CHEMICAL ENGINEERING TRANSACTIONS
VOL. 94, 2022

A publication of
AIDIC
The Italian Association
of Chemical Engineering
Online at www.cetjournal.it

Guest Editors: Petar S. Varbanov, Yee Van Fan, Jiří J. Klemeš, Sandro Nižetić

ISBN 978-88-95608-93-8; **ISSN** 2283-9216

DOI: 10.3303/CET2294235

Effect of Varying Current on *Pseudomonas Aeruginosa* Degradation of Naphthalene

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Polycyclic aromatic hydrocarbons are recognized as pollutants with carcinogenic, teratogenic, and mutagenic properties. Polycyclic aromatic hydrocarbons have therefore become priority pollutants that have attracted researchers' attention to developing remediation strategies. Numerous approaches, including physical, chemical, and biological strategies have been employed. But most of the techniques are associated with complexity issues, high cost and lack of scientific acceptance. Bioremediation making use of bacteria and fungi has also been researched extensively because of its cost effectiveness and environmental compatibility. But bioremediation of polycyclic aromatic hydrocarbons is associated with inefficiencies related to slow processes because of the recalcitrant, hydrophobic, and lipophilic properties of petroleum hydrocarbons. In this research, the possibility of enhancing the removal of naphthalene from contaminated water by the application of current was studied. The current of 1 A and 2 A was applied in an electrochemical cell inoculated with Pseudomonas aeruginosa. Total carbon removal was 87.33 %, 90.14 % and 80 % for 1 A, 2 A and when only biodegradation (0 A) was used respectively. The results showed that the application of current can enhance the degradation of naphthalene if the electrolytic current is not too high to affect cell membranes of the bacteria.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants that end up in the environment through oil spills, incomplete combustion of fossil fuels and industrial effluents (Nguyen et al., 2020). PAHs are mainly produced by anthropogenic activities and are released to the environment where they adsorb to suspended solids in surface waters, soil or the atmosphere (Huang et al., 2020). PAHs including naphthalene, biphenyl, fluorene, anthracene, phenanthrene among others are classified as hazardous due to their carcinogenic, teratogenic, genotoxic, and persistent nature (Zhang et al., 2019). Exposure of PAHs to humans may cause various cancers and several other diseases such as oral cancer, stomach cancer, asthma, and heart diseases (Zhang et al., 2019). Furthermore, PAHs are also regarded as endocrine disrupting chemicals (EDCs) meaning they suppress the immune system (Zhang et al., 2016).

Due to the toxic properties of PAHs, efforts are always made to remove PAHs from water before human consumption (Lawal, 2017). Electrochemical technologies such as electrochemical oxidation have shown great potential for treatment of polluted water in recent years (Rubio-Clemente et al., 2014). Electrochemical oxidation unlike other technologies is preferred by scientists because of its environmental compatibility, versatility, energy efficiency, and cost-effectiveness (Wang and Xu, 2012). Oxidation of the pollutants in an electrochemical system occurs at the anode leading to the formation of radicles while at the cathode hydrogen is mainly produced so may not be involved in pollutant oxidation (Wang and Xu, 2012). The treatment of water is therefore mainly dependent on the anode material to obtain anodic oxidation (McQuillan et al., 2020). Active anodes (such as carbon, graphite, IrO2, Pt, and RuO2) or non-active anodes (such as SnO2, PbO2, and boron-doped diamond) are used to drive anodic oxidation (Rubio-Clemente et al., 2014). Irrespective of the anode type denoted as MOx the first reaction is the oxidation of water that leads to the formation of °OH as described by Rivera et al. (2011) in Eq(1). The °OH formed then binds strongly or weakly to the anode electrode (Zhang et al., 2013). In the active anodes the °OH strongly bounds to the electrode forming metal oxides (MOx + 1) as seen in Eq(2) (Rubio-Clemente et al., 2014). In the presence of PAHs in the medium, the organic pollutants are directly

Paper Received: 17 November 2021; Revised: 24 March 2022; Accepted: 10 May 2022
Please cite this article as: Gidudu B., Calitz D., Chirwa E.M.N., 2022, Effect of Varying Current on Pseudomonas Aeruginosa Degradation of Naphthalene, Chemical Engineering Transactions, 94, 1411-1416 DOI:10.3303/CET2294235

oxidised to the anode (Eq(3) and Eq(4)) or the pollutants are indirectly fully oxidised to CO2 with the aid of electrochemical oxidants such as °OH (Eq(1)), H2O2 (Eq(5)) and O3 (Eq(6)) (Rubio-Clemente et al., 2014).

$$MO_x + H_2O \longrightarrow MO_x(^oOH) + H^+ + e^- \tag{1}$$

$$MO_x(^oOH) \longrightarrow MO_{x+1} + H^+ + e^- \tag{2}$$

$$R + MO_{x+1} \longrightarrow MO_x + RO \tag{3}$$

$$R + MO_x(^oOH) \longrightarrow MO_x + CO_2 + H^+ + e^- \tag{4}$$

$$2MO_x(^oOH) \longrightarrow 2MO_x + H_2O_2 \tag{5}$$

$$3H_2O \longrightarrow O_3 + 6H^+ + 6e^- \tag{6}$$

One of the drawbacks associated with electrochemical oxidation is the relatively high operational costs especially if total mineralisation of the pollutant is to be achieved (Torres et al., 2003). Still, total mineralisation of organic pollutants is usually achieved using non-active electrodes, but these have been reported to corrode leading to the release of toxic ions into the water (Rubio-Clemente et al., 2014). The use of advanced oxidation processes such as electrochemical oxidation to enhance the biodegradation of recalcitrant organic pollutants like PAHs can reduce the treatment operational costs and ensure total mineralisation of the organic pollutants (Torres et al., 2003).

In this study, we investigated the possibility of combining electrochemical oxidation with biodegradation for the removal of naphthalene from water. This involved the use of a batch electrochemical reactor inoculated with a hydrocarbon degrading strain Pseudomonas aeruginosa. The effect of pH, temperature and applied current on naphthalene removal were evaluated.

2. Materials and methods

2.1 Microbial growth medium and growth conditions

The mineral salt medium (MSM) was prepared as reported by Trummler et al. (2003) by dissolving in 1 L of distilled water: 6.0 g (NH4)2SO4; 0.4 g MgSO4×7H2O; 0.4 g CaCl2×2H2O; 7.59 g Na2HPO4×2H2O; 4.43 g KH2PO4; and 2 mL of trace element solution. Plate count agar, nutrient agar and nutrient broth were prepared by dissolving the amounts indicated on the bottle in distilled water followed by autoclaving at 121 oC in order to sterilize for 15 min. The agar was poured on to the agar plates between 40-50 oC. The trace elements solution consisted of 20.1 g L−1 EDTA (Disodium salt), 16 g L−1 FeCl3×6H2O, 0.18 g L−1 CoCl2×6H2O, 0.18 g L−1 ZnSO4×7H2O, 0.16 g L−1 CuSO4×5H2O and 0.10 g L−1 MnSO4×H2O.

2.2 Electrochemical Set up

The electrokinetic reactor was constructed under our directions by Glassware South Africa with the dimensions as seen in Figure 1. Graphite electrodes (100 mm long × 20 mm diameter) were vertically inserted in the reactor at a specified distance apart and connected to the DC power supply (0-30 V,0-3 RS-IPS 303A) using stranded iron wires. The magnetic stirrer was set to stir at 180 rpm. Viable cell count was determined by plate counting, the pH was measured using PL-700 Series bench top meter and temperature was measured using a Thermo Scientific Orion DuraProbe cell.

2.3 Naphthalene degradation studies

A stock solution of naphthalene was prepared by dissolving naphthalene in acetone to achieve a PAH concentration of 1 mg/mL. 100 mL of naphthalene from the stock solution was added to the reactor. The solvent was allowed to evaporate overnight to leave a thin coating of naphthalene at the bottom of the reactor. 500 mL of MSM was added to the reactor to obtain a final PAH concentration of 200 mg/L. The reactor was inoculated with 1 mL of aliquot of hydrocarbon degrading bacteria (Pseudomonas aeruginosa) harvested in its log phase since this is the stage the bacteria can most withstand environmental stress. The electrodes were fixed in position with 80 mm distance between them and connected to a DC Power supply. The experiments were run for 5 days under incubation conditions of 37 oC and constant stirring. The current applied was varied from 1 A to 2 A while the control was the experiment with no current applied to only rely on biodegradation.

2.4 Total carbon analysis as a function of naphthalene

Samples were picked after every 24 hours from each of the experiments. The samples were centrifuged and filtered to remove the bacteria and any solids. Liquid samples were ready for analysis in the Schimadzu Total Organic Carbon Analyzer.

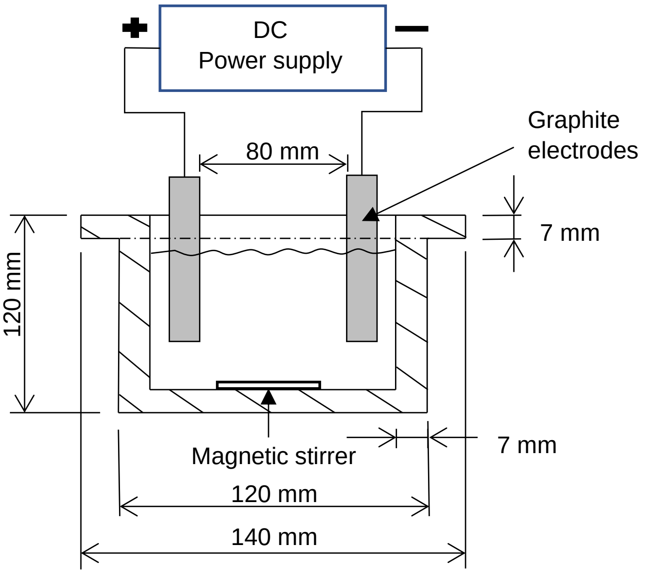

Figure 1: Schematic view of the electrochemical reactor

3. Results and discussion

3.1 Effect of current on pH

In all the experiments the pH was adjusted to 7 just before the experimentation started (Figure 2). In the experiments where 0 A and 2 A was applied the pH remained between 7.6 and 6.6 while in the experiment where 1 A was applied, the pH kept reducing to the end of the experiment. Conventionally the pH in an electrochemical system is influenced by the generation of H^+ ions at the anode electrode and the generation of ^{o}OH ions at the cathode electrode (Jeon et al., 2015). In the experiment in which 2 A was applied there seems to be a balance between the generation of the hydroxyl and hydrogen ions produced which ensures that the pH is still close to 7. In the experiment where 1 A was applied the pH continuously reduced from the beginning of the experiment demonstrating that the electrochemical system was dominated by H^+ ions produced at the anode leading to the continuous reduction in pH. The slight change in pH when no current is applied (0 A) is mainly because of the metabolites produced by the microbes (Sánchez-Clemente et al., 2018).

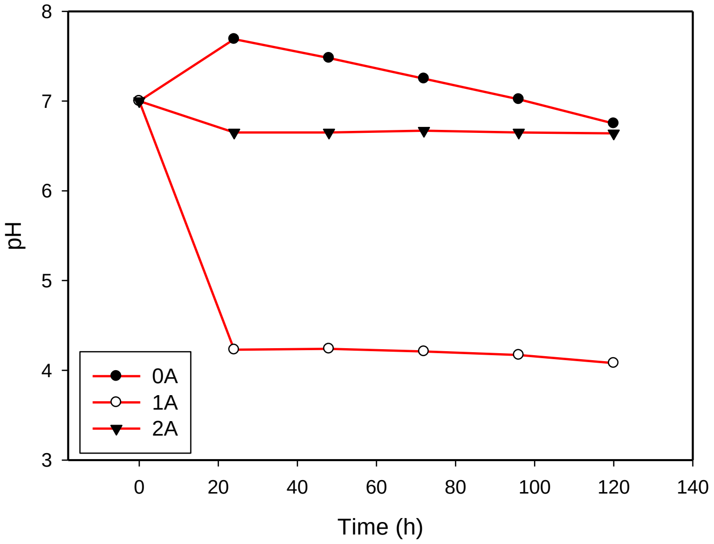

Figure 2: Effect of current on pH variation

3.2 Effect of pH on viable cell count

In this study, active electrodes were used to avoid the production of toxic compounds that are often produced by non-active electrodes to prevent the inhibition of bacterial growth (Rubio-Clemente et al., 2014). In the experiments where current was applied, the variation of pH as a result of the production H^+ ions and ^oOH ions produced at the electrodes was of much significance due to the effect of pH on microbial growth (Gidudu and Chirwa, 2020). Furthermore, the current applied at the electrodes may kill the bacterial by destroying the cell membranes of the cells (Gidudu and Chirwa, 2021). The impact of the intensity of the current on bacteria is observed when the physiology of the membranes and its permeability is compromised (Beretta et al., 2019). In the experiment where 1 A was applied, the low pH resulted in the low growth of the bacteria which compromises the cell membranes of the bacteria (Gidudu and Chirwa, 2021). When 2 A was applied, the pH remained in the range of 7.6 and 6.6 which is close to 7 the optimum pH of *Pseudomonas aeruginosa* (Gidudu and Chirwa, 2020). This facilitated the uninhibited growth of the bacteria throughout the experiment much as the intensity of the current was higher. This also means that current applied did not have a substantial effect on cell growth like the pH did. But still growth without current was way higher than when current was applied as seen in Figure 3.

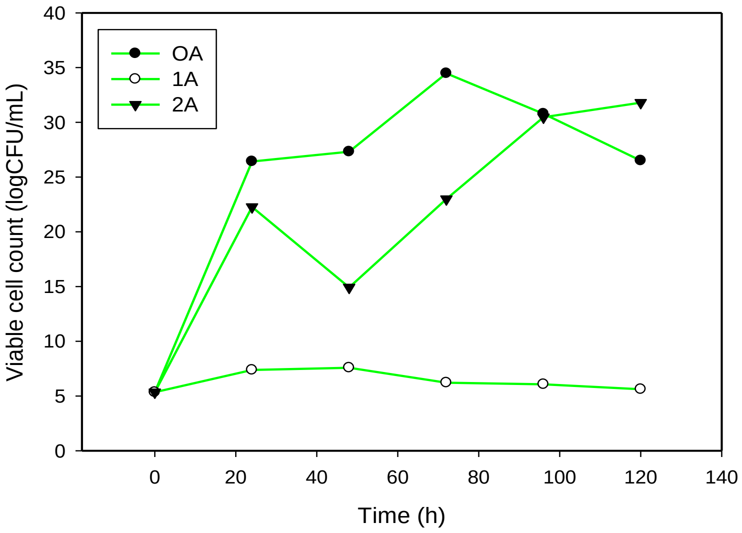

Figure 3: Effect of current on microbial growth

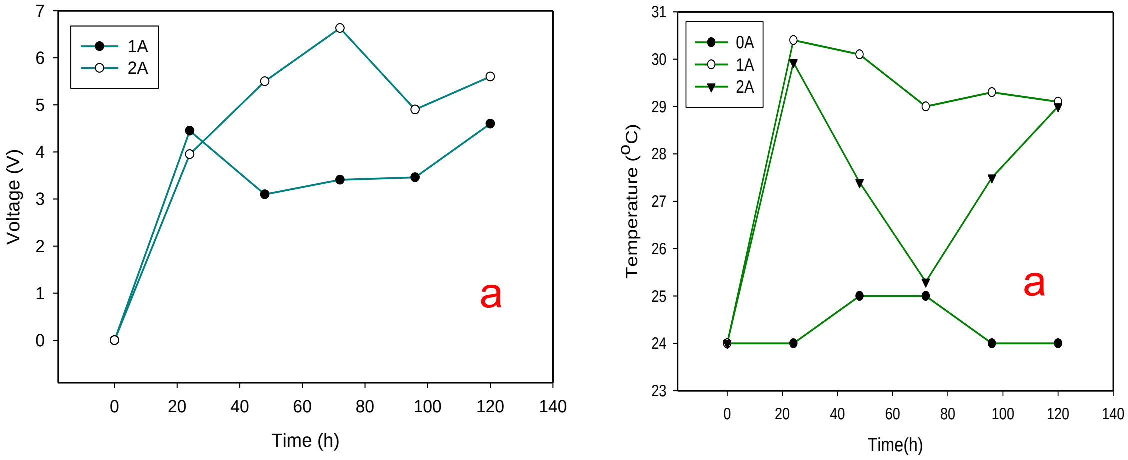

Figure 4: Time course of voltage (a) and the effect of applied current on temperature (b)

3.3 Effect of current on voltage

As seen in Figure 4, the relationship between current and voltage/temperature overtime is shown. The results demonstrate that the voltage fluctuated throughout the experiments in both experiments (Figure 4 (b)). The highest voltage obtained when 1 A was applied was 4.6 V while that obtained when 2 A was applied was 6.6 V. Voltage is directly proportional to the product of current and resistance hence this could be the reason for the fluctuations observed due to the differences in the electrolytic current and the fluctuations in the resistance due to the increase and decrease in the ionic strength of the system. This is observed in Figure 4 (b) where the temperature in 1 A is seen to be higher than that in 2 A. This means that the application of current led to the conversion of electrical energy to thermal energy through Joule heating (Liu et al., 2020). The thermal energy generated is proportional to the product of its resistance, the square of its current and the time the current is allowed to flow (Liu et al., 2020). Therefore, much as the flow of current in the reactor when 2 A was applied was higher than in 1 A, the resistance of the electrolyte in 1 A was higher leading to the generation of more heat than other experiments.

3.4 Effect of current on naphthalene removal

Figure 5 shows the removal of naphthalene from the water. The experiment with the highest current led to the highest removal of the hydrocarbon from water. The degradation of naphthalene was 80 % when only biodegradation was implemented (O A), 87.33 % when 1 A was applied and 90.14 % when 2 A was applied. When electrochemical oxidation is used to degrade polycyclic hydrocarbons using active electrodes such as graphite, it has previously been reported that total mineralisation of the hydrocarbons is often unachievable requiring the use of non-active electrodes (Rubio-Clemente et al., 2014). In this study, the high degradation of naphthalene when 1 A and 2 A is applied is mainly because of the partial degradation of the organic pollutant through direct oxidation of the organic pollutant to the anode as seen in Eq(3) or the indirect total oxidation of the organic pollutant with the aid of electrochemical oxidants as seen in Eq(4), Eq(5) and Eq(6) (Rubio-Clemente et al., 2014). Furthermore, the inoculation of the bacteria aided in the total mineralisation of the partially degraded pollutant especially in 2 A where the temperature, pH and current were favourable enough to allow the growth of the bacteria as it utilises naphthalene as a substrate. Much as the increase in current has been reported not to have a great effect in terms of enhancing the oxidation of PAHs (Rubio-Clemente et al., 2014), the combination of bacteria with the application of high current (that does not inhibit cell growth) had the highest hydrocarbon removal from the contaminated water. The high temperature and low pH in 1 A affected microbial degradation and the electrochemical oxidation of the pollutant since high temperature beyond 21 ^{o}C may affect electrochemical oxidation (Rubio-Clemente et al., 2014), while pH beyond 7 affects cell multiplication of *Pseudomonas aeruginosa* (Gidudu and Chirwa, 2020).

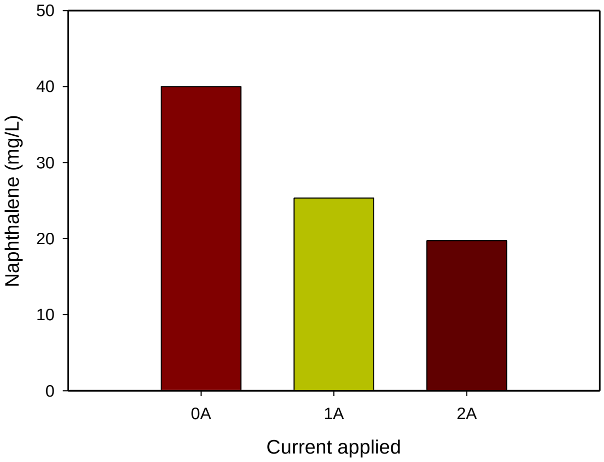

Figure 5: Naphthalene remaining in the water after 120 h of treatment

4. Conclusions

The combination of electrochemical oxidation with biodegradation led to the most efficient removal of naphthalene from the contaminated water but the combined process was affected by the continuous variation of pH in some experiments. The combined process may be arranged to start with electrochemical oxidation to partially degrade the polycyclic aromatic hydrocarbons followed by their total mineralization in the subsequent setup based on microbial biodegradation. Future studies should also involve the degradation of several polycyclic aromatic hydrocarbons to establish the independent effect of biodegradation on the combined bio-electrochemical process under controlled pH.

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