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Biosorption of Manganese in Drinking Water by Isolated Bacteria

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Abstract: Water is an important nutrition for living thing, such as humans, animals and plants. Nowadays, it has been polluted with inorganic contaminants which are discharged from industries. Manganese is one of the inorganics contaminant that causes low hemoglobin level, neurotoxicity, pipes clogging and bad taste if the concentrations in water exceed the regulated limit. A biological treatment process was investigated to treat the manganese through the biosorption mechanism by using Biological Aerated Filter (BAF) system. The microbes were taken from sewage activated sludge and isolated in agar media and identified by using Biolog Microstation System. These microbes included both Gram positive and Gram negative groups and the morphology were rod shape (*Bacillus*). The screening test has been done to select the highest manganese uptake by these strains and further studies under laboratory condition as a function of pH, biosorbent dosages and manganese toxicities were investigated comparison with biosorbent of sewage activated sludge. The biosorption isotherms were fitted with Langmuir to represent the equilibrium of the maximum manganese uptake by bacteria. The screening resulted that HAH1 has a higher manganese uptake capacity than others strain with 13.31 mg Mn²⁺/g biomass at pH 6 and biomass dosage of 0.1 g. The further studies resulted, manganese biosorption increased with rise in pH, biosorbent dosages and manganese toxicities. The Langmuir isotherm model revealed HAH1 was a better biosorbent of manganese than sewage activated sludge with the maximum biosorption capacity (q_{max}) of 55.56 mg Mn²⁺/g biomass and K_d value of 133.44.

Key words: Biosorption, manganese, drinking water, langmuir isotherm

INTRODUCTION

In recent years, the usage of heavy metals such as manganese, iron, zinc, chromium, nickel and arsenic in the industries has increased dramatically. These heavy metals will be pollutants to drinking water if the discharged concentration in the river exceeds the limit. Manganese in drinking water highly toxic to the living thing and environment which is causes gastrointestinal accumulation (Henrik *et al.*, 2004), low haemoglobin levels (Burgoa *et al.*, 2001), neurotoxicity (Veliz *et al.*, 2004), bad taste, water look brown and pipe clogging. This pollutant is released into the environment by industries such as fertilizer, petrochemical, electroplating, tanneries, metal processing and mining (Parvathi *et al.*, 2007). The Ministry of Health Malaysia has regulated that the concentration of manganese in raw water is below than 0.2 mg L⁻¹ while in treated water is below 0.1 mg L⁻¹.

Biological process is a good alternative to treat manganese in drinking water than the conventional

process. The advantages of biological process are such that no chemical usage, low operation and maintenance cost (Pacini *et al.*, 2005), process can be operated in a small scale (Gage *et al.*, 2001) high efficiency in detoxifying effluents and no nutrient requirements (Jianlong *et al.*, 2001). Manganese biosorption in the Biological Aerated Filter (BAF) is an application of the biological process for manganese treatment in drinking water. According to previous studies (Atkinson *et al.*, 1998), bacteria, algae, fungi and yeast are found to be capable of efficiently manganese biosorption. These microorganisms have a higher capacity for manganese removal and the uptake of manganese are selective than the conventional method.

The aim of this study is to isolate the bacteria from Sewage Activated Sludge (SAS) system for manganese biosorption study. The effects of pH, biosorbent dosages and manganese toxicities and on the manganese biosorption capacity of the isolated bacteria are studied extensively. The manganese biosorption isotherms of the strain are fitted with Langmuir isotherm.

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MATERIALS AND METHODS

Isolation and identification procedures: Sewage activated sludge was collected from aeration tank of sewage treatment plant located at Putrajaya, Malaysia and was cultured in reactor 10 L. After a few days, the activated sludge was serially diluted with distilled water from 10^{-1} to 10^{-5} . About 0.1 mL sample were taken and spread on nutrient agar and incubated in a growth chamber (GC 1050, Protech) at 37°C for 2 days. The isolated colonies in the agar plates were taken and grown for a few times to get the pure culture. Gram staining was done to identify the group of strains for start up identification. The strains will be identified by using Biolog Microstation System; Gram Negative 2 (GN2) and Gram Positive (GP2) Microplate for the future work.

Strain cultivation: The strains of each isolate were grown in 250 mL conical flasks containing 150 mL of nutrient broth and cultivated in shaking incubator (S1-600R, Japan) at 150 rpm and 37°C for 24 h. The cultivation was harvested by means of a centrifuge (Kubota 5220, Japan) at 350 rpm for 15 min. After two rinses with distilled water, the cells were suspended in distilled water to prepare the biomass stock solution. The biomass stock concentration was determined gravimetrically by dry weight at 105°C for 24 h.

Manganese biosorption studies: A batch experimental was setup using 250 mL conical flasks containing 100 mL of 50 mg Mn²⁺/L solution. This method was adapted from previous studies about manganese biosorption by yeast and fungi (Parvathi *et al.*, 2007). Manganese solution was prepared using MnCl₂·4H₂O (ChemAR). The biosorbents of cell culture was suspended in the manganese solution and incubated at 150 rpm on shaking incubator (S1-600R, Japan) at 37°C for 24 h. Samples were withdrawn at periodic intervals and were centrifuged (Eppendorf 5804, Germany) at 5000 rpm for 10 min. The supernatant was analyzed by using Adsorption Atomic Spectrometer (AAAnalysat 800, USA) and the manganese uptake by the biosorbents was calculated using the following equation (Vieira and Volesky, 2000):

$$q_e = \frac{V(C_i - C_e)}{X} \tag{1}$$

Where:

- q_e = The manganese uptake (mg Mn²⁺/g biomass)
- V = The volume of the manganese solution (mL)
- C_i = The initial concentration of manganese in the solution (mg Mn²⁺/L)

C_e = The final concentration of manganese in the solution (mg Mn²⁺/L)

X = The dry weight of the biomass (g)

Effect of pH: To study the effect of pH on the manganese biosorption, the initial pH of the solution was fixed to a range of pH 3-9 using either 0.1 M NaOH or 0.1 M HNO₃. The pH of the solution was control using pH meter (CyberScan 510, Singapore) at periodic intervals.

Effect of biosorbent dosage: The concentrations of bacteria used for the study were 0.2, 0.4, 0.6, 0.8 and 1.0% (w/v). The cell suspension was mixed in the manganese solution with a concentration of 50 mg Mn²⁺/L and pH 5.5-6 was maintained.

Effect of manganese toxicities: To investigate the effect of manganese toxicities on manganese uptake by biosorbents, the initial concentration of manganese was set in range of 25-300 mg Mn²⁺/L. A constant of 0.1 g of biosorbent dosage was suspended in the solution and pH 5.5-6 was maintained.

RESULTS AND DISCUSSION

Bacterial identification and enhancement: Six colonies were found and isolated on a fresh nutrient agar plates for the pure culture growth. The start up identification was performed by using Gram staining procedure and observation on the colonies as shown in Table 1. Most of these isolated bacterial were rod morphology and belonged to a wide variety of species including Gram negative and Gram positive. Reported by Kasan and Beacker (1989), these species are several commonly found in activated sludge, especially *Pseudomonas*, *Bacillus* and *Aeromonas*.

Screening test for manganese biosorption: The isolated bacterial and mix culture from SAS were exposed to a few set of manganese solution with 50 mg Mn²⁺/L concentration and were incubated for 24 h retention time. The screening shows species HAH1 has a higher biosorption capacity than the other biosorbents as shown

Table 1: Start up identification of bacterial from SAS

Bacterial	Morphology	Colour	Gram groups	Manganese uptake (mg g ⁻¹)
HAH1	Rod	White	+	13.12
HAH2	Rod	Dark yellow	+	8.00
HAH3	Rod	Light yellow	-	6.35
HAH4	Rod	Light white	-	10.31
HAH5	Rod	Pink	-	6.00
HAH6	Rod	Yellow	+	2.62

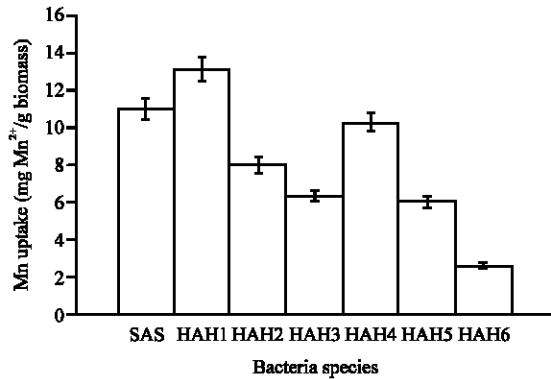


Fig. 1: Manganese biosorption with different isolated bacteria at 24 h retention time

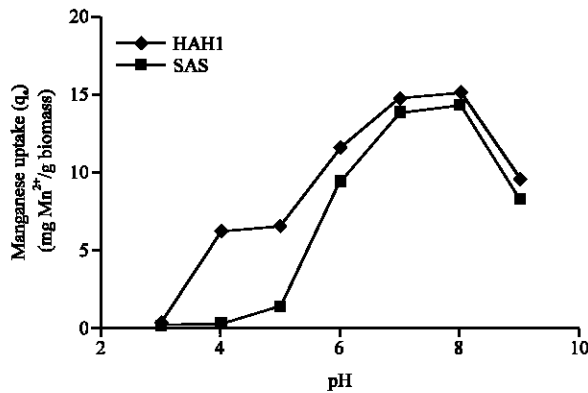


Fig. 2: Effect of pH on manganese biosorption

in Fig. 1. It is shown that the isolated HAH1 produce more extracellular polymers, which provide surface sites for adsorbing and complexing heavy metals (Leung *et al.*, 2001). In addition, all of the cell wall of isolated bacteria have some chemical functional groups which play vital roles in biosorption, including carboxyl, phosphonate, amine and hydroxyl groups (Doyle *et al.*, 1980; Van der Wal *et al.*, 1997; Vijayaraghavan and Yun, 2008).

Effect of pH, biosorbent dosage and manganese toxicities on manganese biosorption

Effect of pH: The favourable pH for metals biosorption by bacterial biomass has been found in a range of pH 3-6 (Vijayaraghavan and Yun, 2008). The manganese biosorption by *Pseudomonas aeruginosa* AT18 had been showing higher biosorption at pH 7 with maximum biosorption capacity of 38.2 mg Mn²⁺/g biomass (Silva *et al.*, 2009). As shown in Fig. 2, manganese biosorption increased with increasing pH of the manganese solution, but not in a liner relationship. For instance, at pH 3 the manganese uptakes were 0.34 and

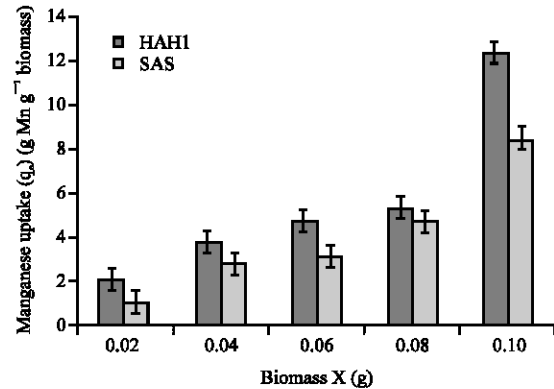


Fig. 3: Effect of biosorbent dosages on manganese biosorption by HAH1 and SAS

0.14 mg Mn²⁺/g biomass for HAH1 and SAS, respectively. In addition, the lowest uptakes could be attributed to competition between ion Mn²⁺ and the abundant ion H⁺ in the solution for attachment to binding sites of the biosorbents (Parvathi *et al.*, 2007). The optimum manganese uptake by these two biosorbents increased to 6.57-14.84 mg Mn²⁺/g biomass and 1.40-13.90 mg Mn²⁺/g biomass at pH 5-7, which are its uptake, seems to be constant at pH 7-8.

Biosorbents dosage: The manganese uptake increased with the concentration of biosorbents HAH1 and SAS dosage as shown in Fig. 3, due to the increased surface area of these two biosorbents, which in turn increases the number of binding sites (Esposito *et al.*, 2001). Biosorbents HAH1 recorded higher manganese uptake than the SAS with 2.1, 3.8, 4.8, 5.4 and 12.4 mg Mn²⁺/g biomass for HAH1 dosage of 0.2, 0.4, 0.6, 0.8 and 1.0%, respectively. Meanwhile, manganese uptakes by SAS were 1.1, 2.8, 3.2, 4.7 and 8.5 mg Mn²⁺/g biomass. Although at low biosorbent dosage, HAH1 adsorbed higher amount of manganese as compared to SAS which were attributed to chemical functional groups (carboxyl, phosphonate, amine and hydroxyl groups) and physical characteristics (moisture, soluble and iorganics contents) in biosorbents.

Manganese toxicities: The initial manganese concentrations have effect on biosorption process, which is higher concentration resulting in a high manganese uptake by biosorbents (Vijayaraghavan and Yun, 2008; Binupriya *et al.*, 2007). As shown in Fig. 4, increased the manganese concentration from 25-300 mg Mn²⁺/L resulted in an increase of manganese uptake by HAH1 and SAS from 6.6-33.8 mg Mn²⁺/g biomass and 4.3-25 mg Mn²⁺/g biomass, respectively. Instead, the manganese biosorption by fungus (*Aspergillus niger*) and yeast

Table 2: Comparison of manganese biosorption capacity of HAH1 with different biosorbents

Biosorbents	Manganese toxicities (mg L ⁻¹)	Biosorbent X (g)	q _e (mg g ⁻¹)	q _{max} (mg g ⁻¹)	References
<i>Aspergillus niger</i>	25-200	0.025-0.1	19.34	-	Parvathi <i>et al.</i> (2007)
<i>Saccharomyces cerevisiae</i>	25-200	0.025-0.1	18.95	-	Parvathi <i>et al.</i> (2007)
<i>Pseudomonas aeruginosa</i> AT18	49-70	0.01	22.39	20.32	Silva <i>et al.</i> (2009)
<i>Riccia xuitans</i>	-	1.00	-	0.254	Chojnacka (2007)
<i>Arthrobacter</i> sp.	10-2000	-	-	406.00	Veglio <i>et al.</i> (1997)
<i>Gleothecae magna</i>	1, 2 and 5	-	175.20	-	Mohamed (2001)
HAH1	25-300	0.02-0.1	2.1-12.4	55.56	Present study

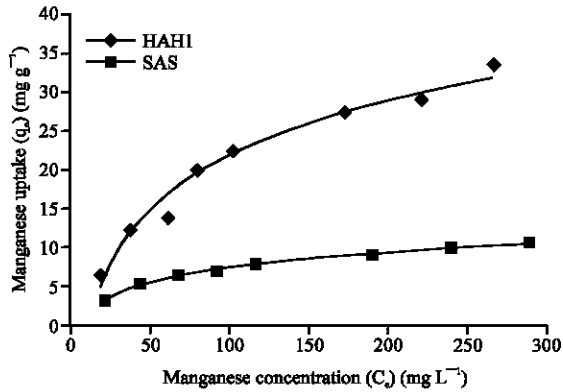


Fig. 4: Effect of manganese toxicities on biosorption by HAH1 and SAS

(*Saccharomyces cerevisiae*) with increasing manganese concentration from 25-200 mg Mn²⁺/L, the uptake were 2.46-19.34 mg Mn²⁺/g biomass and 1.54-18.95 mg Mn²⁺/g biomass (Parvathi *et al.*, 2007), respectively. On the other hand, Table 2 presents the comparison of manganese biosorption capacity of HAH1 with others biosorbent as functional of manganese toxicities and biosorbent dosages.

Biosorption isotherms: Biosorption isotherms provide the surface properties and affinity of the biosorbent and can be used as comparison in biosorptive capacity of the biomass for different heavy metals. The metal uptake by microorganisms has been shown to occur in two stage: an initial rapid stage (passive uptake), followed by a much slower process (active uptake) (Goyal *et al.*, 2003). In line of the rapid equilibrium, the Langmuir isotherm was chosen for fit the experimental data to evaluate the biosorption behavior on biosorbents.

Langmuir isotherm:

$$q = \frac{q_{max} K_d C_e}{(1 + K_d C_e)} \quad (2)$$

where, q_{max} is the maximum manganese specific uptake (mg g⁻¹) and K_d represents the equilibrium constant of biosorption reaction. This model was linearized as followed to obtain q_{max} and K_d values from the plot.

Table 3: Kinetic parameters for Langmuir isotherm with different biosorbents

Biosorbents	Isotherm constants		
	q _{max}	K _d	R ²
HAH1	55.56	133.44	0.987
SAS	10.00	25.28	0.944

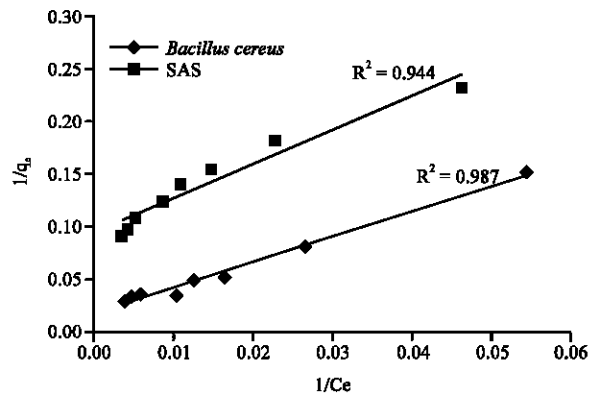


Fig. 5: Langmuir biosorption isotherms of manganese on HAH1 and SAS

$$\frac{1}{q} = \frac{1}{q_{max} K_d C_e} + \frac{1}{q_{max}} \quad (3)$$

The Langmuir equilibrium isotherm of HAH1 was represented on Fig. 5, as comparison with SAS. The model shows that HAH1 was a better biosorbent for manganese biosorption than SAS as shown in Table 3, which was the maximum manganese biosorption; q_{max} was 55.56 mg Mn²⁺/g biomass and K_d value of 133.44.

CONCLUSION

The isolation of mixed culture from the sewage activated sludge showed there were six dominants bacteria. These bacteria were Gram positive and Gram negative groups. Screening test for manganese biosorption resulted that HAH1 had higher manganese uptake than others strains with 13.31 mg Mn²⁺/g biomass. The subsequent studies resulted that increasing the pH, biosorbent dosages and manganese toxicities, the uptakes also increased. The Langmuir biosorption isotherm proved that the HAH1 was a better biosorbents

than the sewage activated sludge, with the maximum manganese biosorption, q_{max} of these two biosorbents were 55.56 mg Mn²⁺/g biomass, 10 mg Mn²⁺/g biomass and K_d value of 133.44 and 25.28, respectively. In future work, the isolated bacteria will be identified by using Biolog Microstation System; Gram Negative 2 (GN2) and Gram Positive (GP2) Microplate.

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