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Interaction between Heavy Metals and Aerobic Granular Sludge

Shuguang Wang[1], Shaoxiang Teng[1] and Maohong Fan[2]
[1]*School of Environmental Science and Engineering, Shandong University*
[2]*Department of Chemical and Petroleum Engineering, University of Wyoming*
[1]*China*
[2]*USA*

1. Introduction

Metals play important roles in the life processes of microbes. Some metals, such as Fe, Zn, Cu, Ni, and Co are of vital importance for many microbial activities when occur at low concentrations. These metals are often involved in the metabolism and redox processes as parts of enzyme cofactors or participators in the electron transfer in microbial respiration (Zandvoort et al., 2006). However, metals at high concentrations are inhibitory or even toxic to living organisms. Essential metals in the enzymes can be displaced by toxic metals which have the similar structure, thus resulting in the enzymes inactivation or damage (Bruins et al., 2000).
Heavy metals contaminants have posed great challenge to wastewater treatment. Large quantity of heavy metals are released from mineral rock weathering and anthropogenic sources such as metalliferous mining and smelting, electroplating, batteries, fertilizers, and pigments industries (Sirianuntapiboon & Ungkaprasatcha, 2007; Ong et al., 2005a). Due to their high toxicity and environmental recalcitrance, remediation of heavy metal is of urgent importance. Many techniques have been tried out to remove heavy metals from wastewater. Physicochemical methods, such as chemical precipitation, ion exchange, adsorption, electrolysis, chemical oxidation/reduction and membrane technologies, are found to be ineffective or rather expensive or generate toxic slurries (Liu et al., 2003; Pamukoglu & Kargi, 2006). Biological treatment is considered a promising technique for bioremediation of heavy metals wastewater, since it can degrade organic pollutant in the wastewater and simultaneously transform heavy metals.
Aerobic granulation is a novel environmental biotechnological technique which draws intensive interest in the last 20 years. Aerobic granules, as defined as self-immobilized microbial aggregates, are usually cultivated in sequencing batch reactors (SBRs). When compared with conventional activated sludge, the aggregation of microorganisms into compact granules brings extra benefits such as excellent settleability, high biomass retention, diverse microbial structure, and the ability to resist high organic and toxic loadings (Su & Yu, 2005; Tay et al., 2001). Due to the excellent abilities of aerobic granular sludge, they have been employed to treat wastewaters containing organic pollutants, N, P, heavy metals, and dyes etc (Beun et al., 2001; Chen et al., 2008; Cheng et al., 2008; Liu et al., 2009; Wang et al., 2007; Wang et al., 2010).

Source: Environmental Management, Book edited by: Santosh Kumar Sarkar,
ISBN 978-953-307-133-6, pp. 258, September 2010, Sciyo, Croatia, downloaded from SCIYO.COM

Heavy metals can be transformed into states with low mobility or toxicity by aerobic granules through physicochemical and biological processes. Cell walls and the extracellular polymeric substances (EPS) provide rich binding sites for metals. Microbial activities of aerobic graules are also capable of metal detoxification. Moreover, the unique layer structure of aerobic granules gives them addiditional advantages in metal-resistance. While bioremediating heavy metals, microbial activities of granules are stimulated or inhibited by metals depending on their concentrations. Aim of this study was to give a detailed description of the interaction between heavy metals and aerobic granular sludge, including possible metal-microbe interplay patterns, main metal uptake behaviour and mechanism, influence of metals on the reactor performance, and the metal-resistance strategies of aerobic granules. Better understanding of these interactions and influence factors helps to raise rational operation strategies for the bioremediation of heavy metals wastewaters.

2. Metal-microbe interaction and metal speciation

Bioavailability of heavy metals highly depends on environmental conditions, such as pH, alkalinity, redox potential, and activities of microorganisms (van Hullebusch et al., 2005a). Heavy metals mainly exist in the forms of Me^{2+}, $MeSO_4$, and $MeCl^-$ at pH<7, while $MeHCO_3^+$ and $MeCO_3$ are dominant at pH>7 (Hietala & Roane, 2009). In the study of Sandrin & Maier (2002), the ionic cadmium (Cd^{2+}) concentration at pH 4 was 44 mg/L while it decreased to 4 mg/L at pH 7. Metal bioavailability is enhanced under acidic conditions, thus increasing the potentional metal toxicity. Metal bioavailability is also influenced by redox potential. High redox potential (800 to 0 mV) favors metal solubility while low redox potential (0 to -400 mV) immobilises metal in precipitated forms (Hietala & Roane, 2009).

Microbial bioremediation affects the fate of heavy metals. Heavy metals can not be degraded in microbial metabolism/co-metabolism process. Bioremediation of heavy metals is accomplished by the conversions between inorganic and organic forms or the inorganic valence changes through redox (Ramasamy et al.,2007). At least four general approaches are involved in the bioremediation of heavy metals, which are summarized in Fig.1.

1. Biosorption

Biosorption between positively charged heavy metals and negatively charged cell walls occurs commonly in the biological treatment system. Moreover, cell walls and EPS secreted by cells consist of complex substances, such as lipopolysaccharides, proteins and carbohydrates. These chemicals contain abundant functional groups which provide sorption sites for metal binding and metal immobilization.

2. Intracellular bioaccumulation

Heavy metal can be accumulated within cells via membrane transport systems. Heavy metals are first bound to extracellular ligands and then get transported through cell wall with these ligands. Once inside the cell wall, metals are inactived, localized within intracellular structures, or participate in biochemical process (Sigg, 1987).

3. Direct enzymatic reduction

The principle of reductive biotransformation of heavy metals lies on the decrease of mobility and toxicity when metals are reduced to lower redox states. In the direct reduction process, metal-reducing microorganisms use the oxidized form of metals (such as Cr (VI), U (VI), and Tc (VII)) as electron accepters and transform them into reduced species (Cr (III), U (IV), and Tc (IV)). Aerobic or anaerobic reduction of Cr (VI) to Cr (III) has been accomplished by a wide range of microorganisms (Kamaludeen et al., 2003).

4. Indirect enzymatic reduction

Reductive biotransformation also facilitates indirect metal immobilization. Metal-reducing and sulfate-reducing bacteria are usually involved in this process. Electrons extracted from the oxidation of organic compounds or hydrogen are used to reduce Fe (III), Mn (IV), and SO_4^{2-} to Fe (II), Mn (III), and H_2S. Heavy metals then interact with these reduced products to form separate or multicomponent insoluble species (van Hullebusch et al., 2005b). Indirect enzymatic reduction often happens in sedimentary and subsurface environments or in anaerobic wastewater treatment reactors. The most active reduced products are Fe (II) and H_2S. Fe (II) is used as electron donor in the reduction of Cr (VI) to Cr (III) by Fe-reducing bacteria, such as *Geobacter, Desulfuromonas, Shewanella*, and *Pelobacter*. (Coates et al., 1996; Wielinga et al., 2001). Heavy metals in up-flow anaerobic sludge bed (UASB) reactors are often precipitated by sulphide produced by sulphate biological reduction, which enables the simultaneous removal of heavy metal, sulphate, and organic pollutants (De Lima et al., 2001; Sierra-Alvarez et al., 2006).

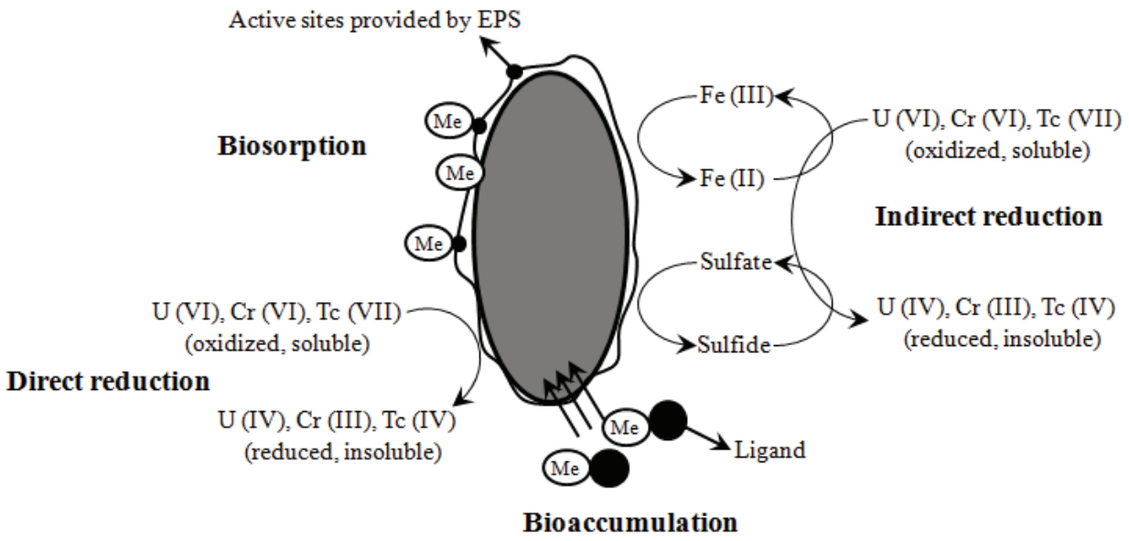


Fig. 1. Possible metal-microbe interaction

The speciation and bioavailability of heavy metals are highly affected by the physicochemical properties of wastewater treatment system and microbial activities. Heavy metals in the system usually can be classified to five groups (Li et al., 2009; van Hullebusch et al., 2006):

1. Exchangeable fraction
2. Carbonate fraction
3. Fe/Mn oxide-combined fraction
4. Organic matter/sulfides bound fraction
5. Residual fraction

Property of each fraction varies, making the total heavy metals concentration a poor indicator of metal bioavailability and toxicity. The residual and exchangeable fractions possess the highest mobility, since these metals can be easily released through ion exchange. Metals trapped in the Fe/Mn oxide will be leached if redox condition in the system changes. Organic matter always has high affinity towards heavy metals. However, decomposition or oxidation of organic ligands occur with time, resulting in the exposure of heavy metals. Sulfide fraction is insoluble and thus exhibits lower toxicity. However, chemical states of

sulfide are dependent on the system conditions. Metal release happens during the oxidation of sulfide precipitate under oxygen-rich conditions (Salomons, 1995).

3. Biosorption of heavy metals by aerobic granular sludge

Bioremediation of heavy metals can occur through several pathways. However, biotic and inactive sludge show limited differences in metal uptake capacity in batch mode studies. Sirianuntapiboon & Ungkaprasatcha (2007) studied the uptake capacity of bio-sludge towards Pb^{2+} and Ni^{2+}. It was found that the uptake capacity decreased by 10-30% after autoclaving, indicating the significant role of adsorption in the remediation process.

3.1 Biosorption behavior of aerobic granular sludge

Various biosorbents, such as fungus, algae, bacteria, and activated sludge, have been used to remove heavy metals. The maximum adsorption capacities for Cd^{2+} of these biosorbents lie in a range of 22-153 mg/g, and those for Zn^{2+} and Cu^{2+} were 14-170 and 5.9-130 mg/g dry biomass (Liu et al., 2004). Table 1 summarizes the maximum adsorption capacities of granular sludge and some commercially available adsorbents. As can be seen from Table 1, aerobic granules have comparable adsorption capacities with other biosorbents. Furthermore, the dense structure and excellent settling ability of aerobic granular sludge make it more feasible than those suspended biosorbents. Aerobic granules also exhibited high removal capacities when compared with some commercially available resins and granular activated carbon (GAC).

Biosorption of heavy metals is a rapid process and usually reaches equilibrium within several hours. The adsorption process experiences a relatively fast initial sorption followed by a slower and longer uptake. At the beginning of adsorption, a large number of vacant active sites are available for heavy metals, and the driving force provided by the metal concentration differences between the granule surface and solution is large. As the active sites are gradually occupied by metals, the adsorption process slows down. Heavy metals can enter the pores within the granules and subsequently get adsorbed. However, the interior adsorption needs to overcome lager mass transfer resistance.

The adsorption capacity depends on various system parameters, such as pH, temperature, and ionic strength. Among them, pH is the most important factor by affecting the chemistry of both the biomass surface and heavy metals. The adsorption capacities of aerobic granules are usually enhanced at higher solution pH. For example, the biosorption capacities of aerobic granules increased from ~20 mg Pb^{2+}/g to 44 mg Pb^{2+}/g when the solution pH was increased from 3.0 to 4.0 (Yao et al., 2008). Cu (II) adsorbed by aerobic granules at pH 3 was 19.25 mg/g and that at pH 5 was 36.72 mg/g (Gai et al., 2008). It is believed that the biomass surface is protonized at low pH. The protonized ligands and metal cations will compete for binding sites. As the pH increases, more functional groups with negative charges become exposed, which results in the biosorption capacity enhancement (Gai et al., 2008; Hawari & Mulligan et al., 2006a). Higher temperature always favors the adsorption process through the increase in surface activity and kinetic energy of the solute (Sag & Kutsal, 2000). However, the influences of temperature are usually insignificant and operating the adsorption system at high temperature is impractical.

Table 1 also shows even in the same system, the adsorption affinity varies for different metals. The metals are adsorbed preferentially in the order of Pb (II)> Zn (II)> Cu (II)> Cd (II)> Ni (II)> Co (II), which maybe related with the covalent index of metal ions (Brady & Tobin, 1995; Leung et al., 2001; Puranik & Paknikar, 1999).

Adsorbent	Metal	q_{max} (mg/g dry biomass)	pH	Reference
Aerobic granules	Cd (II)	566	7	Liu et al, 2003
Aerobic granules	Pb (II)	87.7	5.5	Yao et al., 2008
Aerobic granules	Co (II)	55.5	7	Sun et al., 2008
	Zn (II)	62.5	5	
Aerobic granules	Cd (II)	625	7	Liu et al., 2004b
	Zn (II)	204	6	
	Cu (II)	52.9	5	
Anaerobic granules	Co (II)	8.4	6	van Hullebusch et al., 2004
	Ni (II)	7.9		
Calcium treated anaerobic granules	Pb (II)	255	5.5	Hawari & Mulligan, 2006a
	Cd (II)	60		
	Cu (II)	55		
	Ni (II)	26		
Commercial resins Duolite GT-73	Pb (II)	122	4.5-5.0	Vaughan et al., 2001
	Cd (II)	105		
	Cu (II)	61		
	Ni (II)	60		
Commercial resins Amberlite IRC-718	Pb (II)	290	4.5-5.0	Vaughan et al., 2001
	Cd (II)	258		
	Cu (II)	127		
	Ni (II)	129		
GAC	Pb (II)	26	4.0-4.5	Suh & Kim, 2000

Table 1. Maximum adsorption capacities (q_{max}) of different sorbents towards heavy metals

3.2 Metal speciation in aerobic granular sludge and adsorption mechanism

Several mechanisms have been proposed for the uptake of heavy metals by biomass, including ion exchange, complexation, and precipitation. Table 2 summarizes the contribution of different mechanisms in some biosorption processes. Among them, ion exchange seems to be dominant except in the study by Yao et al. (2009), where complexation is mainly responsible for the heavy metal uptake.

Heavy metal sorption is associated with the simultaneous release of light metal ions (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) in the ion exchange process. Light metal cations, especially Ca^{2+}, are found to enhance sludge granulation, and thus are always added in the granulation process (Jiang et al., 2003; Mahoney et al., 1987). One of the possible promotion mechanisms is the formation of EPS-Ca^{2+}-EPS bridge or cell-Ca^{2+}-cell linkage (Liu et al., 2002). Sites initially occupied by these light cations are substituted by metals in the adsorption process.

Heavy melts biosorption by complexation is accomplished by binding of metals to EPS. EPS is a mixture of macromolecular polyelectrolytes including polysaccharides, proteins, and nucleic acids. EPS is an essential component for the aerobic granulation and the subsequent three-dimensional matrix maintenance (Sheng & Yu, 2006). In addition, the abundant functional groups, such as carboxylate, hydroxyl, amide, and amine groups, create complexation sites for metal binding. EPS produced by *Paenibacillus jamilae* had a maximum complexation capacity of 230 mg Pb/g EPS (Morillo et al., 2006). Loosely bound EPS (LB-

EPS) and tightly bound EPS (TB-EPS) were extracted from aerobic granules and used as biosorbents to remove Zn^{2+} and Co^{2+} (Sun et al., 2009). Results show that LB-EPS was more efficient in heavy metals uptake than TB-EPS. The adsorption capacities of LB-EPS and TB-EPS for Zn^{2+} were 6.9 and 1.1 mg/mg EPS respectively and those for Co^{2+} were 5.5 and 1.5 mg/mg EPS. Xu & Liu (2008) studied the metal-EPS complexation in aerobic granules by fourier transform infrared (FTIR) spectroscopy. Table 3 summarizes the main functional groups on aerobic granules and the FTIR spectroscopy changes after metal adsorption. The main functional groups involved in the metal binding were alcoholic, carboxylate, amine and ether groups. Realizing the important role of functional groups, Sun et al. (2010) used polyethylenimine to enhance amine groups on aerobic granules. This surface modification successfully increased the Cr (VI) uptake capacity of granules by 274%.

Adsorbent	Metal	Adsorption mechanisms			Reference
		Ion exchange (%)	Complexation (%)	Precipitation (%)	
Aerobic granules	Cd (II)	75.51	19.36	5.13	Xu & Liu, 2008
	Cu (II)	71.31	16.19	12.50	
	Ni (II)	82.43	14.20	3.37	
Anaerobic granules	Pb (II)	51	20	29	Hawari & Mulligan, 2006b
	Cu (II)	77	18	0	
	Cd (II)	82	15	0	
	Ni (II)	98	0	0	
Aerobic granules	Cr (III)	11.2	60.3	18.7	Yao et al., 2009
Aerobic granules	Cu (II)	~70	Unknown	Unknown	Gai et al., 2008

Table 2. Contribution of different mechanisms to heavy metals biosorption

Except ion change and complexation, heavy metals can also be removed from aqueous solutions by chemical precipitation. New crystals as $CdCO_3$ and $Cu_2(OH)_3Cl$ were detected in aerobic granules by X-ray diffraction analysis (XRD) after exposure to Cd^{2+} and Cu^{2+} (Xu & Liu, 2008). As compared with ion exchange and complexation, the distribution of precipitation was minor (Table 2).

4. Effects of heavy metals on the aerobic granular sludge system

Heavy metals occurring at low concentrations are essential for many physiological and biochemical processes of microorganisms. Metals are often involved in the enzyme system. Co works as a cofactor in vitamin B_{12} and methyltransferase which play key roles in methylotrophic methanogenic pathway (Beveridge & Doyle, 1989). Ni is essential for methyl-CoM-reductase and uerase while Fe and Cu are of vital importance for NO-reductase, nitrite reductase, and ammoniummonooxygenase (Ensign et al., 1993; Ferguson, 1994; Hausinger, 1994). While acting as essential trace elements for microbial metabolism, metals at high concentrations introduce inhibition and toxicity to living organisms. Sandrin & Maier (2003) presented three inhibition patterns of heavy metals (Fig. 2). In the first pattern (Fig. 2A), the inhibition of heavy metals is proportional to their concentrations. In pattern B, low

concentrations metals simulate microbial activity while inhibition begins to show up at high metal concentrations. This phenomenon is usually found in the mixed consortia system. The simulation at low metal concentrations can be attributed to the differential toxicity effects. Metals may select for a metal-resistant, functional population while inhibiting a metal-sensitive, non-functional population. Differential toxicity effects reduce competition for resource needed by the metal-sensitive, non-functional population, thus resulting in apparent simulation. In pattern C, metals exhibit inhibition at low concentrations. However, inhibition, after a maximum level, becomes milder under higher metal concentrations. This may be the results of microbial community evolution for high metal-resistant microorganisms or more efficient detoxification mechanism induced at high metal concentrations.

Vibration type	Functional type	Wave number (cm^{-1})			
		Granules	Cd^{2+} loaded granules	Cu^{2+} loaded granules	Ni^{2+} loaded granules
Overlapping of stretching vibration of OH and NH	OH into polymeric compounds and amine	3407	3414	3346, 3335	3402
Asymmetric stretching vibration of CH_2		2928	2927	2928	2928
Stretching vibration of C=O	Carboxylic acids	1725	Intensity decrease	1725	Intensity decrease
Stretching vibration of C=O and C–N (amide I)	Protein (peptidic bond)	1648	1648	1648	1648
Stretching vibration of C–N and deformation vibration of N–H (amide II)	Protein (peptidic bond)	-----	1520	1535	1520
C-H bending		1488	1488	1468	1488
Bending of C–O–H	alcoholic group	----	1384	1385	1385
Deformation vibration of C=O	Carboxylic acids	1261	1245	1240	1244
Bending vibration of C–O	Polysaccharides	1082	1082	-------	1082
Stretching vibration of OH	Polysaccharides	1056	1056	1056	1056
"Fingerprint" zone	Phosphate/ sulphur functional groups	<1000	<1000	<1000	<1000

Table 3. Functional groups on aerobic granules and wave number changes in FTIR spectroscopy after heavy metals adsorption (Adapted from Xu & Liu (2008))

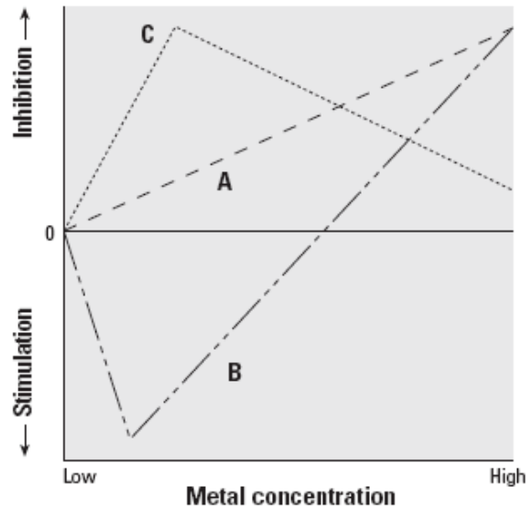


Fig. 2. Inhibition patterns of heavy metals (from Sandrin & Maier (2003))

4.1 Wastewater treatment performance in the presence of heavy metals

Wang et al. (2010) investigated the toxicity of Cu (II) and Ni (II) on aerobic granular sludge in SBRs. The reactors were operated with 5 mg/L Cu (II) or Ni (II) for 26 days and then the metals concentration was increased to 15 mg/L. Results show that biomass growth in the reactor was inhibited by 5 mg/L of Cu (II), while Ni (II) stimulated biomass yield even under the concentration of 15 mg/L. Influences of heavy metals on the SBRs treatment performance were shown in Fig. 3. Chemical oxygen demand (COD) and NH_4^+-N removal were slightly inhibited by 5 mg/L of Cu (II) in the first ten days. However, prolonged metal addition and increased metal concentration decreased COD degradation efficiency to 60%~80%. SBRs exhibited poor nitrification efficiency (~20%) when the concentration of Cu (II) was increased to 15 mg/L. Ni (II) had milder toxicity on aerobic granules activities when compared with Cu (II). Even at the concentration of 15 mg/L, Ni (II) caused slight reduction in COD and NH_4^+-N removal efficiency.

Evident NO_2^--N accumulation was observed in the first ten days of Cu (II) addition. Howver, COD degradation at this time was comparable with the metal-free control, indicating the sensitivity of nitrifying bacteria. The higher sensitivity of nitrifiers to heavy metals than heterotrophs are widely reported. Fluorescent in situ hybridization (FISH) analysis of Principi et al. (2006) revealed dramatic decrease in the abundance of *β-proteobacteria* under metal addition, which comprised ammonia-oxidizing bacteria. Stasinakis et al. (2003) also demonstrated nitrifying bacteria the most sensitive parts in the microbial community. Heavy metal can influence phosphorus removal through affecting alkaline phosphatase activity (APA). Zn^{2+} and Cu^{2+} at the concentration of 5.0 mM had an obvious inhibitive effect on the APA while Mn^{2+}, Co^{2+}, Pb^{2+} and Cr^{6+} ions stimulated the APA (Xie et al., 2010).

Among the metals investigated, Cu (II) always induces higher toxicity while Pb (II) processes the least toxicity (Lin & Chen, 1997; Li & Fang, 2007). Metals exert their toxicity on microorganisms through one or more mechanisms. Metal cations may substitute for physiologically essential cations within an enzyme (e.g., Cd^{2+} may substitute for Zn^{2+} or Ca^{2+}; Ni^{2+} substitutes for Fe^{2+}; Zn^{2+} substitutes for Mg^{2+}), thus inhibiting the function of the

enzyme (Nies, 1999). Metal oxyanions may take place of essential nonmetal oxyanions who are structurally similar with them. For example, arsenate may be used in place of phosphate. Besides the inactivation or damage of enzymes, Cu (II) also cause cytoplasmic membrane disruption. As a redox-active metal, Cu (II) can catalyze the production of free hydroxyl radicals and promote membrane lipid peroxidation (Howlett & Avery, 1997; Hu et al., 2003).

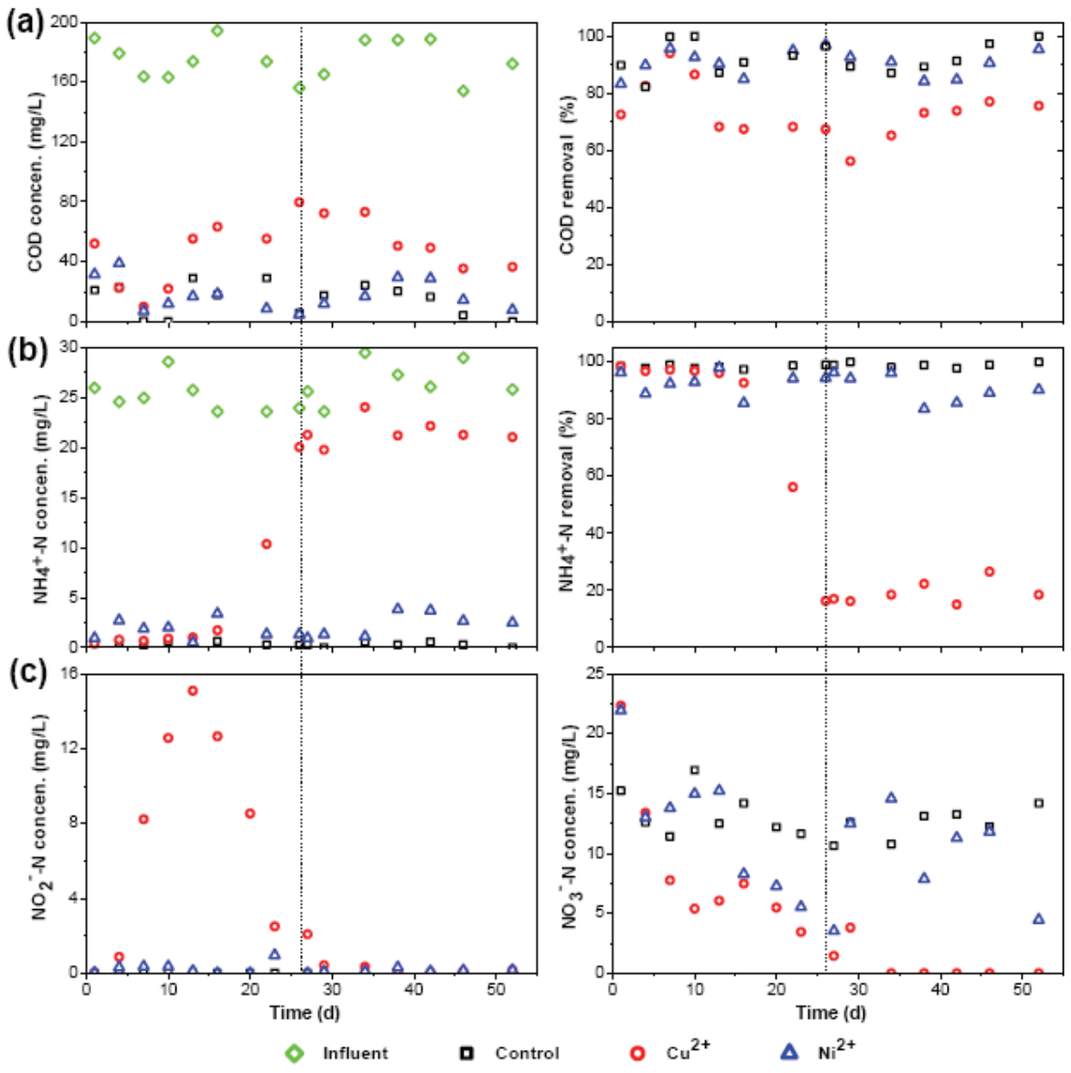


Fig. 3. Influence of heavy metals on wastewater treatment in aerobic granular sludge system: (a) and (b) COD concentration and removal efficiency; (c) and (d) NH_4^+-N concentration and removal efficiency; (e) NO_2^--N concentration and (f) NO_3^--N concentration (from Wang et al. (2010))

4.2 Metal resistance of granular sludge

Many researchers studied the effects of heavy metals on activated sludge system (Ong et al., 2004; Ong et al., 2005b; Santos et al., 2005; Sirianuntapiboon & Ungkaprasatch, 2007; Tsai et al., 2006). However, results from these studies are difficult to be compared due to the

various metal bioavailability caused by different operation modes, substrate compositions, seed sludge sources and concentrations. The total organic carbon (TOC) removal efficiency of activated sludge process decreased from 98% to 88% in the presence of Ni (II) while the same Ni (II) loading rate exerted slight influence on aerobic granules system (Ong et al., 2004; Wang et al., 2010). Table 4 gives the metal/VSS ratios causing 50% inhibition of microbial activity of anaerobic granules and flocculent sludge. Results show that granules always had higher toxicity-resistance than flocculent sludge.

Metal	Specific methanogenic activity [a]		Acetate degradation [b]		Methane production [b]	
	UASB granules	Flocculent sludge	Intact granules	Disintegrated granules	Intact granules	Disintegrated granules
Cd	>400	14.3	660	610	630	480
Cr	310	27.4	770	660	510	380
Cu	180	23.3	580	520	360	260
Ni	120	745	450	300	240	180
Zn	105	29.8	250	210	170	120

[a] Adapted from Lin 1993
[b] Adapted from Bae et al., 2000

Table 4. Metal/VSS ratios (mg/g VSS) causing 50% inhibition of sludge activity

Higher toxicity-resistance of granules is the benifit given by their unique physical, chemical, and biological properties, i.e., their compact sturcture, EPS, and dynamic microbial community. The spatial architecture of granules creates diffusion resistance. Taking dissolved oxygen (DO) as an example, it can only permeate 125 μm beblow the surface of a 1.50 mm phenol-fed granule (Chiu et al., 2007). Through diffusion resistance, granular sludge reduce the heavy metals concentrations within the granules.

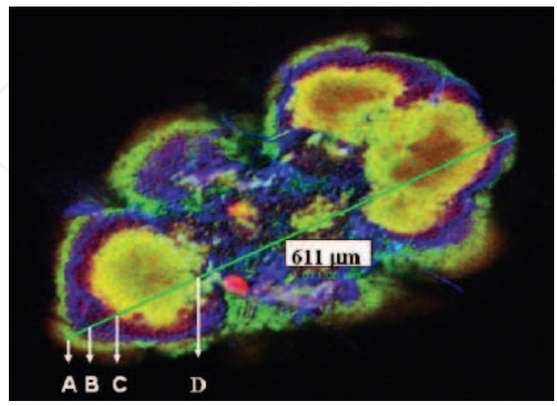


Fig. 4. EPS staining of a phenol-degrading granule. Green: proteins; red: nucleic acids; blue: polysaccharides; yellow: proteins + nucleic acid; purple: nucleic acids+polysaccarides; A-protein + nucleic acid layer, B-Polysaccharide layer, C-nucleic acid layer, D-protein + cells. (from Adav et al., 2007)

EPS acts as protective barrier in metal-resistance of aerobic granules. EPS, on one hand, reduces free metal concentrations by complexation, on the other hand, prevents toxicants from reaching microbes within the granules by diffusion limitation. Fig. 4 shows the EPS distribution in a phenol-degrading granule (Adav et al., 2007). The granule consists of a proteins and nucleic acids rich outer surface, followed by a polysaccharide layer. The inner core was filled with proteins and cells. This EPS matrix protected microorganisms from exposure to high concentrations of metals. Moreover, research also found that microorganisms could regulate EPS synthesis and modify EPS components (Sheng et al., 2005; Wang et al., 2010). They would secrete more EPS in the presence of heavy metals, especially more proteins. In the study of Sheng et al. (2005), the EPS content increased by 5.5, 2.5, and 4.0 times than the control when exposed to 30 mg/L Cu (II), 40 mg/L Cr(VI), and 5 mg/L Cd(II).

Diffusion limitation develops important physic-chemical gradients (e.g., pH, E_h and oxygen) in aerobic granules. These various physic-chemical environments enable diverse microbial community. Heterotrophic and autotrophic bacteria, aerobe and anaerobe coexist in aerobic granules. Microorganisms can alternate their metabolic pathways or redistribute themselves to acclimate to meal toxicity. Viret et al. (2006) studied the influence of Zn (II) and Ni (II) on oxygen consumption of benthic microbial communities. The oxygen consumption at the surface was found to decrease by 60%-90% after Zn (II) and Ni (II) spiking. However, the oxygen consumption zone was stretched, implying the migration of aerobe into inner space to avoid metal toxicity or/and the metabolism switch of facultative aerobic microorganisms to aerobic respiration which is more efficient than the original fermentation. Wang et al. (2010) applied Biolog tests to analyze the substrate utilization patterns of aerobic granules before and after long-term metal addition. Principal component analysis of the Biolog tests and the hierarchical cluster analysis showed different groups based on metal treatment, indicating changes in microbial community structure induced by Cu and Ni (Fig. 5).

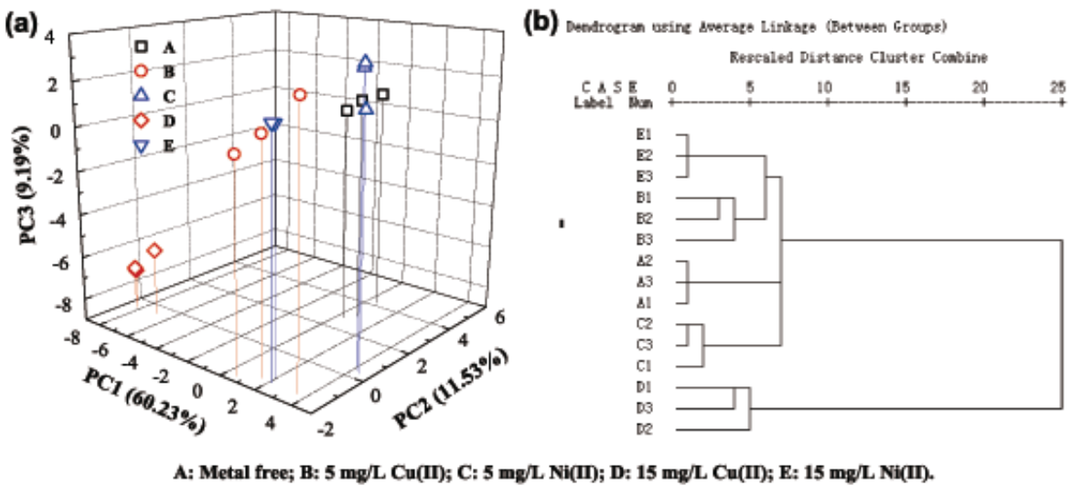


Fig. 5. Effects of heavy metals on the microbial community investigated by (a) principal component analysis and (b) hierarchical cluster analysis of Biolog tests. (from Wang et al. (2010)).

5. Conclusion

The interaction of heavy metals and aerobic granular sludge occurs on the surface and the inner space of sludge. These physical or biochemical processes are mainly based on sludge and are also influenced by environmental conditions and metal characteristics. Many studies have been conducted to uncover metals bioremediation by aerobic granular sludge and the effects of metals on sludge. These results are helpful for understanding the metal transformation and performance of aerobic granular sludge process.

In bioremediation of heavy metals by aerobic granular sludge, adsorption accounts for most metal uptake. The high biomass retention, compact and porous structure, and excellent settling ability of aerobic granules enable them good performance in the biosorption of heavy metals. Aerobic granules show high adsorption capacities when compared with other biosorbents and some commercial adsorbents. Heavy metals can be adsorbed to aerobic granules by replacing the sites of light metal ions (such as Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) which already exist in the high content EPS, or binding to the functional groups on EPS and cell wall. Chemical precipitation on the metal–sludge interface also contributes to metal sorption to granules. Further experiments and characterizations of aerobic granule are needed to interpret space distribution of adsorbed metals and the adsorption mechanisms. Besides, good stability and unique structure of aerobic granules provide opportunity to enhance desired surface functional groups by chemical modification, which leads an important field in biosorption. Aerobic granules possess superior settling ability, so the removal of heavy metals in a continuous reactor similar as UASB or SBR maybe more hopeful.

Effects of heavy metals on the alive microbe are complicated, especially in the aerobic granular sludge reactor. The inhibition of heavy metal on aerobic granules depends on the metal species and concentrations. Organic pollutants degradation and nitrification are both negatively affected by the prolonged addition of high concentration heavy metals. However, higher toxicity-resistance than flocculent sludge has been proved in aerobic granules. This could be attributed to the unique compact structure and diverse microcosm. Diffusion resistance developed by the layer structure functions as barrier and buffer. The responses to heavy metal exposure are also of interest. Microbes in aerobic granules can regulate EPS synthesis and alternate their metabolic pathways to acclimate to meal toxicity. High concentration heavy metals should weaken the stability of whole microorganisms in the aerobic granules. Microbial test is a useful method for exploring sensitivity of different microorganisms to metals. Better understanding of metal biotransformation and responses of aerobic granules is essential for optimizing the aerobic granule reactor treating or exposed to heavy metals.

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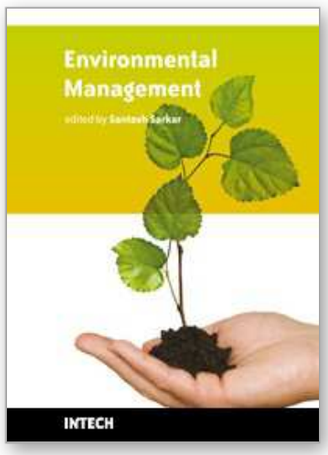
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Environmental Management

Edited by Santosh Sarkar

ISBN 978-953-307-133-6
Hard cover, 258 pages
Publisher Sciyo
Published online 12, August, 2010
Published in print edition August, 2010

There has been a steady increase in anthropogenic pressure over the past few years due to rapid industrialization, urbanization and population growth, causing frequent environmental hazards. Threats of global environmental change, such as climate change and sea level rise, will exacerbate such problems. Therefore, appropriate policies and measures are needed for management to address both local and global trends. The book 'Environmental Management' provides a comprehensive and authoritative account of sustainable environmental management of diverse ecotypes, from tropical to temperate. A variety of regional environmental issues with the respective remedial measures has been precisely illustrated. The book provides an excellent text which offers a versatile and in-depth account of management of wide perspectives, e.g. waste management, lake, coastal and water management, high mountain ecosystem as well as viticulture management. We hope that this publication will be a reference document to serve the needs of researchers of various disciplines, policy makers, planners and administrators as well as stakeholders to formulate strategies for sustainable management of emerging environmental issues.

How to reference

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Shuguang Wang, Shaoxian Teng Teng and Maohong Fan (2010). Interaction between Heavy Metals and Aerobic Granules, Environmental Management, Santosh Sarkar (Ed.), ISBN: 978-953-307-133-6, InTech, Available from: http://www.intechopen.com/books/environmental-management/interaction-between-heavy-metals-and-aerobic-granules

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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
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InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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