



Decolourization and mineralization of Acid Red 27 metabolites by using multiple zoned aerobic and anaerobic constructed wetland reactor

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ABSTRACT

Acid Red 27 (AR27) is widely used in paper processing, textiles, foods, cosmetics, beverages and medicines. Effluents containing AR27 are discharged daily into the environment which can adversely affect human and animals as AR27 is classified as an endocrine disruptor. This study describes the application of an aerobic and anaerobic baffled constructed wetland (ABCW) reactor in the removal of AR27 from a solution of synthetic wastewater with 50 mg/L AR27 concentration. The ABCW reactor planted with *Phragmites australis* was set with intermittent aeration and 1 d hydraulic retention time. This system's performance was evaluated in terms of COD, NH_4^+ , colour and mineralization of intermediates with and without aeration at different concentrations. Based on the performance and analysis of GCMS, FTIR, HPLC a degradation pathway for AR27 was proposed. The system achieved COD removal of 88%, NH_4^+ removal of 98%, complete decolourization and mineralization. FTIR analysis showed that provision of intermittent aeration contributed to the removal sulphonic groups and aromatic amines. The performance of the ABCW is attributed to the long pathway travelled by the wastewater which increased contact time of pollutants with microbes and rhizomes; and the synergistic effect of the combination of rhizomes, microbes and supplementary aeration.

Keywords: Constructed wetland; Azo dye; Aerobic and anaerobic baffled constructed wetland reactor; Synthetic wastewater; Intermittent aeration

1. Introduction

The azo dyes are comprised of a class of synthetic organic compounds that are characterized by the presence of one or more $-\text{N}=\text{N}-$ chromophoric groups conjugated to an aromatic system. Azo dyes are widely used in textile dyeing, paper printing, colour photography, pharmaceuticals and cosmetics [1]. The usage of azo dye in various industries makes azo dye the most used dyes in industries, hence azo

dyes are the largest group of dyes that are released into the environment [2]. There are more than 100,000 commercially available dyes with over 7×10^7 tons of dyestuff produced annually worldwide [3]. Industrial dyeing processes led to the annual discharge of approximately 30,000 to 150,000 tons of dyes into receiving waters, which may be toxic and carcinogenic to living beings [4].

Acid Red 27 (AR27) also known as amaranth dye is a naphthylazo, sulfonic acid based dye which is used to give a red to purple coloration to food stuffs, cosmetics and

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medicines. This dye was banned in 1976 by the US Food and Drug Administration due to its carcinogenic nature [5]. Nevertheless, AR27 is still used as textile dyes for wool, silk, photography and as food dye for caviars, sweets and beverages. Besides that, during the applying process of AR27 to paper, phenol, leather and formaldehyde resins, excess dye would enter into wastewater [6]. Colour in wastewater affects visibility, aesthetics, transparency and gas solubility in water bodies. Moreover, dyes in wastewater reduces penetration of light into water which affects the photosynthetic activity of aquatic plants [7]. Besides that, metabolic cleavage of azo linkage leads to toxic by-products such as aromatic amines [8].

Azo dyes possess a complex structure and synthetic nature which makes them difficult to degrade. Generally, biodegradation of azo dyes require sequential anaerobic and aerobic processes [9]. This is due to the need for aerobic processes to mineralize aromatic amines residues from anaerobic decolourization which resists anaerobic degradation. Aerobic processes are able to remove organic matter but reveals poor colour and nutrient removals whereas; anaerobic processes prove to be great in colour removal but not in organic matter and nutrients [10]. Hence, the development of the aerobic and anaerobic constructed wetland reactor which provides multiple zones of aerobic, anoxic and anaerobic conditions within the system. There were no studies reporting the removal of AR27 using constructed wetland and very limited studies on the removal of azo dyes in general using different types of constructed wetlands [11–13].

In terms of constructed wetlands, the conventional types are vertical and horizontal flow constructed wetlands. The vertical flow constructed wetlands possess a higher oxygen transport capacity which allows ammonia nitrogen to be removed effectively. However, limited denitrification occurs in such a system. Horizontal flow constructed wetland demonstrated insufficient oxygenation of the rhizosphere in previously conducted field measurements which lead to incomplete nitrification and limited nitrogen removal [14]. Hence, the use of one of these constructed wetlands for nitrogen or azo dye removal would not be very efficient due to their inability to provide both aerobic and anaerobic conditions simultaneously. These type of constructed wetlands are usually combined to provide a high removal efficiency especially for nitrogen removal [15]. In this study, both aerobic and anaerobic conditions as well as anoxic conditions were incorporated into a single system by inserting vertical baffles in a horizontal constructed wetland which would allow an upward and downward flow. The bottom region of the reactor is in anaerobic condition while the top is in aerobic condition. Therefore the pollutants would pass through a series of anaerobic and aerobic treatment due to the multiple zoned constructed wetland, to achieve higher removal efficiencies in both nitrogen and azo dye removal.

In light of the above observation, the objective of this study is to evaluate the performance and effectiveness of the Aerobic and Anaerobic Baffled Constructed Wetland (ABCW) reactor in terms of organic compounds, nutrients and colour removal from wastewater containing azo dye. Besides that, this study was also conducted to further

explore decolourization and degradation of AR27 and to propose a degradation pathway.

2. Material and methods

2.1 Reactor setup

The Aerobic-anaerobic Baffled Constructed Wetland (ABCW) reactor as shown in Fig. 1 was fabricated using acrylic plates. The design of the ABCW reactor consisted of 5 planted compartments of dimensions 30 cm × 80 cm × 30 cm with the use of 1 cm thick acrylic plates. *Phragmites australis* also known as common reed which was obtained from Tasik Melati, Perlis. The plants were planted at the middle section of the ABCW just above the supplementary aeration. The root zone was placed approximately 15 cm deep from the water table. Water table starts slightly above the gravel surface as stated in previous study [16].

In order to control the aerobic and anaerobic zones, the first, third and fifth compartment of the ABCW reactor was provided with supplementary aeration. This supplementary aeration was provided intermittently at 3 h interval where the aeration was switched on for 3 h and off for the following 3 h (continuous cycle). Besides controlling the aerobic and anaerobic zones, the provision of aeration intermittently at 3 h interval was also conducted to conserve energy. This interval was selected because the system has been previously tested without any supplementary aeration, and the main aim of the ABCW which is to provide multiple aerobic, and anaerobic zones could not be achieved as the entire system was in an anaerobic state. Moreover, this system was also tested with provision of continuous aeration for 24 h which caused the entire system to be in an aerobic state. (Data not shown). With the 3 h intermittent aeration, the aerobic and anaerobic zones throughout the reactor was controlled. This aeration time fulfilled the purpose of this reactor, which is to provide multiple aerobic and anaerobic zones for decolourization and mineralization purposes. Since the aeration was supplied intermittently, energy saving could also be achieved.

After the reactor was planted and filled with gravel up to 30 cm from the bottom, the void volume was measured. The working volume of ABCW (including wetland media) is 72 L. Approximately 30 L of wastewater is treated every day since the void volume of the ABCW is 29.63 L. Loading rate was an average of 326 mg/L, 352 mg/L and 386 mg/L for before dye addition, 25 mg/L AR27, and 50 mg/L AR27 concentration respectively. Other reactor characteristics are as stated in Table 1.

2.2. Seeding

The gravel was separated according to diameter size of 1 cm before washing, drying and immersing in activated sludge. Activated sludge was retrieved from a glove company, Shorubber (M) Sdn. Bhd. at Jejawi, Perlis. Seeding process was divided into aerobic and anaerobic conditions. To obtain the desired conditions, the following measures were taken: (1) Aerobic condition was prepared by supplying aeration in an open container, containing 150 kg of gravel, and (2) Anaerobic condition was done in a closed container with the equal amount of gravel. Both the containers were

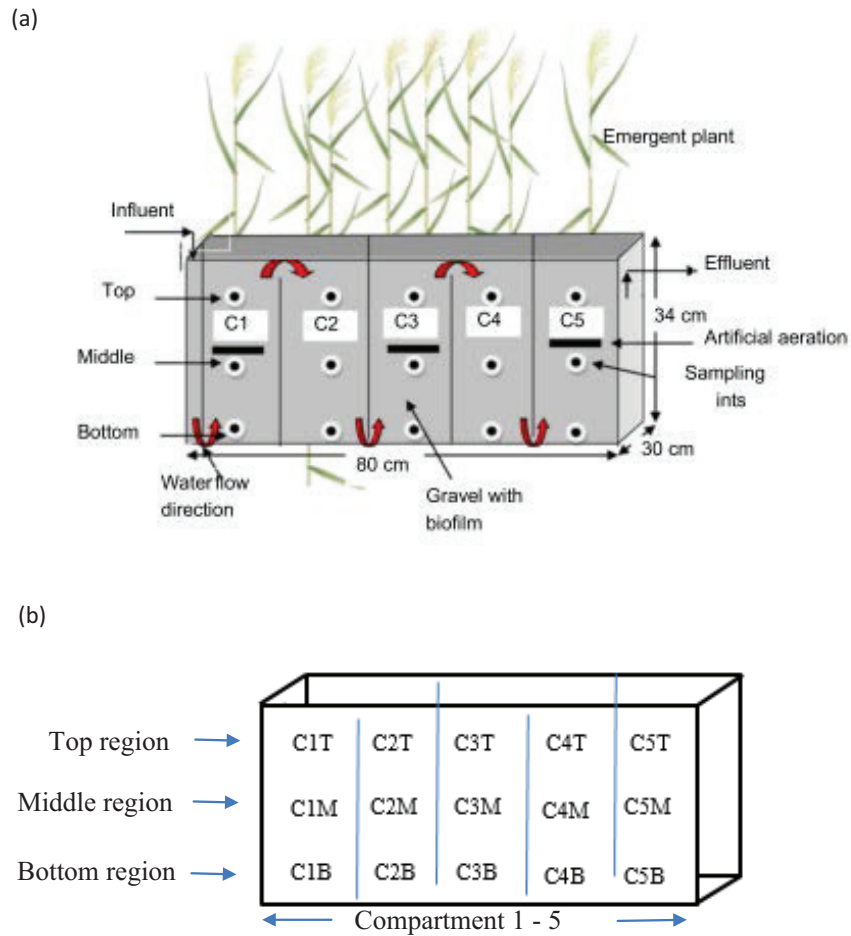


Fig. 1. Aerobic-anaerobic baffled constructed wetland reactor.

Table 1
Reactor characteristics

Reactor characteristics	Specifications
Height of gravel bed	30 cm
Volume of gravel bed	72 L
Average gravel diameter	1 cm
Average gravel bed porosity	37%
Average void volume	29.63 L
Hydraulic Retention Time (HRT)	1 day
Average flow rate	29 mL min ⁻¹
Temperature	28°C ± 4°C

supplied with synthetic wastewater as substrate for the microbes during the entire seeding process.

2.3. Analysis

Synthetic wastewater of the following composition was then pumped into the reactor on a daily basis: C₆H₅COONa 107.1, CH₃COONa 204.9, NH₄NO₃ 176.1, NaCl 7.0,

MgCl₂·6H₂O 3.4, CaCl₂·2H₂O 4.0 and K₂HPO₄·3H₂O 36.7, in mg/L [17,18]. Other ingredients in this composition act as nutrients and buffer solutions. Most researchers used glucose and sucrose as carbon source and model pollutants. However, there are limited studies on sodium benzoate [13,19,20]. Therefore, the authors chose sodium benzoate as model pollutant in this study. In addition to being the carbon source, sodium benzoate is also a preservative for food and pharmaceutical industries.

The following are the characteristics of the waste water composition: COD, 326 mg/L, NO₃⁻, 136 mg/L, NH₄⁺, 39.6 mg/L. After the reactor attained a stable state, 25 mg/L and subsequently 50 mg/L of AR27 was fed daily into the reactor. This system does not have a specific time frame to achieve stabilization. However, signs of stabilization can be observed within 10 to 20 days. Stable state is achieved after the acclimatization period which could be observed through the results obtained. When a parameter change is introduced to the system, the system demonstrates fluctuation in all the results. This is because the plants and microbes require time to adapt and acclimatize to their surrounding environment. Once acclimatization is accomplished, the system demonstrates a stable state which could be observed through the time cost study.

Water samples were collected at the influent, effluent and sampling points while the aeration was switched on and off to analyse for COD, ORP and colour concentration to assess treatment performance of the ABCW reactor. ORP was measured using an ORP meter (HANNA HI 8424 pH meter, USA). All samples were centrifuged (L500 Table top Low Speed centrifuge, China) before preparing for COD analysis. Calorimeter (HACH DR/890 Calorimeter,) was used to analyse for COD concentration. The official method used to determine the COD in this study is the closed reflux, colorimetric method. This method consists of adding a strong oxidant, ($K_2Cr_2O_7$) to the water sample under acidic condition (H_2SO_4) and using silver (Ag) as catalyst. The sample is then digested for 2 h under $150^\circ C$ and its determination is done using a spectrophotometer [21]. NH_4^+ was analysed using the NH_4^+ probe (Martini Instruments Mi 151 pH/ORP/Temperature bench meter, China). Water sample which contained dye was filtered prior to colour concentration and UV-Vis spectra analysis was carried out by using a UV-Vis spectrophotometer (Hitachi U-2800, Japan). Fourier transform infrared spectroscopy (FTIR) analysis was conducted using Perkin Elmer FTIR spectrophotometer at IR region of $4000\text{--}600\text{ cm}^{-1}$. Prior to FTIR analysis, water samples were dried at $50^\circ C$ for 2 d.

High performance liquid chromatography (HPLC, Shimadzu) for the HPLC analysis with 2 solvent delivery pumps, UV detector at 254 nm. 20 μL of filtered sample (45 μm filter) was injected into C18 analytical column (250 mm \times 4.6 mm) for molecule separation. The mobile phases consisted of 1% of acetic acid (I) and methanol (II); with flow rate at 1 mL/min in gradient mode (5% of II at 0–5 min, 5%–40% of II at 5–25 min and 40%–100% at 25–35 min).

The GC-MS (Shimadzu QP2010) was equipped with a BPX-5 capillary column (30 m \times 0.25 mm \times 0.25 μm). The GC-MS analysis was operated in a temperature programmed mode with an initial oven temperature of $40^\circ C$ without hold, ramped to $250^\circ C$ at rate of $10^\circ C/min$, and held for 15 min. Injector temperature was operated at $250^\circ C$ with splitting ratio 1:10. Purified helium was used as the carrier gas at flow rate 1 mL/min. Mass spectra of intermediates detected from sample were interpreted with National Institute of Standards and Technology (NIST) library database. Samples was extracted with dichloromethane at ratio 1:30 of water sample.

3. Results and discussion

3.1. ORP

Redox potentials higher than 100 mV are known to be in aerobic conditions and redox potentials lower than -100 mV are known to be in anaerobic environment [22]. Redox potentials are vast indicators of the activity and diversity of various microbial populations [23]. Through the ORP profile of the ABCW reactor, several processes involving microbial activities in removing nutrients and organic compounds could be explained. The aerobic and anaerobic zones were controlled to provide a wide range of redox conditions which will consequently enhance a variety of biological processes within the same wetland bed to remove multiple pollutants. Organic carbon for instance is removed by all types of respirations; however the rate and efficiency can be enhanced

by promoting aerobic conditions within the system. Besides that, nitrogen species and AR27 removal requires both aerobic and anaerobic/anoxic conditions for nitrification, denitrification, decolorization and mineralization of intermediates. Hence the need to manipulate the redox conditions within the system. This could be achieved by altering organic loading rate, plant species selection, supplementary aeration, design and mode of operation [23,24]

Fig. 2 a) and b) demonstrates the oxidation reduction potential (ORP) trend throughout the ABCW reactor according to the flow of the wastewater. Generally both profiles, with and without aeration for all concentrations portrayed the same trend where redox potentials were lower at the bottom region of the reactor, indicating anaerobic environment and higher at the middle and top region of the reactor, indicating aerobic and anoxic environment. This is attributed to the unavailability of air at the bottom region; presence of rhizomes and supplementary aeration at the middle region; and convection from atmospheric air at the top region. Besides that, the ORP profile proved that the intermittent aeration (3 h on: 3 h off) managed to control and stabilize aerobic and anaerobic zones within the ABCW reactor as there were insignificant difference between both aeration conditions.

As could be observed in the figures below, ORP values increased as the dye concentration was increased. Both ORP profiles demonstrated the lowest redox range when AR27 concentration was the least and on the contrary when AR27

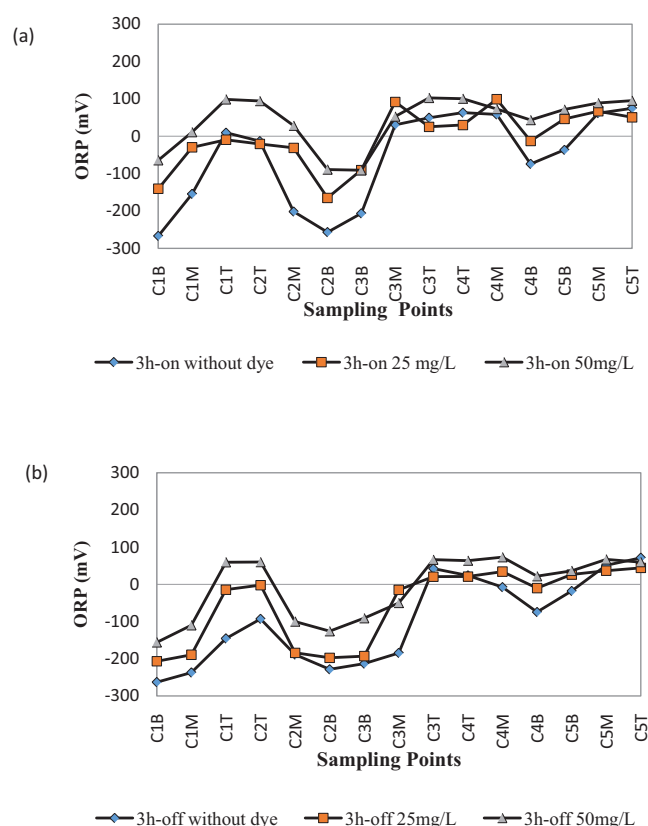


Fig. 2. ORP profile of ABCW reactor for different concentrations with aeration (a) and without aeration (b) according to the flow of wastewater.

concentration was the highest. This could be due to the toxicity of AR27 which may have inhibited microbial activity. This phenomenon could be observed throughout the reactor. However, it is more obvious in compartments 1 and 2 because most of the decolourization took place in the first compartment of the reactor, resulting in the production of intermediate compounds which is toxic to the microbes.

3.2. COD

Fig. 3a is an illustration of the chemical oxygen demand (COD) of the ABCW reactor at the influent and effluent together with the average removal efficiency of COD concentration. Based on Fig. 3a, it could be observed that the ABCW reactor exhibited a much stable performance as it matured. When fed with 50 mg/L of AR27, at an average COD influent of 386 mg/L the ABCW managed to achieve 88% of COD removal producing an average effluent of 46 mg/L. Removal efficiency decreased from 98% to 91% and subsequently to 88%. The decrease in removal efficiency could be due to the presence of some intermediates which could be mineralized under a longer hydraulic retention time. Besides that, toxicity of AR27 which affected microbial activity also contributed in the decrease of COD removal efficiency. Treatment performance could be attributed by well distribution of supplementary aeration throughout the ABCW reactor which boosted mineralization of organic matter by both aerobic and anaerobic heterotrophic microorganisms. COD removal in the ABCW reactor is mainly due to biodegradation rather than macrophyte uptake as no significant difference was shown between planted and unplanted wetlands during the initial phase of the study (data not shown). Although macrophytes exclusively did

not contribute directly in the COD removal of the ABCW reactor, the synergism of plants and microbes where root exudates of plants proliferates microbes by offering a rich source of nutrients for their growth while microbes reduce abiotic stress, increase metal ion uptake, induce systemic resistance, synthesize fungal cell wall degrading systems and produce antibiotics did contribute in the COD removal of the ABCW reactor [25,26].

Fig. 3b shows the full profile of the COD removal within the ABCW reactor according to the flow of the wastewater at 50 mg/L AR27 for both with and without aeration at 3 h interval. Generally COD concentration decreased throughout the system for both aeration times producing an average of 42 mg/L and 66 mg/L at the effluent for with and without aeration respectively. COD removal for both aeration times did not show much difference as the aeration (3 h on: 3 h off) managed to control and stabilize both aerobic and anaerobic zones over time in the system as indicated by the ORP profile. However, the system achieved a higher COD removal during aeration compared to without aeration. This is due to the activity of heterotrophic microbes which would consume more organic matter for metabolism with the presence of more oxygen. Denitrification according to Eq. (1) is irreversible and occurs with the availability of organic substrate, only under anaerobic and anoxic conditions where instead of oxygen, nitrogen is used as an electron acceptor [14].



Therefore, the rapid decrease of COD at the beginning of the reactor was due to denitrification as the ABCW reactor was in an anaerobic, reductive state indicated by the ORP profile causing the microbes to utilize organic matter to perform denitrification.

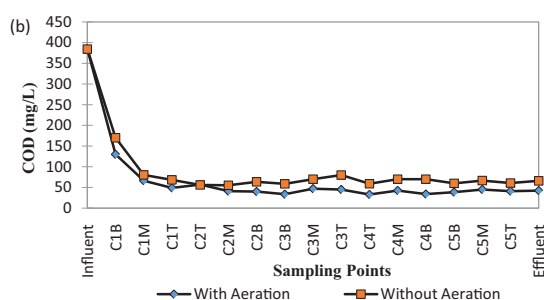
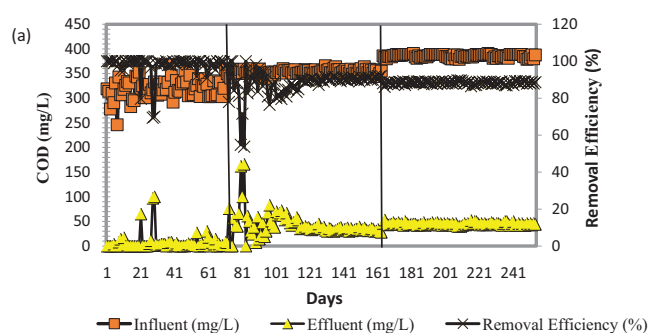


Fig. 3. COD removal efficiency a) and COD concentration profile (b) throughout the ABCW reactor according to the flow of wastewater in the ABCW reactor.

3.3. NH_4^+ removal

Removal mechanisms of nitrogen in constructed wetlands include volatilization, ammonification, nitrification/denitrification, plant uptake and matrix adsorption. However it has been proven by numerous studies that major mechanisms for nitrogen removal in constructed wetlands is microbial nitrification/denitrification [27]. Nitrification is a chemoautotrophic process where ammonium is biologically oxidized to nitrate with nitrite as an intermediate in the reaction sequence as indicated in Eq. (2):



Fig. 4a depicts the ammonium concentration in the influent, effluent and the removal efficiency that was achieved by the ABCW reactor. Overall the efficient nitrification in this system is due to the significant amount of oxygen supplied by the intermittent aeration and rhizomes of *P. Australis*. The significant amount of O_2 supplied could be observed from the ORP profile. The rhizomes of *P. Australis* also promoted the growth of nitrifiers by providing root exudates and enhancing the establishment of microbes especially the chemolithotrophic bacteria. These type of bacteria strictly depends on the oxidation of ammonia for energy generation and growth [28].

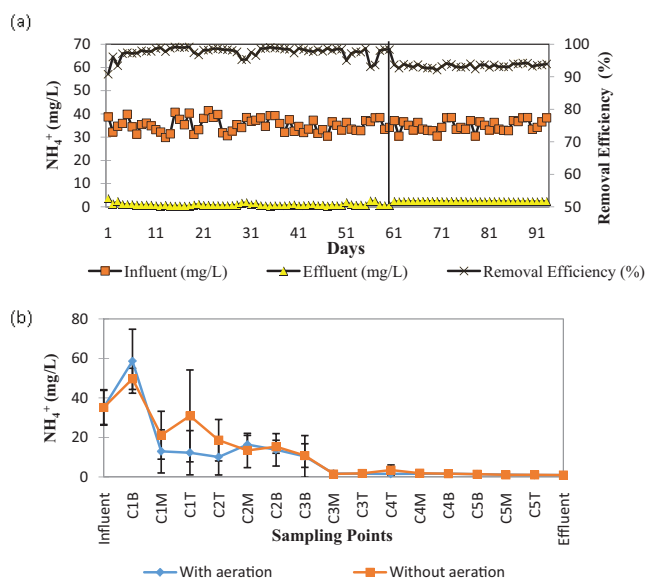


Fig. 4. Removal efficiency of NH_4^+ concentration (a) and NH_4^+ concentration profile (b) throughout the ABCW reactor according to the flow of the wastewater.

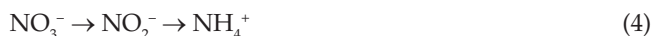
As could be observed in Fig. 4a the system demonstrated an average of 98% efficiency in nitrate removal producing an average effluent of 0.85 mg/L before the addition of AR27 and 93% removal efficiency with the addition of 50 mg/L AR27 producing an average effluent of 2.3 mg/L. Removal efficiency of NH_4^+ decreased with the increase in dye concentration by 5% which could be attributed to the inhibition of azo dye to the activity of ammonium oxidizing bacteria [29].

Fig. 4b is an illustration of the NH_4^+ concentration within the system according to the flow of the wastewater. Generally, the system showed insignificant difference in NH_4^+ concentration with and without aeration except in the first and second compartment. This phenomenon reflected the ORP profile which did not show much difference in redox potentials towards the effluent of the ABCW reactor for both aeration times and on the contrary in the first two compartments. The low removal efficiency of NH_4^+ at the first two compartments could also be attributed to the dye concentration in the first 2 compartments which was higher compared to compartments 3, 4, and 5. The higher dye concentration caused inhibitory effects on the ammonium oxidizing bacteria at compartments 1 and 2.

An increase of NH_4^+ concentration was observed in the bottom region of compartment 1 for both aeration times which could be due to the conversion of part of the azo bonds caused by the reductive environment as reflected in the ORP profile to ammonia (NH_3). Azo linkage of AR27 could either be transformed into ammonia (NH_3) and nitrogen (N_2) in the atmosphere or incorporated into biomass [29]. Ammonia deprotonates a fraction of water to produce ammonium and hydroxide ions when in aqueous solution according to Eq. (3), which caused an increase of NH_4^+ concentration C1B.



Another possibility of an increased NH_4^+ concentration at C1B is due to the Dissimilatory Nitrate Reduction to Ammonia (DNRA) as shown in Eq. (4). DNRA occurs under anaerobic conditions and limited electron acceptors which is carried out by anaerobic and facultative anaerobic bacteria [30].



Compartment 3 onwards had a more efficient ammonium removal for both aeration times which could be attributed to the complete decolourization of azo dye which no longer posed an inhibitory effect on the ammonium oxidizing bacteria; more oxidative environment compared to the first half of the reactor.

3.4. Fate of AR27 in ABCW reactor

The intermediate compounds were analysed and identified based on the UV-Vis, FTIR, HPLC and GCMS. UV-Vis and FTIR results are as shown in Figs. 5a and 5b respectively.

3.4.1. UV-Vis spectrum analysis

It could be seen in the visible absorbance that the AR27 azo bond peak at 520 nm disappeared completely indicating complete decolourization. This reflects the colour concentration at the effluent which could not be detected. Azo bond peak disappeared completely for both concentrations as mentioned in previous study for 25 mg/L and Fig. 5a for 50 mg/L [31]. The system showed rapid decolourization at the bottom region of compartment 1 due to the reductive condition as indicated by the ORP profile. The rapid decolourization of AR27 is also due to the more biodegradable nature of co-substrates which will donate electrons from its biodegradation to support azo bond cleavage. The breakdown of azo bond results in the formation of aromatic amines within the system.

Based on the peaks obtained from the UV-Vis spectrum, two other peaks formed in the effluent of 25 mg/L AR27 concentration after the degradation of the visible absorbance peak. Theoretically the peaks at 225 nm and 320 nm represents 4-aminonaphthylene-1-sulfonic acid and 1-aminonaphthylene-2-hydroxy-3,6-disulphonic acid. At 50 mg/L of AR27, no peak at 320 nm was found indicating degradation of one of the sulfonic compounds. However there were peaks at 260 nm and 220 nm which represents the spectrograms of 1-aminonaphthylene-2-hydroxy-3,6-disulphonic acid. There was no presence of peak 320 nm at 50 mg/L AR27 indicating mineralization of one of the main sulfonic group produced after breakdown of azo bond. The system managed to mineralize this compound at 50 mg/L instead of 25 mg/L due to the maturity and establishment of the microbial community within the system.

3.4.2. FTIR analysis

FTIR spectroscopy was conducted on the influent and effluent samples of the ABCW reactor, ranging from 4000 cm^{-1} to 650 cm^{-1} , to identify the change in structure and

Table 2
Intermediate compounds identified at various sampling points of the ABCW reactor

Intermediate compounds	Sampling points
4 - aminonaphthylene-1-sulphonic acid	C1B
1,4-naphthalenedione-2-hydroxy	C1T, C2T, C3T, C4T, C5T
1,4-naphthalenedione	C1B,C1T,C4T
1,2-naphthalenedione	C1B,C4T
Phenol	C5T
Propanoic acid	C1B, C2B
Oxalic acid	C4T
1,4-butanediol	C1T, C2B
1-butanol	C1T,C5B

stage which is the process after the symmetrical cleavage of the azo bond. The symmetrical cleavage would result in the formation of two main compounds which could be identified theoretically. These two main compounds were 4-aminonaphthylene-1-sulphonic acid and 1-aminonaphthylene-2-hydroxy-3,6-disulphonic acid [4]. These compounds then go through primary oxidation and form naphthalene groups. This prediction was made based on the UV-Vis spectra obtained by analysing each sampling point. The proposed degradation pathway was reported after obtaining results from HPLC and GCMS. The reported degradation pathway ends at degradation of harmless aliphatic acids such as propanoic acid into carbon dioxide and water.

The first step is the symmetrical cleavage of the azo bond, at C1B producing two main compounds as a result of azo bond breakdown. One of the main compounds, 4-aminonaphthylene-1-sulphonic acid was detected by HPLC.

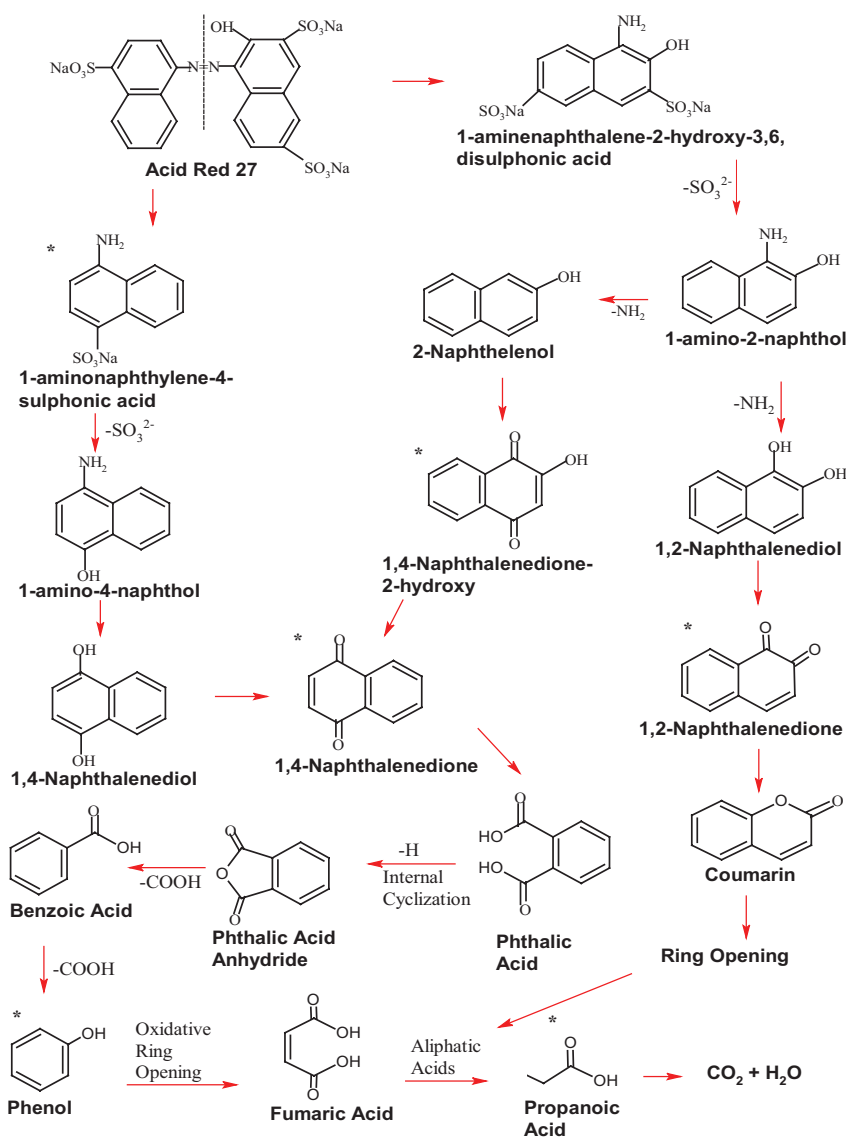


Fig. 6. Proposed degradation pathway of AR27 in the ABCW reactor. (*indicates compounds that were detected in this study)

1-aminonaphthylene-2-hydroxy-3,6-disulphonic acid is the other product of the symmetrical cleavage of the azo bond. These naphthalene compounds then go through desulphonation resulting in the formation of a mixture of isomers named 1-amino-4-naphthol and 1-amino-2-naphthol. The isomers then went through oxidative deamination and hydroxylation process which formed the subsequent compounds (1,4-naphthalenediol; 2-naphthalenol; 1,2-naphthalenediol). These compounds were not detected through GCMS, however was in accordance with several studies on degradation of azo dye AR27 such as [37,38,29].

The next step involves oxidation of these proposed compounds to 1,4-naphthalenedione; 1,4-naphthalenedione-2-hydroxy and 1,2-naphthalenedione which were identified by GCMS from the samples at the top region of the reactor as stated in Table 2. After this stage, the compounds go through ring opening producing phthalic acid. Phthalic acid was proposed as the GCMS analysis detected 1,2-Benzenedicarboxylic acid which is also known as phthalic acid dibutyl ester. Phthalic acid can be converted to phthalic anhydride (internal cyclization) and subsequently to benzoic acid after decarboxylation and hydroxylation processes [38]. Phenol is then identified by the GCMS at $m/z = 66$. Oxidative ring opening occurs at this point of the degradation pathway resulting in the formation of fumaric acid which was proposed because fumaric acid is the oxidative product of succinate acid which was detected at $m/z = 28$. Further oxidation of the remaining benzene rings in the system would lead to the formation of short chain aliphatics and alcohols such as propanoic acid, oxalic acid, 1-butanol and 1, 4-butanediol which were some of the compounds detected in the system. The final step in this degradation pathway is the decomposition of these aliphatics and alcohols to carbon dioxide and water.

The identification of the intermediate compounds in the ABCW reactor till the final stage as shown in the proposed degradation pathway by the GCMS provides an evidence that the ABCW reactor could achieve complete degradation and mineralization due to the aerobic, anoxic and anaerobic conditions conducive for the complete mineralization of AR27. However there were presence of a naphthalene compound in the effluent as indicated by the UV-Vis and FTIR analysis which could probably be solved by increasing hydraulic retention time to achieve complete mineralization of AR27 intermediates.

4. Conclusion

As conclusion supplementary aeration and baffles in the ABCW reactor controlled and provided multiple aerobic, anoxic and anaerobic zones (as indicated by ORP profiles) which enabled complete decolourization and degradation of Amaranth dye. Intermediate compounds identified from HPLC, FTIR and GCMS suggested that the system could achieve complete decolourization and mineralization. FTIR analysis presented the benefits of intermittent aeration to the system which showed that effluent with aeration lack the peaks of sulphonic groups and aromatic amines. High COD and NH_4^+ removal efficiency is attributed to the provision of intermittent aeration and the longer pathway travelled by the contaminants.

Supplementary data

E-supplementary data of this work can be found in online version of the paper.

Acknowledgement

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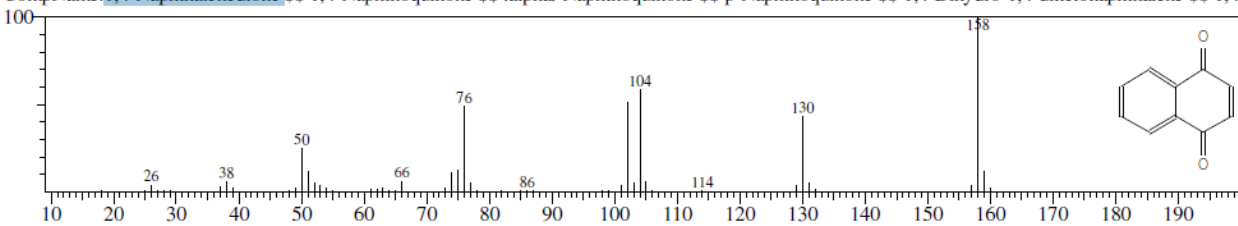
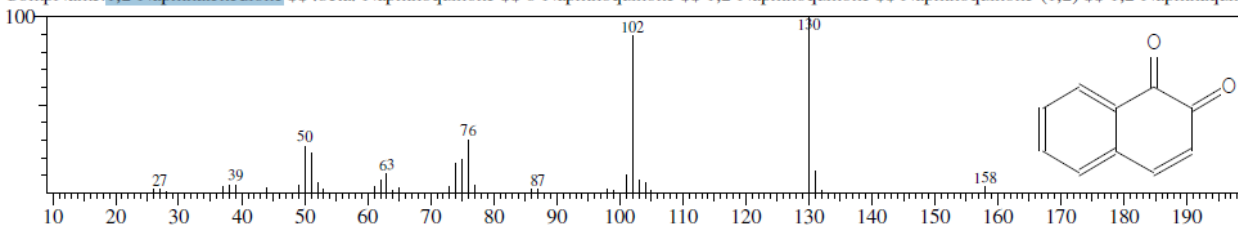
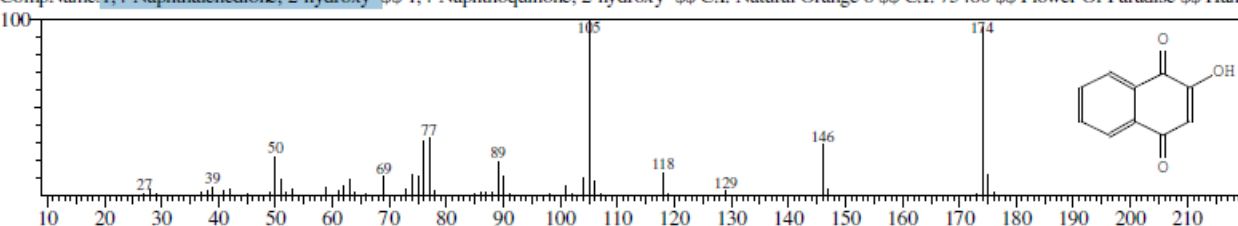
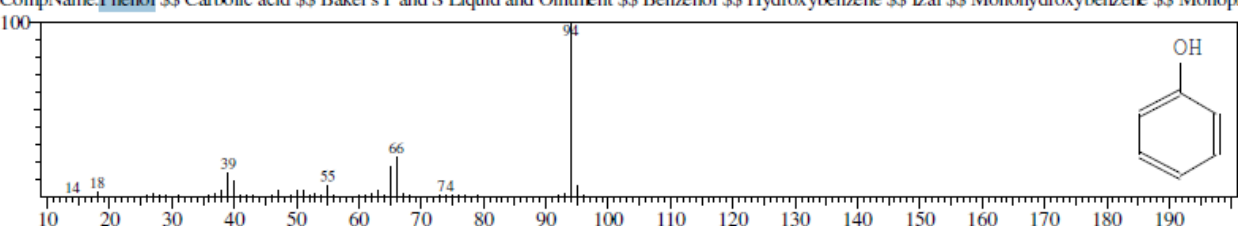
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Supplementary data

Table 1

GCMS spectrum for intermediates related to the proposed degradation pathway of AR27

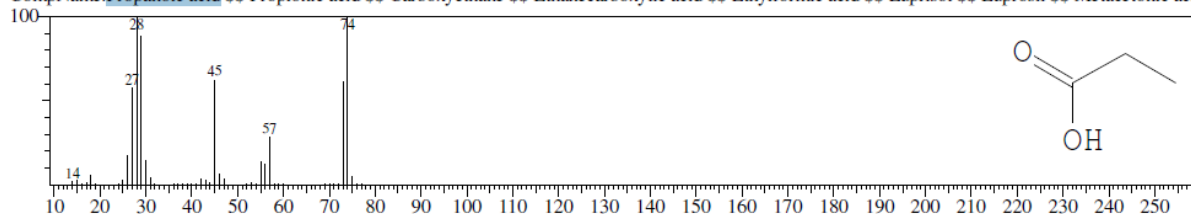
GCMS spectrum
<p>1,4-Naphthalenedione</p> <p>Hit#:1 Entry:10243 Library:NIST08s.LIB SI:89 Formula:C10H6O2 CAS:130-15-4 MolWeight:158 RetIndex:1491 CompName:1,4-Naphthalenedione \$\$ 1,4-Naphthoquinone \$\$.alpha.-Naphthoquinone \$\$ p-Naphthoquinone \$\$ 1,4-Dihydro-1,4-diketonaphthalene \$\$ 1,4-</p> 
<p>1,2-Naphthalenedione</p> <p>Hit#:3 Entry:10240 Library:NIST08s.LIB SI:72 Formula:C10H6O2 CAS:524-42-5 MolWeight:158 RetIndex:1491 CompName:1,2-Naphthalenedione \$\$.beta.-Naphthoquinone \$\$ o-Naphthoquinone \$\$ 1,2-Naphthoquinone \$\$ Naphthoquinone-(1,2) \$\$ 1,2-Naphthoquinone</p> 
<p>1,4-Naphthalenedione, 2-hydroxy</p> <p>Hit#:1 Entry:12808 Library:NIST08s.LIB SI:72 Formula:C10H6O3 CAS:83-72-7 MolWeight:174 RetIndex:1621 CompName:1,4-Naphthalenedione, 2-hydroxy- \$\$ 1,4-Naphthoquinone, 2-hydroxy- \$\$ C.I. Natural Orange 6 \$\$ C.I. 75480 \$\$ Flower Of Paradise \$\$ Hana</p> 
<p>Phenol</p> <p>Hit#:1 Entry:1301 Library:NIST08s.LIB SI:80 Formula:C6H6O CAS:108-95-2 MolWeight:94 RetIndex:901 CompName:Phenol \$\$ Carboic acid \$\$ Baker's P and S Liquid and Ointment \$\$ Benzenol \$\$ Hydroxybenzene \$\$ Lzal \$\$ Monohydroxybenzene \$\$ Monop</p> 

Propanoic acid

Hit#:2 Entry:428 Library:NIST08s.LIB

SI:54 Formula:C₃H₆O₂ CAS:79-09-4 MolWeight:74 RetIndex:676

CompName:Propanoic acid \$\$ Propionic acid \$\$ Carboxyethane \$\$ Ethancarboxylic acid \$\$ Ethylformic acid \$\$ Luprisol \$\$ Luprosil \$\$ Metacetonic acid

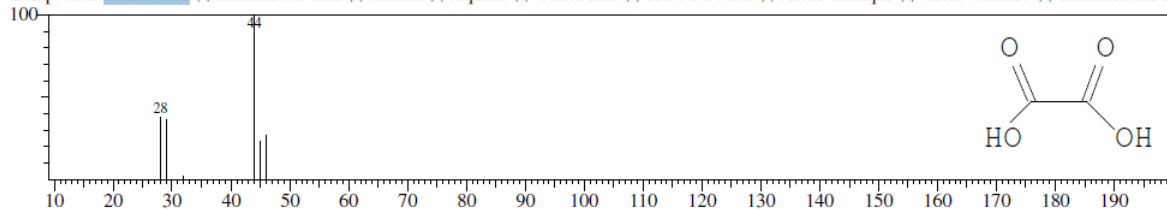


Oxalic acid

Hit#:2 Entry:1120 Library:NIST08s.LIB

SI:59 Formula:C₂H₂O₄ CAS:144-62-7 MolWeight:90 RetIndex:933

CompName:Oxalic acid \$\$ Ethanedioic acid \$\$ Aktisal \$\$ Aquisal \$\$ Oxiric acid \$\$ HOCCOOH \$\$ Acide oxalique \$\$ Acido ossalico \$\$ Ethanedionic ac

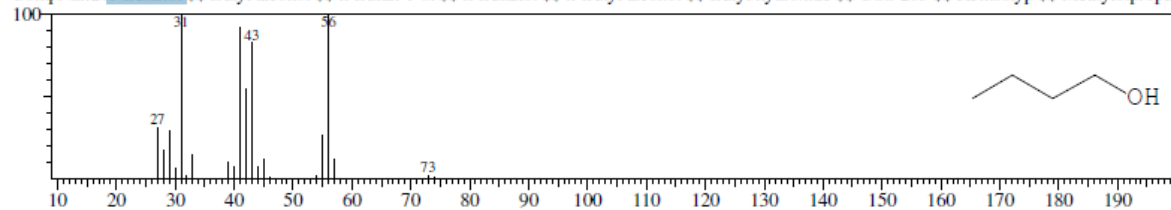


1-Butanol

Hit#:5 Entry:443 Library:NIST08s.LIB

SI:72 Formula:C₄H₁₀O CAS:71-36-3 MolWeight:74 RetIndex:662

CompName:1-Butanol \$\$ Butyl alcohol \$\$ n-Butan-1-ol \$\$ n-Butanol \$\$ n-Butyl alcohol \$\$ Butyl hydroxide \$\$ CCS 203 \$\$ Hemostyp \$\$ Methylpropan

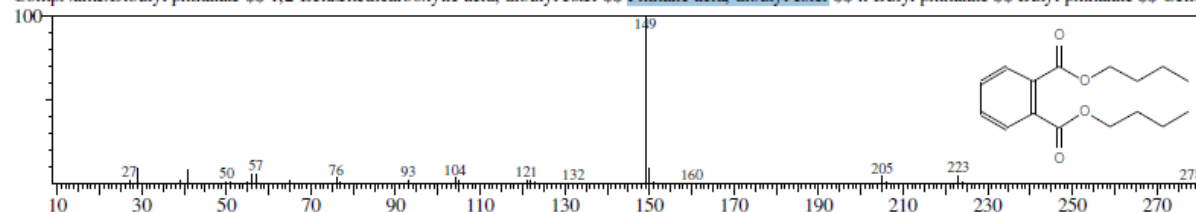


Phthalic acid,dibutyl ester

Hit#:2 Entry:23200 Library:NIST08s.LIB

SI:84 Formula:C₁₆H₂₂O₄ CAS:84-74-2 MolWeight:278 RetIndex:2037

CompName:Dibutyl phthalate \$\$ 1,2-Benzenedicarboxylic acid, dibutyl ester \$\$ Phthalic acid, dibutyl ester \$\$ n-Butyl phthalate \$\$ Butyl phthalate \$\$ Cellu

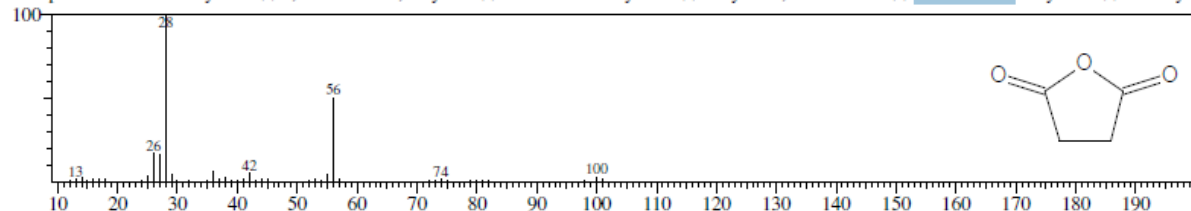


Succinic acid anhydride

Hit#:3 Entry:1694 Library:NIST08s.LIB

SI:66 Formula:C₄H₄O₃ CAS:108-30-5 MolWeight:100 RetIndex:996

CompName:Succinic anhydride \$\$ 2,5-Furandione, dihydro- \$\$ Butanedioic anhydride \$\$ Dihydro-2,5-furandione \$\$ Succinic acid anhydride \$\$ Succinyl a

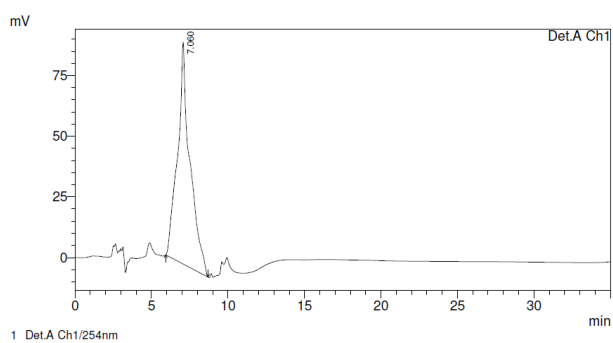
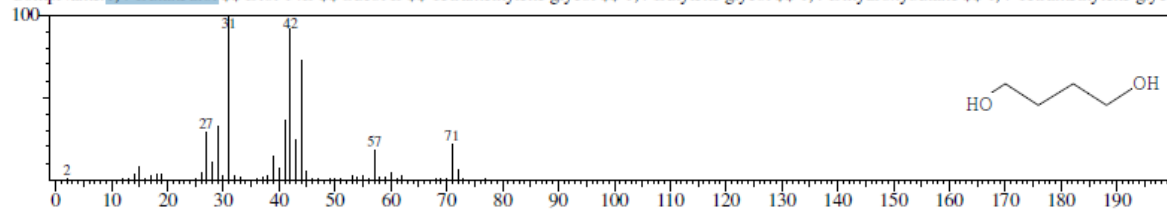


1,4-Butanediol

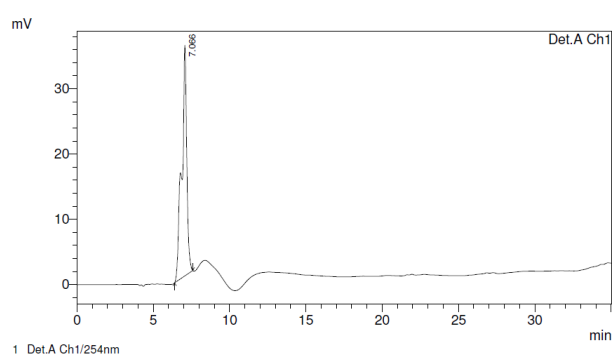
Hit#:4 Entry:1156 Library:NIST08s.LIB

SI:72 Formula:C4H10O2 CAS:110-63-4 MolWeight:90 RetIndex:904

CompName:1,4-Butanediol \$\$ Diol 14B \$\$ Suco1 B \$\$ Tetramethylene glycol \$\$ 1,4-Butylene glycol \$\$ 1,4-Dihydroxybutane \$\$ 1,4-Tetramethylene glycol



(a)



(b)

Fig. S1. HPLC analysis of sampling point C1B at 254 nm (a) proving the existence of compound 4-aminonaphthylene-1-sulphonic acid as retention time matched standard (b).