

Influence of Anoxic Selectors on Heavy Metal Removal by Activated Sludge

Jay H. Niec and Daniel K. Cha*

Department of Civil and Environmental Engineering, University of Delaware, Newark, DE 19716, USA

Abstract The goal of this research is to compare the metal binding characteristics of an anoxic selector activated sludge system and a conventional activated sludge system. Metal biosorption by biomass harvested from experimental systems was determined by a series of batch experiments. Heavy metals studied in this research were zinc, cadmium, and nickel. The sorption isotherm showed that the selector sludge had significantly higher sorption capacity than did the control sludge. Metal biosorption behavior closely followed a Freundlich isotherm model for equilibrium concentrations. ECP contents of biomass estimated by alkali extraction technique showed that ECP levels in the selector sludge significantly higher than that in the sludge harvested from the conventional system, indicating that the higher metal sorption capacity of selector sludge may be due to the selection of the ECP-producing bacteria (*i.e.*, *Zoogloea* sp.) by the selector system.

Keywords: heavy metals, activated sludge, anoxic selector, extracellular polymers

INTRODUCTION

A selector is defined as a separate initial mixing zone upstream of the aeration basin in which recycled activated sludge (RAS) and influent wastewater are mixed. An anoxic selector provides an environment where dissolved oxygen (DO) is not present and therefore nitrate (NO_3^-) in RAS streams is utilized as the electron acceptor. Anoxic selector technology has been successfully used to control activated sludge bulking and foaming which is caused by the proliferation of filamentous bacteria [1,2]. A distinctive feature of the activated sludge morphology in anoxic selector systems is the presence of large amorphous colonies that can be easily separated from the liquid phase by gravity [3]. Although these floc-forming bacteria are important for efficient sludge settling, they may have secondary effects that are not well known. For example, floc-forming bacteria may adsorb more heavy metals than the other bacteria present in activated sludge, producing biosolids containing greater quantities of heavy metals and these higher concentrations of metals in biosolids could create disposal problems.

A large body of environmental literature demonstrates that activated sludge processes can remove substantial quantities of soluble metals [4-6]. Metal removal occurs as a result of interaction with microbial flocs of activated sludge. Extracellular polymers (ECPs) of microorganisms have been shown to play a significant role in metal removal [7-10]. Floc-forming bacteria,

Zoogloea ramigera, isolated from activated sludge plants produce substantial quantities of negatively charged extracellular polymers [11] that can adsorb a variety of metal ions [12,13]. Friedman and Dugan [14] reported that *Z. ramigera* 115, which produce an extensive extracellular zoogloal matrix, can adsorb twice as much metals as *Z. ramigera* 116M, which produces no zoogloal matrix. A distinctive feature of the activated sludge morphology in anoxic selector systems is the presence of large amorphous zoogloal colonies [3]. These forms of growth have been related to the presence of ECP-producing bacteria, *Z. ramigera* [15]. Su *et al.* [6] using synthetic wastewater, showed that the biomass harvested from an aerobic selector activated sludge unit had significantly higher sorption capacity than did the biomass from a conventional system (an aerobic selector is aerated and contains dissolved oxygen).

As a follow-up to the laboratory-scale aerobic selector study with synthetic wastewater, the objective of this research was to evaluate whether an anoxic selector can enhance biosorption of heavy metals, thus increasing the metal removal efficiency of an activated sludge system treating a domestic wastewater. A series of pilot-scale experiments was conducted at a full-scale treatment plant to compare metal (zinc, cadmium, and nickel) removal efficiencies of anoxic selector sludge to that of sludge from a conventional reactor operated in parallel. The metal sorption data were fit to the Freundlich isotherm model to compare the sorption capacity of two sludges.

*Corresponding author

Tel: +1-302-831-2435 Fax: +1-302-831-3640

e-mail: cha@ce.udel.edu

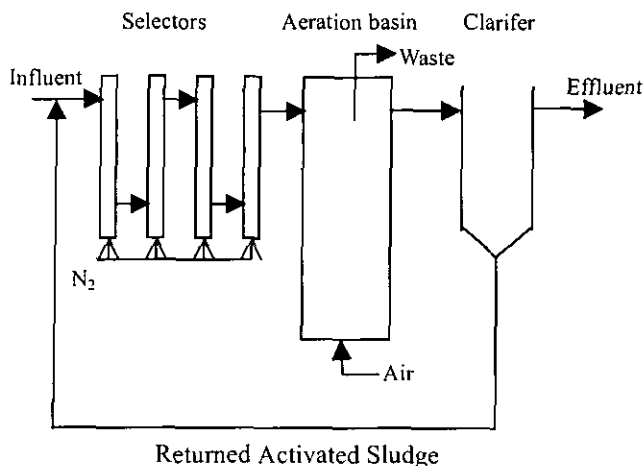


Fig. 1. Schematic diagram of anoxic selector system.

MATERIALS AND METHODS

Description of Pilot-scale Activated Sludge System

Activated sludge cultures were established in continuous-flow pilot-scale activated sludge units on a continuous feed of primary effluent of the Michigan City Sanitary District Wastewater Treatment Plant (Michigan City, IN, USA). Primary effluent feed for the pilot-scale units was taken continuously from the effluent weir area of the full-scale plant primary clarifiers and transferred directly by gravity to a constant head tank next to the pilot-scale units. Each experimental unit consisted of an aeration basin and a secondary clarifier. Flow from the aeration basin to the secondary clarifier was by gravity. Settled sludge in the clarifier was continuously pumped back to the aeration basin using a peristaltic pump. Aeration basins were cylindrical, completely stirred tank reactors (CSTR) with a liquid volume of 60 L. The aeration basins were constructed using a 12-in. ID PVC pipe. A Sanitaire 9-in. dia. fine-bubble diffuser was anchored at the base of each aeration basin for mixing and aeration. The pH of the aeration basins was controlled to desired values using a pH recorder/controller and strong acid (HCl) or strong base (NaOH) addition. The dissolved oxygen (DO) concentration in the aeration basins was maintained at 5 mg/L to prevent the development of low DO filamentous microorganisms. The anoxic selector consisted of four 1.5-L upflow column reactors in series (Fig. 1). All four anoxic selector reactors were provided with a small gas vent on top and were bubbled with nitrogen gas for mixing and stripping of dissolved oxygen (DO).

To investigate the influence of an anoxic selector system on metal removal efficiency of an activated sludge system, an anoxic selector unit and a conventional activated sludge unit were operated in parallel at an MCRT of 15 days. A constant MCRT was maintained in both systems by daily batch wasting of mixed liquor from the aeration basins. The performance of pilot-scale

Table 1. Operating parameters and average values for the bench-scale activated sludge systems

Parameter	CSTR	Selector System
Influent Flow, L/day	140	140
MCRT, day	15	15
Average MLSS, mg/L	2,770	2,390
pH	7.0	7.0
Temperature, °C	21	21
HRT: Aeration Basin, h	5	4.7
First Selector, min	–	12
Total Selector, min	–	49

units was assessed and the reactors were controlled by routine measurements of influent total suspended solids (TSS) and chemical oxygen demand, effluent TSS and COD, aeration basin TSS and volatile suspended solids (VSS), and clarifier TSS. Standard Methods [16] techniques were used for all analyses. Operating parameters for the bench-scale activated sludge systems are summarized in Table 1.

ECP Contents of Activated Sludge

ECP concentrations in the harvested mixed liquor samples were determined using an alkali extraction technique [17]. Hundred mL of mixed liquor sample with known TSS was centrifuged at $3,000 \times g$ for 20 min and after decanting the supernatant, 40 mL of 2 N NaOH was added to the pellet. The samples were gently agitated on an orbital shaker at room temperature for 5 h and then centrifuged at $10,000 \times g$ for 15 min. Following the centrifugation, viscous supernatant was collected and appropriate amount of ethanol was added to give a final concentration of 60% (v/v). The supernatant-ethanol mixture was thoroughly mixed and stored overnight in a refrigerator. After refrigeration, white cotton-like precipitate was separated from the liquid by centrifugation at $3,000 \times g$ for 10 min and collected by filtration through a glass fiber filter.

Metal Uptake by Activated Sludge

Mixed liquor samples from the control and the selector systems were harvested for the batch sorption experiments to compare the biosorption characteristics of biosolids. Metals tested in this study were zinc (Zn), cadmium (Cd), and nickel (Ni) and all three metals were added as nitrate salts.

High-density polyethylene bottles were used for batch metal sorption experiments to minimize metal sorption to the bottle surfaces. To obtain sorption isotherms for each metal, 75-mL cell suspensions in 100-mL bottles were spiked with 5 different initial metal concentrations and the bottles were shaken on an orbital shaker for 12 h at the ambient temperature. Su *et al.* [6] showed that the metal sorption by activated sludge reached equilibrium in 5–6 h.

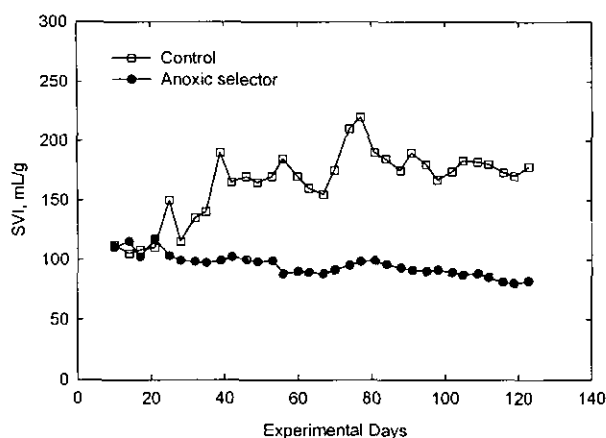


Fig. 2. Comparison of SVI between the selector and the conventional system.

Triplicate bottles were set-up for each metal concentration. In addition, control bottles were prepared by spiking the cell-free solution (pH 7; 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 with a 0.45 μm membrane filter (Millipore, Bedford, MA, USA) and soluble metal concentrations in the filtrate were determined with a Perkin-Elmer atomic adsorption spectrophotometer (Norwalk, CT, USA). The difference between the soluble metal concentration in bottles with and without cells was used to calculate the mass of metals sorbed to the biomass. The filtered biomass was dried and weighed to determine the total biomass concentration in the metal sorption bottles.

RESULTS AND DISCUSSION

The anoxic selector was judged to be functioning properly as a selector (i) nitrate removal in the selector, (ii) improvement in settling characteristics of mixed liquor, and (iii) presence of amorphous zoogloal colonies in the flocs. Over the 120-day experimental period, the average nitrate removal in the selector was 4.9 mg/L. This nitrate removal was coupled to the soluble COD uptake in the selector. Sludge volume index (SVI) measurements of activated sludge were used to monitor settling characteristics of mixed liquors in the experimental reactors. The conventional activated sludge system (control) began to bulk (SVI > 150 mL/g) in 20 days (Fig. 2) and the bulking persisted throughout the experimental period. SVI values in the anoxic selector unit, on the other hand, remained at or below 100 throughout the experiment. Microscopic examination of the mixed liquors revealed large amounts of filamentous bacteria in the control system. Conversely, large amounts of amorphous zoogloal colonies were observed in the anoxic selector sludge, which have been related to the presence of *Zoogloea* sp. [15]. Amorphous

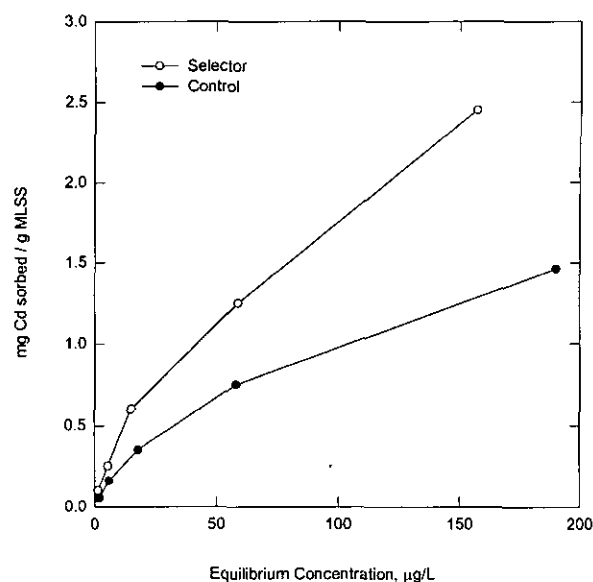


Fig. 3. Comparison of cadmium sorption isotherms for selector and control biomass.

zoogloal colonies observed in the selector sludge were not observed in the control sludge, confirming that the anoxic selector was functioning properly. All the batch sorption experiments were conducted after 50 days of activated sludge operation, avoiding the period of severe fluctuation in SVI.

Biosorption Isotherms

To determine the amount of metals sorbed to sludge at various equilibrium metal concentrations, five different initial concentrations were used for the batch sorption experiments. Fig. 3 compares the cadmium biosorption isotherms for both selector and control sludges. The selector sludge had significantly higher biosorption amounts than the control sludge. Sorption isotherms for nickel and zinc revealed similar behaviour; the amount of metal sorbed by the selector sludge was higher than the control sludge (data not shown). The difference increases with increasing equilibrium concentration. In all biosorption isotherms, the curves are L-shaped indicating a reduction in the number of available sorption sites as free metal ion concentration increases. Fig. 4 shows that metal binding by anoxic selector sludge was highest for Zn, followed by Cd and then Ni. The same sequence of affinity was observed with biomass harvested from the control unit. This result is consistent with the sequence reported by Su *et al.* [6] where they noted that the same sequence of affinities for metals (Zn > Cd > Ni) by activated sludge from laboratory activated sludge units. Brown and Lester [7] reported that the sequence of preferential uptake of metals by activated sludge from laboratory activated sludge units, pilot plants, and full-scale treatment plants was Fe > Zn > Cu, Cr > Pb > Hg > Cd > Ni > Mn.

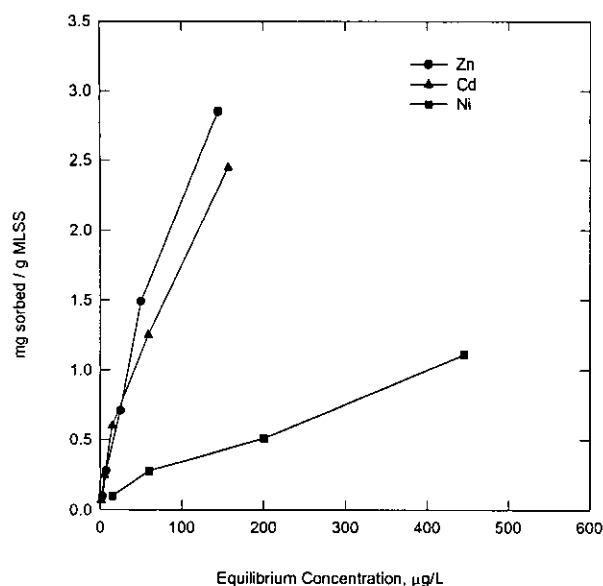


Fig. 4. Comparison of biosorption of metals by the selector biomass.

Freundlich Isotherms of Metal Biosorption

The Freundlich isotherm, which is commonly used to model metal adsorption by biomass, is described by the equation:

$$X_b / M = k C^{1/n}$$

where: X_b/M = mg metal biosorbed per g of MLSS.
 C = Equilibrium concentration of metals in solution (mg/L)
 k = Constant related to capacity
 $1/n$ = Constant related to affinity

Freundlich adsorption isotherms were calculated for all three metals, for both selector and CSTR sludges. Fig. 5 illustrates the Freundlich isotherm for cadmium.

The constants k and $1/n$ obtained from the Freundlich isotherm are summarized in Table 2 for cadmium, nickel, and zinc. Values of k , the constant related to adsorption capacity, were consistently higher for the selector sludge than they were for the control sludge for all three metals (Table 2). A statistical test (F-test) revealed that this difference was significant ($\alpha = 0.05$) for all three metals. There was no statistically significant difference in the values of $1/n$ obtained for the different sludges. Since the $1/n$ constant is related to sorption affinity, this result suggests that the metal sorption mechanism is the same for both control and selector sludges. A likely common removal mechanism is sorption to ECP. The higher k values for the selector sludge indicate that there is more ECP thus more sorption sites available for the metal uptake.

In order to compare the ECP contents of the selector and control sludges, ECP in the harvested mixed liquor

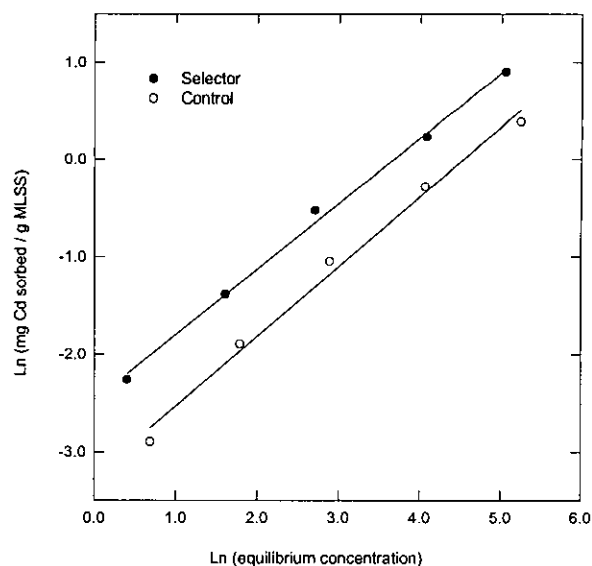


Fig. 5. Freundlich isotherm of cadmium.

Table 2. Freundlich isotherm constants for metal biosorption by the anoxic selector and the control sludges

	Cd		Ni		Zn	
	Selector	Control	Selector	Control	Selector	Control
k	0.084	0.039	0.015	0.005	0.18	0.058
$1/n$	0.67	0.71	0.70	0.79	0.56	0.59
R^2	0.99	0.99	0.99	0.98	0.94	0.96

samples was extracted using an alkali extraction technique. Fig. 6 shows that the ECP levels in the selector sludge are always greater than the levels in the control sludge. This result indicates that the higher metal sorption capacity of selector sludge may be attributed to higher ECP contents, which provides more sorption sites for metal uptake.

Relative to the conventional activated sludge system, biosolids in an anoxic selector reactor configuration have a greater adsorption capacity for metals. This increased capacity may be attributed to higher concentration of the ECP-producing bacteria (*i.e.*, *Zoogloea* sp.) in the selector system. Freundlich isotherms developed from the data suggest that there is no significant difference in metal sorption mechanisms between microbial populations in a selector system and populations present in a conventional activated sludge system.

These results also suggest some interesting implications for activated sludge plants contemplating process modifications to include selector reactors. Although an enrichment of ECP-producing bacteria can suppress the growth of filamentous organisms and enhance settling of biosolids, secondary effects should be considered. For example, higher concentrations of metals in biosolids could create disposal problems. In contrast, other systems could benefit from these secondary effects. For

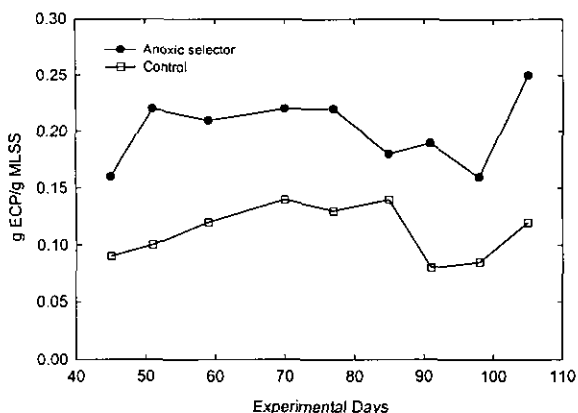


Fig. 6. Comparison of ECP contents of selector and control biomass.

treatment plants facing stringent treatment requirements, additional metal removal from ECP-producing bacteria would be a welcome secondary effect.

CONCLUSION

Conclusions from this research include:

- Sorption isotherms showed that the selector sludge has significantly higher sorption capacity than does the control sludge.
- The order of metal affinity for activated sludge is: Zn > Cd > Ni
- Metal biosorption data followed Freundlich isotherm model.
- ECP levels in the selector sludge are always greater than the levels in the control sludge.

REFERENCES

[1] Jenkins, D., M. G. Richard, and G. T. Daigger (1993) *Manual on the Causes and Control of Activated Sludge Bulking and Foaming*. Lewis, Ann Arbor, MI, USA.

[2] Cha, D. K., D. Jenkins, W. P. Lewis, and W. H. Kido (1992) Process control factors influencing *nocardia* populations in activated sludge. *Water Environ. Res.* 64: 37-43.

[3] Cha, D. K. (1990) *Process Control Factors Influencing Nocardia Population in Activated Sludge*. Ph. D. dissertation, University of California at Berkeley.

[4] Brown, H. G., C. P. Hensley, G. L. McKinney, and J. L.

Robinson (1973) Efficiency of heavy metals removal in municipal sewage treatment plant. *Environ. Lett.* 5: 103-114.

[5] Tien C. T. and C. P. Huang (1987) Adsorption behavior of $cu(ii)$ onto sludge particulate surfaces. *J. Environ. Eng.* 113: 285-299.

[6] Su, M. C., D. K. Cha, and P. R. Anderson (1995) Influence of selector technology on heavy metal removal by activated sludge: secondary effects of selector technology. *Wat. Res.* 29: 971-976.

[7] Brown, M. J. and J. N. Lester (1979) Metal removal in activated sludge: the role of bacterial extracellular polymers. *Wat. Res.* 13: 817-837.

[8] Brown, M. J. and J. N. Lester (1982) Role of bacterial extracellular polymers in metal uptake in pure bacterial culture and activated sludge: I. Effect of metal concentration. *Wat. Res.* 16: 1539-1548.

[9] Lawson, P. S., R. M. Sterritt, and J. N. Lester (1984) Adsorption and complexation mechanisms of heavy metal uptake in activated sludge. *J. Chem. Tech. Biotechnol.* 34B: 253-262.

[10] Geesey, G. G. and L. Jang (1989) Interactions between metal ions and capsular polymers. pp. 325. In: T. J. Beverage and R. J. Doyle (eds.). *Metal Ions and Bacteria*. Wiley Interscience, NY, USA.

[11] Dugan, P. R. and Pickrum H. M. (1972) Removal of mineral ions from water by microbial produced polymer. pp. 1019-1038. In: *Proceedings of the 27th Annual Purdue Ind. Waste Conf., Purdue Univ., West Lafayette, IN, USA*.

[12] Rudd, T., R. M. Sterritt, and J. N. Lester (1983) Stability constant and complexation capacities of complexes formed between heavy metals and extracellular polymers from activated sludge. *J. Chem. Tech. Biotechnol.* 33A: 374-380.

[13] Noberg, A. B. and H. Persson (1984) Accumulation of heavy-metal ions by *Zoogloea ramigera*. *Biotechnol. Bioeng.* 26: 239-246.

[14] Friedman, B. A. and P. R. Dugan (1968) Concentration and accumulation of metallic ions by the bacterium. *Zoogloea*. *Dev. Ind. Microbial.* 9: 381-388.

[15] van Niekerk, A. M., D. Jenkins, and M. G. Richard (1987) The competitive growth of *Zoogloea ramigera* and type 021N in activated sludge and pure culture: A model for low F/M bulking. *J. Wat. Pollut. Control Fed.* 59: 262-273.

[16] APHA (1992) *Standard Methods for the Examination of Water and Wastewater*, 18th ed. American Public Health Association, Washington, DC, USA.

[17] Brown, M. J. and J. N. Lester (1980) Comparison of bacterial extracellular polymer extraction methods. *Appl. Environ. Microbiol.* 40: 179-185.

[Received July 14, 2000; accepted October 7, 2000]