ORIGINAL ARTICLE

# Biosorption of heavy metal by thermotolerant polymer-producing bacterial cells and the bioflocculant

**Saithong Kaewchai[1] and Poonsuk Prasertsan[2]**


## Abstract

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**Biosorption of heavy metal by thermotolerant polymer-producing bacterial cells and the bioflocculant**
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**Three strains of thermotolerant polymer-producing bacteria; *Bacillus subtilis* WD 90, *Bacillus subtilis* SM 29, and *Enterobacter agglomerans* SM 38 as well as their biofloculants were used to investigate on the adsorption of heavy metal, nickel and cadmium. The effects of pH and concentrations of heavy metal were investigated. The optimum pH for nickel and cadmium adsorption by the dried cells of *E. agglomerans* SM 38 were found to be 7.0 (25.5% removal) and 8.0 (32% removal), respectively. For *B. subtilis* WD 90 and *B. subtilis* SM 29, the optimum pH at 8.0 exhibited the nickel removal of 27% and 25%, respectively, and cadmium removal of 28% and 28.5%, respectively. The heavy metal adsorption by the dried cells and wet cells of *E. agglomerans* SM 38 were slightly increased with increasing initial concentrations of nickel and cadmium up to 60 and 30 ppm, respectively. The bioflocculant of *B. subtilis* WD 90 and *B. subtilis* SM 29 showed the highest nickel removal of 90.7% and 87.0% respectively, while the cadmium removal was 90.9 and 91.4%, respectively. The optimum pH for adsorption of both nickel and cadmium by the bioflocculant of *E. agglomerans* SM 38 was 7.0 with the removal of 92.8 and 84.2%, respectively. The optimum nickel con-**


**[1]B.Sc. (Agriculture), Master Student, Joint Graduate School of Energy and Environment, King Mongkut's University of Technology Thonburi, Bangkok 10140 [2]Ph.D. (Biotechnology), Assoc. Prof., Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112 Thailand.**
**Corresponding e-mail : ppoonsuk@ratree.psu.ac.th**
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**centration for adsorption by the bioflocculant of *E. agglomerans* SM 38 was 10 ppm, with the removal of 92.5%, and rather stable up to 60 ppm. The optimum cadmium concentration for adsorption by the bioflocculant of *B. subtilis* SM 29 was 60 ppm at pH 8.0 with the removal of 85.7%. Therefore, the bioflocculant of the three isolates gave higher heavy metal adsorption than the cells.**

**Key words :** bioflocculant , biosorption, heavy metal, thermotolerant, polymer, bacterial cells

## บทคัดย่อ

**สายทอง แก้วฉาย[1] และ พูนสุข ประเสริฐสรรพ์[2]**

**การดูดซับโลหะหนักทางชีวภาพด้วยเซลล์แบคทีเรียทนร้อนที่ผลิตสารช่วยตกตะกอนและสารช่วยตกตะกอนชีวภาพ**

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**ศึกษาการดูดซับโลหะหนัก (นิเกิลและแคดเมียม) โดยใช้แบคทีเรียทนร้อนที่ผลิตพอลิเมอร์ จำนวน 3 สายพันธุ์ ได้แก่ *Bacillus subtilis* WD90 , *Bacillus subtilis* SM29 และ *Enterobacter agglomerans* SM 38 รวมทั้งสารช่วยตกตะกอนจากเชื้อเหล่านี้ จากการศึกษาผลของพีเอชและความเข้มข้นของโลหะหนัก พบว่าพีเอชที่เหมาะสมต่อการดูดซับนิเกิลและโครเมียมโดยใช้เซลล์แห้งของ *Enterobacter agglomerans* SM 38 คือ พีเอช 7.0 (ค่าลดลง 25.5%) และพีเอช 8.0 (ค่าลดลง 32%) ตามลำดับ สำหรับแบคทีเรียอีก 2 สายพันธุ์ ค่าพีเอชที่เหมาะสม คือ พีเอช 8.0 สามารถดูดซับนิเกิลได้ 27% และ 25% ตามลำดับ ดูดซับแคดเมียมได้ 28% และ 28.5% ตามลำดับ การดูดซับโลหะหนักด้วยเซลล์แห้งและเซลล์เปียกของ *Enterobacter agglomerans* SM 38 เพิ่มขึ้นเล็กน้อย เมื่อความเข้มข้นเริ่มต้นของนิเกิลและแคดเมียมเพิ่มขึ้นถึง 60 และ 30 พีพีเอ็ม ตามลำดับ สารช่วยตกตะกอนชีวภาพของ *Bacillus subtilis* WD90 และ *Bacillus subtilis* SM29 สามารถดูดซับนิเกิลได้สูงสุดเท่ากับ 90.7% และ 87.0% ตามลำดับ ในขณะที่ดูดซับแคดเมียมได้ 90.9% และ 91.4% ตามลำดับ พีเอชที่เหมาะสมต่อการดูดซับนิเกิลและแคดเมียมโดยสารช่วยตกตะกอนของ *Enterobacter agglomerans* SM 38 เท่ากับ 7.0 ซึ่งได้ค่าที่ลดลงเท่ากับ 92.8% และ 84.2% ตามลำดับ ความเข้มข้นที่เหมาะสมของนิเกิลสำหรับการดูดซับด้วยสารช่วยตกตะกอนชีวภาพของ *Enterobacter agglomerans* SM 38 คือ 10 พีพีเอ็มโดยลดลง 92.5% และมีความคงตัวจนถึงระดับ 60 พีพีเอ็ม สำหรับการดูดซับแคดเมียมโดยสารช่วยตกตะกอนของ *Bacillus subtilis* SM29 ค่าความเข้มข้นที่เหมาะสมเท่ากับ 60 พีพีเอ็ม ที่พีเอช 8.0 โดยลดลง 85.7% ดังนั้นสารช่วยตกตะกอนชีวภาพของเชื้อที่แยกได้ทั้ง 3 สายพันธุ์ สามารถดูดซับโลหะหนักได้ดีกว่าตัวเซลล์**

[1]**บัณฑิตวิทยาลัยร่วมด้านพลังงานและสิ่งแวดล้อม มหาวิทยาลัยเทคโนโลยีพระจอมเกล้าธนบุรี บางมด ทุ่งครุ กรุงเทพฯ 10140**
[2]**ภาควิชาเทคโนโลยีชีวภาพอุตสาหกรรม คณะอุตสาหกรรมเกษตร มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112**

The problem associated with heavy metals in wastewater entering natural waters have been well documented (Florence and Morrison, 1992). Heavy metals (arsenic, cadmium, copper, lead, zinc) presents a possible human health risk (Sag and Kutsal, 1989). The increasing problem of heavy metal contamination of soil, water and other environment has stimulated a search for new mechanisms to remove these pollutants. Recent research in the area of heavy metal removal from wastewater and sediments has focused on the development of materials with increased affinity, capacity, and selectivity for target metal (Parirandeh, *et al*., 1998). The existing methods for the removal of metals from the environment can be grouped into biotic and abiotic. Biotic methods are based on the accumulation of the heavy metal by plants or microor-

ganisms; abiotic methods include physiochemical processes such as precipitation, coprecipitation, and adsorption of the heavy metal by a suitable adsorbent (Celis, *et al*., 2000). The use of bacteria to uptake heavy metal by the range of bacterial components, such as extracellular polysaccharide and cell wall component was investigated (Geddie and Sutherland, 1993). Extracellular polysaccharide already plays important roles in controlling heavy metal pollution in the sewage treatment process. There are many species of microorganisms that are used for study of uptake of heavy metal.

Biopolymer from microbial source has received much attention recently due to the awareness of environmental problem which inevitably affects the health of human being (Kurane, *et al*., 1994). Although they are widely applied as several functions, the function as "flocculant" was also interesting and should be developed for wastewater treatment and water treatment, in fermentation industries to replace the generally used chemically synthetic polymers which are not friendly to the environment (Yokoi, *et al*., 1997). Besides possessing the flocculating activity, the biopolymer may be able to adsorb heavy metals or function as water-absorbent.

Three strains of thermotolerant flocculant-producing bacteria were selected from our previous study (Kaewchai and Prasertsan, 2002). They grew very well in palm oil mill effluent. This paper aims to investigate on the ability of these bacteria and their bioflocculants for adsorption of heavy metals which are normally present in wastewater treatment.

## Materials and Methods

### Microorganisms

Three thermotolerant-polymer producing bacterial strains; *Bacillus subtilis* WD 90, *Bacillus subtilis* SM 29, and *Enterobacter agglomerans* SM 38 used in this experiment were selected from previous studies (Kaewchai and Prasertsan, 2002). They were maintained in the polyglutamic acid producing medium (PGA medium).

### Medium

The PGA medium contained 2% glucose, 0.05% yeast extract, 5% glutamic acid and 0.05% $MgSO_4.7H_2O$, pH was adjusted to 7.0 (Yokoi, *et al*., 1995)

### Preparation and analysis of heavy metal

Heavy metal tested for adsorption by microorganism and biopolymer were nickel and cadmium. They were prepared from $NiSO_4.6H_2O$ (as $Ni^{2+}$) and $CdSO_4.8H_2O$ (as $Cd^{2+}$) at the concentrations of 10, 15, 30, 60 and 100 ppm. The metal concentration was analyzed using the inductively coupled plasma atomic emission spectroscopy (ICP-AES) at the Central Equipment Division, Prince of Songkla University, Hat Yai.

### Heavy metal adsorption by the cells

The adsorption of heavy metal was carried out using the cells of the thermotolerant flocculant-producing bacteria. Cell suspension was prepared by cultivating the three selected polymer-producing bacteria in PGA medium on a shaker (200 rpm) at 45°C. After 24 h cultivation, the cells were harvested by centrifugation at 12,735*xg* (10,000 rpm) for 15 min at 4°C, then washed with sterile 0.5% (w/v) NaCl solution followed by sterile distilled water. The cells were dried at 105°C about 2 h.

#### Preparation of samples

The dried cells (200 mg/l) were suspended in distilled water and homogenized in a mixer to destroy aggregation of cells. The cell suspensions were added into 10 ppm of nickel (Ni) and cadmium (Cd) solution. The wet cells were suspended in 100 ml of 0.5% (w/v) NaCl solution at room temperature in order to obtain a suspension with equivalent to the dried cell concentration of 200 mg/l. Sodium chloride was included to prevent cell damage due to osmotic pressure. These suspensions were used as samples for the study on the effects of the following factors on heavy metal adsorption.

**Effect of pH**

The effect of pH (5.0, 6.0, 7.0, and 8.0) was investigated by adjusting pH of the sample using dried cells of the three strains. The adsorption test was conducted on a shaker (100 rpm) at 30°C for 6 and 24 h. The samples were taken and centrifuged at 12,735*xg* (10,000 rpm) for 15 min. The supernatants were analyzed for the remaining heavy metal in the solution (Sag and Kutsal, 1989) and the percentage of each metal removal calculate based on its initial concentration (Gourdon *et al*., 1990a). The optimum pH and bacterial strain were selected for further studies.

**Effect of heavy metal concentrations**

The adsorption of heavy metal at the concentrations of 10, 15, 30, 60, and 100 ppm using the dried and wet cells suspension of the selected strain (200 mg/l) at the optimum pH were compared. The adsorption test was conducted in an incubator shaker (100 rpm) at 30°C. The samples were taken after 6 h incubation for the dried cell suspension. For wet cell suspension, 0.5% (w/v) NaCl was included and the samples were taken after 2 h incubation (Gourdon *et al*., 1990a). The supernatants of the samples were analyzed and the percentage of each metal removal calculated as mentioned above.

**Heavy metal adsorption by the bioflocculant**

The biopolymer of the three selected strains were recovered from the PGA medium according to the procedure as described by Dermlim *et al*. (1999) after 24 h cultivation on a shaker (200 rpm) at 45°C (Kaewchai and Prasertsan, 2002). Their polymers were dialyzed in a dialysis bag with a molecular weight cut-off 12,000-14,000 to remove low molecular weight impurities, then dried under vacuum (Rudo, *et al*., 1984). They were used in the following studies.

**Effect of pH**

For adsorption study, 0.1 g of dried polymer of the three strains was added in a flask containing 100 ml of 10 ppm metal solution of nickel and cadmium. The sample pH was adjusted to pH 5, 6, 7 and 8 and the flasks were shaken (100 rpm) for 24 h at 30°C. The samples were taken and filtered through a 0.2 μm micropore cellulose acetate filter to separate the soluble and colloidal forms. The soluble samples were analyzed for the remaining metal concentrations (Sag and Kutsal, 1989) and the percentage of each metal removal calculated (Gourdon *et al*., 1990a). The optimum pH and bacterial strain(s) were selected for further studies.

**Effect of heavy metal concentration**

The effect of heavy metal concentrations (10, 15, 30, 60, and 100 ppm) on the metal adsorption by the biopolymer (0.1% w/v) of the selected strain(s) at the optimum pH was investigated. The adsorption test procedure and analysis of samples as well as the calculation of percentage of each metal removed were the same as described above.

## Results and Discussions

**Heavy metal adsorption by the cells**

Ion uptake by the range of bacterial component, such as cell wall component and extracellular polysacchride, plays important roles in controlling heavy metal pollution in the sewage treatment process (Gourdon, *et al*., 1990b). In this study, *B. subtilis* WD 90, *B. subtilis* SM 29, and *E. agglomerans* SM 38 were used to adsorb the heavy metal by the cell component and the biopolymer. These three strains were polyglutamic acid polymer producing bacteria. Cadmium and nickel were chosen as a model because they are among the most toxic heavy metals and could be found in different industries such as metal-processing industries, steel foundries, motor vehicles, aircraft industries, and paint chemical industries, textile production, printing, pigment work, electroplating, ceramics, metallurgical alloying, etc.

**Effect of pH**

The effect of pH on nickel removal by the dried cells of the three strains after 6 h incubation is shown in Figure 1A. The highest nickel adsorption was obtained from *B. subtilis* WD 90 and *B. subtilis* SM 29 at pH 8, while *E. agglomerans* SM 38 gave the highest value at pH 7. The nickel adsorption by *B. subtilis* WD 90 and *B. subtilis*

SM 29 increased with increasing pH over the range test, whereas *E. agglomerans* SM 38 increased with increasing pH until pH 7.0 and decreased thereafter. Nickel adsorption at 24 h incubation gave similar result (Figure 1B). It was observed that nickel adsorption by *E. agglomerans* SM 38 was slightly different within the pH range 5-7 at both 6 and 24 h incubation and higher than those of *B. subtilis* WD 90 and *B. subtilis* SM 29.

The removal of cadmium by the dried cells of the three strains at different pH after 6 and 24 h incubation time is shown in Figure 2. Results illustrated that all three strains gave the highest cadmium uptake at pH 8 both after 6 and 24 h incubation time. It was observed that *B. subtilis* WD 90 and *B. subtilis* SM 29 removed cadmium at 6 h incubation time higher than at 24 h incubation. At the same pH, *E. agglomerans* SM 38 showed higher cadmium adsorption than that by *B. subtilis* WD 90 and *B. subtilis* SM 29 except at pH 5.0 after 6 h incubation.

The optimum pH for nickel and cadmium adsorption by the dried cells of *E. agglomerans* SM 38 was found to be 7.0 (25.5% removal) and 8.0 (32% removal), respectively. For *B. subtilis* WD 90 and *B. subtilis* SM 29, the optimum pH

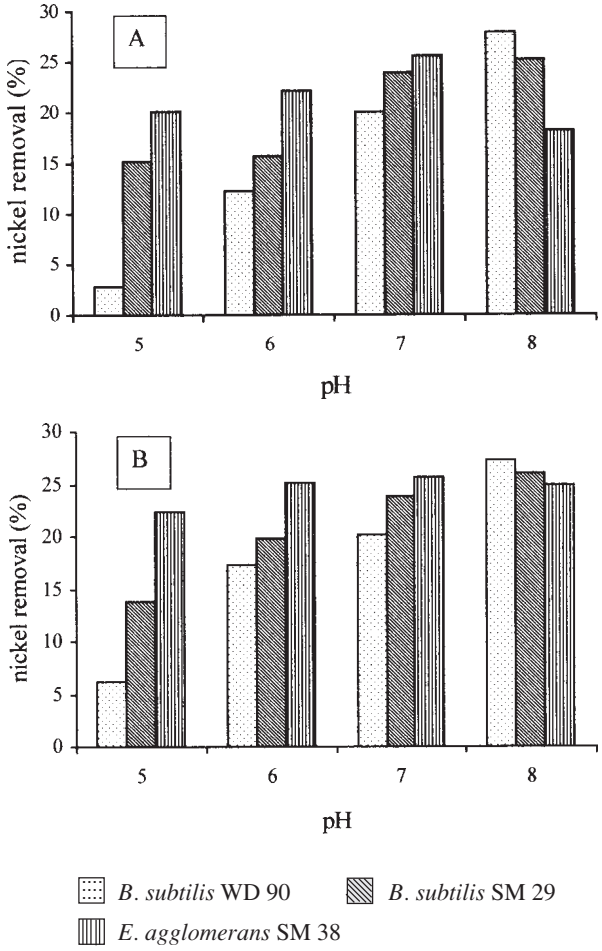


**Figure 1. Effect of pH on nickel (10 ppm) removal by dried cells of the three thermotolerant polymer-producing bacteria at 6 h (A) and 24 h (B) cultivation**

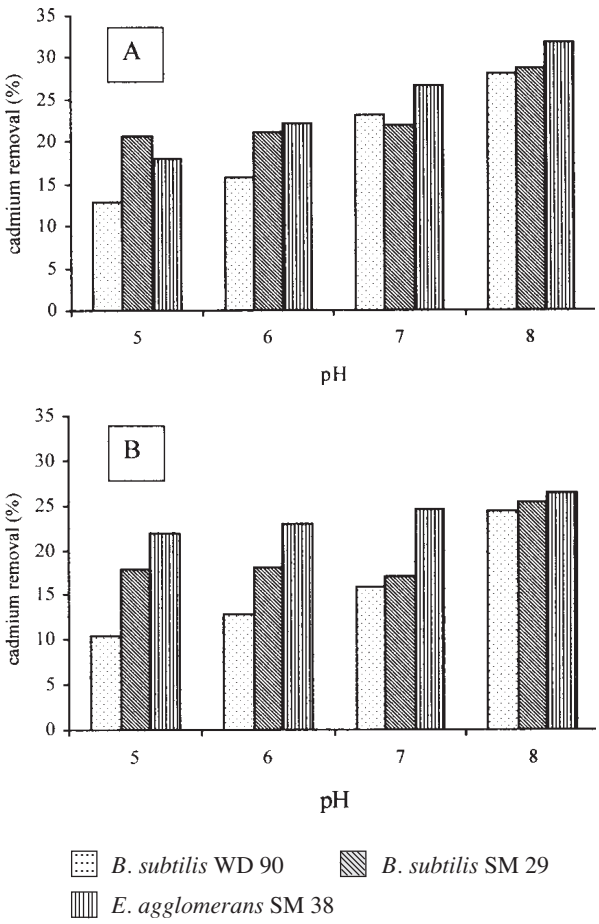


**Figure 2. Effect of pH on cadmium (10 ppm) removal by dried cells of the three thermotolerant polymer-producing bacteria at 6h (A) and 24h (B) cultivation**

at 8.0 exhibited nickel removal of 27% and 25%, respectively and cadmium removal of 28% and 28.5%, respectively. The increase of pH resulted in an increased negative charge on the surface of the cell which favored electrochemical attraction and adsorption of metal (Gourdon *et al*., 1990a). This pH was lower than that (pH 9.1) used for cadmium removal (> 99%) by *Pseudomonas aerugenosa* (Wang, *et al*., 1997) but fell within the pH range of 4.0-8.0 that is widely accepted as being optimal for metal uptake for almost all types of biomass (Blackwell, *et al*., 1995). The biosorptive capacity of the cells was sensitive to pH (Simine, *et al*., 1998). The pH was reported to be the most important factor for all ions uptake, and the removal capacity was shown to increase with pH but the upper limit of working pH was limited by hydroxide precipitation (Geddie and Sutherland, 1993).

Increase of incubation time had a little effect on the nickel uptake by these three selected strains. These results corresponded to that of Delgado, *et al*. (1996) in which there were no significant differences for metal uptake within 24 h to 4 days incubation. Cadmium adsorption by *B. subtilis* WD 90, *B. subtilis* SM 29 decreased during 6 h to 24 h incubation. The slight decrease is cadmium adsorption after 6 h may be related to the not occurring or occurring at a very low rate of the metabolic mediation (Gourdon, *et al*., 1990b).

The cell wall structure plays an important role for adsorption of heavy metal (Gourdon, *et al*., 1990a) and Gram positive and Gram negative bacteria have significant differences in their cell wall structure. In this work *E. agglomerrans* SM 38 was Gram negative while *B. subtilis* WD 90 and *B. subtilis* SM 29 were Gram positive. Results (Figures 1 and 2) showed that the Gram negative bacterium had higher metal biosorption capacity than the two Gram positive bacteria over the pH range tested except at pH 8. The Gram negative bacterium was able to accumulate higher amounts of cadmium than Gram positive bacteria. However, it was reported that Gram positive bacteria had a higher cadmium biosorption capacity than Gram negative bacteria (Gourdon *et al*., 1990a). This phenomenon did not imply that all Gram negative bacteria would accumulate heavy metal higher than all Gram positive bacteria. Nevertheless, the Gram negative *E. agglomerans* SM 38 was selected for further studies.

**Effect of heavy metal concentration**

The effect of metal concentrations on metal removal by the dried and the wet cells of *E. agglomerans* SM 38 were investigated. The concentrations of nickel and cadmium (10, 15, 30, 60 and 100 ppm) at the optimum pH (7.0) were studied. The nickel and cadmium adsorption by the dried cells of *E. agglomerans* SM 38 are shown in Figure 3. It was found that the nickel removal decreased at 15 ppm but above after that they remained stable as the concentrations increased up to 60 ppm and decreased thereafter. The cadmium adsorption, however, increased as the metal concentrations increased up to 60 ppm and dropped sharply at 100 ppm. Therefore, under the concentrations tested, the maximum adsorption of nickel and cadmium adsorption by the dried cells of *E. agglomerans* SM 38 were 10 ppm and 60 ppm, respectively. This result was similar to the maximum adsorption of cadmium (II) ion at 50 ppm on the dried cells of *Zoogloea ramigera* (Norberg, 1984).

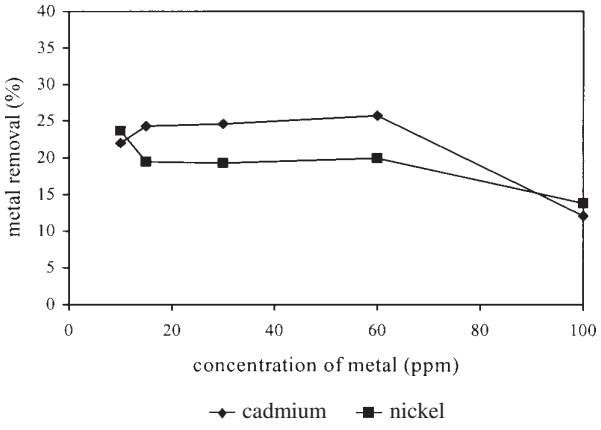


**Figure 3. Effect of metal concentration on nickel and cadmium removal by the dried cells of *Enterobacter agglomerans* SM 38 at pH 7.0 after 6 h incubation at 30°C**

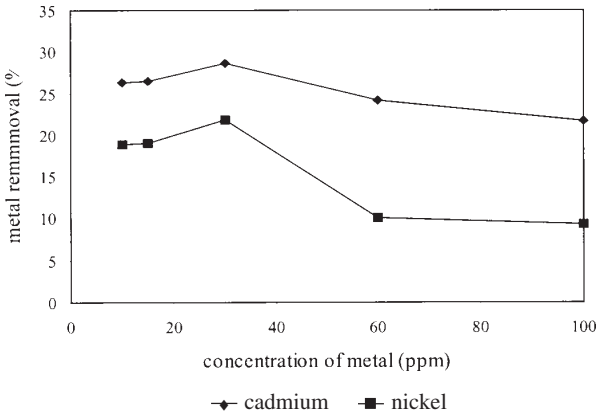


**Figure 4. Effect of metal concentration on nickel and cadmium removal by the wet cells of *Enterobacter agglomerans* SM 38 at pH 7.0 after 6 h incubation at 30°C**

Nickel and cadmium removal by the wet cells of *E. agglomerans* SM 38 at pH 7.0 is shown in Figure 4. The nickel and cadmium concentrations were varied the same as those used by the dried cells. Results showed that the adsorption of both metals were slightly increased with the increase of initial concentrations up to 30 ppm, and decreased thereafter. Therefore, the maximum adsorption of nickel and cadmium by the wet cells of *E. agglomerans* SM 38 occurred at 30 ppm. This value was higher than that by the wet cells of *Klebseilla aerogenes* in which the adsorption of both metals gradually decreased with increasing initial concentrations from 0.01 to 10 mg/l (ppm) (Brown and Lester, 1982). It was observed that at low initial metal ion concentration, moderately high adsorption yield was obtained. In this experiment, the cadmium adsorption was higher than the nickel adsorption in both cases.

Comparison on using dried and wet cells for the adsorption of two heavy metals indicated that the dried cells exhibited higher adsorption for cadmium than nickel while the wet cells could adsorb nickel more than cadmium. This should be taken into account on the application of the cells for metal adsorption.

**Heavy metal adsorption by bioflocculants**

**Effect of pH**

Bioflocculant from the three selected isolates were used for adsorption of nickel. The effects of pH were examined at pH 5.0, 6.0, 7.0, and 8.0. The results showed that all three selected isolates could adsorb more than 70.0% of the nickel (Figure 5). *E. agglomerrans* SM 38 gave the highest nickel adsorption at pH 7.0 but *B. subtilis* WD 90 and *B. subtilis* SM 29 gave the highest values at pH 8.0. It was observed that *E. agglomerrans* SM 38 could adsorb more nickel than *B. subtilis* WD 90 and *B. subtilis* SM 29, except at pH 8.0.

Studies on the effect of pH on cadmium adsorption by the three strains (Figure 6) illustrated that *E. agglomerran*s SM 38 had the maximum cadmium adsorption at pH 7.0, while it was at pH 8.0 for *B. subtilis* WD 90 and *B. subtilis* SM 29. The cadmium adsorption by *B. subtilis* WD 90 and *B. subtilis* SM 29 was higher than that by *E. agglomerrans* SM 38. It was observed that the cadmium adsorption increased slightly when the pH increased from pH 5.0 to 7.0. This was

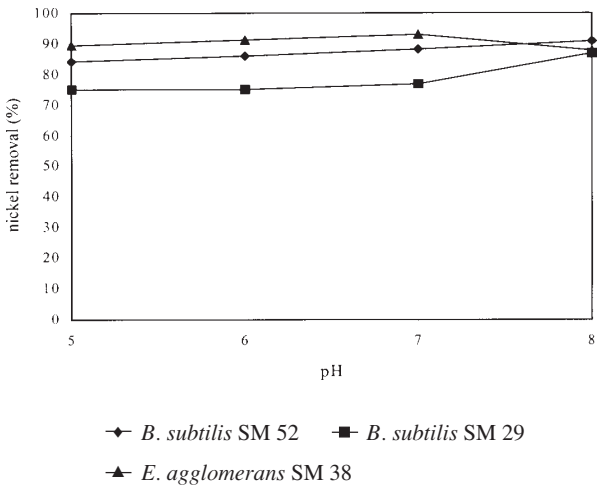


**Figure 5. Effect of pH on nickel (10 ppm) removal by the bioflocculant of the three thermotolerant polymer-producing bacteria at pH 7.0 after 24 h incubation at 30°C**

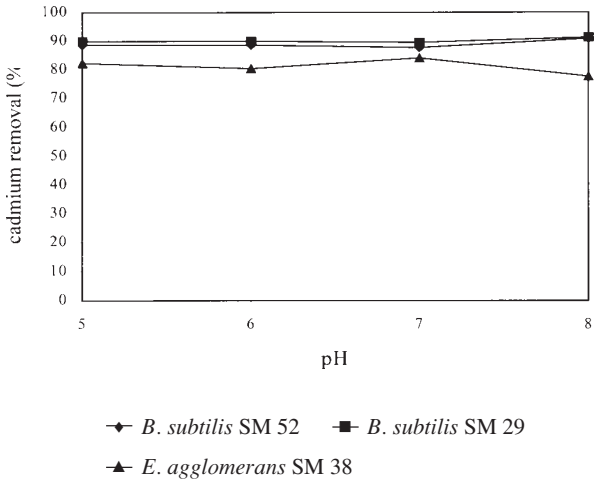


**Figure 6. Effect of pH on cadmium (10 ppm) removal by the bioflocculant of the three thermotolerant polymer-producing bacteria at pH 7.0 after 24 h incubation at 30°C**

similar to the adsorption of $Ca^{2+}$ by *Zooglea* polysaccharide which increased gradually with increasing pH from 3.0 to 7.0, whereas adsorption of $Mg^{2+}$ increased gradually until pH 7.0 (Geddie and Sutherland, 1993). This phenomenon illustrated that at low pH, a high concentration of protons competes for the same anionic sites on the polymer as the divalent cations. The mass of protons leads to their preferential binding and thus divalent cation binding is low. As the pH increases, the $H^+$ concentration falls, and the cations can compete to a greater extent at the binding sites.

*E. agglomerrans* SM 38 was selected for further study on nickel concentration and *B. subtilis* SM 29 was selected for the effect of cadmium concentration, since they showed the highest nickel and cadmium adsorptions, respectively.

**Effect of heavy metal concentration**

Effect of nickel concentration on the adsorption by the bioflocculant of *E. agglomerrans* SM 38 at pH 7.0 is illustrated in Figure 7. The nickel removal was still higher than 70%. Results illustrated that the nickel adsorption did not increase with the increase of initial concentrations. The nickel adsorption maintained around 90% up

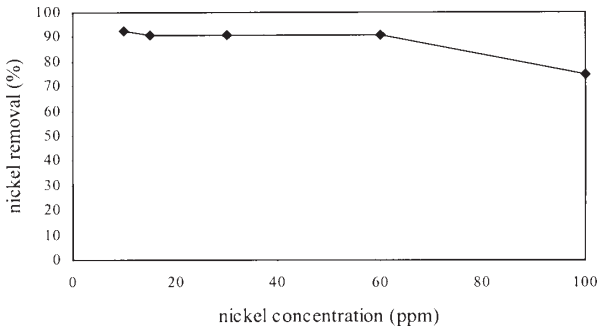


**Figure 7. Effect of nickel concentration on the removal by the bioflocculant of *Enterobacter agglomerans* SM 38 at pH 7.0 after 24 h incubation at 30°C**

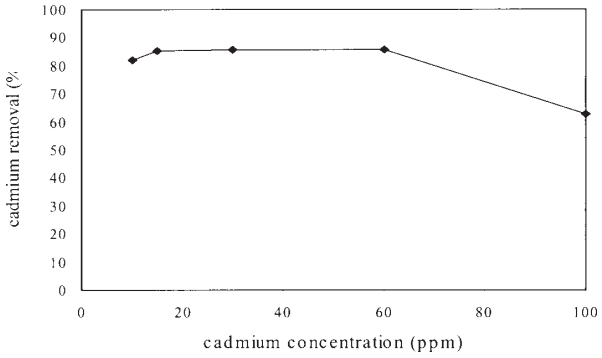


**Figure 8. Effect of cadmium concentration on the removal by the bioflocculant of the *Bacillus subtilis* SM 29 at pH 8.0 after 24 h incubation at 30°C**

to 60 ppm and decreased to about 70% at 100 ppm. This means that if the initial concentration of nickel was higher than 60 ppm, the adsorption of nickel decreased.

The effect cadmium concentration on the adsorption by the bioflocculant of *B. subtilis* SM 29 at pH 8.0 is shown in Figure 8. The cadmium removal maintained at 85% up to 60 ppm and decreased to 62% at 100 ppm. At low initial metal ion concentrations, moderately high yield was obtained. Increasing initial metal concentration from 0.01 to 100 mM, the metal removal by *Bacillus simplex* ZAN-044 increased and decreased at

1000 mM (Polmung and Prasertsan, 1997).

## Conclusion

The highest uptake of nickel and cadmium by the dried cells of *E. agglomerans* SM 38 was at pH 7.0 and 8.0 respectively. For *B. subtilis* WD 90 and *B. subtilis* SM 29, the highest value of both metal adsorption was at pH 8.0. The nickel and cadmium adsorption by the dried and the wet cells of *E. agglomerans* SM 29 at pH 7.0 was slightly increased with increasing initial concentration up to 60 and 30 ppm, respectively. The removal of nickel and cadmium by the bioflocculant from the three selected strains was higher than 70%. The highest adsorption of both metals by the bioflocculant of *B. subtilis* WD 90 and *B. subtilis* SM 29 were both at pH 8.0 while it was at pH 7.0 for *E. agglomerans* SM 38. The optimum concentration of nickel and cadmium adsorption by the bioflocculant of *E. agglomerans* SM 38 and *B. subtilis* SM 29 was 60 ppm. Adsorption of nickel and cadmium by the bioflocculant from the three selected strains was higher than that by the cells.

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