Removal of Sulphate and Manganese on Synthetic Wastewater in Sulphate Reducing Bioreactor Using Indonesian Natural Zeolite

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ABSTRACT

The present research was conducted to investigate sulphate and manganese removal from synthetic wastewater. The continuous laboratory scale of down-flow fluidized-bed reactor (DFBR) using sulphate reducing bacteria (SRB) consortium and Indonesian natural zeolite as a bacterial support material was designed. At 9 days operation, maximum sulphate and manganese removal was observed to be 23% and 15.4%, respectively. The pH values were also changed to neutral. The population of SRB increased which effect on the raising of their activity for removing sulphate and manganese. Using the scanning electronic microscopy (SEM), it was observed that natural zeolite possesses excellent physical characteristics as a bacterial support material in DFBR. The imaging SEM result of SRB consortium on zeolite surface clearly showed the developed SRB biofilm on that particle. Analysis result of EDX confirmed that manganese was precipitated as manganese–sulfides.

Keywords: *DFBR; SRB consortium; natural zeolite; SEM-EDX; manganese-sulfides*

ABSTRAK

Penelitian dilakukan untuk menyelidiki penghilangan sulfat dan mangan dalam limbah cair sintetik. Desain penelitian berupa down-flow fluidized-bed reactor (DFBR) skala laboratorium dengan sistem kontinyu menggunakan konsorsium sulphate reducing bacteria (SRB) dan zeolite alam dari Indonesia sebagai material penyangga bakteri. Pada pengoperasian hari ke-9, penghilangan sulfat dan mangan secara maksimum teramati masing-masing sebesar 15,4% dan 23%. Nilai pH juga berubah menjadi netral. Populasi SRB meningkat sebagai akibat kenaikan aktivitas bakteri dalam penghilangan sulfat dan mangan. Pengamatan menggunakan scanning electronic microscopy (SEM) terlihat bahwa zeolit alam mempunyai karakter fisik unggul sebagai material penyangga bakteri dalam DFBR. Hasil pencitraan SEM terhadap konsorsium SRB pada permukaan zeolit secara jelas memperlihatkan terjadi pembentukan SRB biofilm pada partikel tersebut. Hasil analisis EDX menegaskan bahwa senyawa mangan mengalami presipitasi dalam bentuk mangan sulfida.

Kata Kunci: *DFBR; konsorsium SRB; zeolite alam; SEM-EDX; mangan sulfida*

INTRODUCTION

Due to rapid industrialization, particularly from mining industries have been generated acid mine drainage (AMD). Those polluted waters occur when sulphide rock are exposed to oxygen and water in the absence of sufficient neutralizing minerals. The AMD is characterized by a low pH and high concentrations of heavy metals and sulphate [1-2]. The presence of elevated concentrations of manganese and other heavy metals in wastewater can exert detrimental effect on human health and environment due to their toxicities [3-5]. In order to remove those pollutants from industrial effluents, many attempts related to both heavy metal and sulphate remediation have been carried out all around the world.

Anaerobic sulphate reducing bioreactors are becoming the biological alternative treatment of acidic metal-containing wastewaters due to low cost and high efficiency. These reactors contain sulphate reducing bacteria (SRB) that have the ability to reduce sulphate to sulphide and then this sulphide reacts with certain metals dissolved, such as manganese, copper, iron and zinc, forming insoluble precipitates [1]. Numerous reactor designs for microbial sulphate reduction have been reported [4-8]. The applying of those reactor designs have also been reported to be used for the treatment of industrial effluents, particularly from acid

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mine drainage (AMD) [9-12]. However, there are two problems for applying those anaerobic sulfate reducing bioreactors: (1) lack of bacterial resistance to metals, and (2). the loss of bacteria biomass in the systems with high hydraulic loading rates. In the literatures, zeolites have reported shown a great capacity for adsorption of heavy metal (Mn, Cu, Cd, Pb and Zn) [13-15], aflatoxins [16], and cationic dye [17]. In addition, previously researchers reported that zeolites can be useful as a microbial support in anaerobic processes of different wastewaters [18-20]. Therefore the bioreactor could be operated at higher heavy metal concentration by immobilization using natural zeolites. This immobilized SRB could lead to its biofilm formation. Moreover, SRB cells have better stability and higher metabolism [21]. Hence, that formation may reduce metals toxicity and the loss of that SRB biomass. This process may further stimulate their activities in the sulphate reducing bioreactor. Due to highly abundance of natural zeolites obtained in some area of Indonesia. It is important to evaluate possibilities for utilizing Indonesian natural zeolite as a carrier for SRB immobilization in the sulphate reducing bioreactor. The main objective of this research was to operate the sulphate reducing consortium in a controlled bioreactor system using natural zeolite as supporting materials, to determine the sulfate reduction rate, manganese removal and pH profile. The laboratory scale reactor was selected which can be control all the influential environmental parameters in well-organized manner. The optimized conditions for that reactor were obtained from batch experiments conducted previously.

EXPERIMENTAL SECTION

Materials

The mixed culture of SRB used in the present study was obtained from sheep manure samples. The culture was placed in a glass bottle with Postgate B medium and incubated at 35 °C [22]. This medium contained (in g/L): K_2HPO_4 0.5, NH_4Cl 1.0, $CaSO_4$ 1.0, $FeSO_4.7H_2O$ 0.5, sodium lactate 8 mL, $MgSO_4.7H_2O$ 2.0, yeast extract 1.0 and ascorbic acid 0.1. The medium was aerated with nitrogen gas to maintain anaerobic conditions before inoculating. Every week 20% of the volume of the culture in the bottle was replaced by fresh medium. After three months, a culture containing high density of bacteria of different morphological types was achieved.

The synthetic wastewater (pH 5 and 2500 mg/L sulphate) contained (in g/L): $MgSO_4.7H_2O$ 2.563, Na_2SO_4 1.479; KH_2PO_4 0.056; NH_4Cl 0.11, ascorbic acid 0.011, yeast extract 0.05 and sodium lactate 8 mL. The heavy metal of $MnSO_4.H_2O$ was added separately to the bottles reach to final concentration of 10 mg/L.

The natural zeolite used as SRB biomass support was obtained from a deposit located in the Province of Gunung Kidul, Yogyakarta, Indonesia. The chemical composition (%, w/w) of the zeolite used was SiO_2 86.3%; Al_2O_3 13.7%; Fe_2O_3 2.4%; CaO 2.3%; MgO 0.4%; Na_2O, 1.7%; and K_2O 1.8%. It has mineral composition as clinoptilolite; mordenite and montmorillonite. The surface area, pore volume and pore size of zeolite was 20.0 m^2/g, 12.4 cc/g, and 23.8 Å, respectively. It was powdered and grouped into three groups based on its diameter size (R1, R2 and R3 Groups). Diameter size of group R1, R3 and R3 were 0.4-0.6 cm, 0.6-0.8 cm, and 0.8-1.2 cm, respectively. Before utilization it was washed twice and soaked with deionized water for 24 h at 30 °C. After this treatment, zeolite was dried for 24 h at 80 °C and used as a carrier for immobilization of mixed SRB cultures in both batch and continuous experiments.

Instrumentation

Centrifuged (Eppendorf, 5810R) apparatus was used for separation between bacterial cells and medium. For analysis of sulphate and manganese removals in DFRB were used a spectrophotometer (Shimadzu UV-1601) and Atomic Absorption Spectroscopy (AAS) apparatus (Hitachi, Z-2000), respectively. Decreasing of pH values in the bioreactor was measured using pH meter (Metrohm). The characteristics of biofilm and the manganese interacted on zeolite surface were observed using Scanning Electron Microscope- Energy Dispersive- X-ray Spectroscopy (SEM-EDX) (JEOL JSM-T300).

Procedure

Anaerobic batch experiments

The anaerobic batch tests were performed in sealed flasks (250 mL). The flask experiments were seeded with 20 mL of the mixed SRB cultures into 180 mL synthetic wastewater under the anaerobic condition. Three groups of zeolites in different diameter size (R1, R2 and R3) in the amount of 20 g were added to each batch SRB cultures. Therefore the inoculated flasks were incubated at 30 °C for 14 days. During the incubation, samples were collected at 0, 1, 3, 7 and 14 days and were further analyzed.

Anaerobic continuous experiments

The zeolite of R1 added in the batch experiment previously which demonstrate the highest removing pollutants (sulphate and manganese metal), increasing pH and population number of SRB obtained, was selected for biomass carrier of continuous experiments. The continuous laboratory scale of down-flow fluidized-bed reactor (DFBR) inoculated with mixed SRB was used in this study [23]. The DFBR was fed with synthetic wastewater that prepared freshly every day. The volume of the DFBR reactor is 1.2 L and it is filled with 1.13 kg natural zeolite and 0.67 L synthetic medium with the result that the surface of zeolite in the reactor is completely covered. The reactor is then inoculated with 40 mL of SRB culture from batch experiments. The DFBR was operated in a temperature controlled at 30 °C for 9 days. During this operation, samples of influent and effluent were collected at 0, 3, 5, 7 and 9 days and were further analyzed.

Chemical analysis

At certain intervals, 10 mL samples of both batch experiment and continuous experiment were collected using a syringe, and centrifuged at 5000 rpm to prepare the cell free supernatant at 4 °C. This supernatant was used for analysis of concentrations of sulphate and manganese metal. Sulphate concentration was measured according to the turbidimetric method using a Shimadzu UV-1601 spectrophotometer, and the absorbance of the sample was measured at wavelength of 420 nm [24]. For determination of the dissolved manganese the supernatant samples were acidified with nitric acid and analyzed by Atomic Absorption Spectroscopy (AAS) using a Hitachi, Z-2000 model spectrometer. The pH samples were measured immediately without centrifugation using pH meter (Metrohm). The number of SRB was enumerated by the three-tube Most Probable Number (MPN) assay with serial dilutions [22]. Performance of down-flow fluidized-bed reactor (DFBR) on the pollutants removal (sulphate and manganese concentration) was evaluated at the inlet (initial concentration) as well as the outlet (final concentration). The pH value during that bioreactor operation was also measured.

Characterization of developed SRB biofilms population

The developed SRB biofilms populations were taken at 5 and 9 days DFRB operation. Samples on the zeolites surface of DFBR were enumerated by the three-tube Most Probable Number (MPN) assay with serial dilutions. One cm^2 biofilm samples on zeolite surface of DFBR were scraped from the substrates using a sterile scalpel and dispersed in 10 mL of sterile water. Samples were then diluted serially and inoculated in Postgate B medium [22]. The cultures furthermore were incubated at 30 °C for 5 days. The characteristics of biofilm attachment on zeolite surface were observed using Scanning Electron Microscope- Energy Dispersive-X-ray Spectroscopy (SEM-EDX). For SEM the biofilms samples were fixed with 2.5% glutaraldehyde, ethanol (dehydrated) and coated with gold under vacuum in an argon atmosphere [25]. The surface morphology of the gold coated samples was visualized by a SEM (JEOL JSM-T300). The surfaces elemental analyses of a Mn interacted in zeolite were carried out by Energy dispersive X-ray.

Statistical analysis

The experiments were done in triplicates. All values are expressed as means ± standard deviation. Mean values of treatments were compared by the analysis of variance (one-way ANOVA) followed by Duncan Multiple Range Test (DMRT) method for mean differences testing. Differences were considered significant at $p < 0.05$.

RESULT AND DISCUSSION

Effect of Different Diameter Size of Natural Zeolite on Mixed SRB Activity

Effects of different diameter size of natural zeolite (R1, R2 and R3) on the mixed SRB activities were investigated for 14 days in anaerobic batch experiments. During those incubations, all mixed SRB cultures revealed the black precipitates. Those precipitates were indicated on mixed SRB activity that can utilize sulphate (SO_4^{2-}) as an electron acceptor, therefore generating hydrogen sulfide (H_2S). Synthetic acid mine drainage containing lactate was used by SRB as organic substrate for generating bicarbonate ions and hydroxides [1]. Furthermore, those sulfides, carbonates and hydroxides can react with manganese metal and increase pH value in the AMD. However their activity rates were influenced significantly by different size of natural zeolite addition ($P < 0.05$) and the highest result was analyzed on the zeolite R1, followed by zeolite R2, R3 and control (Fig. 1). Since zeolite R1 had the smallest diameter compared with zeolite R2 and R3, therefore increasing the surface area of zeolite for attachment and colonization of SRB. These enhancements influenced microbial and enzymatic transformation of a variety of substances for sulphate reducing bacterial during their attachment and colonization in the zeolite surface [26-28]. In addition zeolites have been reported to absorb manganese and other heavy metals [29-30]. As the result, number SRB cells of zeolite R1 was highest compared with zeolite R2 and R3. Hence, that SRB metabolism for biological sulfate reduction process was also highest. Their sulphate reducing process induced reducing sulphate and manganese concentrations that lead to higher pH values.

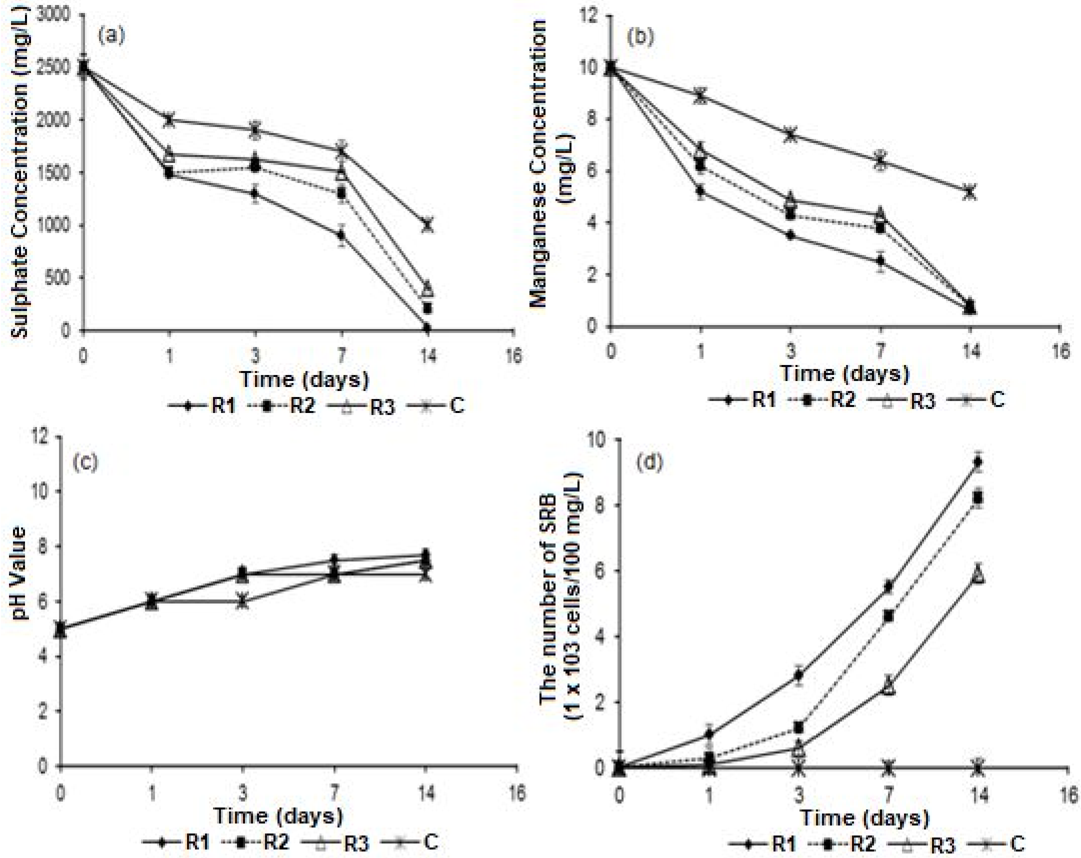

Fig 1. Variation sulphate concentrations (a), manganese concentrations (b), pH values (c) and the number of SRB cells (d) during batch experiments of bioreactor using synthetic wastewater which treated by zeolite R1: diameter size 0.4-0.6 cm, R2: 0.6-0.8 cm, R3: 0.8-1.2 cm and C: without treatment

The average of both sulphate and manganese removals in these batch experiments of zeolite R1 were 50.3% and 56.4%, respectively. As can be seen, sulphate concentration droplet rapidly to 148 mg/L on the first day of incubation and then decreased to 28 mg/L on the end day of incubation (14 days). The reduction of sulfate concentration during that incubation was followed by a decrease in the concentration of manganese. Consequently the pH values were also changed to neutral. The reduction manganese concentration during this process was influenced by the occurrence of precipitation reactions between manganese metal and some compounds which produced during SRB activities (biological sulfate reduction process), such as sulfides, carbonates and hydroxides. Decreasing manganese concentration in the anaerobic batch culture caused reduction manganese toxicity in SRB cell. Furthermore the numbers of SRB cells as biofilms in the zeolite surface were significantly increased during 14 days incubation.

Sulphate Reductions, Mn Removals and Ph Values of Effluent on Anaerobic Continuous Experiments

Based on the results from the batch experiments, zeolite R1 was applied in the continuous laboratory scale of down-flow fluidized-bed reactor (DFBR) in 1.2 L volume. The performance of DFBR in terms of sulfate reductions, Mn removals and pH values were presented in Fig. 2. The reactor started with 5.5% sulfate reduction, 1.9% Mn removal and pH increased in 3 days operation without any lag period due the inoculation with active SRB biomass from an optimum batch experiment. The active SRB may have increased over time and the bacteria may have been adapted to the operating conditions [1-2, 31]. The performance of a sulfate removal increased maximum to 23% in 9 days operation. Therefore, metals were precipitated with the sulfide produced by sulfate reduction process. This performance was followed by a manganese removal increased maximum to 15.4%. The sulfate reduction rate and manganese removal rate of the DFBR operations were 3.2 and 6.4% day^{-1}, respectively.

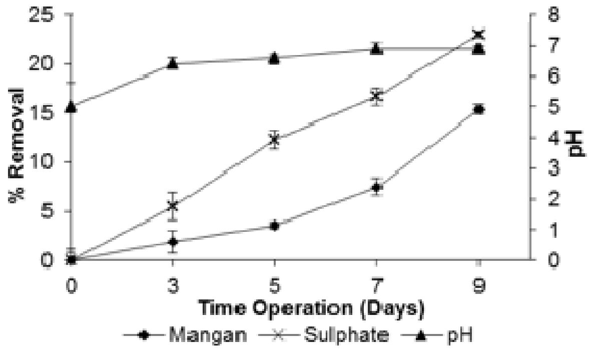

Fig 2. Removal of sulphate, manganese concentrations and pH values of effluent during anaerobic continuous bioreactor operation

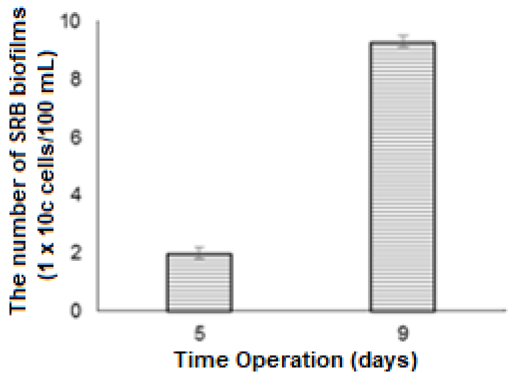

Fig 3. The Number of SRB Biofilms attached on zeolite surface in anaerobic continuous bioreactor operation

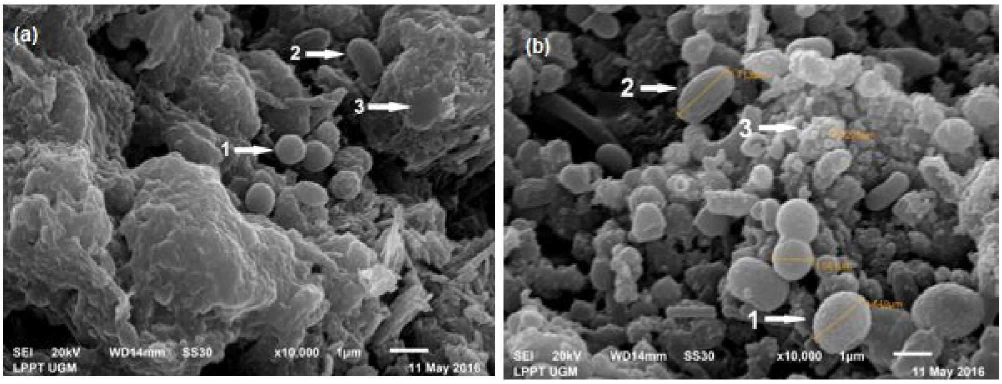

Fig 4. Developed biofilm of SRB on the zeolite surface in the continuous reactor after a 5 days (a) and 9 days (b) operation, 1: coccoid form bacterium cell; 2: bacillary form bacterium cell; 3: exopolysaccharide (EPS). Photographs were taken at 10,000× amplification

Previously reported sulfate and manganese reduction rate in an ethanol-fed DFBR using zeolite obtained from Turkey in 9 days operation were 0.4 and 0.3%, respectively. It was also clearly demonstrated that the bioreactor that feed by synthetic AMD with pH value of 5 .0 could change pH values of effluent were tend to neutral due to alkalinity production during sulfidogenic electron donor oxidation [32-33].

This reactor was only setup for 9 days operation at optimum conditions 30 °C with sodium lactate as carbon source, whereas the application this bioreactor in the field scale reported in several literatures was operated in the longer operation than 9 days. Longer operation of the bioreactor could remove sulphate and manganese concentration more efficient and effective, thus can be applied in the wastewater. Previously researchers reported that sulfate reduction and metal precipitation in the bioreactor operation of 250 days were 86% and 97%, respectively. [34]. Similar results also have been reported by Yamashita et al. that denitrification reactor packed with wood as a carbon source in the operation of 1500 days could remove of sulphate and manganese almost of 90% and 99%, respectively [35].

Characteristics of Developed SRB Biofilms Population

The number of SRB cells attached to zeolite in the bioreactor that operated in 5 and 9 days were presented in Fig. 3. During bioreactor operation, the population density of SRB increased approximately 5 times from 5 days to 9 days operation. This raising altered of cross-feeding, co-metabolism, interspecies hydrogen and proton transfer between SRB cells [36-37]. Furthermore, its condition may further stimulate the growth of micro colonies which effect on the increasing of their activity for removing sulphate and manganese.

Those developed SRB biofilms on the zeolite surface were also observed in the bioreactor using SEM (Fig. 4). A large microorganism accumulation in both the interior of the ruggedness and in the superficial zones of natural zeolite was observed during bioreactor operation. The attachment and colonization of SRB cells on the zeolite surface caused they were more protected from friction [38]. It was also observed that the support surface formed by a compact mass of microorganisms, principally with coccoid and bacillary

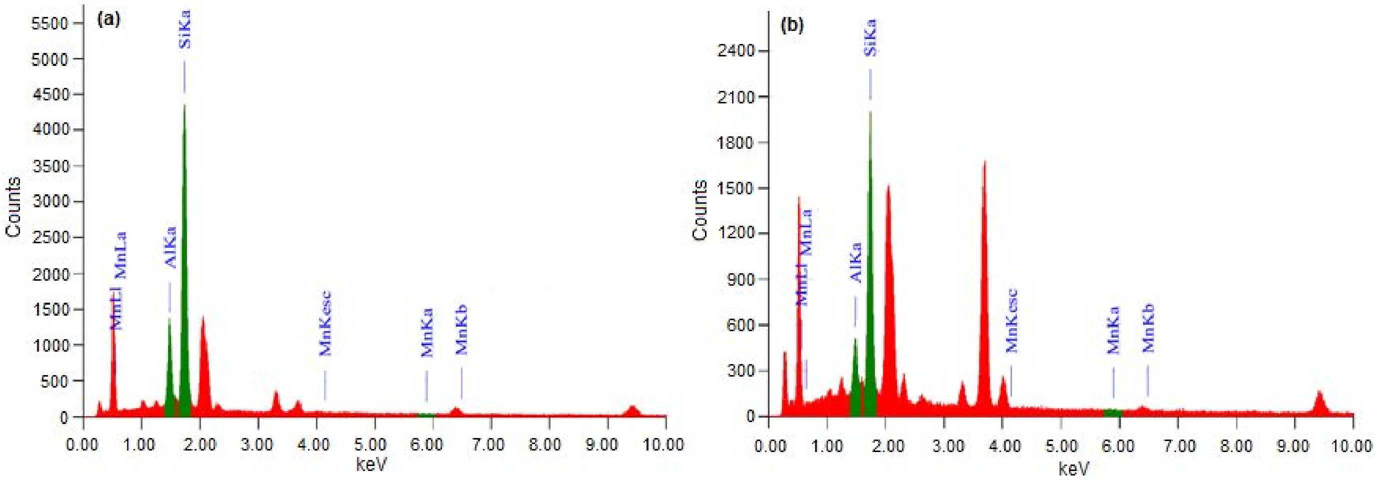

Fig 5. EDX spectra of SRB biofilm attached on zeolite particle after interacting with manganese in the continuous reactor after 5 days operation (a) and 9 days operation (b)

forms. These cells were directly attached to the zeolite surface and cells were immersed and covered with an exopolysaccharide (EPS) which keeps them together. EPS is produced by that bacterium which forms a dense and sticky glycocalyx on the surface of bacterial cells [39-40].

The composition of the metal precipitates which were accumulated from the surface of zeolite in the bioreactor was determined by SEM-EDX. Those analysis of the zeolite surface indicated that the precipitated compounds were most probably MnS (Fig. 5), this suggests that the removal of metal is mainly via sulfide precipitation. It is possible to determine that sulfide precipitation is the dominant metal removal mechanism in bioreactor operations. Therefore, two main sub-processes in bioreactors process can be referred to as diffusion and anaerobic reduction of sulfate, and the reaction of biogenic sulfide with metallic ions and precipitation of metal sulfide. Detection via EDX confirmed the mechanism of the precipitation of metal sulfides occurred due to the certain sulfide-rich layer around the zeolite surface. Therefore, metals ions might not be inhibit SRB activities even in high concentrations. The precipitation formed during their activities resulted higher percentage of manganese concentration that attached to the natural zeolite in reactors at 9 days operation than 5 days. These percentages of manganese concentrations present on 5 and 9 days incubation were 0.15% and 0.88%. As these bacterial consortiums could develop into biofilms on the Indonesian natural zeolite that adapted to higher sulphate and manganese concentrations, therefore, the application of zeolite in the bioreactor might held a critical role in reducing sulphate and manganese of AMD.

CONCLUSION

Indonesian natural zeolite was effective as a sulphate reducing bacterial support material which designed in continuous laboratory scale of down-flow fluidized-bed reactor (DFBR) for removing sulphate and manganese. It has excellent physical characteristics and the adequate environment for developing of SRB biofilms on its surface particle material. The highest efficiency of sulphate reduction and manganese in this bioreactor were found to be 23% and 15.4%, respectively at 9 days operation. The imaging SEM of SRB consortium on zeolite surface clearly showed the developed SRB biofilm on that particle. Analysis of EDX confirmed that manganese was precipitated as manganese-sulfides.

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