International Journal of Energy and Sustainable Development
Vol. 3, No. 4, 2018, pp. 105-115
http://www.aiscience.org/journal/ijesd

Anaerobic Biodegradation of Benzene in Microbial Fuel Cells: Influence of Inoculum Type on MFC Performance

Oluwaseun Adelaja[1, 2, *], Tajalli Keshavarz[1], Lukman Bello[2], Godfrey Kyazze[1]

[1]Department of Life Sciences, Applied Biotechnology Research Group, University of Westminster, London, UK
[2]Department of Chemistry, Federal University of Technology, Akure, Nigeria

Abstract

Petroleum hydrocarbon contamination in the environment resulting from anthropogenic activities has posed a significant health threat. Microbial fuel cell (MFC) could be used as one of the biotechnological tools in hydrocarbons removal especially in the subsurface environment. In this present work, the biodegradation of benzene using a range of inocula (*Shewanella oneidensis* MR1 14063*, Pseudomonas aeruginosa* NCTC 10662, mixed cultures and combinations thereof) in MFCs was investigated with respect to degradation rate, degradation efficiency and power production. Results indicated that all inocula tested could degrade benzene effectively. The best performing inocula, AMC (adapted anaerobic digested sludge) gave a benzene degradation rate of 64µM/h (which is 2 fold higher than the anaerobic (non-MFC) control), a maximum power density of 0.82mW/m^2 and a COD removal of 87.3%. Ecotoxcity testing based on *Vibrio fischeri* indicated that the treated solution was three times less toxic than the original solution. This work highlights the possibility of using microbial fuel cells to achieve efficient benzene biodegradation through co-metabolism with concomitant bioelectricity production. MFCs could be employed for treatment of benzene-contaminated subsurface environments or oil refinery wastewater.

Keywords

Benzene, Biodegradation, Microbial Fuel Cells, Bioelectricity Production, Cometabolism, Inocula

Received: November 28, 2018 / Accepted: December 17, 2018 / Published online: January 19, 2019

1. Introduction

Benzene is one of the most important industrial chemicals which serves as a precursor for the manufacture of other petrochemical products [1]. As a result of accidental leakages from storage facilities and improper disposal of spilled and spent petroleum products, benzene is commonly found in refinery wastewater, groundwater and sediments. Due to the its mobility, solubility and toxicity, benzene tends to persist in the natural environments thus posing serious threats to ecosystems and human health e.g. cancerous diseases like leukaemia among others [2-3]. Existing clean-up methods involving physical and chemical methods such as skimming, electro-kinetics and use of chemical dispersants are capital intensive and usually incur high maintenance costs [4]. Bioremediation methods that use microorganisms for degrading petroleum contaminants into less toxic forms constitute an attractive alternative to conventional techniques. In anoxic environments, biodegradation proceeds slowly (in terms of biokinetics) due to the absence of efficient electron terminal acceptors (TEA) like oxygen. Although microbes could use alternative TEAs such as nitrates, sulphates and metallic oxides during substrate metabolism [5]; in practice they are usually found in low quantities in such environment and wash off easily due to their solubility thus resulting into ineffective remediation

* Corresponding author
E-mail address: seunadelaja28@gmail.com (O. Adelaja)

processes aside from high material cost [6].

Recently, microbial fuel cells (MFCs) have attracted significant attention because of their unique electricity generation coupled with the removal of organic contaminants which success is greatly attributed to the presence of an inexhaustible and insoluble electron acceptor (acting as a solid-state anode) compared to soluble electron acceptors used by conventional anaerobic technologies [7]. Hence, MFCs could enhance degradation kinetics and support low biomass yield [8]. Many published works have reported the use of MFC technology for treating a diverse range of substrates [9-11]. Wolin et al.[12] investigated the biodegradation of benzene in MFC using a mixed culture. They reported complete degradation of 10.87mg/L benzene within 22 - 24.5h using potassium ferriccyanide as catholyte. However, the use of potassium ferriccyanide as an electron acceptor in the cathode is not sustainable from environmental impact and cost perspectives. Also, Rakoczy et al. [13] recently demonstrated benzene and sulphide removal from contaminated groundwater in a dual MFC over a period of 770 days. Hitherto, the use of MFCs for degradation of petroleum contaminants such as BTEX compounds has not been explored extensively.

During the degradation of organic contaminants, biotransformation products are formed. Most of these biodegradation products are often acidic metabolites resulting from incomplete biodegradation by pollutant-degrading microorganism [14]. However, some of these biotransformation products may be more toxic than the parent pollutant. Since the essence of bioremediation is to ensure that degradation products are harmless or non-toxic such that they meet regulatory discharge limits, there is a need to perform toxicity assessment of degradation products by using microtox acute toxicity test at the end of MFC operation. The toxicity profiles of degradation products resulting from anaerobic benzene degradation (especially in MFC system) have not been widely characterized in earlier studies.

The nature of microorganism has a pivotal role to play in the effective biodegradation of recalcitrant compounds such as benzene in the environment. Most studies have reported the use of both adapted pure culture, coculture and undefined mixed culture in the anaerobic degradation of benzene in different environments [1, 15, 16]. The interactive effect between these microorganisms and their combination thereof could have a significant impact on MFC performance (both in terms of substrate degradation and power production). In line with the foregoing, two pure strains namely, *Pseudomonas aeruginosa* and *Shewanella oneidensis* have been selected in the present study together with an undefined mixed culture and an adapted mixed culture. *P.aeruginosa* is a benzene-degrading bacteria that produces biosurfactant-which could serve as redox mediators for electron transports to the anode while *S.oneidensis* is an electrochemically-active bacteria that can transfer electrons both directly or indirectly (via the secretion of flavins) to the anode. Mixed cultures have been reported to show good process stability in MFCs due to the presence of versatile metabolic capabilities. Combination of different degrading species (i.e. coculture and undefined mixed culture) could be advantageous as interactions between microbial community could enhance degradation rates and MFC performance (in terms of current generation). These interactions could either exhibit additive, inhibitory or synergistic effects [17]. Such strains or their combinations could serve bioaugmentation purposes in treatment of benzene-contaminated MFC reactors.

However, systematic studies are required to better understand the degradation of benzene using different bacteria strains for future application of MFC technology in the treatment of petroleum hydrocarbons. To the best of authors' knowledge, studies on the influence of microorganism such as pure cultures, undefined mixed culture and adapted mixed culture in MFC systems has hardly been reported. Therefore, the current study focused on the degradation of benzene in MFCs in the presence of different microbial catalysts. The criteria for evaluation of microbial catalysts were based on electrochemical and degradation performance at the end of MFC operations. In this study, it was shown that benzene could be degraded under anaerobic conditions in mfcs using different inoculum type.

2. Material and Methods

2.1. Chemicals

98.0% pure Benzene was procured from Acros (UK), D-glucose, Sodium pyruvate and other reagents used were gotten from Sigma Aldrich (UK). Also, Ficodox Plus™ mixed COD reagent were obtained from Fisher Scientific (Loughborough, UK). All chemicals were of analytical grade and used without further purification**.**

2.2. Biocatalyst and Cell Media

Shewanella oneidensis (MR1 14063) and *Pseudomonas aeruginosa* (NCTC 10662) alongside an undefined anaerobic consortium were used in this study as cell inoculum for MFC operation. The two pure strains were procured from microbial culture collections in our laboratory while the anaerobic digested sludge samples were collected from Mogden Sewage Treatment Works London (UK). For the adapted anaerobic digested sludge, it was taken from a working MFC which had been running in a fed-batch mode for more than six months. *S.oneidensis* was initially grown in

minimal medium supplemented with sodium pyruvate (500mg L^{-1}) and 500mg L^{-1} casein hydrolysate (Sigma Aldrich UK) while *P.aeruginosa* was sub-cultured in minimal medium supplemented with 300 mg L^{-1} D-glucose. The minimal medium consisted of: 8.24 g L^{-1} Na_2HPO_4, 5.08 g L^{-1} NaH_2PO_4, 1.0 g L^{-1} NH_4Cl, 0.5 g L^{-1} NaCl, 0.25 g L^{-1} $MgSO_4$, 12.5mL Wolfe vitamins solution (Adelaja et al., 2014), 12.5mL Trace mineral solution (Wolin et al., 1963). Anaerobic digested sludge samples were also grown anaerobically in minimal medium supplemented with D-glucose at 30°C for 48h. *V.fischeri* for bioluminescence toxicity assays was grown in *oceanibulbus* growth medium (NCIMB growth media catalogue).

2.3. MFC Configuration and Operation

A double-chambered MFC comprising two identical glass bottles with a working volume of 200mL in a chamber was separated by a cation exchange membrane (20cm^2, CMI-7000 Membranes International NJ, USA). The glass bottles were held together by a metallic holder with two rubber gaskets placed in-between to secure a water-tight seal between the glass walls and the membrane. The electrodes (both cathode and anode) were made of carbon paper felt (C-TEX 27; surface density 110g/m^2; surface area 1100m^2/g, Mast Carbon Inc, Basingstoke, UK) with projected surface area of 20cm^2. Both sides of the cathode were loaded with a platinum (Pt) catalyst (0.50mg/cm^2). The catholyte was 100mM phosphate buffer solution (pH 7), aerated continuously with air (at a flow rate of 100mL air/min) using an aquarium pump (KOI Air, UK). An external resistance of 1000Ω was used in all experiments and the potential across the external load was recorded in real-time using LabView data acquisition software (LabView instruments Texas, USA). The MFCs were sterilised by autoclaving at 121°C for 15mins while anolyte additions were done aseptically. The MFCs were seeded with 20mL of actively growing culture at its exponential stage. Anaerobic conditions were maintained in the anode chamber by sparging with 100% Nitrogen gas for 15 mins before the start-up. All experiments were conducted a temperature regulated at 30±0.5°C Stuart 160D incubator (Fisher Scientific, UK).

2.4. Experimental Design

Benzene degradation using different strains and/or bacterial consortia.

The influence of inoculum type on benzene degradation and MFC performance was investigated using *S.oneidensis* (S. O), *P.aeruginosa* (P. A), a coculture of *S.oneidensis* and *P.aeruginosa* (CO), anaerobic digested sludge (MC), anaerobic digested sludge with the coculture (MCO), anaerobic digested sludge with *S.oneidensis* (MCS), anaerobic digested sludge with *P.aeruginosa* (MCP) and lastly adapted anaerobic digested sludge, AMC (Table 1). Each inoculum was 10% v/v of the working volume of the anode (200mL) while the anodic chamber (of 250mL total capacity) had a working volume of 200mL which contains the minimal medium (as described in section 2.2), 100mg L^{-1} cosubstrate (pyruvate or glucose), 200mg L^{-1} benzene and the inoculum (20mL). Also, in each treatment, three controls were employed namely; an abiotic MFC, disconnected MFC and MFC with no benzene present. All experiments were carried out in triplicates. The experiment was carried out in dual chamber MFCs assembled and operated as described in Section 2.4.

Table 1. Summary of biocatalyst (s) used in each experiment.

	Strain used			Inoculum type
Experimental run	*P.aeruginosa*	*S.oneidensis*	Undefined mixed culture	
1	-	+	-	S. O*
2	+	-	-	P. A
3	+	+	-	CO (P. A & S. O)
4	-	-	+	MC
5	+	+	+	MCO
6	-	+	+	MCS
7	+	-	+	MCP
8	-	-	+[a]	AMC
9	-	-	-	Abiotic control

*Glucose (100ppm) was used as co-substrate in all experiments except for *S.oneidensis* (S.0) where pyruvate (100ppm) was used in lieu of glucose. [a] Adapted mixed culture.+ means present, - means absent

2.5. Toxicity Assay for Biodegradation Products

Toxicity assays were performed according to the Microtox standard acute toxicity testing procedure. Toxicity assessments were conducted for both pre- and post- treatment MFC operations at 200ppm benzene concentration. All samples analysed were centrifuged at 13.2 *x g*, filtered through 0.22 µm PTFE filters to remove suspended biomass. Exactly 2% NaCl was added to all samples prior to the test procedure for osmotic adjustment of samples. The luminescent intensity measurements of samples were taken using Fluostar Optima (BMG Labtech, Ortenburg, Germany) luminometer. The sample incubation temperature was set to

25°C and samples incubated for 15mins prior measurement. The half-maximal effective concentration, EC_{50} (indicating the concentration at which a 50% reduction in luminescent intensity is observed) was expressed as a COD equivalent of the analysed samples.

2.6. Analytical Methods

2.6.1. Benzene Degradation: Extraction and HPLC Analysis

Anolyte samples containing benzene were analysed by high-performance liquid chromatography (HPLC, Dionex GS50), USA) using a Photo-diode Array (PDA) detector (DIONEX, PDA-100) at 254nm. The conditions of HPLC operated for analysis of benzene degradation taken from the anode chamber were similar as earlier reported by [11]. The analytical column was a reversed phase column, Supelcosil™ LC-PAH column (150mm × 4.6mm) using acetonitrile. The benzene concentrations in the gaseous phase were calculated with Henry's law using the constants at 25°C of 0.25 for benzene [6]. For accurate quantification of total benzene used up, the amount of benzene present in the electrode was determined by soaking the anode electrodes in 10mL methanol at the end of each experiment and subsequently placed on a shaker for 1h at 200rpm. Aliquots were transferred into 2ml eppendorf, immediately followed by centrifugation at 13.2 *xg* for 10mins. Degradation efficiencies and rates were determined based on the remaining benzene in solution and that adsorbed on the electrode at the end of MFC operation.

2.6.2. COD Removal Measurement During MFC Operation

The chemical oxygen demand (COD) of the samples was determined using the closed reflux titrimetric method described by Environment Agency (UK) Standard method 5220D [18]. Appropriately diluted 1mL samples were used for each determination.

The COD of samples was expressed as percentage COD removal. The percentage COD removal was calculated as follows:

$$\text{Percentage COD removal (\%)} = \frac{\text{COD}_\text{i} - \text{COD}_\text{f}}{\text{COD}_\text{i}} \text{X } 100 \quad (1)$$

where COD_i and COD_f are initial COD and final COD values respectively.

2.6.3. Electrochemical Monitoring

The efficiency of the MFCs for all inoculum types was determined based on voltage and current outputs. In order to obtain the polarization curve, the external resistance was changed from 10Ω to 1MΩ. Maximum power density and internal resistance of MFCs were obtained using the power density curve method. The current flowing through each external load and power produced were calculated as described by [18]. Power density (Wm^{-2}) and current density (Am^{-2}) were normalized to the projected total surface area of the anode (40 cm^2). Coulombic efficiency (CE) was calculated as $CE = \int I\,dt / C_t \times 100\%$, where $\int I\,dt$ is the coulombs calculated by integrating the current over time, C_t (C) is the theoretical amount of coulombs that is available from COD, which was calculated as $C_t = FbV\Delta COD/M$, where F is the Faraday's constant, b is the number of moles of electrons produced per mol of substrate (b = 4), V (L) is the liquid volume, ΔCOD (mg/L) is the change in COD concentration, and M is the molecular weight of the substrate (M=32).

2.7. Statistical Analysis

All data are presented as means of triplicate experiments unless otherwise stated and the error bars represent the standard deviation of the mean (SD). One - way analysis of variance (ANOVA) was used to compare MFC performances and degradation rates among all treatments. Statistical significance of differences was determined by one-way analysis of variance using Prism Graph Pad 5.0 ($P < 0.05$ was considered statistically significant). Correlation analyses were also conducted (using Prism Graph Pad 5.0) to measure the degree of association between data collected.

3. Results and Discussion

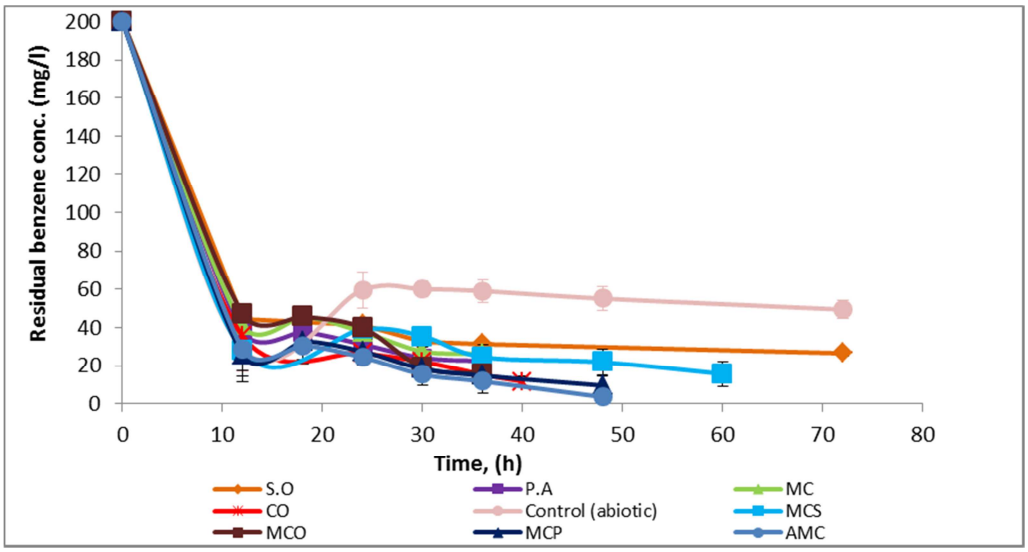
3.1. Benzene Biodegradation During MFC Operation

3.1.1. Effect of Inoculum Type on Benzene Degradation

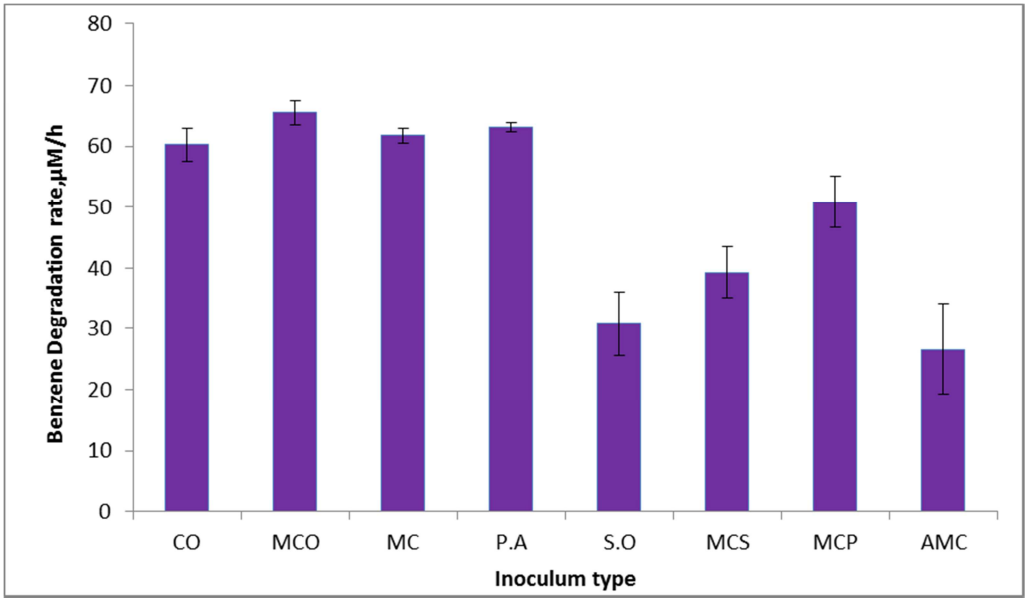
Benzene removal during MFC operation involves two processes namely; adsorption and microbial degradation. The first step is adsorption (which accounts for rapid disappearance of benzene in aqueous phase) followed by microbial degradation. Notably, a spike in benzene concentration in aqueous phase was observed few hours (*ca.* 10-12hrs) after start up (Figure 1A). The observed spike in benzene concentration, as apparent for all microbial consortia tested, could be attributed to the fact that desorption rates of adsorbed benzene were higher than degradation rates in the course of the whole biodegradation process [19]. In Figure 1, the degradation rates and removal of benzene during MFC operation are represented. All the seven inocula showed very high potential for benzene removal with minimum degradation efficiency of 86% compared to the controls (Figure 2). This suggests a better degradation performance using MFCs compared to other anaerobic conventional systems. Enhanced anaerobic oxidation of benzene was achieved with the anode serving as electron acceptor, hence

making the metabolic process more favorable thermodynamically for the microorganism to meet their energy requirements for cell synthesis and maintenance.

Furthermore, there is a statistical significant difference (p<0.05) in the degradation performances of different inocula tested which suggests benzene degradation in MFCs is a function of inoculum type. In this present study, AMC gave the highest degradation rate of 64µM/h with *S.oneidensis* giving the lowest degradation rate of 31µM/h. This indicates that all the inocula tested may possess the aromatic hydrocarbon degrading enzymes (though at comparatively varying degree of enzymatic activity) which facilitated the cometabolic degradation of the recalcitrant compound under anaerobic conditions. Degradation rates recorded in this study are relatively higher than those reported in the literature where other alternative electron acceptors (such as nitrates, sulphates, etc.) were used to enhance anaerobic degradation of benzene in different contaminated environments [15, 16, 20]. Cervantes et al. [21] reported the degradation rates of 2.78 x10^{-3}mM/h (0.116µM/d) for benzene anaerobic degradation using a humic model compound, anthraquinone-2, 6-disulfonate (AQDS), as terminal electron acceptor in a contaminated soil environment. In another studies, Chakraborty et al.[22] demonstrated anaerobic degradation of BTEX by *Dechloromonas* strain RCB using different TEAs. They reported degradation rates of 6µM/d which also far lower than that (using MFC system) reported in our study.



A



B

Figure 1. (A) Benzene concentration in the bulk anode solution as a function of time and (B) benzene (average) degradation rates by different inocula used in Experiment 1. The error bars represent the SD of the mean.

Anaerobic degradation of benzene in MFC generally involves extracellular transfer electrons resulting from microbial oxidation of the substrate to an anode (an insoluble TEA). Extracellular electron transfer by anode respiring bacteria could be either by direct use of pilus-like assemblages known as *nanowires*, which have been reported found in *Geobacter spp* and *S. oneidensis* or electron transfer mediated by the use of soluble redox electron shuttles - indirect electron transfer [8, 23]. *P.aeruginosa* and *S. oneidensis* have been widely reported in the literature to secrete phycocyanin and flavin-like molecules respectively [24]. Adapted-anaerobic mixed cultures also have been reported to contain diverse anodophilic microorganisms that could mediate electrons transfer vis-a-vis substrate oxidation.

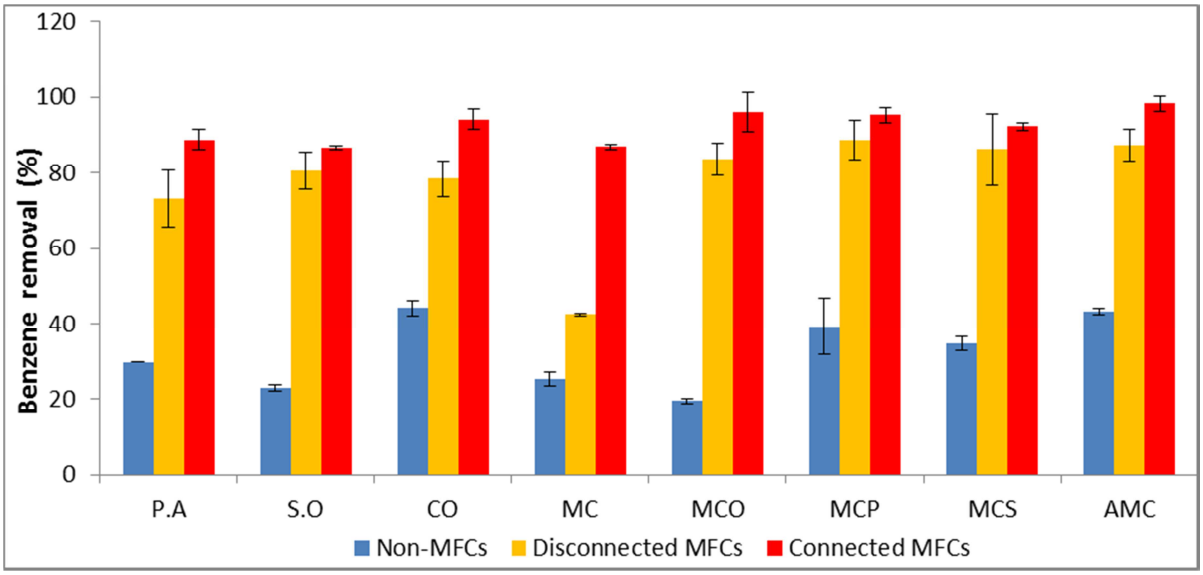


Figure 2. Degradation efficiencies for benzene removal by different inoculum types.

Both mixed culture and axenic culture that possess benzene degrading enzymes have been reported extensively in the literature [15, 16, 21, 25]. Pure strains of *Pseudomonas species* have been reported by some authors to have a potential to degrade aromatic hydrocarbons – including BTEX compounds [25-27]. *S.oneidensis* MR1 used in this study may also possess benzene degrading enzymes that enabled cometabolic biodegradation of the hydrocarbon pollutant under anaerobic conditions. To the best of authors' knowledge, this is the very first report that demonstrated that pure strains such as *P.aeruginosa* and *S.oneidensis* can oxidise benzene in a microbial fuel cell. In a study conducted by Coates et al.[28] on the enhanced anaerobic degradation of diesel using MFCs, *S.oneidensis* was found among electrochemically active bacteria present in the anode of the MFCs as revealed by phylogenetic analysis. Similarly, Cervantes et al. [21] reportedly found *Shewanella spp.* among facultative genera present in benzene contaminated sediments. Thus, this suggests *S.oneidensis* could, in principle, potentially degrade petroleum hydrocarbons coupled with concomitant electricity generation via the anode. This proposition has been supported by findings reported in this study.

Mechanism of anaerobic degradation of benzene is unclear. Anaerobic biodegradation of benzene have been reported previously to undergo several possible mechanisms such as benzene methylation, carboxylation, alkyation, reduction, or hydroxylation reactions followed by ring cleavage. Whichever the case, they are all channeled through a central intermediate metabolic pathway called the benzoate metabolic pathway in the presence of benzoyl-CoA enzymes [2]. In this study, some acidic intermediates were formed as indicated by decreased in measured pH (from 7.0 to 5.6) during benzene degradation for all inocula tested. However, identification of these degradation products with the hope of identifying metabolic pathway for benzene degradation is difficult owing to the presence of co-substrate, nature and range of inocula examined in this present study. Nevertheless, further work is ongoing to identify these metabolites.

Bioaugumentation is one of the bioremediation techniques employed in the enhanced biodegradation of contaminated sites where natural attenuation are too slow and proves ineffective [17]. However, the success of these techniques relies solely on the adaptability of the bioaugmented species to the environmental conditions that exists in the site of application. Moreover, in this study, all the seven inocula gave different degradation rates and efficiencies suggesting microbial interactions existing among them could be additive, synergistic or inhibitory as represented in Figure 1&2. Among the range of inocula tested, the bacteria

consortia-AMC happens to show the best degradation performance. Therefore, our findings suggest AMC as a possible choice of strain for bioremediation of benzene in contaminated environments.

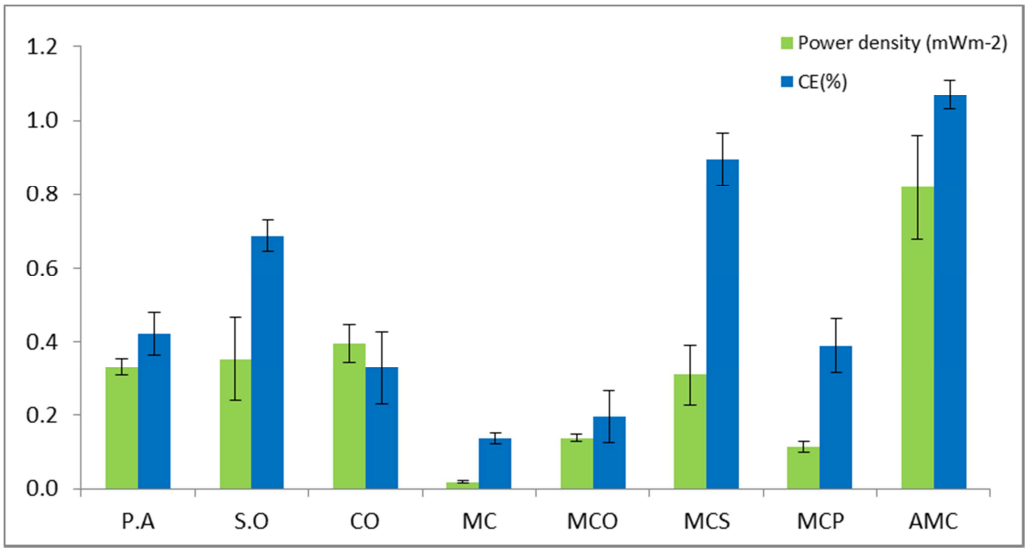
3.1.2. Influence of Adsorption Process on Benzene Degradation in a Bioelectrochemical Reactor

In this study, biodegradation of benzene in a microbial fuel cell was investigated using different inocula. Rapid disappearance of benzene from the aqueous solution was observed as shown in Figure 1A. The reason for this could be attributed to the adsorption of significant amount of benzene by the anode. As previously mentioned, the anode was made up of a carbon-felt paper which is a common type of carbon material widely used in MFC study. Carbon materials (e.g. carbon-felt used as anode) have been reported previously to possess high adsorption capacity and this could be probably due to the nature of material and its specific surface area [6, 18]. On the other hand, the model hydrocarbon (benzene) used in this study is generally classified as hydrophobic compounds and therefore exhibits high affinity to solid materials [18]. Nevertheless, electrochemically active microorganisms could still utilize adsorbed benzene on the anode. The findings reported in the present work is also in agreement with Zhang et al. [2] who also demonstrated that adsorbed benzene on the electrode were subsequently metabolized by microorganisms.

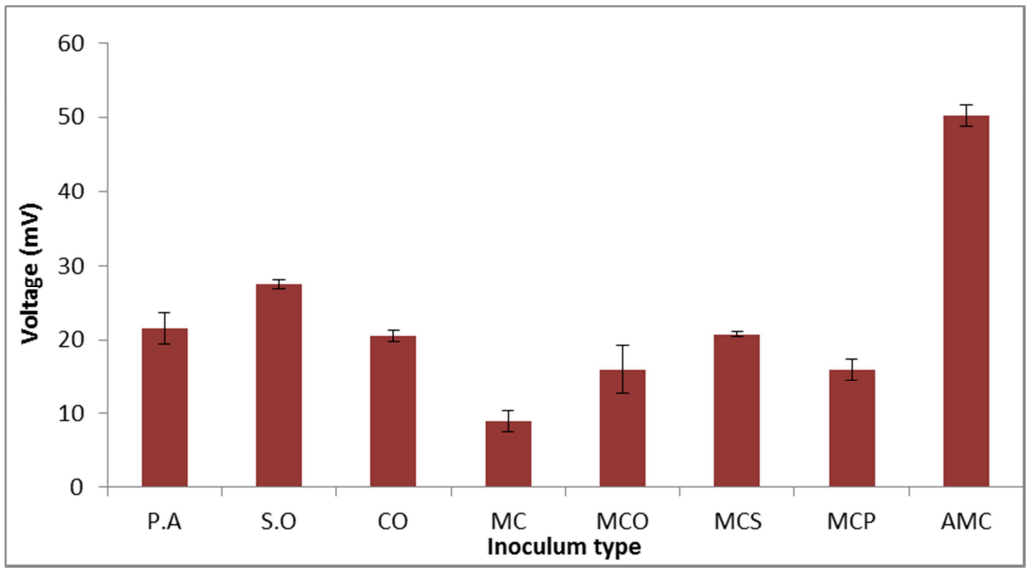
Possible mechanisms for microbial metabolism of the adsorbed benzene could be mediated by the presence of anode-respiring bacteria or exoelectrogenic microorganisms. Benzene adsorption on the anode probably had no detrimental effect on electron transfer to the anode since degradation efficiency was better in MFCs than non-MFCs (anaerobic control) as shown in Figure 2. Benzene adsorption onto the electrode thus enhances the removal efficiency in general, assuming the adsorbed benzene is being degraded as postulated in this study [26]. Therefore, it is suggested that the presence of such carbon-like electrode (acting as electron acceptors) would further enhance removal of petroleum hydrocarbons pollutants from contaminated site, especially in sub-surface environments where accessibility of microorganisms to contaminants poses one of the major challenges in the deployment of conventional remediation technologies.

3.2. Electrochemical Performances of Different Inoculum Type in MFCs

As shown in Figure 3, there is a marked variation in the electrochemical performances exhibited by different inocula which is a function of inoculum type. The bacteria consortia - AMC gave the highest power density of 0.82mW/m^2 which is 40-fold compared to MC (0.02mW/m^2). In terms of maximum power densities, there is no significant difference among SO, PA and MCO ($p>0.05$). High power density recorded for AMC might probably be due to the combined effect of presence of both electrogenic and benzene-degrading bacteria which could have enhanced MFC performance as reported. Second to AMC in terms of power density is CO which shows a higher electrochemical performance than other inoculum types. This reason for CO's performance might be due to synergistic interactions that exist between PA and SO. PA and SO have reported to be an electrochemically active microorganism [8]. PA excretes redox molecules such like phenazines and phycocyanin which act as electron shuttling agent by transporting electrons across the cell membrane to the anode while SO produces flavin-like molecules. Increase in concentrations of such redox mediators could have facilitated high electron transfer rates which have reflected in high peak power density produced as observed in this study for the co-culture (CO).



A



B

Figure 3. (A) Maximum power densities, coulombic efficiencies and (B) Peak voltage outputs for co-metabolic degradation of benzene for different inocula during MFC operation. The error bars represent the SD of the mean.

Interestingly, a good electrochemical performance might not necessarily translate to better degradation efficiency or rate under certain conditions. In this study, we observed that there is disparity between biodegradation rates and power generation for different inocula tested in the MFCs. Many factors could be responsible for this; one of the critical factors influencing system performance could be electrochemical capability of the microorganism. Andrew et al. [29] reported similar observation where relationship between power density and degradation rates using different substrates were investigated. Coulombic efficiency recorded for all inocula tested were generally low (within 0.14-1.04%). The observed low coulombic efficiency may be due to ingression of oxygen into the anode, soluble substrate loss due its diffusion into the cathode (in case of dual MFCs), incomplete substrate oxidation or presence of other alternative electron acceptors such as sulfates (0.25 g L^{-1} $MgSO_4$ was used in this study) which make up the anolyte medium [7]. The nature of microorganisms or their consortia (either pure or mixed culture) may also contribute to the low efficiency observed in the study. The presence of non-exoelectrogenic species such as fermentative bacteria and methanogens (e.g. in mixed consortia) could be a limiting factor to coulombic efficiency yield [30].

3.3. Assessment of MFC Performance for Benzene Degradation

In this study, MFC performances of all inocula were assessed based on their degradation performances and electrochemical performances. The system performance index, K is calculated as;

$$K = xyz /1000 \quad (2)$$

where x, y and z represents degradation rate (µM/h), maximum power density (mW/m^2) and COD removal (%) respectively. A high K value is an indication good system performance.

As indicated in Table 2, bacteria consortia - AMC was found to be the best performing inocula with MC being the least in order of overall system performance. Findings from this study recommend the use of AMC for bioaugmentation purposes in the remediation of benzene-contaminated sites based on MFC technology.

Table 2. Evaluation of system performances by different inocula based on three main parameters.

Inoculum type	Degradation rate, x (µM/h)	Max. power density, P_{max}, y (mW/m^2)	COD removal, z (%)	System performance index, K = xyz/1000
S.0	31.85±5.20	0.33±0.02	63.05±0.42	0.65
P. A	63.12±0.70	0.35±0.01	63.38±3.69	1.40
CO (P. A&S. O)	60.29±2.70	0.39±0.05	61.76±1.56	1.45
MC	62.71±1.20	0.02±0.01	66.67±7.07	0.08
MCO	66.54±2.10	0.14±0.02	70.00±1.69	0.65
MCS	39.30±4.20	0.31±0.08	26.32±0.71	0.32
MCP	51.82±7.30	0.12±0.01	50.00±0.74	0.31
AMC	64.62±7.20	0.82±0.14	87.25±2.21	4.58

3.4. Toxicity Reduction During MFC Operation

Microbial degradation of pollutants often results in incomplete mineralization and hence the formation of degradation products with unknown chemical and toxicological characteristics. In this study, toxicity assays were conducted for both pre-treated and treated liquid samples at 200ppm benzene concentration (Figure 4). Result indicates that degradation products are less toxic than the parent pollutant at the benzene concentration tested. The half maximal luminescence inhibition value (EC_{50}) for the treated and pre-treated samples were 149.4 mg COD/L and 50.3 mg COD/L respectively. The EC_{50} values for treated sample is three-fold higher than the pre-treated sample suggesting a significantly ($p< 0.05$) lower cytotoxicity effect based on bioluminescent organism used (*V.Fischeri*). Increase in EC_{50} values is an indication of decreasing toxicity compared relatively with the pre-treatment samples. Result suggests the possible formation of degradation products mixtures of lower molecular weight compounds like volatile fatty acids or other simpler acid metabolites which have may have lower toxic effects than the parent pollutant - benzene [31]. Proof of some acid metabolites present in samples was further supported by decrease in pH of samples compared to pre-treatment samples (i.e. from pH 7 to 5.6). The findings of this study further reinforces the evidence reported in the previous sections about the effective degradation of benzene-contaminated synthetic wastewater using MFC as it is the ultimate goal of any remediation strategy is to convert toxic contaminants into less toxic or non-toxic forms [32].

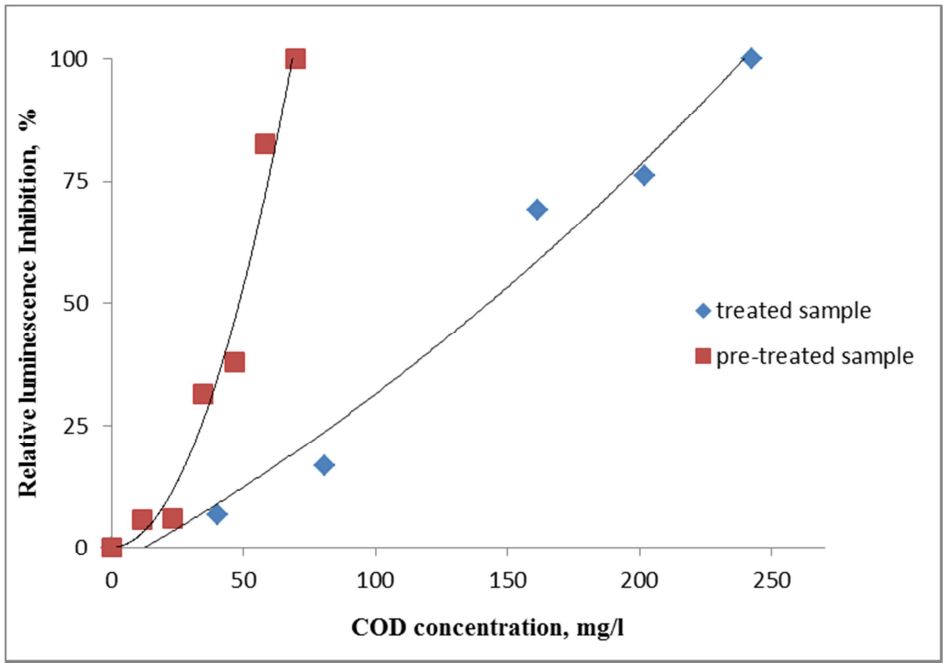


Figure 4. *Vibrio fischeri* bioluminescence based toxicity determinations of pre-treated and treated samples at 200ppm benzene concentration.

4. Conclusion

This study demonstrated the feasibility of using MFC technology in the co-metabolic degradation of a model BTEX compound with concomitant electricity production in the presence of different inoculum type as microbial catalyst. The bacteria consortia- AMC was the best performing inocula. The culture gave a benzene degradation rate, maximum power density and COD removal of 64µM/h, 0.82mW/m^2 and 87.3% respectively. Hence, these findings suggest its potential use for bioaugmentation purposes in MFCs. The EC_{50} values, based on *Vibrio fischeri* ecotoxicity testing, for post treatment is three-fold higher than the pre-treatment suggesting a lower cytotoxicity effect. This work suggests MFCs could potentially be employed as an efficient system for treatment of benzene-contaminated subsurface environments or wastewater. Finally, considering that only the effect of inoculum type was performed in this work, more investigation on benzene degradation in MFC at different environmental conditions are necessary to assess the robustness of the MFC system.

References

[1] Li Hui, Qian Zhang, Xiao-Li Wang, Xing-Yuan Ma, Kuang-Fei Lin, Yong-Di Liu, Ji-Dong Gu, Shu-Guang Lu, Lei Shi, Qiang Lu, Ting-Ting Shen (2012) Biodegradation of benzene homologues in contaminated sediment of the East China Sea. *Bioresource Technology* 124:129–136.

[2] Coates J D., Chakraborty R., McInerney M. J. (2002) Anaerobic benzene biodegradation—a new era. *Research in Microbiology* 153:621–628.

[3] Suflita J. M, Caldwell M. E. (2000) Detection of phenol and benzoate as intermediates of anaerobic benzene biodegradation under different terminal electron-accepting conditions, *Environ. Sci. Technol*. 34:1216–1220.

[4] Rico-Martínez Roberto, Terry W. Snell, Tonya L. Shearer. (2013). Synergistic toxicity of Macondo crude oil and dispersant Corexit 9500A® to the Brachionus plicatilis species complex (Rotifera) *Environmental Pollution*, 173:5–10.

[5] Yan, Z., Song, N., Cai, H., Tay, J.-H., Jiang, H. (2012). Enhanced degradation of phenanthrene and pyrene in freshwater sediments by combined employment of sediment microbial fuel cell and amorphous ferric hydroxide. *Journal of Hazardous Materials,* 199–200 (0):217-225.

[6] Zhang, T., Gannon, S. M., Nevin, K. P., Franks, A. E., Lovley, D. R. (2010). Stimulating the anaerobic degradation of aromatic hydrocarbons in contaminated sediments by providing an electrode as the electron acceptor. *Environmental Microbiology,* 12 (4):1011-1020.

[7] Morris, J. M., Jin, S. (2012). Enhanced biodegradation of hydrocarbon-contaminated sediments using microbial fuel cells. *Journal of Hazardous Materials, 213–214* (0):474-477.

[8] Hawkes, F. R., Kim, J., Kyazze, G., Premier, G. C, Guwy, A. (2010). Feedstocks for BES conversions. *In:* Rabaey, K., Angenent, L. T., Shroeder, U., and Keller, J. *Biolectrochemical systems: From extracellular electron transfer to biotechnological application.* London: IWA Publishing, pp- 369-388.

[9] Liu, H., Logan, B. E. (2004). Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. *Env. Sci. Tech.* 38:4040–4046.

[10] Patil, S. A., Surakasi, V. P., Koul, S., Ijmulwar, S., Vivek, A., Shouche, Y. S., Kapadnis, B. P. (2009). Electricity generation using chocolate industry wastewater and its treatment in activated sludge based microbial fuel cell and analysis of developed microbial community in the anode chamber. *Bioresour. Technol.* 100:5132–5139.

[11] Adelaja O. A, Keshavarz, T., Kyazze, G. (2014). Enhanced biodegradation of phenanthrene using different inoculum types in a microbial fuel cell *Eng. in Life Sci., 14* (2):1-11.

[12] Wolin, E. A., Wolin, M. J., Wolfe, R. S., (1963). Formation of methane by bacterial extracts. *Journal of Biological Chemistry* 288:2882-2886.

[13] Rakoczy, J., Feisthauer, S., Wasmund, K., Bombach, P., Neu, T. R., Vogt, C. and Richnow, H. H. (2013), Benzene and sulfide removal from groundwater treated in a microbial fuel cell. Biotechnol. Bioeng., 110:3104–3113.

[14] Foght, J. (2008). Anaerobic Biodegradation of Aromatic Hydrocarbons: Pathways and Prospects. *Journal of Molecular Microbiology and Biotechnology,* 15 (2-3):93-120.

[15] Dou Junfeng, Xiang Liu, Zhifeng Hu, Dong Deng (2008). Anaerobic BTEX biodegradation linked to nitrate and sulfate reduction. *Journal of Hazardous Materials* 151:720–729.

[16] Meckenstock Rainer U. Michael K. Jahn, Stefan B. Haderlein. (2005). Anaerobic Degradation of Benzene, Toluene, Ethylbenzene, and *o*-Xylene in Sediment-Free Iron-Reducing Enrichment Cultures. *Applied and Environmental Microbiology*, l71 (6) 3355–3358.

[17] Thompson, I. P., Van Der Gast, C. J., Ciric, L., Singer, A. C. (2005). Bioaugmentation for bioremediation: the challenge of strain selection. *Environmental Microbiology, 7* (7):909-915.

[18] APHA (1997) Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association, Washington DC. [Online] Available at: (http://www.norweco.com/html/lab/test_methods/5220bfp.htm) Accessed on Febuary 4, 2013.

[19] Xia, X., Li, Y., Zhou, Z., Feng, C. (2010). Bioavailability of adsorbed phenanthrene by black carbon and multi-walled carbon nanotubes to Agrobacterium. *Chemosphere, 78* (11):1329-1336.

[20] Schreiber M. E. Bahr J. M. (2002). Nitrate-enhanced bioremediation of BTEX contaminated groundwater: parameter estimation from natural-gradient tracer experiments. *J. Contam. Hydrol.* 55:29–56.

[21] Cervantes Francisco J., Ana Rosa Mancilla, E. Emilia Ríos-del Toroa, Ángel G. Alpuche Solís, Lilia Montoya-Lorenzana. (2011) Anaerobic degradation of benzene by enriched consortia with humic acids as terminal electron acceptors. *Journal of Hazardous Materials* 195 (0): 201– 207.

[22] Chakraborty R., Susan M. O., Emily C., Coates J. D. (2005) Anaerobic Degradation of Benzene, Toluene, Ethylbenzene, and Xylene Compounds by *Dechloromonas* Strain RCB. *Appl. & Environ. Microbiol.*, 71 (12): 8649–8655.

[23] Rozendal, R., Hamelers, H. V. M., Rabaey, K., Keller, J., Buisman, C. J. N. (2008) Towards practical implementation of bioelectrochemical wastewater treatment. *Trends in Biotechnol.,* 26: 450-459.

[24] Rabaey, K., Boon, N., Höfte, M., Verstraete, W. (2005). Microbial Phenazine Production Enhances Electron Transfer in Biofuel Cells. *Environmental Science & Technology, 39* (9):3401-3408.

[25] Brusa, T., Borin, S., Ferrari, F., Sorlini, C., Corselli, C., Daffonchio, D., (2001). Aromatic hydrocarbon degradation patterns and catechol 2,3-dioxygenase genes in microbial cultures from deep anoxic hypersaline lakes in the eastern Mediterranean sea. *Microbiol. Res*. 156:49–58.

[26] Ridgeway, H. F., Safarik, J., Phipps, D., Carl, P., Clark, D. (1990) Identification and catabolic activity of well-derived gasoline-degrading bacteria and a contaminated aquifer. Appl. Environ. Microbiol. 56:3565–3575.

[27] Sahar S., Zeinab B., Ghasem D. N., Esmaeel K., Hassan A. R. (2010) Biodegradation of Toluene and Xylene in an UAPB bioreactor with fixed film of *Pseudomonas putida*. American-Eurasain J. Agric. & Environ. Sci., 9 (1); 1-7.

[28] Coates J. D., R. T. Anderson, J. C. Woodward. (1996) Anaerobic hydrocarbon degradation in petroleum contaminated harbor sediments under sulfate reducing and artificially imposed iron-reducing conditions. *Environ. Sci. Technol*. 30:2784–2789.

[29] Andrew J. Schuler. (2010) Ultraviolet Treatment and Biodegradation of Dibenzothiophene: Identification and Toxicity of Products. *Environ Toxicol Chem*. 29 (11):2409–2416.

[30] Ball H. A., Reinhard M., (1996) Monoaromatic hydrocarbon transformation under anaerobic conditions at Seal Beach, California: laboratory studies, *Environ. Toxicol. Chem.* 15:114–122.

[31] Dalton, H., Stirling, D. I., (1982). Co-metabolism. Philosophical Transactions of the Royal Society 297:481-496.

[32] Xiong Wenhui, Chris Mathies, Kris Bradshaw, Trevor Carlson, Kimberley Tang, Yi Wang (2012) Benzene removal by a novel modification of enhanced anaerobic biostimulation, *Water Research*, 46 (15) 4721-4731.