparger (5) magnetic mixer

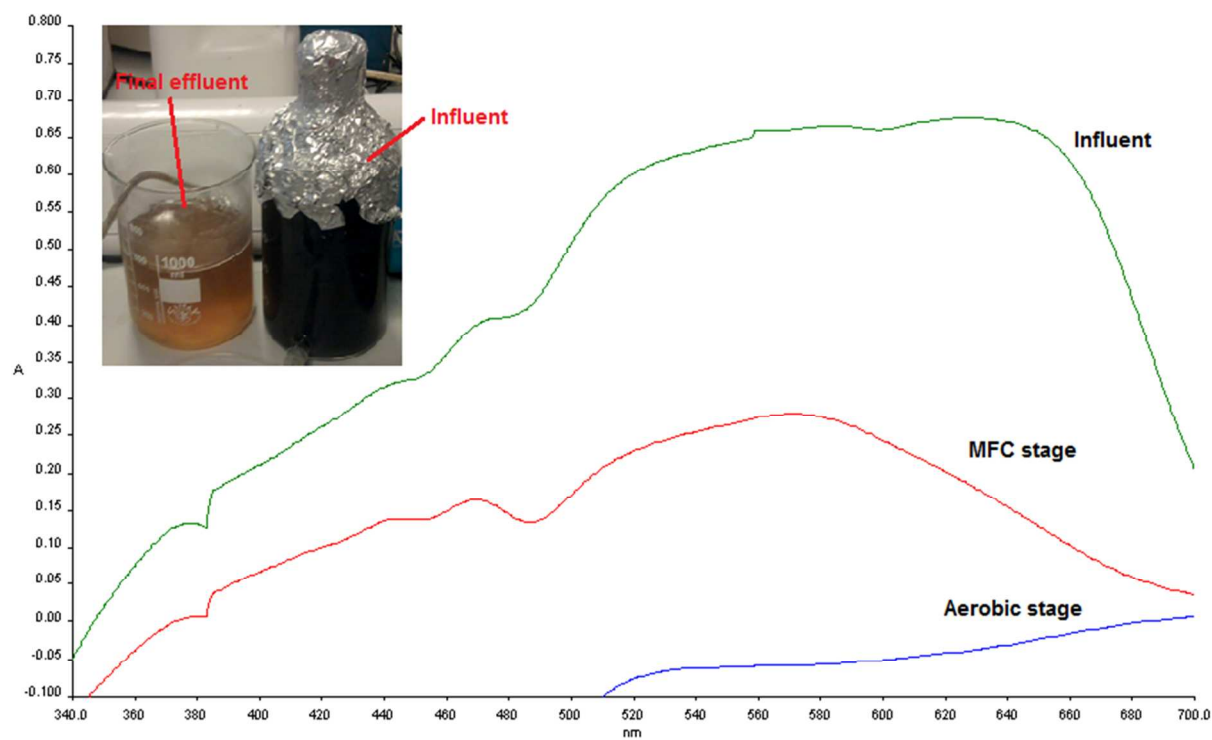




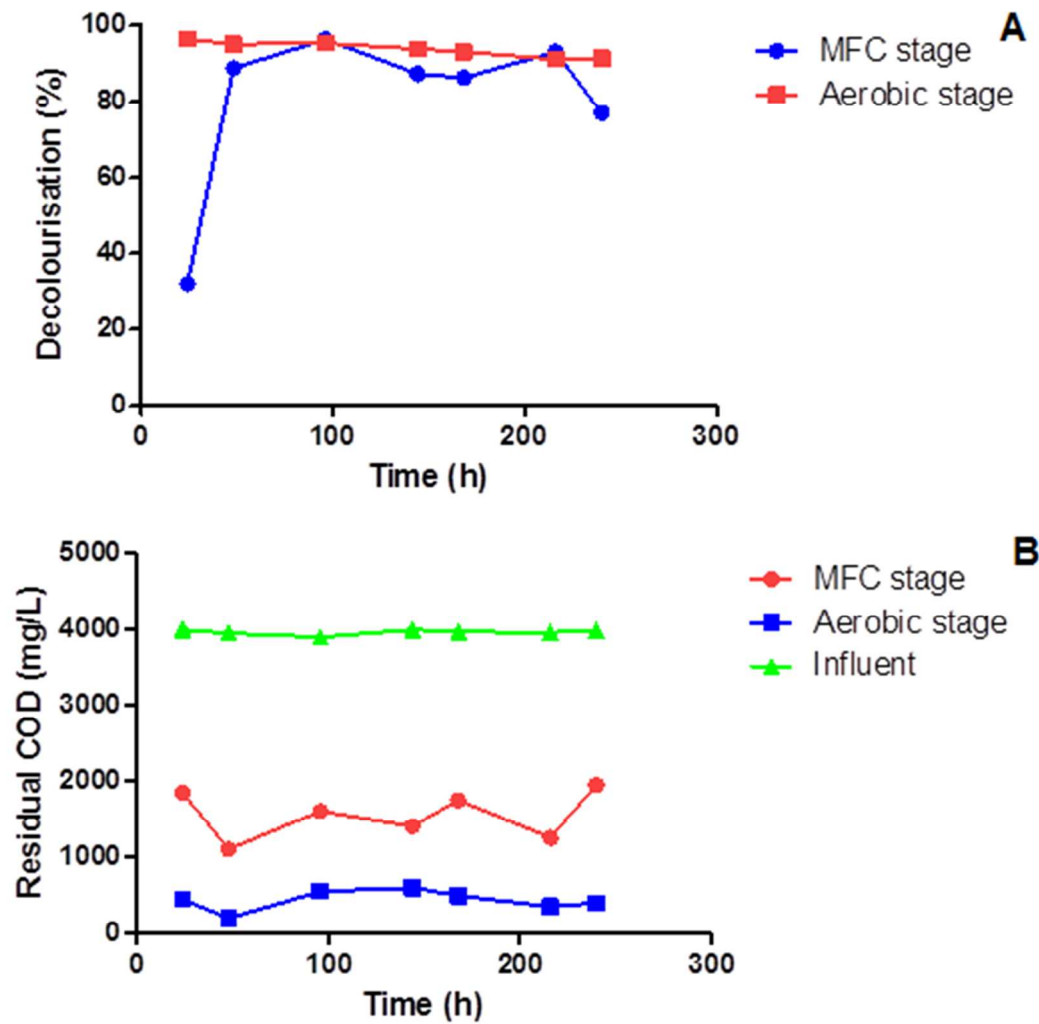
**Figure-2:** The parallel configured external electrical circuit of the three combined MFC modules used in this study.



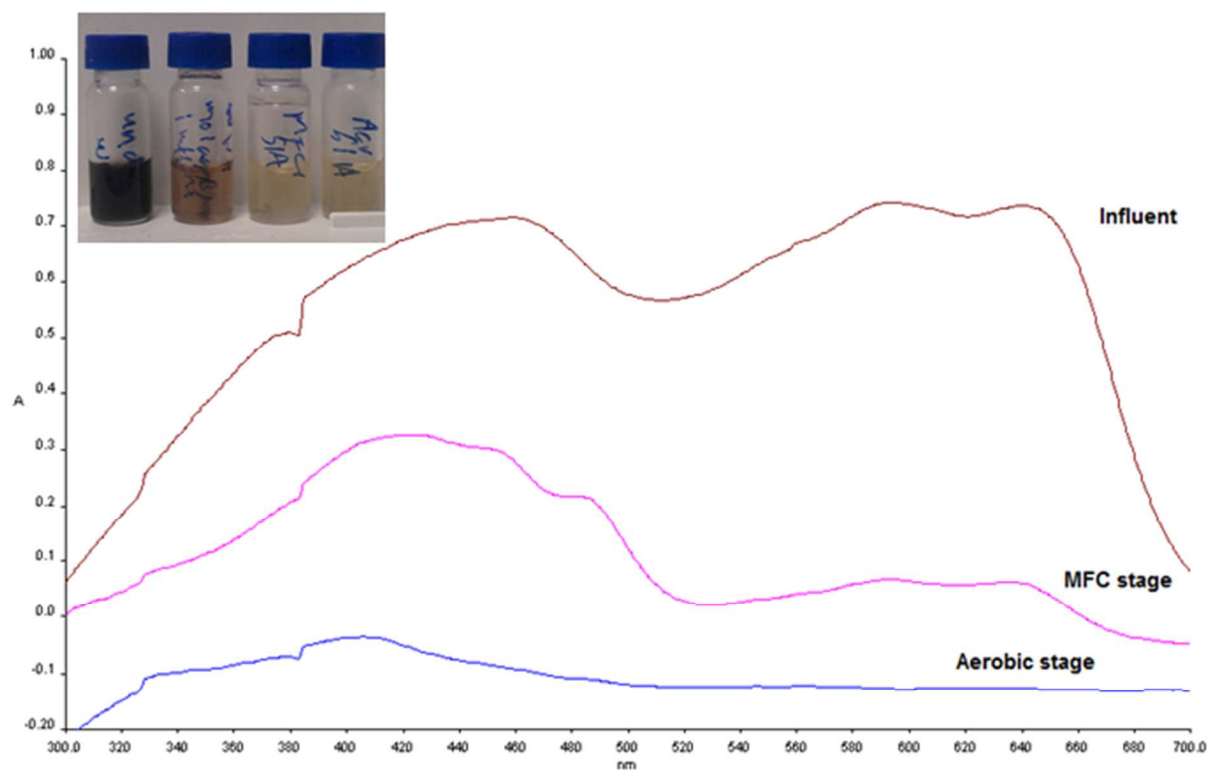
**Figure - 3:** (A) Colour removal of the model wastewater containing AO-7 ( $35 \text{ mgL}^{-1}$ ) fed into the scaled-up MFC system in the MFC and aerobic stages and (B) residual COD of the samples taken in the MFC and aerobic stages



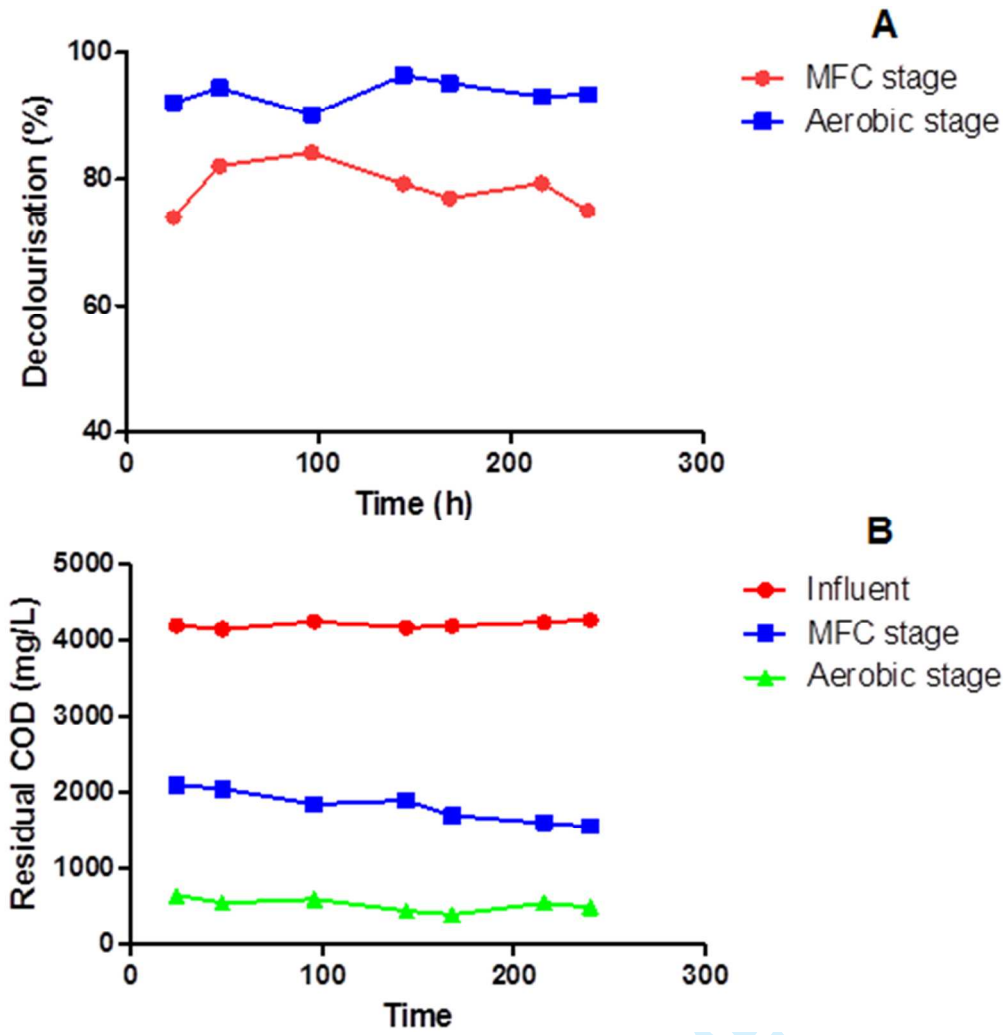
**Figure – 4:** Decolourisation of colour industry wastewater from an acid dyebath for wool in the scaled-up MFC system at the MFC stage and the aerobic stage. Inset – clear visible colour difference between the influent dye wastewater and the final effluent.



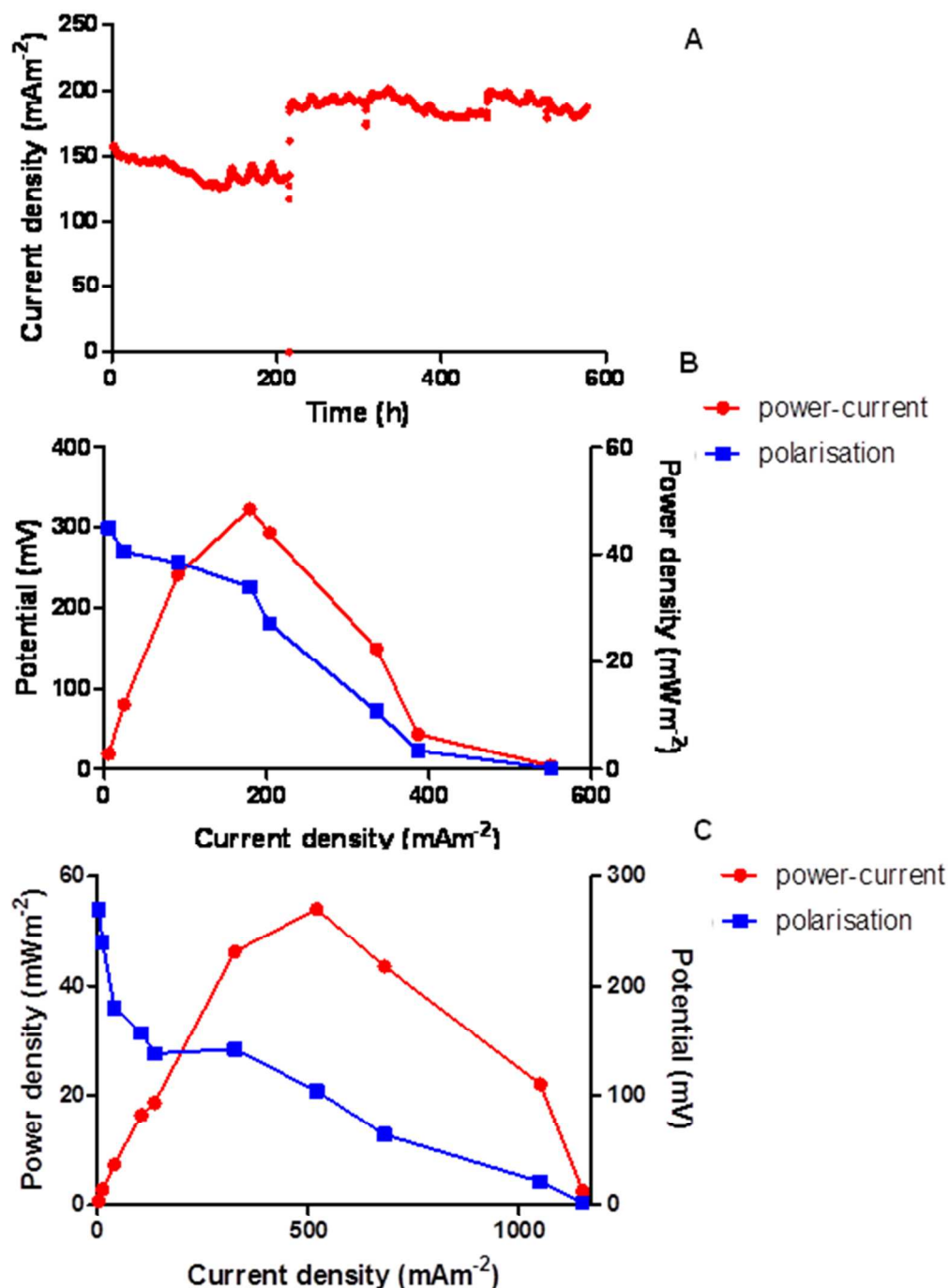
**Figure – 5:** (A) Colour removal performance of scaled up MFC reactor system treating wastewater originating from an acid dye bath for wool at the MFC stage and aerobic stage (B) residual COD of the samples obtained from the MFC stage



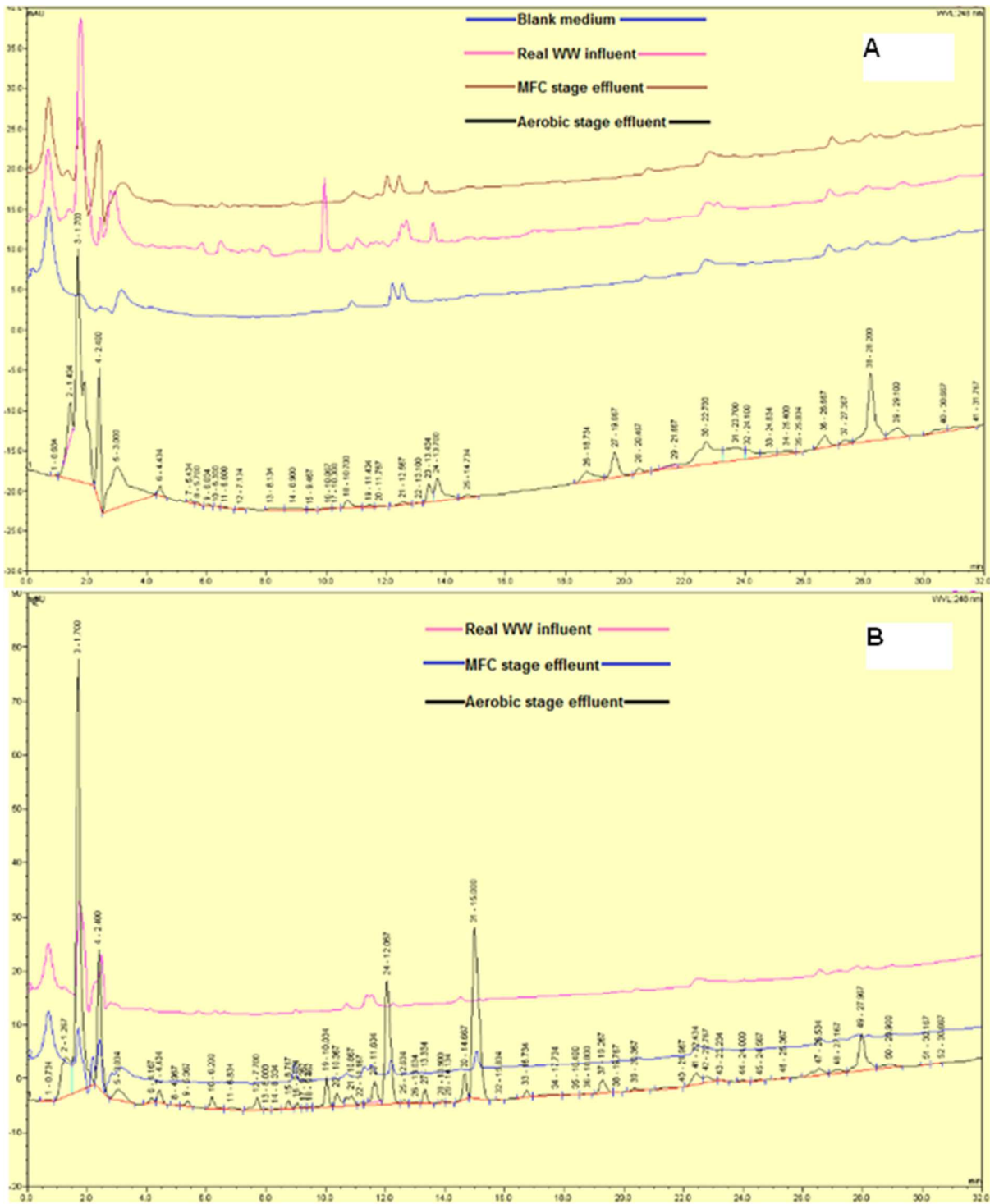
**Figure – 6:** Decolourisation of colour industry wastewater from leather tanning in the scaled – up MFC – aerobic bioreactor during the MFC stage and aerobic stage. Inset – comparison of vials from left to right containing leather tanning wastewater influent, MFC stage effluent, aerobic stage effluent and model wastewater containing only molasses



**Figure – 7:** (A) Decolourisation performance of the scaled up MFC-aerobic reactor system when using colour industry wastewater originating from leather tanning during the MFC stage and aerobic stage (B) COD removal performance during MFC stage and aerobic stage.



**Figure – 8:** (A) Current production in scaled – up MFC modules during AO-7 containing simulated wastewater treatment ( $R_{\text{ext}} = 500 \, \Omega$ ) (B) The average individual electrochemical performance of the three parallel connected MFC modules during real dye wastewater treatment (C) The power – current plot and polarisation curve of the parallel connected combined MFC module while treating colour industry wastewater from an acid dyebath for wool



**Figure – 9:** (A) overlay of HPLC chromatograms of colour industry wastewater from leather tanning. Compared chromatograms indicate the blank media (i.e. molasses without colour industry wastewater), leather tanning wastewater supplemented with molasses (influent), the effluent from MFC stage and the effluent from the aerobic stage of the integrated scaled – up bioreactor system (B) Overlay of HPLC chromatograms of real colour industry wastewater from wool colouring. Compared chromatograms indicate



real colour industry wastewater from wool colouring, MFC stage effluent and aerobic stage effluent of the integrated scaled- up bioreactor system

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**Table- 1:** Typical characteristics of the two colour industry wastewaters used in this study at unmodified state.

Parameter	Acid dyebath wastewater for wool	Acid dyebath wastewater for leather
pH	7.75	7.2
Total dissolved solids (TDS)	161 mgL <sup>-1</sup>	146 mgL <sup>-1</sup>
Conductivity	3.15 ± 1.2 mScm <sup>-1</sup>	289 ± 33 µScm <sup>-1</sup>
Oxidation/reduction potential (ORP) vs Ag/AgCl	(-)53 mV	(-)18.9 mV
COD	1000 ± 60 mgL <sup>-1</sup>	1280 ± 40 mgL <sup>-1</sup>