การกำจัดแคดเมียมออกจากสารละลายโดยใช้ Exopolymers ที่สร้างจาก *Ralstonia* sp. TAK1

Removal of Cadmium from Aqueous Solution by Exopolymers Produced by *Ralstonia* sp. TAK1

นวลนภา ชมพูธวัช[1] จารุวรรณ วงค์ทะเนตร[1] อัจฉรา อัศวรุจิกุลชัย[2] และเบญจภรณ์ ประภักดี[1]

Nuannapa Chompoothawat[1], Jaruwan Wongthanate[1], Achara Ussawarujikulchai[2] and Benjaphorn Prapagdee[1]

บทคัดย่อ

Exopolymers ของแบคทีเรียมีบทบาทสำคัญในการดูดซับโลหะหนัก โดยเกิดจากปฏิกิริยาระหว่างอิออนบวกของโลหะหนัก และอิออนลบของหมู่ฟังก์ชั่นของ exopolymers *Ralstonia* sp. TAK1 เป็นแบคทีเรียที่มีความสามารถในการต้านทานความเป็นพิษของแคดเมียมโดยการผลิต exopolymers ซึ่ง *Ralstonia* sp. TAK1 คัดแยกมาจากดินที่มีการปนเปื้อนแคดเมียมบริเวณที่มีการทำเหมืองแร่สังกะสี ในอำเภอแม่สอด จังหวัดตาก การศึกษาครั้งนี้ได้ทำการศึกษาเกี่ยวกับชนิดของคาร์บอนที่สามารถชักนำการสร้าง exopolymers ของ *Ralstonia* sp. TAK1 รวมทั้งยังศึกษาประสิทธิภาพของเซลล์แบคทีเรียและ exopolymers ในการกำจัดแคดเมียมจากสารละลาย ผลการศึกษาพบว่า *Ralstonia* sp. TAK1 ที่เพาะเลี้ยงในอาหารเลี้ยงเชื้อที่เติมกลูโคสความเข้มข้น 2 เปอร์เซ็นต์สามารถสร้าง exopolymers สูงสุดในระยะ stationary (24 ชม.) ของการเจริญ (5.39 กรัมต่อลิตร) หลังจากนั้น ปริมาณ exopolymers ลดลงเหลือ 5.08 กรัมต่อลิตรในช่วงกลางของระยะ stationary (32 ชม.) และพบว่าเซลล์ในระยะ stationary (24 ชม.) ของ *Ralstonia* sp. TAK1 มีประสิทธิภาพสูงสุดในการกำจัดแคดเมียม (41.73 เปอร์เซ็นต์) และความสามารถในการดูดซับแคดเมียมสูงสุด (15.02 มิลลิกรัมของแคดเมียมต่อกรัมของน้ำหนักแห้งเซลล์) ในขณะที่ exopolymers มีค่าประสิทธิภาพในการกำจัดแคดเมียมเท่ากับ 1.91 เปอร์เซ็นต์ และมีค่าความสามารถในการดูดซับแคดเมียมเท่ากับ 0.69 มิลลิกรัมของแคดเมียมต่อกรัมของน้ำหนักแห้ง exopolymers จากผลการศึกษาประสิทธิภาพของเซลล์ *Ralstonia* sp. TAK1 และ exopolymers ในการกำจัดอิออนของแคดเมียมสามารถนำไปสู่การประยุกต์ใช้เซลล์ *Ralstonia* sp. TAK1 และ exopolymers ในการบำบัดแคดเมียมหรือโลหะหนักอื่นๆ ที่ปนเปื้อนในสิ่งแวดล้อมได้

คำสำคัญ : การกำจัดแคดเมียม, exopolymers, *Ralstonia* sp.

[1] ศูนย์ปฏิบัติการเทคโนโลยีชีวภาพสิ่งแวดล้อม มหาวิทยาลัยมหิดล ศาลายา จ. นครปฐม

Laboratory of Environmental Biotechnology, Mahidol University, Salaya, Nakhon Pathom

[2] คณะสิ่งแวดล้อมและทรัพยากรศาสตร์ มหาวิทยาลัยมหิดล ศาลายา จ. นครปฐม

Faculty of Environment and Resource Studies, Mahidol University, Salaya, Nakhon Pathom

ABSTRACT

Bacterial exopolymers have an important role for adsorption of heavy metals. The adsorption mechanism occurs by the interaction between cations of heavy metals and anions of functional groups on exopolymers. *Ralstonia* sp. TAK1 is a resistant cadmium bacterium that can produce exopolymers. This bacterium was isolated from Cd-contaminated soil from a zinc mine in Maesot district, Tak province. This study focused on the types of carbon sources that were able to induce exopolymer production by *Ralstonia* sp. TAK1. The ability of *Ralstonia* sp. TAK1 cells and its exopolymers for removal of cadmium ions in aqueous solution was investigated. The results showed that the amount of exopolymers produced by *Ralstonia* sp. TAK1 peaked at the stationary phase (24 hr) (5.39 g/l) in culture medium amended with 2% glucose. Then, the amount of exopolymers declined when cells entered the mid-stationary phase, approximately at 32 hr (5.08 g/l). The highest cadmium removal efficiency (41.73%) and cadmium adsorption capacity (15.02 mg Cd/ g dry weight biomass) were found in the stationary phase cells of *Ralstonia* sp. TAK1. In the case of exopolymer biosorbent, it showed 1.91% of the cadmium removal efficiency and 0.69 mg Cd/ g dry weight biomass of cadmium adsorption capacity. These findings suggested that the efficiency of *Ralstonia* sp. TAK1 cells and its exopolymers for cadmium ions removal could be applied for bioremediation of cadmium or other heavy metals from contaminated environments.

Keywords : Cadmium removal, exopolymers, *Ralstonia* sp.
E-mail : ariesmemo@hotmail.com

INTRODUCTION

In recently, cadmium has been wildly used in industries. These activities are causing of increase cadmium contaminated into the environment. River located near industrial areas is one of the natural reservoirs that received the effluent containing of cadmium. Thus, the pollution of reservoirs will be negative effects not only environmental quality but it was more dangerous for living organisms. Biological treatment technologies are the new strategy has received increasing attention in recent time. The advantages of these technologies are undisturbed environment. It can potentially save a large percentage of the cost when compared to other alternatives. Exopolymers are biosynthesis polymers produced by microorganisms those able to immobilize metal outside microbial cells (Brown and Lester, 1979. Exopolymers have been recommended as efficiency biosorbent for the removal of metal contaminants in environmental (Kim et al., 1996; Weiner, 1997 and Kazy et al., 2002). Thus, this study was emphasized on the study of the carbon induction of exopolymers production by cadmium resistant bacterium; *Ralstonia* sp. The ability of bacterial cells and exopolymers to carry or bind cadmium ion in aqueous solution was investigated.

METHODS

1. Microorganism and Cultivation

Ralstonia sp. TAK1 was isolated from Cadmium contaminated soil in zinc mine at Maesot district, Tak province (Prapagdee and Watcharamusik, 2009). This bacterium was batch cultured in mineral salts medium (MSM) (2% glucose, 0.6% yeast extract, 0.12% K_2HPO_4, 0.03% KH_2PO_4, 0.01% $MgSO_4$, 0.01% $FeCl_3$, 0.1% NH_4NO_3 and 0.01% $CaCl_2$) at 28°C with 120 rpm shaking.

2. Determination of exopolymer production at various growth phases

Overnight cells of *Ralstonia* sp. TAK1 were subcultured into the MSM supplement with glucose and incubated at 28°C with continuous shaking at 120 rpm. Cell samples were collected for determination of cell dry weight and the levels of exopolymer production during growth phase until 72 hr. For quantitative exopolymers, the exopolymers were extracted by boiling in water bath at 100°C for 15 min. Bacterial cells were completely removed from the medium by centrifugation at 8,000 rpm, 4°C for 20 min. The supernatant was added to an equal volume of ice-cold absolute ethanol for exopolymers precipitation. The precipitated exopolymers were harvested by centrifugation at 8,000 rpm, 4°C for 20 min (Kunito et al., 2001). Amounts of exopolymers were analyzed in according to the phenol-sulfuric method (Dubois et al., 1956). The absorbance of the sample, which represented the concentration of glucose, was measured by using spectrophotometer at wavelength 490 nm. The exopolymer production was calculated in glucose equivalents from a glucose standard curve and exopolymer concentration was expressed as g/l.

3. Investigation of the appropriate carbon source on the enhancement of exopolymer production

To select the optimum carbon source on the enhancement of exopolymer production, *Ralstonia* sp. TAK1 was subcultured into the MSM supplement with various carbon sources instead 2% glucose as follows:

Medium A: MSM + 2% sucrose

Medium B: MSM + 2% galactose

Medium C: MSM + 2% mannitol

Cell cultures in various carbon sources were incubated at 28°C with continuous shaking. Cell samples were collected for determination of cell dry weight and the levels of exopolymers when incubation period entered to the growth phase which gave the maximum yield of exopolymers as described above. The medium that provided the highest level of exopolymers was selected for using in the further study.

4. nvestigation of the efficiency of exopolymers and bacterial cells for removal of cadmium ions from queous solution

For study biosorption of cadmium ions by *Ralstonia* sp. TAK1 and its exopolymers, this experiment was divided into 7 treatments with three replications as follows:

T1: Control experiment (only 20 ml of 5 mg/l $Cd(NO_3)_2$)

T2: 20 ml of 5 mg/l $Cd(NO_3)_2$ + 0.3% of exopolymers

T3: 20 ml of 5 mg/l $Cd(NO_3)_2$ + 0.3% of cell-free culture broth

T4: 20 ml of 5 mg/l $Cd(NO_3)_2$ + 0.3% of the mid-exponential normal cells

T5: 20 ml of 5 mg/l $Cd(NO_3)_2$ + 0.3% of the stationary normal cells

T6: 20 ml of 5 mg/l $Cd(NO_3)_2$ + 0.3% of exopolymers-free exponential cells

T7: 20 ml of 5 mg/l $Cd(NO_3)_2$ + 0.3% of exopolymers-free stationary cells

All experiments were performed in 125-Erlenmeyer flasks. The mixtures were adjusted the pH to 5.5 and shaken at 150 rpm at room temperature. The mixture was equilibrated for 90 min. Following equilibration, the metal-bearing solution was separated from biomass by filtering of cell suspension through the membrane filters. Concentration of unadsorbed cadmium ion in filtrate was determined by flame atomic absorption spectroscopy (FAAS) (Variance spectra model AA-220 FS) at wave length of 228.8 nm.

5. The metal uptake capacity and sorption efficiency

The sorption efficiency was calculated by this following equation.

$$\text{Removal efficiency (\%)} = \frac{(C_i - C_{eq}) \times 100}{C_i}$$

Where; C_i is initial cadmium concentration (mg/l)

C_{eq} is equilibrium cadmium concentration (mg/l)

The results obtained from all the biosorption experiments were calculated for the metal adsorption capacity for construction of adsorption isotherms as followed (Aloysius et al., 1999):

$$q = \frac{(C_i - C_{eq})}{X} \quad \text{(mg/g)}$$

Where; q is cadmium adsorption capacity (mg of Cadmium/ g dry weight of biosorbent)

X is biosorbent concentration in solution (g/l)

RESULTS AND DISCUSSION

1. Production of exopolymers at various growth phases

The results found that *Ralstonia* sp. TAK1 produced exopolymers in the range between 0.72-5.39 g/l (Figure 1). The yield of the exopolymers started at a low rate early during growth (4 hr) (0.72 g/l). Exopolymers were sharply increased when cells entered the exponential growth ended at approximately 12 hr (4.79 g/l) and continued maximally at the stationary phase (24 hr) (5.39 g/l) before decreasing. *Ralstonia* sp. TAK1 produced the highest exopolymers at the stationary phase of growth (24 hr) that was similar to the study of Prasertsan et al. (2008) reported that exopolymer production in *Enterobacter cloacae* was highest during the stationary phase. Moreover, Aslim et al. (2005) found that total exopolymers of *Lactobacillus delbruckii* subsp. *bulgaricus* B3 *L. delbruckii* subsp. *bulgaricus* and *Streptococcus thermophilus* produced the highest exopolymers at exponential phase of growth.

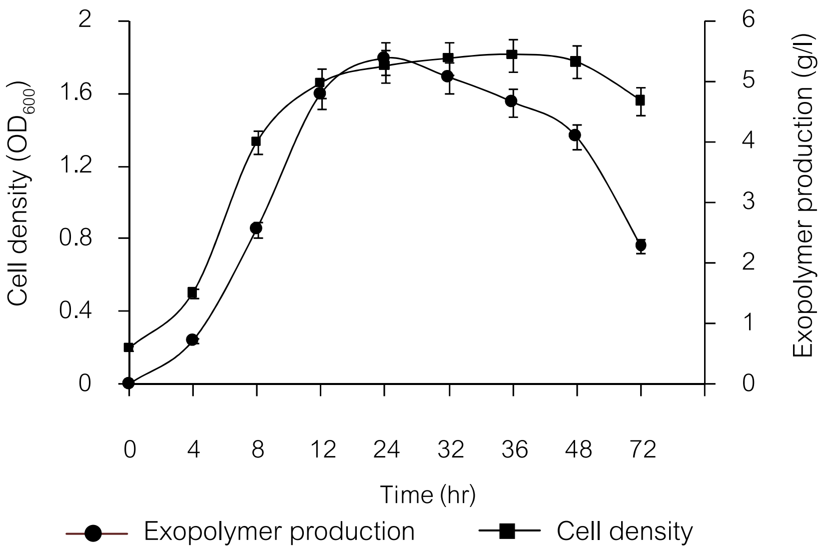

Figure 1 Exopolymer production in *Ralstonia* sp. TAK1 at various growth phases

2. Effects of types of carbon source on the exopolymer production

The effects of various carbon substrates on exopolymer production by *Ralstonia* sp. TAK1 were shown in Figure 2. The basal medium which addition of 2% glucose had significant influent on the highest yield of exopolymers (5.39 g/l) at stationary phase of growth (24 hr). Exopolymer production of *Ralstonia* sp. TAK1 when cultivated in medium containing 2% galactose (4.98 g/l) and 2% sucrose (4.86 g/l) was not significantly different at $p < 0.05$. However, cultivation of *Ralstonia* sp. TAK1 in medium amended with 2% mannitol provided the lower exopolymer production (2.85 g/) than other carbon sources.

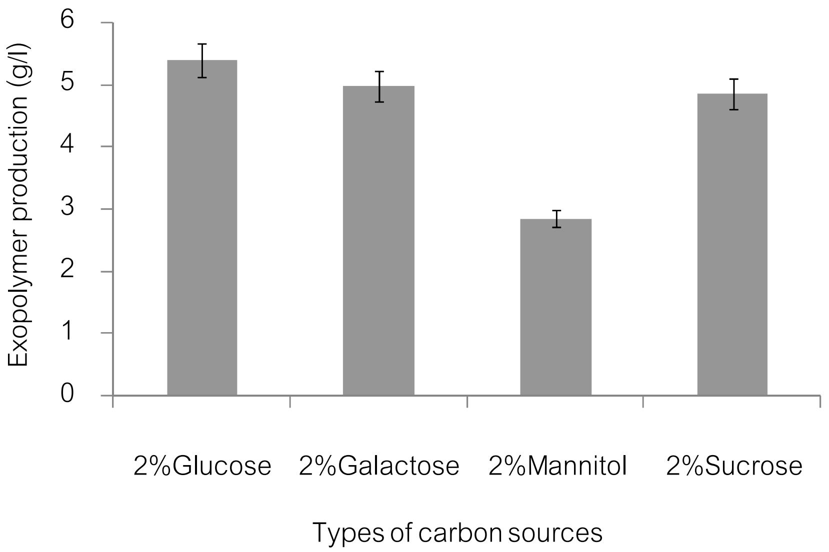

Figure 2 Exopolymer production at stationary phase of growth of *Ralstonia* sp. TAK1 cultivated in MSM supplemented with various carbon sources

The production of exopolymers by bacteria depends on various factors e.g. microbial species, phase of growth, medium composition and environment conditions (Pal and Paul, 2008).

3. Efficiency of bacterial cells and exopolymers for removal of cadmium ions from aqueous solution

The highest cadmium removal efficiency and cadmium adsorption capacity was found in T5 (stationary phase cells of *Ralstonia* sp. TAK1) by 41.73% and 15.02 mg Cd/ g dry weight biomass, respectively (Figure 3). Whereas, T4 or mid-exponential phase cells showed the removal efficiency and cadmium adsorption capacity by 12.72% and 4.58 mg Cd/ g dry weight biomass, respectively. These results indicated that yields of exopolymers could effect on the efficiency of cadmium removal from aqueous solution. In addition, exopolymers as biosorbent exhibited 1.91% of the cadmium removal efficiency and 0.69 mg Cd/ g dry weight biomass of cadmium adsorption capacity. However, exopolymer-free cells showed the less potential of removal efficiency and cadmium adsorption capacity that was no significantly different at p <0.05 between exopolymer-free mid-exponential phase cells (0.37%, 0.13 mg Cd/ g dry weight biomass, respectively) and exopolymer-free stationary phase cells (0.74%, 0.27 mg Cd/ g dry weight biomass, respectively).

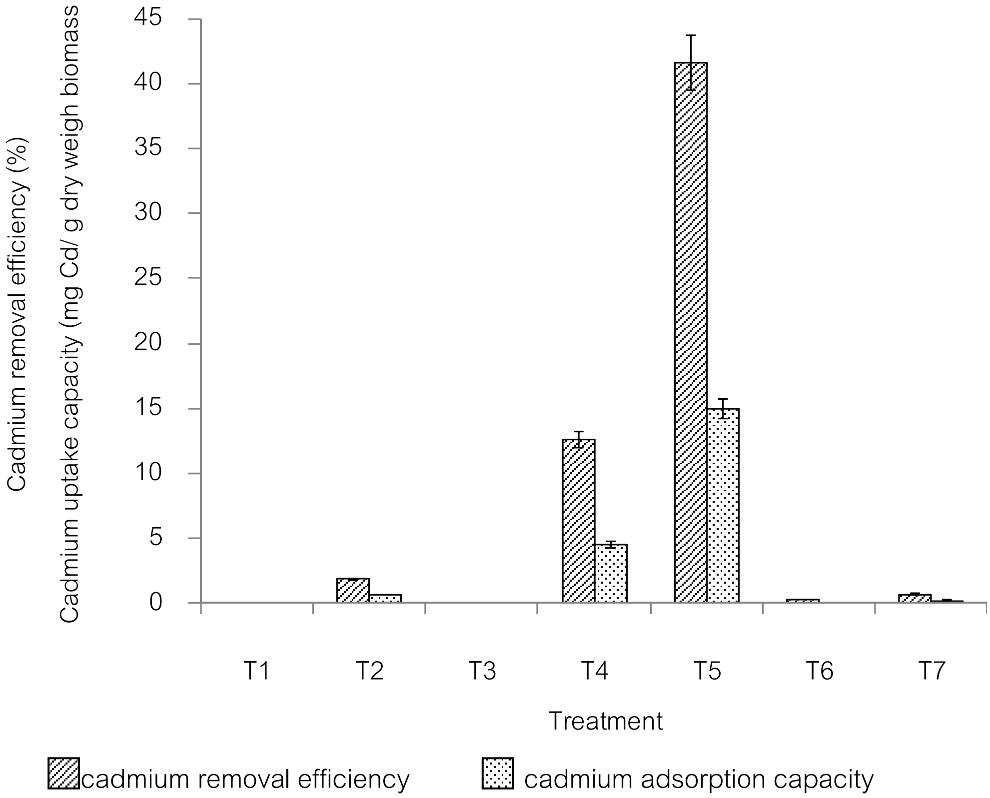

Figure 3 ficiency of bacterial cells and exopolymers for removal of cadmium in aqueous solution

The adsorption efficiency of heavy metal on biosorbent can be influenced on various parameters including metal concentration, biomass dose, temperature, contact time and solution pH. (Ahalya et al., 2003; Xie et al., 1996). Exopolymers exhibit an excellent metal-binding property. The binding of heavy metal cations to bacterial exopolymers generally occurs through electrostatic interaction with negatively charged functional groups of exopolymers (Santamaria et al., 2003).

CONCLUSION

The production of exopolymers in *Ralstonia* sp. TAK1 was highest at the stationary phase (24 hr). Then, the quantity of exopolymers declined when cells entered the mid stationary phase approximately 32 hr. For investigated the efficiency of bacterial cells and exopolymers for removal of cadmium ion from aqueous solution The highest cadmium removal efficiency and cadmium adsorption capacity was found in T5 (stationary phase cells of *Ralstonia* sp. TAK1). In addition, exopolymer-free cells as biosorbent showed the less potential for cadmium removal.

ACKNOWLEDGEMENT

This research was partially supported by the grant from the Center for Toxicology, Environmental Health and Management of Toxic Chemical under Science and Technology Postgraduate Education and Research Development Office (PERDO) of the Ministry of Education to N. Chompoothawat.

REFERENCES

Ahalya N, Ramachandra TV, Kanamadi RD. Biosorption of heavy metals. Res J Chem Environ. 2003; 7: 71-79.

Aloysius R, Karim MIA, Ariff AB. The mechanism of cadmium removal from aqueous solution by nonmetabolizing free and immobilized live biomass of *Rhizopus oligosporus*. World J Microb Biot. 1999; 15: 571-578.

Aslim B, Yuksekdag ZN, Beyatli Y, Mercan N. Exopolysaccharide production by *Lactobacillus delbruckii* subsp. *bulgaricus* and *Streptococcus thermophilus* strains under different growth conditions. World J Microb Biot. 2005; 21: 673-677.

Brown MJ, Lester JN. Metal removal in activated sludge: the role of bacterial extracellular polymers. Water Res. 1979; 13: 817-837.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem. 1956; 28, 350-356.

Kazy SK, Sar P, Singh SP, Sen AK, D'Souza SD SF. Extracellular polysaccharides of a copper-sensitive and a copper-resistant *Pseudomonas aeruginosa* strain: synthesis, chemical nature and copper binding. World J Microb Biot. 2002; 18: 583-588.

Kim SY, Kim JH, Kim CJ, Oh DK. Metal adsorption of the polysaccharide produced from *Methylobacterium organophilum*. Biotechnol Lett. 1996; 18: 1161–1164.

Kunito T, Saeki K, Nagaoka K, Oyaizu K, Mutsumoto S. Characterization of Cu-resistant bacterial communities in Cu-contaminated soils. Eur J Soil Biol. 2001; 37: 95-102.

Pal A, Paul AK. Microbial extracellular polymeric substances: central elements in heavy metal bioremediation. Indian J Microbiol. 2008; 48: 49-64.

Prapagdee B, Watcharamusik A. Adaptive and cross-protective responses against cadmium and zinc toxicity in cadmium-resistant bacterium isolated from a zinc mine. Bra J Microbiol. 2009; 40: 838-845.

Prasertsan P, Wichienchot S, Doelle H, Kennedy JF. Optimization for biopolymer production by *Enterobacter cloacae* WD7. Carbohyd Polm. 2008; 71: 468-475

Santamaria M, Diaz-Marreto AR, Hernandez J, Uutierrez-Navarro AM, Corzo J. Effect of thorium on the growth and capsule morphology of *Bradyrhizobium*. Environ Microbiol. 2003; 5: 916-924.

Weiner RM. Biopolymers from marine prokaryotes. Trends Biotechnol. 1997; 15: 390–394.

Xie JZ, Chang HL, Kilbane JJ. Removal and recovery of metal ions from wastewater using biosorbents and chemically modified biosorbents. Bioresour Technol. 1996; 57: 127-136.