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# International Journal of Applied Sciences and Biotechnology

A Rapid Publishing Journal

ISSN 2091-2609

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**CODEN (Chemical Abstract Services, USA): IJASKD**

Vol-3(2) June, 2015

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SEM-Biotech  
Publishing

Impact factor\*: **1.422**  
Scientific Journal Impact factor#: **3.419**  
Index Copernicus Value: **6.02**

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Research Article

## GENERATION OF RENEWABLE POWER FROM BIODEGRADATION OF ANTHRACENE IN A MICROBIAL FUEL CELL REACTOR USING DIFFERENT BACTERIAL INOCULA

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### Abstract

Microbial fuel cells (MFCs) are increasingly attracting attention as a sustainable technology as they convert chemical energy in organic pollutants to renewable electricity. Anthracene is a polycyclic aromatic hydrocarbon (PAH) that presents a high pollution and health risk. In this study, anthracene degradation with electricity production in Single – chamber air cathode MFC was investigated with respect to values of its biodegradation and MFC performance using different inocula combinations (Anaerobic sludge (AS), *Pseudomonas putida* (PP), *Geobacter sulfurreducens* (GS), *Shewanella putrefaciens*(SP), mixed cultures, and combinations thereof). All the inocula showed high potentials for anthracene degradation efficiency and power density, ranged 41 – 98 % within 120 – 216h and 110.08 – 156.06 mW/m<sup>2</sup>, respectively. The best overall performing inoculum was anaerobic sludge supplemented with *P. putida* (AS+PP), having a degradation rate, degradation efficiency, COD removal, maximum power density and coulombic efficiency of 38 μM/d, 98 %, 83 %, 156.06 mW/m<sup>2</sup> and 21, respectively. Effect of initial anthracene concentration was also investigated. Results indicated that increasing of initial anthracene concentration to 40 mg/L has a positive effect on both the anthracene degradation rate and the power density by 79 and 83.93 %, respectively, which attained by the best inoculum AS+PP (degradation rate of 41 μM/d and a maximum power density of 287.04 mW/m<sup>2</sup>). This study highlights the possibility of using MFCs technology to generate renewable electricity and achieve high degradation rates of anthracene simultaneously, through co-metabolism.

**Keyword:** Renewable Power; Biodegradation; Anthracene; Microbial Fuel Cell; Polycyclic Aromatic Hydrocarbon.

### Introduction

The demand for energy is increasing rapidly worldwide. Currently, the required world energy mainly depends on fossil fuel in which its resources are declining, and their ignition due to carbon dioxide production has serious negative effects on the world climate (Davis and Higson, 2007; Lee *et al.*, 2008; Venkata *et al.*, 2008). On the other side, the rapid process of industrialization has led to the production of large amounts of pollutants which has to undergo treatment. Therefore, it consumes a lot of energy (Min *et al.*, 2005; Venkata *et al.*, 2007). In order to resolve the problems due to energy deficiency, the world climate changes, the environment pollution and the increasing cost of pollution treatment, new green technology has been needed as new energy sources to substitute fossil fuels (Ghangrekar and Shinde, 2007; Sleutels *et al.*, 2009). The term renewable bioenergy is very topical nowadays because of fast depletion of natural power resources. The production of power or electricity from renewable resources (organic material) which do not cause any net carbon dioxide emission is very much desired (Lovely, 2006; Davis and Higson, 2007; Du *et al.*, 2007). Microorganisms can

produce electricity from organic material by biotechnology of microbial fuel cells (MFCs) which has many benefits including cleanliness, effectiveness, recyclability and are not producing toxic by-products (Logan, 2004; Liu *et al.*, 2005; Moon *et al.*, 2006). Microbial fuel cells (MFCs) are devices that use microorganisms to catalyze the conversion of chemical energy in organic compounds into electrical power. They have great potential as a technology for sustainable bioenergy production due to their ability to generate electricity from wastewater and organic pollutants while simultaneously decontamination (Logan and Regan, 2006). The MFCs are considered a promising sustainable technology that can be used for organic pollutant biodegradation with simultaneous electricity generation (Liu *et al.*, 2004). It was reported that electricity could be generated in MFCs from various of readily degradable compounds, including sugars, such as monosaccharides (Liu and Logan, 2004; Catal *et al.*, 2008), carboxylic acids, such as acetate, butyrate, propionate (Liu *et al.*, 2005), alcohols, such as ethanol, methanol (Kim *et al.*, 2007) proteins, such as bovine serum albumin (Heilmann and Logan, 2006), biomass hydrolysate (Zuo *et al.*, 2006), and

wastewater streams (Rabaey *et al.*, 2005). In a few cases, some recalcitrant compounds, such as petroleum contaminants, were also used as the fuel in MFCs for both biodegrading pollutants and for generating electricity (Morris and Jin, 2008; Ren *et al.*, 2007; Morris *et al.*, 2009; Alshehri, 2015a; 2015b). Based on wide literature review, anthracene has not been reported as a MFC fuel for biodegrading the pollutant and generating electricity previously (Li *et al.*, 2013). Anthracene is a polycyclic aromatic hydrocarbon (PAHs) composed of three fused benzene rings, and it has been identified as priority pollutant by the United States Environmental Protection Agency (USEPA) (White, 1986). As a result of incomplete combustion of organic matter in automobile exhaust, petrochemical industry, or accidental spills during the transportation of petroleum, PAHs become ubiquitous contaminants in the environment (Sartoros *et al.*, 2005; Jacques *et al.*, 2008). Since PAHs exhibit carcinogenic, mutagenic and other toxic properties (Crisafulli *et al.*, 2008), as well as their characteristics such as bioaccumulation, biomagnification and persistent toxicity, PAHs have posed serious risks to the environment and human health. Consequently, PAHs have raised a great environmental concern all over the world (Yuan *et al.*, 2000). Biodegradation is an economic and environmentally friendly technology for removal of PAHs (Giraud *et al.*, 2001). Anaerobic biodegradation is an important removal mechanism for PAHs (Boopathy, 2004). Anaerobic biodegradation may require the presence of terminal electron acceptors (TEAs) such as nitrate, sulphate or metallic oxides but even then it is a slow process. The deployment of TEAs for in situ treatment is also not without its problems, for example, the high solubility of TEAs in water makes them too easily diffuse away from the point of application due to hydrodynamic forces (Zhang *et al.*, 2010). Also, as anaerobic degradation proceeds, the amount of TEAs depletes and thus becomes a rate-limiting factor for degradation. Continuous supply of TEAs is not sustainable due to high cost resulting from maintenance and energy costs. MFCs provide solution as a new technique in enhancing biodegradation of recalcitrant contaminants (Aulenta and Majone, 2010; Mu *et al.*, 2011; Bin *et al.*, 2013; Alshehri, 2015a; 2015b). MFCs are unique in term of that the microorganisms are able to transfer electrons extracellularly to a solid material like an anode electrode. The presence of these insoluble and inexhaustible electrodes allows continuous transfer of electrons to the cathode where they are consumed by oxygen. This use of oxygen as an indirect TEA would be expected to enhance hydrocarbon degradation compared to degradation via anaerobic respiration. Most studies investigating the feasibility of treating petroleum hydrocarbons used

undefined mixed cultures or indigenous microbes as inocula (Luo *et al.*, 2009; Morris *et al.*, 2009; Yan *et al.*, 2012). Most of the researchers recorded prolonged experimental durations, unfortunately, this could limit the potential application of this unique technology in real scenarios. The use of pure or defined co-cultures in the presence of co-substrates could reduce the period required to degrade hydrocarbons. In the case of co-cultures, there could be potential for synergistic utilization of the metabolic pathways from the microorganisms involved (Bader *et al.*, 2010). Such synergy may involve one organism reducing available oxygen in the anode thus enhancing growth of another microaerophilic microorganism or strict anaerobe. Alternatively, by-products of one microorganism may be used by another microorganism as substrate, redox mediator, surfactant etc. In present work, anthracene has been chosen as a model compound of the PAHs family to study its biodegradation as fuel in MFC to generate bioelectricity. The investigation was primarily focused on the effect of different bacterial inocula and the initial anthracene concentration.

## Materials and methods

### Chemicals

Anthracene (purity  $\geq 97\%$ , crystalline blue-violet fluorescence flakes; molecular weight: 178.23; melting point: 216 – 218 °C; boiling point: 340 °C; density: 1.099 g/cm<sup>3</sup>, octanol-water partition coefficient: 4.456; solubility: 0.065 mg/l) and methanol (HPLC-grade) were purchased from Sigma-Aldrich. All other reagents and chemicals were purchased from Merck, India. All chemicals were of analytical grade and used without further purification.

### Microbial inocula and culture medium

Anaerobic sludge (AS) was obtained from Makkah Sewage Treatment Plant (KSA). Three pure strains as *Pseudomonas putida* (PP), *Geobacter sulfurreducens* (GS) and *Shewanella putrefaciens* (SP) were purchased from the German collection of microorganisms and cell cultures, Braunschweig, Germany. Anaerobic sludge, *P. putida*, *G. sulfurreducens* and *S. oneidensis* were grown anaerobically separately in mineral salts medium (MSM) supplemented with 100 mg/L of D-glucose and subsequently incubated at 30°C for 48 h. Table 1, summarizes different types of the used inocula combinations. The Mineral salts medium (MSM) was composed of (g/L of deionized water) 3 NH<sub>4</sub>Cl, 0.5 KH<sub>2</sub>PO<sub>4</sub>, 0.5 K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, 0.008 MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.002 CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.002 MnSO<sub>4</sub>·H<sub>2</sub>O, 0.002 FeSO<sub>4</sub>·7H<sub>2</sub>O and 0.002 CaCl<sub>2</sub>·2H<sub>2</sub>O. The pH was adjusted to 7.0 with either HCl or NaOH solutions. Culture medium was sterilized in an autoclave at 121°C for 15 min.

**Table 1:** Summary of the used inocula combinations in the experiments

Inoculum	Inocula combinations	Symbol
1	Anaerobic sludge	(AS)
2	<i>Pseudomonas putida</i>	(PP)
3	<i>Geobacter sulfurreducens</i>	(GS)
4	<i>Shewanella putrefaciens</i>	(SP)
5	<i>S. putrefaciens</i> + <i>G. sulfurreducens</i> + <i>P. putida</i>	(SGP)
6	Anaerobic sludge + (SGP)	(AS+SGP)
7	Anaerobic sludge + <i>S. putrefaciens</i>	(AS+SP)
8	Anaerobic sludge + <i>G. sulfurreducens</i>	(AS+GS)
9	Anaerobic sludge + <i>P. putida</i>	(AS+PP)

### MFC set up and operation

Single – chamber air cathode MFCs were constructed as described previously (Liu and Logan, 2004) with some modification. Briefly, the anode and cathode were placed in parallel on the opposite side of the chamber (total volume is 200 mL, working volume is 100 mL) with distance of 5cm. Non – wet proofed carbon cloth (type A,E – TEK, Somerset, NJ, USA, 4cm<sup>2</sup>) which was used as anode. Wet – proofed (30%) carbon cloth (type B, E – TEK, Somerest, NJ, USA, 10cm<sup>2</sup>) was used as cathode pressed to proton exchange membrane (Nafion 117, Dupont CO., USA) on the water – facing side. The anode chamber was filled with anolyte medium (MSM) (pH 7.0). The MFCs were sterilized by autoclaving at 121°C for 15 min, followed by addition of anolyte to the anode chamber which was done aseptically. All experiments conducted in this study were operated in fed-batch mode whereas the MFCs were inoculated with 10 mL of one type of the inoculum per cycle. Anaerobic conditions were maintained in the anode chambers by purging them with 100% N<sub>2</sub> for 15 min before MFC operation began. The pH was adjusted by adding NaOH or HCl. All experiments were conducted at 30 ± 0.5°C using an incubator (LAB – LINE ® AMBI – USA). The net volume of the anolyte was 100 mL for each experiment. Immediately after adding the fuel and inoculum, MFCs were hooked up to a data acquisition system to start monitoring the voltage generation (150Ω).

### Biodegradation of anthracene in MFC

The influence of inoculum type on anthracene degradation at different concentrations (10 – 80 mg/L) and MFC performance was investigated using anaerobic sludge (AS), *P. putida* (PP), *G. sulfurreducens* (GS), *S. putrefaciens* (SP), a mix-culture of *S. putrefaciens* + *G. sulfurreducens* + *P. putida* (SGP), anaerobic sludge with the mix-culture (AS+SGP), anaerobic sludge with *S. putrefaciens* (AS+SP), anaerobic sludge with *G. sulfurreducens* (AS+GS) and anaerobic sludge with *P. putida* (AS+PP) (Table 1). Each inoculum was 10% v/v of the working volume of the anode chamber (100 mL). The anolyte medium consisted of 100 mg co-substrate (glucose) per liter of MSM, 30 mg/L anthracene (taken from a 1000-fold concentrate in 100% methanol) and the inoculum (10 mL). In each treatment, a control was employed as an abiotic MFC. The used

methanol in dissolving the anthracene (0.1% v/v of the working volume) is considered to be nontoxic since the used concentration is far below the minimum inhibitory concentration for microorganisms (Caldwell, 1989; Wadhvani *et al.*, 2009).

### Anthracene analysis

Anolyte samples containing anthracene were analyzed by high performance liquid chromatography (HPLC Agilent 1100) using a Photo-diode Array (PDA) detector (DIONEX, PDA-100) at 254 nm. The injected volume was 20 µL. The analytical column was a reversed phase column, Supelcosil™ LC-PAH column (150 mm × 4.6 mm). The mobile phase (80% acetonitrile and 20% deionized water) flow rate was 0.5 mL/min. The column oven temperature was set at a constant temperature of 25°C. The minimum detectable concentration for anthracene was 5 µg/L. Anthracene extraction procedures as follow: 1 mL of aliquots were withdrawn at intervals from the MFC and transferred to 2 mL eppendorf tubes. Subsequently, 1 mL of methanol was added to make up to 2 mL, and the eppendorf tubes (which were placed in a 200 mL glass beaker) were incubated in an incubator shaker for 1 h at 25°C and 150 rpm. The tubes were then centrifuged at 10,000 g for 10 min, and 500 µL of the supernatant was carefully transferred into 1.5 mL HPLC glass vials prior to analysis by HPLC. In order to quantify the total amount of anthracene degraded, the amount of anthracene adsorbed on the anode was determined by soaking the anode electrodes in 20 mL methanol at the end of each experiment for 1 h at 200 rpm. Aliquots were transferred into 2mL eppendorf, immediately followed by centrifugation at 10,000 g for 10 min. All liquid samples were immediately analyzed within few hours after sampling in order to minimize adsorption onto the wall of the sample vials. Biodegradation efficiencies and rates were determined based on the remaining anthracene in solution and that adsorbed on the anode at the end of MFC operation

### COD removal measurement

The chemical oxygen demand (COD) of the samples was determined using the closed reflux titrimetric method as described in the Environment Agency (USA) Standard method 5220D (APHA, 1997). Appropriately diluted 1 mL samples were used for each determination. COD removal was calculated as:  $COD (mg/L) = (K_b - K_s) * DF * M *$



8000, where:  $K_b$  and  $K_s$  are ferrous ammonium sulphate (FAS) titrant volumes for blank and the sample, respectively. DF is the sample dilution factor, and  $M$  is the molarity of the FAS solution. The COD of samples was expressed as percentage COD removal and COD removal rate. The percentage COD removal was calculated as: percentage COD removal (%) =  $(\text{COD}_i - \text{COD}_f) / \text{COD}_i \times 100$ , where:  $\text{COD}_i$  and  $\text{COD}_f$  are initial COD and final COD values respectively.

### Electrochemical analysis

Voltage was measured after the MFC has reached the steady state by a digital multimeter (Sanwa CD800a, Japan) which was connected to a personal computer. Data was automatically recorded every second via Picolog software (Pico Technology Limited). The corresponding current was based on equation  $I = E/R_{ext}$ , where:  $I$  is current (mA),  $E$  is voltage (mV), and  $R_{ext}$  is external resistance. The power ( $P$ ) was obtained by  $P = IE$ . The current density and the power density have been normalized based on the projected surface area of the anode via equations  $I_{An} = I/A_{An}$ , where  $I_{An}$  is current density and  $A_{An}$  is the surface area of anode,  $P_{An} = E^2/A_{An}R_{ext}$ , where  $P_{An}$  is power density. The polarization curve was obtained at different external resistance (50 - 1000 $\Omega$ ). Internal resistance was derived from the polarization curve as the slope. Coulombic efficiency ( $CE$ ) was derived from the equations  $C_p = It$ ,  $C_{max} = FfS_{COD}V_{An}$ , and  $CE = C_p/C_{max}$ , where  $C_p$  is the coulombs of energy produced,  $t$  is the time of stable voltage output,  $C_{max}$  is the theoretical maximum coulombs,  $F$  is Faraday's constant (96.485 C/mol of electrons),  $f$  is a factor of 1mol electrons/8g COD,  $S_{COD}$  is substrate concentration g COD/l, and  $V_{An}$  is a net volume of anolyte (mL). Statistical analyses were performed with  $\alpha = 0.05$ . All data are presented as means of duplicate experiments. The standard deviation of the mean (SD) ranged between 0.1 - 0.5%.

## Results and Discussion

### Anthracene biodegradation in MFC

Anthracene concentrations in the bulk solution for all nine inocula decreased gradually by different percentages ranged 41 - 98 % within 120 - 216 h (Fig. 1 and 2). The disappearance of anthracene from solution depended mainly on a microbial action. Zhang *et al.* (2010) and Xia *et al.* (2010) reported that microorganisms can degrade aromatic hydrocarbons on an electrode and in solution. The degradation of anthracene for different inocula is shown in Fig. 1 and 2. The reported degradation rates for anthracene using different inocula were determined based on their total concentrations in comparison to the starting concentration. All the nine inocula showed potential for anthracene degradation with minimum degradation efficiency of 41% compared to the control (Fig. 2). Similar observations were made by Huang *et al.* (2011) that high COD and contamination of phenol has removed in a soil MFC. A

marked variation in the anthracene biodegradation rate was observed as a function of inoculum type during the MFC operation. AS+PP gave the highest values of anthracene removal, degradation rate, COD removal, coulombic efficiency, voltage, power density and current density as 98 %, 38  $\mu\text{M/d}$ , 83 %, 21 %, 306V, 156.06  $\text{mW/m}^2$  and 0.51  $\text{mA/m}^2$ , respectively (Fig. 2 - 5 ). Followed by AS+SGP with 96 %, 36  $\mu\text{M/d}$ , 74 %, 18 %, 289 V, 139.20  $\text{mW/m}^2$  and 0.481  $\text{mA/m}^2$ , respectively. SP gave the lowest values as 41 %, 19  $\mu\text{M/d}$ , 56 %, 11 %, 257 V, 110.08  $\text{mW/m}^2$  and 0.428  $\text{mA/m}^2$ , respectively. The strain, *P. putida* was shared in the inocula (AS+PP and AS+SGP) which gave highest values, it may possess PAH degrading enzymes that enabled it to co-metabolically biodegrade anthracene. *Pseudomonas* species have been reported by several authors to have a potential to degrade PAHs compounds (Nasseri *et al.*, 2010; Ma *et al.*, 2011). Ma *et al.* (2011) successively isolated *P. aeruginosa* strain PAH-1 that had the ability to anaerobically degrade phenanthrene with anthraquinone-2, 6-disulfonate as the sole electron acceptor, the authors reported 56.7% phenanthrene removal in the presence of a co-substrate, fructose. *Pseudomonas* species have also been found in MFC anodes and can be classified as electrochemically active bacteria (Logan, 2008). It respire anaerobically via the production of phenazines and pyocyanin, electron shuttling compounds, which they aid in transferring of electrons to the anode (Logan, 2008). These redox shuttling compounds aid in facilitating enhanced microbial oxidation of organic compounds like anthracene via electron transfer to the anode. Since these redox electrons shuttling compounds enhance electron transfer, high degradation rates and power densities would be expected. High degradation rates were observed as presented in this study. Higher degradation rates observed with inoculum of AS+PP might be due to the synergistic effect of its PAH degrading enzymes, biosurfactant self-production and the involvement of soluble shuttlers for the redox powers. Anaerobic biodegradation of PAH has previously been reported to occur via carboxylation followed by cleavage of the aromatic ring (Meckenstock *et al.*, 2004). Degradation efficiencies recorded in this study are relatively higher than those reported in the literature (Rockne and Strand, 1998; Jacquesa *et al.*, 2005; Tsai *et al.*, 2009; Wan *et al.*, 2012). For the first time, this study demonstrated biodegradation of anthracene in a microbial fuel cell. It is clear that the mixed cultures inocula (AS+PP, AS+SGP, AS+GS, AS+SP and SGP) achieved high values in comparison to a single pure microorganism (GS and SP) except the inoculum PP which recorded high rate of anthracene removal (93%). It could interpreting that cooperation of the microorganisms played vital role in achieving the high values. In this study, it has demonstrated that AS+PP gave a high degradation rate and biodegradation efficiency of 38  $\mu\text{M/d}$  and 98% respectively in a MFC reactor.

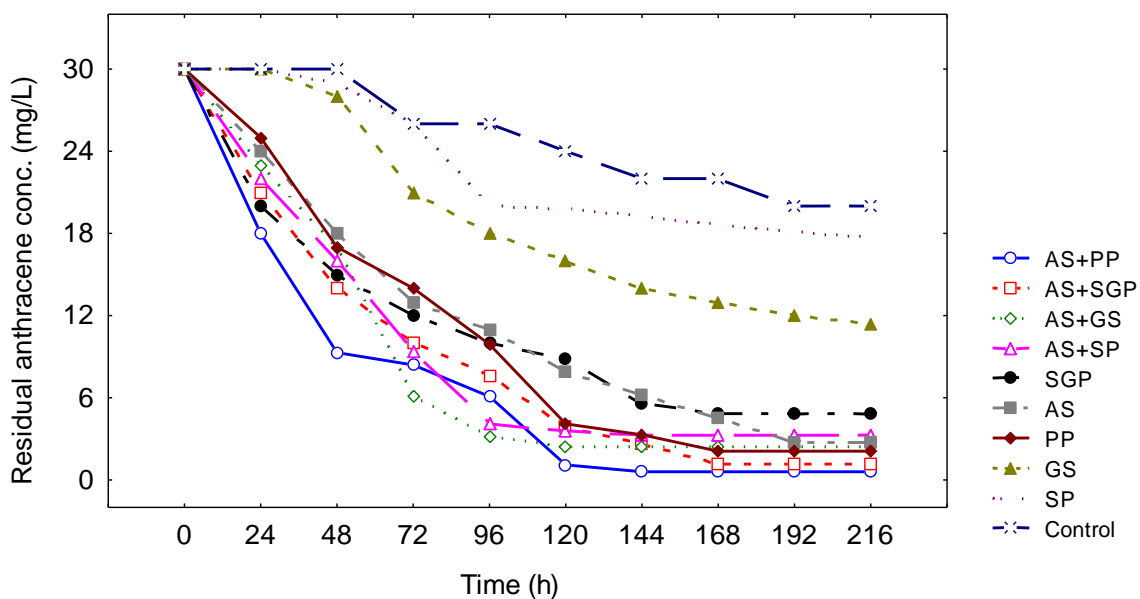


Fig. 1: Anthracene concentration in the bulk anode solution as a function of time by different inocula.

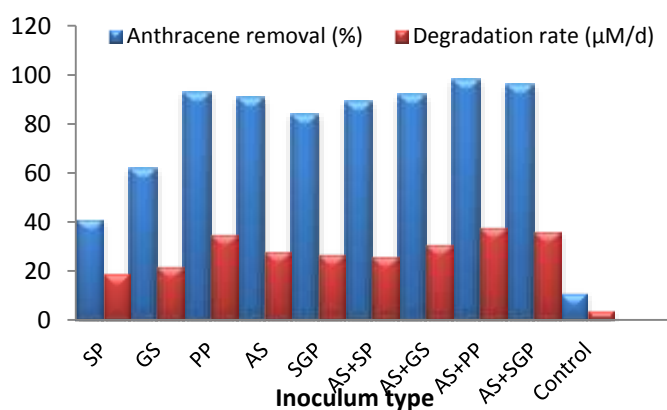


Fig. 2: Anthracene removal (%) and degradation rate (µM/d) by different inocula.

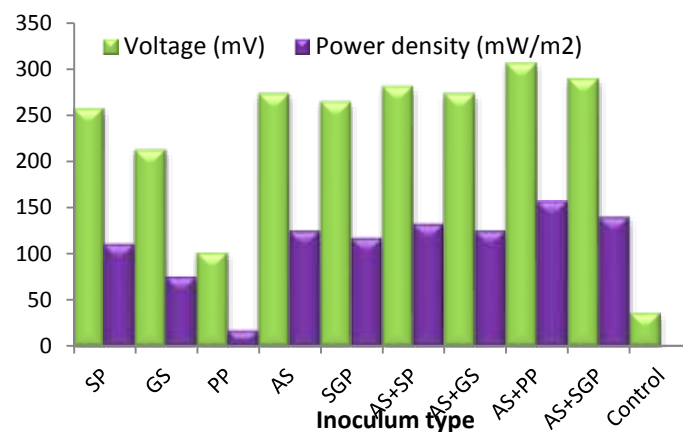


Fig. 4: Voltage (mV) and power density (mW/m²) by different inocula.

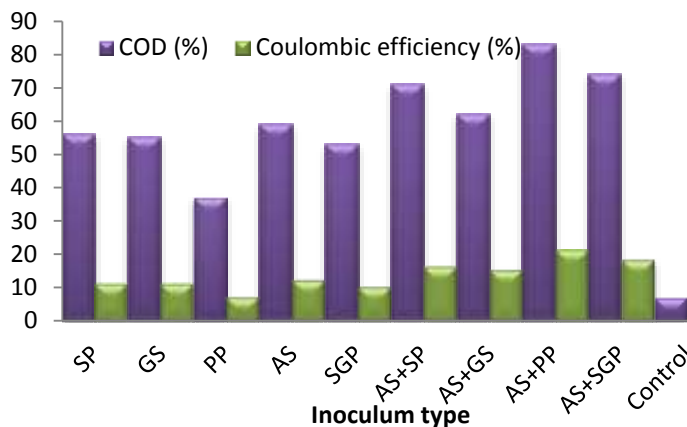


Fig. 3: COD removal (%) and coulombic efficiency (%) by different inocula.

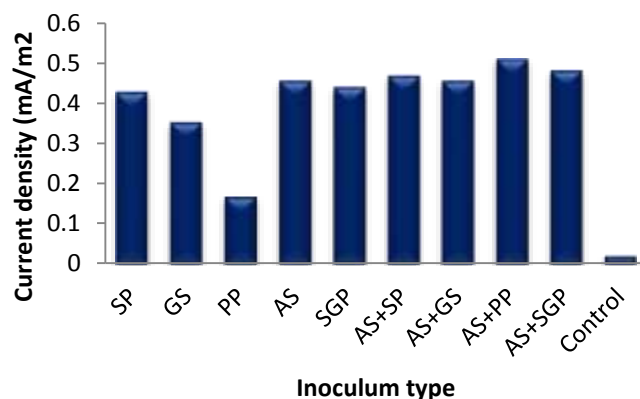


Fig. 5: Current density (mA/m²) by different inocula.

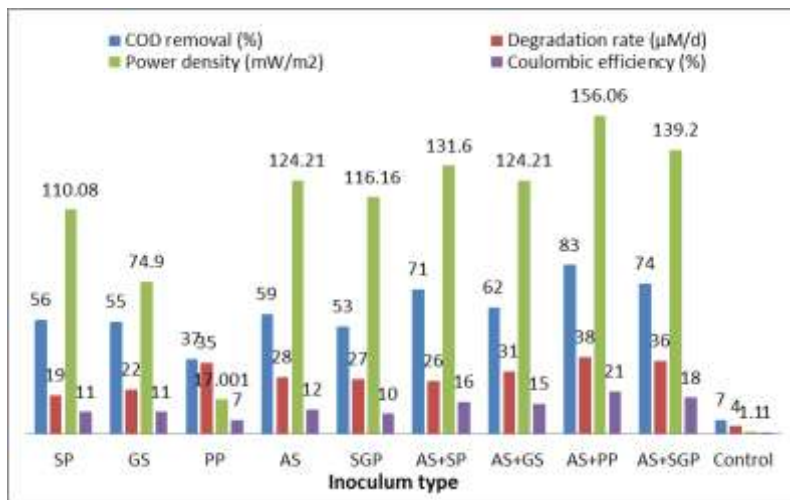


Fig. 6: Comparison between the inocula with respect to COD removal, degradation rate, power density and coulombic efficiency.

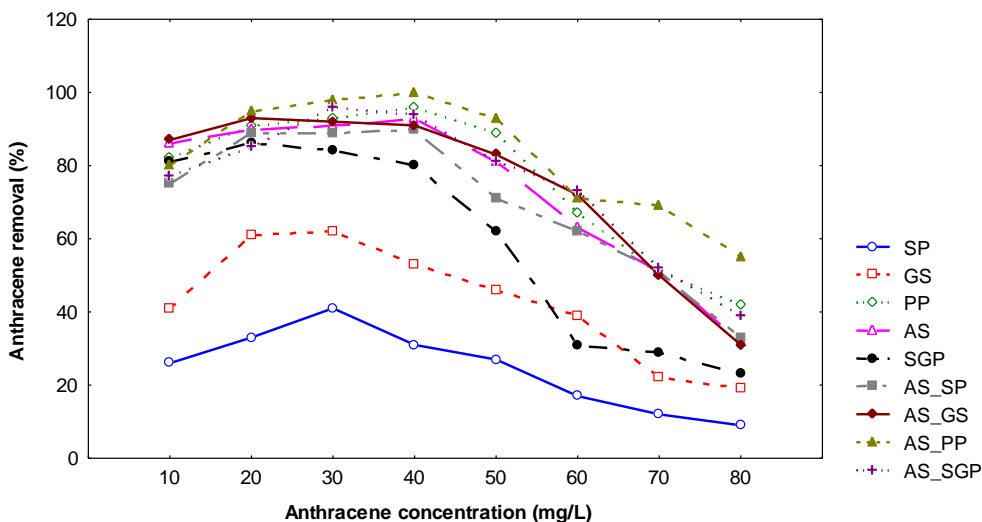


Fig. 7: Effect of initial anthracene concentration on its removal by different inocula.

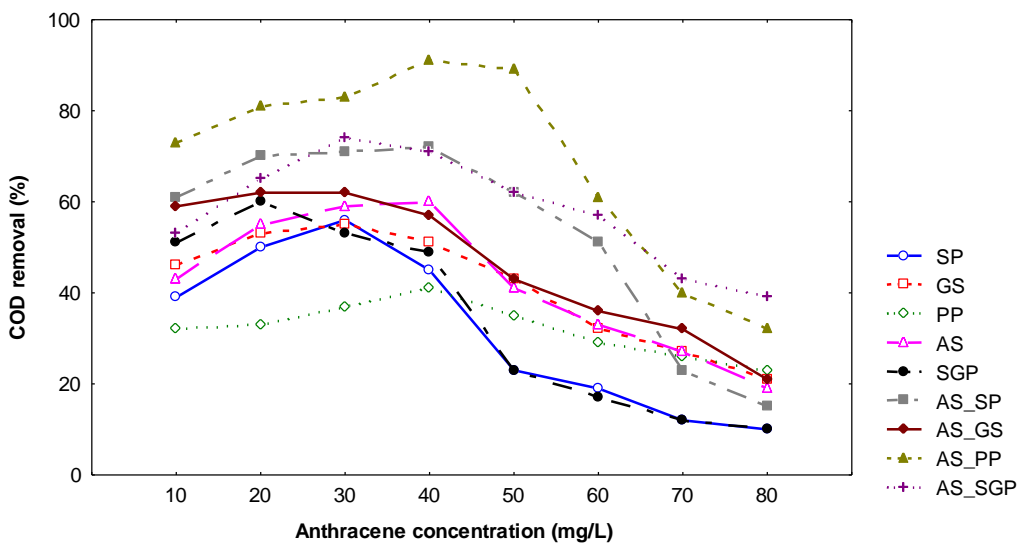


Fig. 8: Effect of initial anthracene concentration on COD removal by different inocula.

### MFC performance during anthracene degradation

A marked variation in electrochemical performances of different inocula (Fig. 3 – 5) is an implication of differences in the electrochemical behavior of each inoculum type. The bacteria consortia AS+PP gave the highest power density of 156.06 mW/m<sup>2</sup> followed by AS+SGP and AS+SP were recorded power densities of 139.20 and 131.60 mW/m<sup>2</sup>, respectively. Power densities from MFCs with co-substrate (glucose) was high, this is likely as a result of the presence of readily oxidisable compounds like glucose and their non-toxic. PP inoculum recorded lower power density relatively (17.001 mW/m<sup>2</sup>), and it is normal because of *P. putida* strain has electrochemical activity lower than SP (*S. putrefaciens*) and GS(*G. sulfurreducens*) which they recorded power densities 110.08 and 74.90 mW/m<sup>2</sup>, respectively. Positive interaction *P. putida* with electrochemically active microbes present in the anaerobic sludge could have likely contributed to high power density observed for bacteria consortia AS+PP. In light of this, findings from this study suggest bacteria consortia (AS+PP) could play an important role in enhancing electrochemical performances of MFCs. Coulombic efficiency recorded for all inocula tested were generally moderate (11 – 21 %). The observed moderate coulombic efficiency may be due to oxygen diffusion into the anode chamber or a result of presence of other alternative electron acceptors such as sulfates (0.008g/L MgSO<sub>4</sub> was used in this study) that make up the anolyte medium.

The criterion for the assessment of MFC performance (using different inocula) is based on the degradation performance and electrochemical performance. Fig. 6 shows a summary of performances of the different inocula. Performance index consists of degradation rate (µM/d), COD removal (%), maximum power density (mW/m<sup>2</sup>) and coulombic efficiency (%). Fig. 6 identifies AS+PP to be the best inoculum while SP is least in order of overall system

performance. This suggests the use of AS+PP for treatment of anthracene contamination and generation electricity simultaneously based on MFC technology.

### Performance of the inocula and MFC at different concentrations of anthracene

Effect of initial anthracene concentration has been studied upon degradation rate and MFC performance over the range 10 to 80 mg/L with constant glucose concentration (100mg/L), for all inocula. The results in Fig. 7 – 13 showed anthracene degradation levels were the highest at initial concentration 40 mg/L by 90 – 100 % for the inocula, AS+PP, AS and AS+SP. The others inocula showed anthracene degradation no more than 96 % at 30 mg/L of initial anthracene concentration. These results indicated that the inoculum AS+PP attained improvement 2 % of anthracene degradation when initial anthracene concentration increased to 40 mg/L. It could be interpreted that increasing of initial anthracene concentration stimulated present bacteria in the inoculum to produce more of specific enzymes to utilize anthracene. It is explicit of Fig. 7 – 13, that the best result was at 40 mg/L of initial anthracene concentration, which it was achieved by AS+PP as anthracene degradation rate, COD removal, voltage, power density, current density and coulombic efficiency of 41 µM/d, 91 %, 415 mV, 287.04 mW/m<sup>2</sup>, 0.691 mA/m<sup>2</sup> and 39 %, respectively. Increasing of initial anthracene concentration to 40 mg/L has a positive effect on both the anthracene degradation rate and the power density by 79 and 83.93 %, respectively. The inoculum AS+PP has attained the highest values at all. It might be result of that anaerobic sludge has consortium of bacteria possess diversity of enzymes as well as, *Pseudomonas* species have been reported by several authors to have a potential to degrade PAHs compounds (Nasseri *et al.*, 2010; Ma *et al.*, 2011).

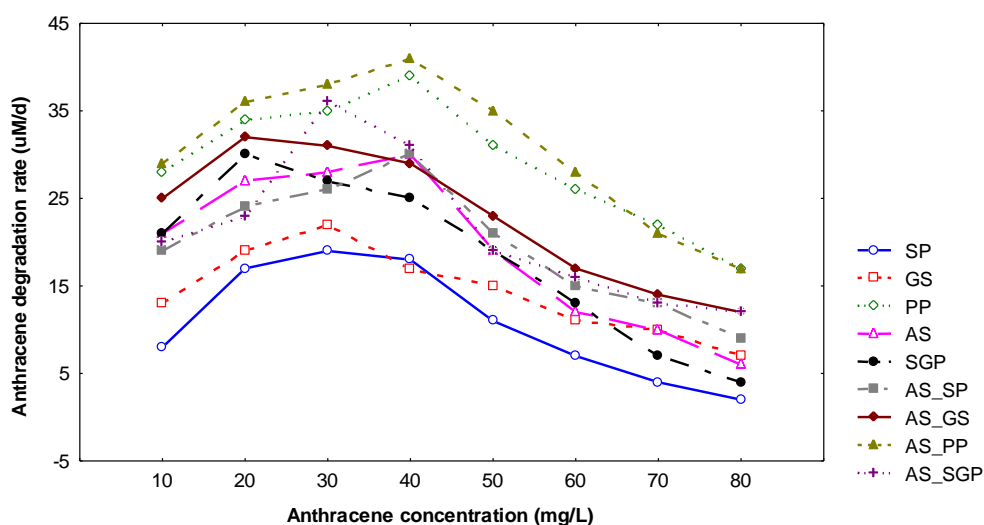


Fig. 9: Effect of initial anthracene concentration on degradation rate by different inocula.



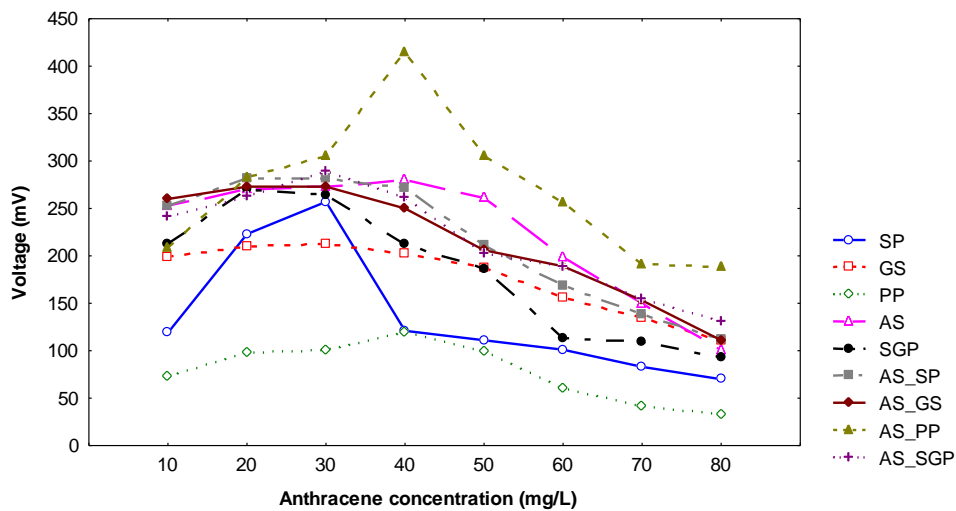


Fig. 10: Effect of initial anthracene concentration on voltage by different inocula.

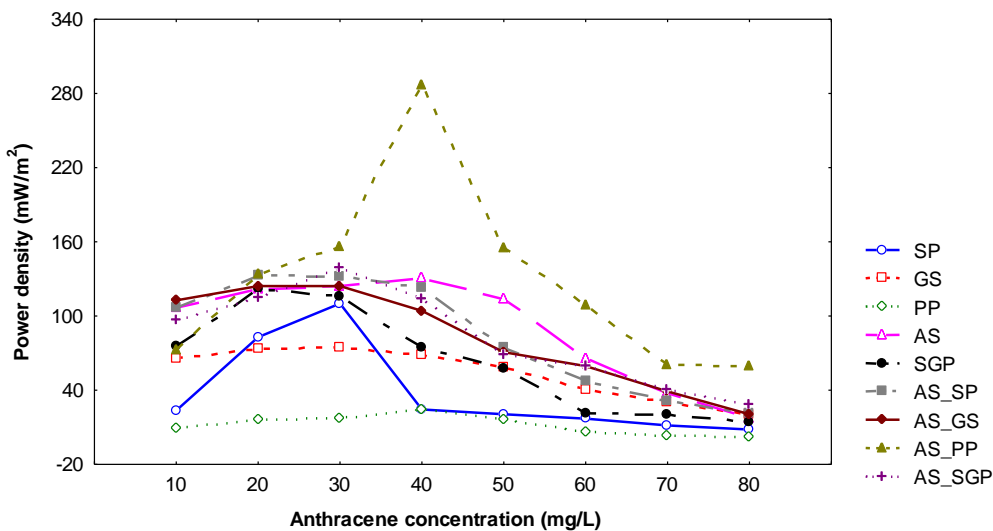


Fig. 11: Effect of initial anthracene concentration on power density by different inocula.

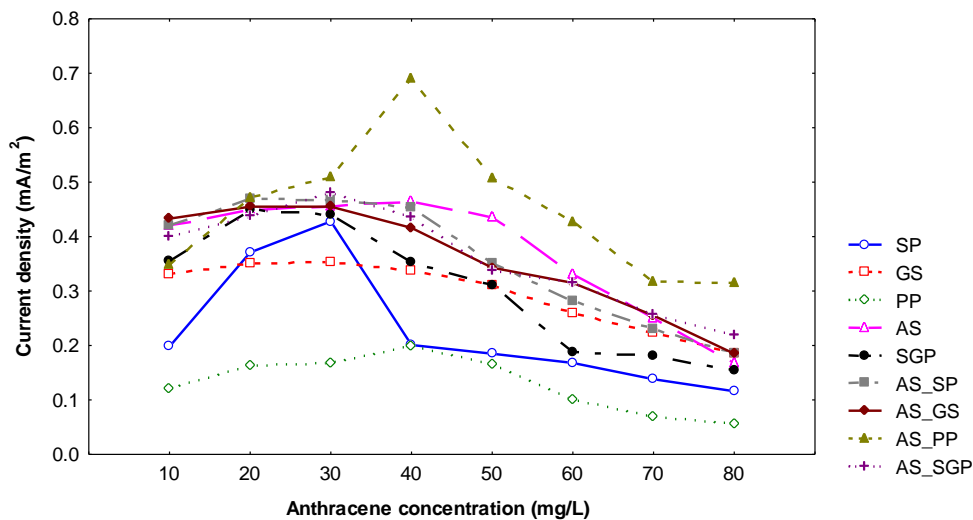


Fig. 12: Effect of initial anthracene concentration on current density by different inocula.

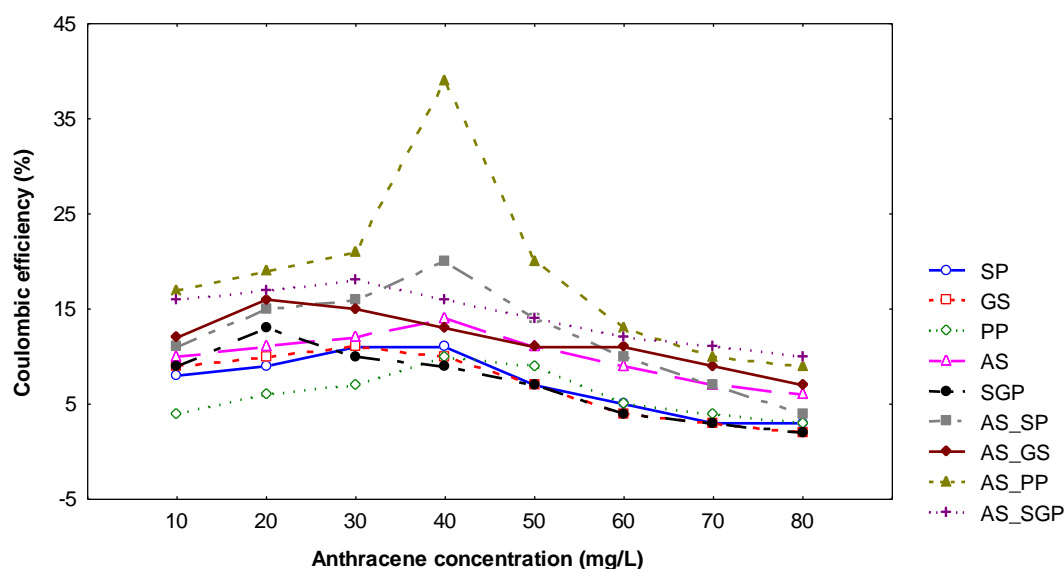


Fig. 13: Effect of initial anthracene concentration on coulombic efficiency by different inocula.

## Conclusions

This study demonstrated the possibility of using MFC technology, utilizing a range of inocula, to enhance the biodegradation of anthracene through co-metabolism with concomitant of electricity production. The best overall performing inoculum was AS+PP, a Anaerobic sludge supplemented with *P. putida*. The inoculum gave an anthracene degradation rate of 41  $\mu\text{M}/\text{d}$ , a maximum power density of 287.04  $\text{mW}/\text{m}^2$  and a COD removal of 91%, at initial concentration 40 mg/L of anthracene. It is suggested that AS+PP may offer good prospects for bioremediation of hydrocarbons in MFCs as well as generation of renewable power. The present work has demonstrated the feasibility of using an innovative technology (MFC) in the generation clean and renewable bioelectricity meanwhile the treatment of a model PAH compound (anthracene). This MFC technology can potentially be used as a bioremediation technology for the treatment of petroleum hydrocarbons in contaminated environments, as well as providing green and sustainable solutions to the global energy crisis, carbon dioxide emissions and climate changes

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