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Removal of Heavy Metals from Rainwater in Batch Reactors with Sulphate Reducing Bacteria (SRB)

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***Abstract*—**The main objective of this research was to investigate the biosorption capacity for biofilms of sulphate reducing bacteria (SRB) to remove heavy metals, such as Zn, Pb and Cd from rainwater using laboratory-scale reactors containing mixed support media. Evidence showed that biosorption had contributed to removal of heavy metals including Zn, Pb and Cd in presence of SRB and SRB were also found in the aqueous samples from reactors. However, the SRB and specific families (*Desulfobacteriaceae and Desulfovibrionaceae)* were found mainly in the biomass samples taken from all reactors at the end of the experiment. EDX-analysis of reactor solids at end of experiment showed that heavy metals Zn, Pb and Cd had also accumulated in these precipitates.

***Keywords*—**Sulphate reducing bacteria (SRB), biosorption capacity.

I. Introduction

A. Sulphate Reducing Bacteria

SULPHATE reducing bacteria exert a significant ecological and environmental impact and it has a great importance in mineralization of organic matter in anaerobic environment [8]. Technologies using (SRBs) are attractive removing heavy metals, such as Cu, Zn and Pb, from contaminated water by the use sulphate as an electron acceptor with sulphate, usually being reduced to sulphide as a final product. Hydrogen sulphides may be formed by which it can combine with heavy metals to form metal sulphides, such as ferrous sulphide, zinc sulphide or lead sulphide, all these are formed as insoluble precipitates [16],[11].

B. Biosorption (Biomass and Biofilms)

Biofilms have been successfully used in the treatment of water and wastewater for over a century. Biofilms and biomass have been investigated by many researchers in different fields including in biofouling, biocorrosion, bioconversion, medicine and limnology [2]. Infact biofilms occur in any system where microorganisms are present, given the composition and activity of a biofilm.

Biofilms are important for the successful operation and control of fixed film processes [7],[2]. Furthermore, a biofilm can be composed of microorganisms immobilized on a substratum of support surface materials (e.g. soil, plastic, activated carbon etc), and generally in association with an organic polymer matrix [2], however, biofilms are multiphase systems that consist of solids and a liquid in the void space between the solids [9]. In addition, biomass growth increases the thickness of the biofilm on the support surface material, and microbial conversions in biofilms are also controlled by the support surface materials, growth kinetics and mass transport processes [10],[14]). Biofilms and Biomass systems can be in two main types . The first is non-living biomass which is prone to saturation at relatively low levels of metal. The second type is a live biomass which may fail due to metal toxicity [5],[13].

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II. Materials and Method

A. Collection of Rainwater

Rainwater was collected from the roof of Moor Bank Garden greenhouse, using the existing roof gutter. The collected water was stored in a 1000 litre, plastic tank wrapped in black polyethylene to exclude light.

B. Bacteria Inocula (SRB) for All Reactors

Bacteria inocula were prepared in the laboratory using soil and sediment as the original SRB source, and rainwater at a ratio of 1:4 (W/V). This ratio was obtained by adding 2 kg of soil to 8 litres of rainwater. The mixture has been heated to 35 ^{o}C under anaerobic conditions in a static water bath with continuous A source of carbon (glucose), sulphate (K_2SO_4), were added at a concentration of 100mg C/l and 100 mg $SO_4^{=}$/l every five days respectively, and trace metals, (Fe, Mn, Mo and Ni) were added at a concentration 1mg/l, 62, 19, 15 µg/l respectively [17].

C. Experimental Design

This experiment involved four laboratory-scale reactors (RA, RB, RC and RD) constructed from 10 litre glass bottles which were completely air-tight. All the four bottles were placed in a water bath to control the temperature at (30 ± 2) ^{o}C. Each bottle has been supplied with a rubber tube linking from the middle for sampling purposes. Support media in the form of a bottom layer of gravel and upper layer of plastic pall-rings were placed in two the reactors, RC and RD, (see Fig. 1).

D. Operation of Reactors

All reactors (RA, RB, RC and RD) were operated under anaerobic condition at (30 ± 2) ^{o}C. 3 litres of a culture of bacterial inocula was added to all four reactors. The remaining part of each reactor was filled with rainwater. At the beginning of the experiment the pH in two of the reactors (RA and RC) was readjusted from 1.5 to 2.5. RC contained support material while RA did not. The pH of the other two reactors RB and RD were that of rainwater (4.5-5.5) at the beginning of the experiment.

Also, RD contained support material while RB did not. In addition, during experiment, glucose and K_2SO_4 were added to each reactor at 24 mg/l each as sources of carbon and sulphate respectively (see Table I).

- RA reactor contained only feed (rainwater) and bacteria inocula with no support material with a pH ranging from1.5-2.5.
- RB reactor the same as RA with pH ranging from 4.5-5.5.
- RC reactor contains both feed (rainwater), bacterial inocula and support material with pH as in RA reactor.
- RD reactor same as RC with pH as in RB reactor.

(NB: All pH values were readjusted at the beginning of experiment)

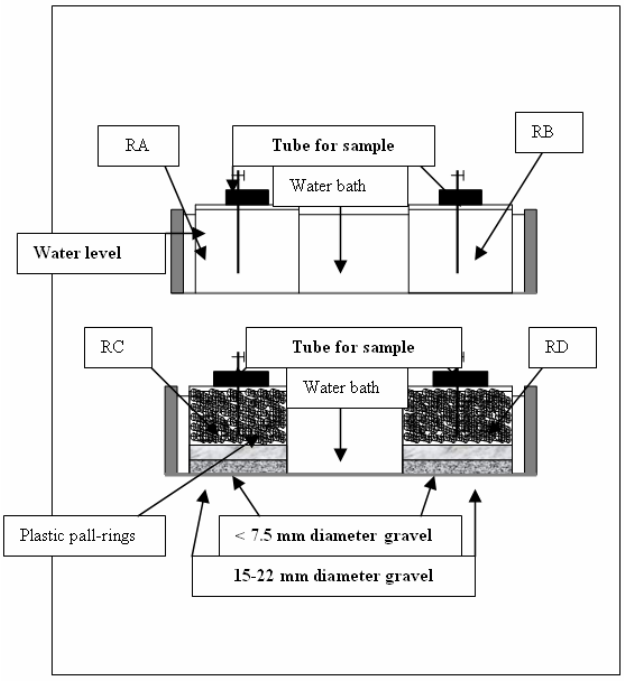


Fig. 1 Schematic diagram of the cross-section of laboratory-scale reactors in the experiment

TABLE I
OPERATING CONDITIONS IN THE LABORATORY-SCALE REACTORS

Reactor	$SO^{=}_4$ mg/l	C mg/l	Ratio C:$SO^{=}_4$	Media Type Bottles	Source of (SRB)	Temperature ^{o}C
RA	24(±2)	24(±2)	1:1	W.S	(BI)	30(±2)
RB	24(±2)	24(±2)	1:1	W.S	(BI)	30(±2)
RC	24(±2)	24(±2)	1:1	PPR+G	(BI)	30(±2)
RD	24(±2)	24(±2)	1:1	PPR+G	(BI)	30(±2)

$ZnCl_2$, $Pb(NO_3)_2$ and $CdCl_2$ were the source of Zn, Pb and Cd respectively. W.S: without support material, Media types (support material) were plastic pall-rings (PPR) and gravel (G). SRB: sulphate-reducing bacteria. BI: bacterial inocula prepared before the start of the experiment.

F. Estimation of Biosorption Capacity

The accumulated biomass in the reactor RA had pH levels ranging between 1.5-2.5 (at the beginning of experiment) and in reactor RB pH levels ranging between 4.5-5.5 (at the beginning of the experiment) i.e without support materials. These biomass sample were collected separately and then each was washed three times with distilled water. However, as for reactor RC pH levels raining between 4.5-5.5 (at the beginning of the experiment) and reactor RD pH levels between 4.5-5.5 (at the beginning of the experiment) i.e with support material. The biofilms that formed on the support material were collected and washed with distilled water in the same manner. The biomass was separated from the water by centrifuging 2500 x g x 30 minutes [3],[6]. The biomass was then left in an oven for 24 hours at 105 ^{o}C [4]. To test the biosorption capacity of the biomass, a 500 ml solution was prepared containing Zn, Pb, and Cd at 5, 0.5 and 0.05 mg/l concentration respectively for each reactor. The pH of the solution was readjusted to match the pH of reactors at the time of collection of samples (see Table II). The dry biomass prepared above was added to the solution to give concentration of 1.24, 1.35 and 0.94 g dry solid per litre for reactors RA, RB, RC and RD respectively, and shaken for 24 hours at 30 ^{o}C [6],[13]. Then the solution was filtered off using, Whatman, NO 1, Qualitative Filters 125 mm Dia, (produced by Whatman International. Ltd. Maidstone, England). The filtrate was then used to measure the concentration of the three heavy metals Zn, Pb, and Cd using an atomic spectrophotometer (AAS), i.e UNICAM 929 AASpectrometer, produced by AII Unicam Analytical Tenchnology, Inc, England. The solid part of the filtered solution was used for the EDX-trace analysis [13].

TABLE II
PH LEVEL RELATED TO BIOSORPTION CAPACITY IN DIFFERENT REACTORS EXPERIMENT, PH VALUES SHOWN ON THE TABLE WERE THE FINAL PH VALUES OF THE REACTORS AT WHICH BIOSORPTION CAPACITY WAS ESTIMATED

Reactor	pH
RA	4.5
RB	5.2
RD	6.9
RC	7.1

The biosorption capacity, i.e the amount of metal ion (mg) bioadsrbed per (g) (dry mass) of biomass can be calculated using the following equation:

$$Q = \frac{(C_i - C_f)\, V}{M}$$

Where:
Q = mg metal ion biosorbed per (g) of biomass.
C_i = initial metal ion concentration (mg/l).
C_f= Final metal ion concentration (mg/l).
M= dry mass of biomass in the reaction (g).
V= volume of the reaction (l).

III. Results and Discussion

According to [11], in the presence of SRB the removal of heavy metals may not only take place by sulphide precipitation, but possibly through biosorption as well. Nonethless, other types of bacteria in the biomass such as *Bacillus subtillis* [1].

Results shown in Tables II and III indicate that the increase in pH can be compared to the initial levels of pH for the aqueous samples of 21 days from the beginning of experiment, the increase in pH levels was higher in reactors containing support materials (plastic pall-rings and gravel), namely reactor RC and RD. On the other hand, the numbers of SRB estimated MPN method in aqueous samples was found to be equal in all reactors 10 days from the beginning of experiment. Also after 21 days, the number of SRB reduces in all reactors except in reactor RD. The high number of SRB in RD can found due to high pH levels and the presence of support materials. These results are shown in Table IV. However, the number of SRB and specific families (*Desulfobacteriaceae and Desulfovibrionaceae)* as estimated by Fluorescence in-situ hybridization (FISH) method were found to be very close in almost reactors. Yet, these number were slightly higher in reactor RD for possibly same reason mentioned above. The results are shown in Table V. Given the above results, the biosorption capacity of SRB for Zn, Pb and Cd was highest in reactor RD, (2.3, 023 and 0.02 mg/g biomass for Zn, Pb and Cd respectively) whereas it was almost the same in RB and RC, and less in RA. This could be due to the fact that reactor RA lacked support materials and the pH was found to be lower than the others during the estimation of the biosorption capacity (see Table III and Figs. 2 and 3). However, these results appear to be in agreement with previous studies. For example, [3] highlighted the significance of an increase in pH in removal of heavy metals such as Zn and Cu through biosorption in presence of SRB strain *Desulfovibrio desulfuricans* of the family *Desulfovibrionaceae* at temperature between 25 and 30 ^{o}C. However, [12] studied biosorption of palladium and platinum using different strains of SRBs *Desulfovibrio frctosivorans and Desulfovibrio vulgaris* of the family *Desulfovibrionaceae*. The maximum biosorption was obtained from *Desulfovibrio desulfuricans* at pH 3.

TABLE III
Biosorption-related pH and Biosorption Capacity for Zn, Pb and Cd (mg/g Biomass in the Different Reactors in the Experiment (NB: the pH values shown on the table were the pH values of the reactors at which biosorption capacity was estimated).

Reactor	pH	B.C Zn mg/g biomass	B.C Pb mg/g biomass	B.C Cd mg/g biomass
RA	4.5	1.09	0.13	0.01
RB	5.2	1.5	0.15	0.012
RD	6.9	1.4	0.2	0.018
RC	7.1	2.3	0.23	0.02

B.C: Biosorption capacity

TABLE IV
Number of SRB using (MPN) Method in the Different Reactors of the Experiment

Reactor	Test 1 Number SRB Cell/ml	Test 2 Number SRB Cell/ml
RA	1600	540
RB	1600	920
RC	1600	920
RD	1600	1600

TABLE V
Number of SRB Cells and the Specific Families (DSB) and (DSV) using (FISH) Method in the Different Reactors in the Experiment

Reactor	SRB Cell/ml	DSR Cell/ml	DVS Cell/ml
RA	$3.3x10^7$	$1.9x10^7$	$2.3x10^7$
RB	$3.4x10^7$	$1.8x10^7$	$2.7x10^7$
RD	$3.6x10^7$	$1.9x10^7$	$2.4x10^7$
RC	$4.1x10^7$	$1.8x10^7$	$2.6x10^7$

DSR: *Desulfobacteriaceae*, DVS: *Desulfovibrionaceae*

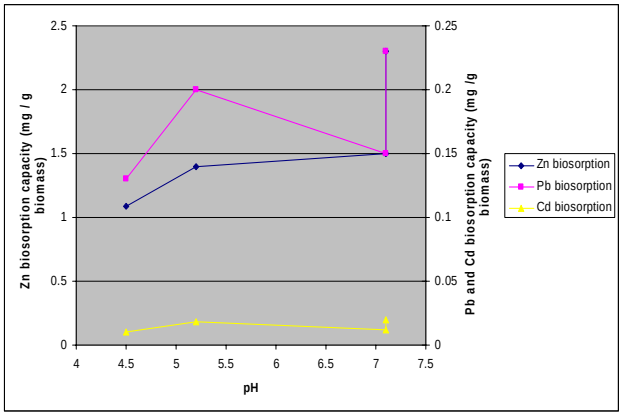


Fig. 2 Relationship between pH and biosorption capacity of Zn, Pb and Cd by SRB biomass (mg /g biomass) in different reactors in the experiment

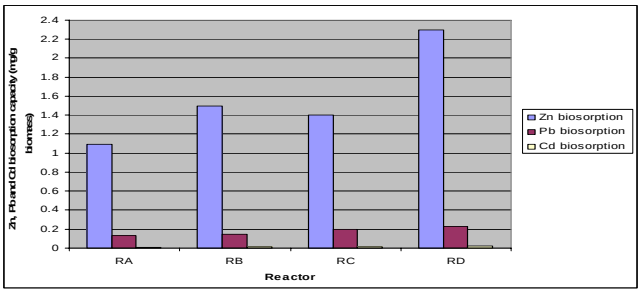
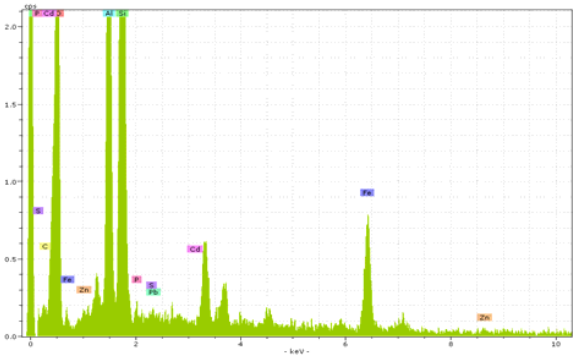
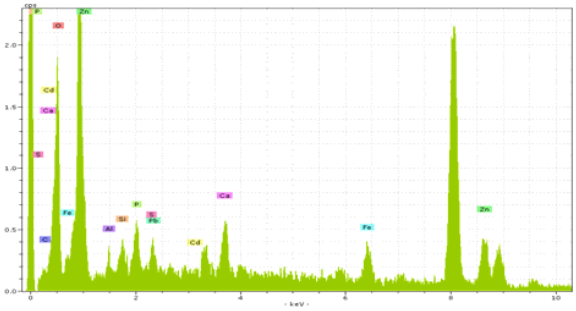


Fig. 3 Zn, Pb and Cd biosorption capacity of biomass in different reactors in the experiment

With regard to the EDX-traces shown in Figs. 3 and 5 of precipitate obtained from the filtration of biomass solution obtained during the process of estimation of biosorption capacity, the result indicate the existence of all three metals i.e Zn, Pb and Cd in all reactors with slight variations in concentration. However, it was noticed that the higher the pH the higher the heavy metal (Zn, Pb and Cd) concentration. Moreover, the EDX-traces also indicated that S was present in all reactors.

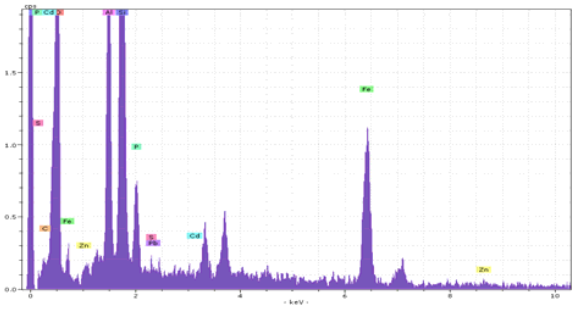


(a)

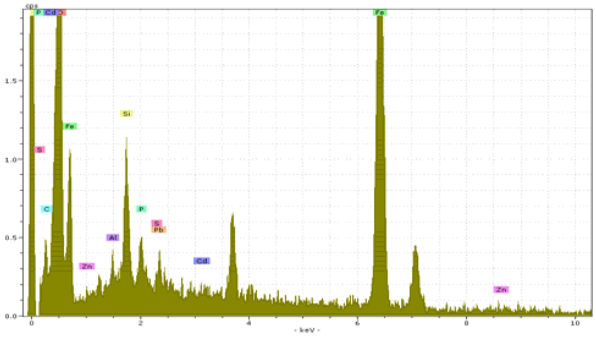


(b)

Fig. 4 EDX-traces showing the signature of heavy metals in biosorption precipitates for reactors (a) RA and (b) RB



(c)



(d)

Fig. 5 EDX-traces showing the signature of heavy metals in the biosorption precipitates for reactors (c) RC (d) RD

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