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Influence of Growth Rate on Biosorption of Heavy Metals by *Nocardia amarae*

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Abstract The goal of the current research was to assess the influence of the growth rate of *Nocardia amarae* on its overall metal binding capacity. Batch sorption isotherms for cadmium (Cd), copper (Cu), and nickel (Ni) showed that *Nocardia* cells harvested from chemostat cultures at a dilution rate of 0.33 d^{-1} had a significantly higher metal sorption capacity than cells grown at 0.5 and 1 d^{-1}. The cell surface area estimated using a dye technique indicated that pure *N. amarae* cells grown at a lower growth rate had a significantly more specific surface area than cells harvested from a higher growth rate operation. Accordingly, this difference in the specific surface area seemed to indicate that the higher metal sorption capacity of the slowly growing *Nocardia* cells was due to their higher specific surface area.

Key words: Biosorption, continuous culture, heavy metals, *Nocardia amarae*, specific surface area

In recent years, an increased emphasis has been placed on studies related to the aqueous-phase separation of toxic heavy metal ions by biosorption [2, 5, 6, 10, 14]. The most abundant source of potentially metal-sorbing biomass in wastewater treatment plants is bacteria [3, 12, 13]. There are various factors to influence the metal removal process by bacteria, and one of the most important factors is related to the bacterial growth conditions. Since different components possess different abilities to remove metals, it is necessary to create an optimal environment favorable to bacterial growth, thereby providing the bacteria with the maximum ability to remove the metals in the wastewater.

Filamentous bacteria, including *Nocardia* spp., are known to be dominant in the microbial flocs of the activated sludge process in wastewater treatment [4] and have a greater surface-to-volume ratio than other microbial cells growing in the flocs. The proliferation of *Nocardia amarae* cells in activated sludge treatment plants has often been associated with unwelcome foaming incidents that lead to a deterioration of the effluent quality, increased time for plant maintenance, hazardous working conditions, and severe anaerobic digester operating problems [9]. However, in addition to causing various operational problems in treatment processes, Kim *et al.* [8] demonstrated that the presence of *Nocardia* may influence the fate of heavy metals in activated sludge treatment plants. They showed that a pure culture of *N. amarae* exhibited a significantly higher metal sorption capacity than the activated sludge biomass and that the metal sorption capacity of the activated sludge increased proportionally with the amount of *Nocardia* cells present in the mixed liquor.

To further examine the influence of *Nocardia* on the fate of metals in activated sludge treatment plants, it is important to assess the effect of the growth rate on the metal binding capacity of *N. amarae*. In various activated sludge plants, the growth rates of the biomass, including *Nocardia*, vary with the mean cell residence time (MCRT), therefore, depending on the MCRT, the growth rate of *Nocardia* would seem to vary from plant-to-plant. Cha *et al.* [1] reported that the presence and density of *Nocardia* in activated sludge is dependent on the operating MCRT, because the growth rate of *Nocardia* is relatively slow compared to other microorganisms present in activated sludge. Accordingly, the objective of the current study was to determine whether *Nocardia* cells growing under different growth conditions exhibited different metal sorption characteristics, by comparing the metal sorption capacity of *Nocardia* cells harvested from chemostat reactors operated at various dilution rates under steady state conditions.

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MATERIALS AND METHODS

Microorganism and Growth

The pure culture of *N. amarae* used in the current study was established with an *N. amarae* strain originally isolated from the activated sludge at the Regional Wastewater Treatment Plant (Sacramento, CA, U.S.A.). A New Brunswick Bioflow C30 (New Brunswick Scientific, Edison, NJ, U.S.A.) fermentor was used for all the chemostat experiments. The chemostat consisted of a 2-l culture vessel with working volumes of 1.5 l, break tube and attached tubing, air lines and filters, feed medium, and an effluent bottle. *Nocardia* cells were grown in a 2-l culture vessel containing a buffered mineral salt medium with 2,000 mg/l sodium acetate as the sole carbon source. A peristaltic pump was used for feeding the culture medium and withdrawing the cells. Pure oxygen was provided in the vessel headspace to deliver the required oxygen to the cells by surface aeration. Mixing was achieved with three height-adjustable magnetic impellers set at 200-300 rpm to prevent any settling of the microbial cells, ensure a rapid distribution of the added feed, and promote a dissolved oxygen transfer from the headspace. The culture temperature in the reactors was maintained at 25±0.5°C. The TSS and COD were determined daily as an indicator of the proximity to a steady state.

To prevent the wash-out of cells from the system, the chemostats were operated at three net growth rates ranging from 1.0 to 0.33 d^{-1}. Once a steady state was reached for each dilution rate (1.0, 0.5, and 0.33 d^{-1}), the biomass was harvested for metal sorption and dye adsorption studies. The *Nocardia* cells were separated from the liquid by centrifugation at 7,000 ×g for 10 min, and washed twice with distilled water to remove any remaining growth media. The washed cells were then resuspended in 0.01 M $NaNO_3$ solution.

Biosorption of Heavy Metals

The heavy metal sorption to the *Nocardia* biomass harvested at various growth rates was evaluated using three metals: cadmium (Cd), copper (Cu), and nickel (Ni). All metals were added as nitrate salts. The pH of the cell suspension was adjusted to 6.0 with 0.1 M HCl or 0.1 M NaOH. High-density polyethylene bottles were used for the batch metal sorption experiments to minimize any metal sorption to the bottle surfaces. To obtain sorption isotherms for each metal, 20 ml cell suspension in 30-ml HDPE bottles was spiked with 9 different initial metal concentrations and the bottles were shaken on an orbital shaker for 12 h at ambient temperature. A preliminary kinetic study indicated that the metal sorption by the *N. amarae* cells and activated sludge reached equilibrium after approximately 6 h [7]. Triplicate bottles were set up for each metal concentration. In addition, control bottles were prepared by spiking the cell-free solution (pH 6; 0.01 M $NaNO_3$) with the test metals to assess losses due to precipitation and sorption to the bottle surfaces. After equilibration, the cell suspension was filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA, U.S.A.) and the soluble metal concentrations in the filtrate were determined with a Perkin-Elmer atomic adsorption spectrophotometer (Norwalk, CT, U.S.A.). The difference between the soluble metal concentration in the bottles with and without the cells was used to calculate the mass of the metals sorbed to the biomass. The filtered biomass was used to determine the total biomass concentration.

Specific Surface Area

The specific surface area of the biomass was determined by the dye adsorption method [11]. An anionic dye solution, Lissamine Scarlet 4R (new coccine acid red #18, Aldrich Chemical Co., St. Louis, MO, U.S.A.), was added to a 25 ml cell suspension and the biomass-dye mixtures were shaken on a rotary shaker for 2 h at 200 rpm to achieve equilibrium. According to Wang [15], dye adsorption to sludge particles reaches equilibrium after 30 min. Following equilibration, each mixture was centrifuged at 12,000 ×g for 10 min and the dye concentration remaining in the supernatant was spectrophotometrically measured (Hach model DR/2000, Hach Co., Loveland, CO, U.S.A.) at 505 nm wavelength. The centrifuged biomass was collected to determine the total biomass concentration. The specific surface area of the cells (m^2/g) was determined by calculating the maximum dye adsorption density, q_m, using the Langmuir adsorption model.

RESULTS AND DISCUSSION

Metal Sorption Study

The influence of the biologically hydraulic retention time on metal sorption was investigated using *Nocardia* cells harvested from a continuous culture at 1.0, 0.5, and 0.33 d^{-1} growth rates. To determine the amount of metals sorbed to the *Nocardia* cells at various equilibrium metal concentrations, 9 different initial concentrations were used for the batch sorption study. The Langmuir isotherm model was fitted to the Cu biosorption for the *N. amarae* cells harvested from different growth rates (Fig. 1). The *Nocardia* cells harvested from the chemostat with a dilution rate of 0.33 d^{-1} exhibited significantly higher Cu sorption capacity than the cells growing at 0.5 and 1 d^{-1}. Similar trends were obtained when comparing the Cd and Ni isotherms. Accordingly, these results suggest that the maximum metal adsorption potential was consistently higher for the slow growing cultures than the fast growing cultures for all three metals.

Table 1 presents the calculated maximum metal adsorption capacity (q_{max}) of Cd, Cu, and Ni to *Nocardia* cells at

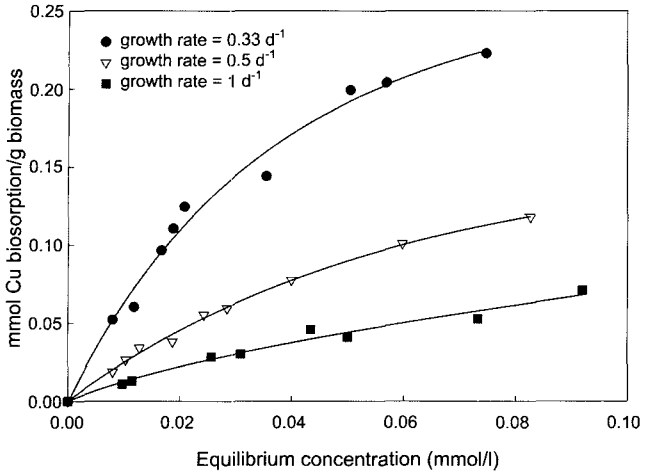

Fig. 1. Copper sorption isotherms for *N. amarae* cells harvested from different growth rates.

various growth rates. The *Nocardia* cells at all growth rates exhibited the highest metal sorption capacity for Cu, followed by Ni and then Cd.

In addition to equilibrium studies, the rate of metal sorption by *N. amarae* was assessed by quantitatively evaluating the fate of metals in mixed cultures. In continuous flow bioreactor experiments, metal solution (0.05 mM; pH 7) was pumped into the 50-ml reactor containing 25 mg of *Nocardia* biomass at a flow rate of 10 ml/min. The value of maximum metal uptake rate (g^{-1} biomass) by *Nocardia* cells grown at 0.33 d^{-1} growth rate is given in Table 2. The maximum metal removal rate was found to be in the following order: Cu>Cd>Ni. This observation was in close agreement with the trend observed from the batch metal sorption study.

Specific Surface Area

The specific surface area of the *Nocardia* cells harvested from different growth rates was determined using a dye adsorption method [11]. The dye adsorption isotherms for the biomass were fitted to a Langmuir isotherm, which is commonly used to model adsorption to microbial surfaces described by the equation:

$$q=\frac{q_m bC_e}{1+bC_e}$$

Table 1. Langmuir isotherm constants for metal sorption by *N. amarae* biomass in continuous culture.

Metal	Constant	Growth rate		
		1 d^{-1}	0.5 d^{-1}	0.33 d^{-1}
Cu	q_m (mmol/g)	0.14	0.26	0.49
Ni	q_m (mmol/g)	0.08	0.10	0.17
Cd	q_m (mmol/g)	0.049	0.065	0.096

Table 2. Maximum metal uptake rate by *Nocardia* cells in a continuous flow bioreactor.

Metal	Maximum metal uptake rate (mmol/l/min)
Cu	0.98
Cd	0.58
Ni	0.37

where

q=mole of dye adsorption per g mass of biomass (mol/g)
C_e=equilibrium concentration of dyes in solution (mol/l)
q_m=maximum dye adsorption per gram of biomass (mol/g)
b=constant related to affinity (l/mol)

The constant, q_m, obtained from the Langmuir isotherm was used to determine the specific surface area of the solids according to the following equation:

$$S=q_m NA$$

where S is the specific surface area of the solids (m^2/g), q_m is the maximum dye adsorption capacity (mole/g), N is Avogadro's number (6.023×10^{23} molecules/mole), and A is the area occupied by a single dye molecule (1.96×10^{-18} m^2/molecule of the dye used in the current study).

Figure 2 compared the dye adsorption isotherms for the *N. amarae* cells harvested from different growth rates in the chemostat cultures. The results revealed that the dye adsorption capacity of the *Nocardia* cells growing at a dilution rate of 0.33 d^{-1} was higher than that of the cells growing at a dilution rate of 1.0 d^{-1}, thus indicating that the specific surface area of the *N. amarae* cells varied with the growth rate. The values of maximum dye adsorption capacity (q_m) and calculated specific surface area (S) at different growth rates are summarized in Table 3. The highest specific surface area of 199 m^2/g was obtained from *N. amarae* cell harvested at a growth rate of 0.33 d^{-1},

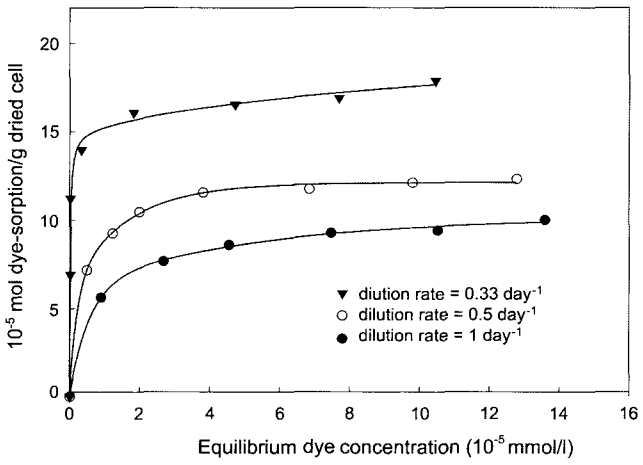

Fig. 2. Dye adsorption isotherms for *N. amarae* cells harvested from different growth rates.

Table 3. Specific surface area (S) and maximum dye adsorption capacity (q_m) for *N. amarae* cells harvested from different growth rates.

Growth rate (d^{-1})	q_m (10^{-5}×mole/g)	S (m^2/g)
1	9.66	114
0.5	12.46	147
0.33	16.86	199

which demonstrates that the higher metal sorption capacity of the slower growing *Nocardia* cells may have been due to their higher specific surface area.

The examination of the specific surface area for the different *Nocardia* growth rates revealed that the values corresponding to the metal sorption capacity were related to the specific surface area, suggesting that the growth rate appeared to be an important factor for metal removal in a continuous system, and that *Nocardia* present in long HRT plants may have a greater capacity to accumulate metals.

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