CO-TREATMENT OF ACID MINE DRAINAGE WITH MUNICIPAL WASTEWATER USING THE ACTIVATED SLUDGE PROCESS: PRELIMINARY TREATABILITY STUDIES[1]

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Abstract: Co-treatment of acid mine drainage (AMD) and municipal wastewater (MWW) using the activated sludge (AS) process is an innovative approach to AMD remediation that utilizes the alkalinity of MWW and the adsorptive properties of AS biomass to remove acidity and metals from AMD-impacted waters. Preliminary treatability studies have been conducted to simulate the effects of introducing AMD to different stages of the AS process. Mixing high-strength synthetic AMD (pH 2.8, 540 mg L^{-1} Fe, 200 mg L^{-1} Al, 30 mg L^{-1} Cu, 60 mg L^{-1} Zn, 30 mg L^{-1} Mn) with settled MWW over a range of dilutions, simulating conditions in the primary sedimentation stage of the AS process, led to rapid and significant removal of metals from solution (average removal: 38 mg L^{-1} Al, 5 mg L^{-1} Cu, 56 mg L^{-1} Fe, 4 mg L^{-1} Mn, and 7 mg L^{-1} Zn). Mixing synthetic AMD with AS biomass at different solids concentrations, simulating AMD loading to the secondary (biological) treatment stage and/or to a subsidiary mixing tank, also removed significant fractions of metals from solution (average removal: 28 mg L^{-1} Al, 5 mg L^{-1} Cu, 25 mg L^{-1} Fe, 3 mg L^{-1} Mn, and 4 mg L^{-1} Zn at a mixed liquor suspended solids concentration of 6 g L^{-1}). Activated sludge respiration inhibition tests indicated high tolerance to spiked additions of synthetic AMD, demonstrating that acute toxicity is mediated by the neutralization and metal removal reactions that occur during mixing. Acclimatization studies indicated that AS sensitivity to spiked additions of AMD decreased after a 25-day period of continuous AMD loading. Laboratory-scale process evaluation studies are underway to demonstrate the feasibility of co-treatment for full-scale application in the treatment of AMD.

Additional Key Words: Acid rock drainage, toxicity, acclimatization, sewage

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Introduction

Wastewater treatment plants (WWTPs) receive wastewater from many sources, including domestic, industrial, and institutional effluents, septic tank wastewater, sewer infiltration, stormwater, and leachate. Municipal wastewater (MWW) is a broad term referring to a mixture of wastewaters derived from these sources, and its composition is highly varied. In general, MWW is a dilute, alkaline mixture containing a wide variety of organic (e.g. carbohydrates, proteins, fats) and inorganic compounds (e.g. inorganic salts and trace elements). Treatment of MWW is primarily required to reduce concentrations of chemical and biochemical oxygen demand (COD and BOD), suspended solids, and nutrients to levels suitable for discharge.

The activated sludge (AS) process is a biological wastewater treatment process widely used for domestic, municipal, and industrial wastewaters (Gray, 1990). It is used to treat a wide variety of recalcitrant and potentially toxic wastewaters, normally in admixture with domestic sewage. In the AS process, solids are removed from influent raw wastewater by screen filtering and settling in a primary sedimentation tank. Settled wastewater is then mixed in suspension under aerobic conditions with AS, a low-density (typically 1.5-3.5 g solids L^{-1}) sludge that is comprised of a diverse population of bacteria, fungi, protozoa, rotifers, and nematodes (Gray, 2004). The metabolic activity of these microorganisms naturally degrades organic matter and nutrients, which are removed from wastewater by various mechanisms, e.g. adsorption onto sludge flocs, mineralization, assimilation, and oxidation (Comeau, 2008). A key element of the biomass structure of AS is the flocculent nature of the solids, resulting from the bacterial formation of extracellular polymers (Brown and Lester, 1979). A fraction of the AS is displaced from the main aeration basin into a settlement tank, where the sludge solids (primarily flocculated biomass) settle and the clarified effluent may then be discharged. The settled sludge (returned AS, or RAS), is returned to the aeration basin, where it serves as an inoculum. Any excess sludge is disposed of, and receives further treatment if required.

Removal of acidity, metals, and sulphate from acid mine drainage (AMD) in active and passive treatment systems is accomplished by dosing with alkaline substances (e.g. CaO) or generating alkalinity either abiotically via passive dissolution of limestone or biotically via bacterial sulphate reduction. These processes lead to an increase in pH and subsequent removal of dissolved metals by precipitation and/or adsorption. Co-treatment of AMD and MWW using the AS process is an innovative approach to AMD remediation that utilizes the alkalinity of MWW and the adsorptive properties of AS to remove acidity and metals from AMD-impacted waters. In theory, co-treatment of AMD and MWW should be highly effective, because compounds that are high in one effluent stream tend to be low in the other. For example, (i) sewage effluent with relatively high concentrations of suspended solids may enhance iron oxyhydroxide precipitation by encouraging iron, which is often present in high concentrations in AMD, to form flocs (Johnson and Younger, 2006), and (ii) phosphate, which is present in high concentrations in sewage effluent, can be sorbed onto the iron oxyhydroxide precipitates (Sibrell et al., 2009; Wei et al., 2008) or react with Al to form hydroxyl-phosphates (Omoike and Vanloon, 1999). Rao et al. (1992) evaluated AMD as a coagulant and reported that it was as effective as the commercial coagulant $FeCl_3$ for turbidity removal from MWW. Passive co-treatment of MWW and AMD in a multi-stage system consistently removed significant concentrations of dissolved Al, As, Cd, Fe, Mn, Pb, and Zn, and resulted in a net-alkaline effluent (Strosnider et al., 2011a), as well as achieving high BOD and nutrient removal efficiency (Strosnider et al., 2011b). Other recent reports of passive co-treatment indicated that AMD enhanced coagulation, sedimentation, and pathogen removal during wastewater treatment (Neto et al., 2010; Winfrey et al., 2010). Johnson and Younger (2006) reported removal of Fe and Mn from net-alkaline coal mine AMD, as well as phosphate, nitrate, and suspended solids removal, in a wetland co-treating AMD with secondary sewage effluent. Metal removal by AS is believed to occur mainly by entrapment and settlement of particulate non-settleable metal-containing solids in the sludge floc matrix, as well as by binding of soluble metal to extracellular polymers (Brown and Lester, 1979; Santos and Judd, 2010). Pamukoglu and Kargi (2009) demonstrated that Cu concentrations up to 30 mg L^{-1} could be tolerated by an AS system with no detrimental effects on COD removal efficiency or sludge settling, by using a pre-mixing stage to remove some Cu by biosorption onto dried waste sludge. Finally, MWW is alkaline, and therefore has a high acid neutralizing capacity (ANC); furthermore, dilution of AMD H^+ concentrations on mixing with MWW causes the pH to increase, thereby decreasing the pH-dependent solubilities of many metals (Strosnider et al., 2011c).

Successful co-treatment depends on the capacity of the AS process to withstand significant loadings of acidity, heavy metals, and sulphate, whilst maintaining high biological wastewater treatment efficiency. Metals can exert toxic effects on the AS microorganisms that lead to inhibition of microbial activity and growth (Alkan et al., 2008; Pai et al., 2009), thus inhibiting aerobic and anaerobic processes that are critical for plant performance (e.g.

removal of BOD, COD, suspended solids, and nutrients) (Chua et al., 1999; Ong et al., 2003; Ong et al., 2004; Xie and Nakamura, 2002; You et al., 2009). Microbial inhibition may cause decreased viability, changes in community structure, and loss of floc structure, leading to poor settling and loss of biomass from the system (Kelly et al., 2004; Love and Bott, 2000; Neufeld, 1976). However, the presence of trace amounts of heavy metals may stimulate microbial growth and have no detrimental effect on the system (Cabrero et al., 1998; Gikas, 2008; Jefferson et al., 2001). The sensitivity of AS to different toxic substances can be influenced by previous exposure, and the population of microorganisms can be modified and selected owing to the presence of industrial effluents (Christofi et al., 2003). Acclimatization of AS to high loads of metals (Neufeld and Hermann, 1975; Sorour and Sayed-Ahmed, 2005), and sulphur (Burgess and Stuetz, 2002) have been reported; furthermore, adding trace concentrations of heavy metals may actually improve biological treatment of wastewater (Wang et al., 2010; Yetis and Gokcay, 1989).

Since mining operations ceased in 1982, AMD has been emanating from the abandoned copper and sulphate mines and tailings piles near Avoca, Co. Wicklow, SE Ireland. The main area impacted by mining is divided by the Avoca River into East and West Avoca, and is comprised of spoils heaps, open pits, and numerous shaft openings. Two major adits, the “Deep” and “Road” Adits, discharge AMD directly into the Avoca River. Other adits (with seasonal flows), surface runoff from spoil, groundwater discharge and bank intrusion also pollute the river, resulting in severe contamination (Gaynor and Gray, 2004; Gray, 1998). Ecosystem destruction, caused primarily by ochre deposition and an associated thick layer of precipitated metals, is obvious in the reaches of the river immediately downstream of the mines, and the extremely poor river quality caused by AMD has resulted in significant losses of macroinvertebrate species, fish, and other native biota, with impacts evident in the entire river downstream of the mine site (Gray and Delaney, 2010). No remediative measures are in place at the Avoca mine site. A sustainable, reliable, and long-term treatment system for Avoca AMD is needed.

In these preliminary studies, two important aspects of the proposed co-treatment process were examined; namely, the acute and chronic toxicity of AMD to AS microorganisms, and the capacity of AS and settled MWW to remove metals from solution. To assess acute toxicity, synthetic AMD containing Fe, Al, Cu, Zn, Pb and Mn (the chief metals of concern at Avoca) was used in batch experiments with AS from WWTPs receiving municipal and industrial effluents. Oxygen uptake rate (OUR) inhibition was chosen as the screening test to evaluate toxicity (Kilroy and Gray, 1992; Madoni et al., 1999). To examine chronic toxicity, acclimatization of AS to continuous loading of AMD (simulating the adit drainage at Avoca) was examined using standard AS biomass assessments (i.e. mixed liquor suspended solids (MLSS), sludge volume index (SVI), and floc morphology), and OUR inhibition tests. The metal removal capacities of AS and settled MWW upon mixing with synthetic AMD were also determined. The effects of contact time, metal concentration, and AS biomass concentration on metal removal were considered. These studies were conducted in support of developing a co-treatment process for AMD and MWW using the AS process.

Materials and Methods

Activated Sludge and Wastewater Sampling

For the OUR inhibition and acclimatization studies AS samples were taken from the aeration tanks of four WWTPs with different influent characteristics. Three WWTPs (labelled M-1, M-2, and M-4 in this report) receive municipal influent, and one WWTP (labelled I-3 in this report) receives industrial influent (70% industrial/30% domestic, with moderately high concentrations of Cu, Pb, As, Sn, Cr, and Ni).

For the metal removal studies, samples were obtained from WWTP M-1. AS samples were taken from the aeration tanks, RAS was collected from the return flow of settling tanks, and settled MWW samples were collected from the primary sedimentation tanks.

All AS and RAS samples were cooled during transport to the laboratory, and kept aerated with porous ceramic air diffusers at 20 ± 2 °C until use. Mixed liquor suspended solids (MLSS) concentrations of AS and RAS were determined according to standard methods (APHA, 1995). Settled MWW samples were immediately refrigerated at 4 °C until use.

Preparation of Synthetic AMD and Wastewater Solutions

Synthetic AMD was used in these studies because real AMD has a highly variable composition and is unstable over long storage periods, thus requiring frequent sampling. Synthetic AMD containing the major metals of concern in Avoca, SE Ireland (i.e. Fe, Al, Cu, Zn, Mn, Pb, and Cd), was prepared at a range of compositions for the different studies.

For the OUR inhibition studies, a severe AMD formulation (Table 1) was used because AMD with sufficient toxicity to AS to cause significant OUR inhibition was required. The severe AMD formulation was identified by multivariate analysis of a large dataset (n=290) of AMD case studies, using the methods described in (Hughes and Gray, *in review*). For the acclimatization studies, a synthetic AMD (Table 1) simulating the AMD emanating from two major adits in Avoca, SE Ireland, was used (Gray and O'Neill, 1995). For the metal removal studies, synthetic AMD was prepared which was similar to the severe AMD formulation in terms of the relative mass loadings of each metal, but which excluded Pb (Table 2).

Synthetic AMD was prepared fresh daily from stock metal solutions of 1000 mg L^{-1} made using $Fe(SO_4)\bullet 7H_2O$, $Al_2(SO_4)_3\bullet 18H_2O$, $Cu(SO_4)\bullet 5H_2O$, $Zn(SO_4)\bullet 7H_2O$, $Mn(SO_4)\bullet H_2O$, $PbCl_2$, and $CdCl_2\bullet H_2O$ and distilled, deionized water. Where necessary, pH was adjusted by adding sulphuric acid H_2SO_4 (1 M).

Table 1. Physicochemical characteristics of synthetic AMD used in OUR inhibition and acclimatization studies.

Parameter (units)	Severe AMD	Simulated Avoca AMD (Gray and O'Neill, 1995)
pH (s.u.)	2.7	3.1
Fe (mg L^{-1})	620	130
Al (mg L^{-1})	200	150
Cu (mg L^{-1})	30	5
Zn (mg L^{-1})	70	90
Mn (mg L^{-1})	30	6
Pb (μg L^{-1})	60	1500
Cd (mg L^{-1})	-	0.2
SO_4 (mg L^{-1})	5120	1670

Using synthetic wastewater ensures that microbial growth and metabolic processes are not limited by deficient supplies of nutrients or trace elements. The synthetic wastewater used in the OUR inhibition tests and the acclimatization study was prepared as a 100-fold concentrate solution, by dissolving peptone (16 g), meat extract (11 g), urea (3 g), sodium chloride (NaCl) (0.7 g), calcium chloride ($CaCl_2\bullet 2H_2O$) (0.4 g), magnesium sulphate ($MgSO_4\bullet 7H_2O$) (0.2 g), and potassium diphosphate (K_2HPO_4) (2.8 g) in 1 L distilled water, according to OECD (1984).

Toxicity and Acclimatization Studies

Toxicity Studies. The Organization for Economic Co-Operation and Development (OECD) Method 209, "Activated Sludge, Respiration Inhibition Test" (OECD, 1984) was used to measure the toxic effect of AMD on AS from the different WWTPs. This test measures the effect of a test substance on microorganisms by comparing the OUR of AS under defined conditions in the presence of five different concentrations of the test substance with the OUR of a control sample made up with water added instead of the test substance. After a 3 h incubation period, a 20 mL subsample was taken from each incubation flask using a pipette, and the Strathtox® respirometer (Strathkelvin Instruments, Glasgow, UK) was used to measure the OUR of each sample. Oxygen concentration was measured over a 10 min period, and the dissolved oxygen concentration was plotted against time. For each concentration of the test substance, the OUR was estimated as the slope of a regression line fitted to a series of data points, and this value was used to calculate the percentage inhibition:

$$\%\ \text{I (percentage inhibition)} = 100 * \left(1 - \frac{2 * R_S}{R_{c1} + R_{c2}}\right) \tag{1}$$

where R_S= OUR at test concentration

R_{c1}= OUR of Control 1

R_{c2}= OUR of Control 2

The percentage inhibition was calculated for each test concentration, and the EC_{50} was determined after plotting the percentage inhibition against toxicant concentration on a log-normal graph. If the OUR of the controls differed by more than 15%, fresh samples were prepared and the entire test was repeated. A solution of 0.5 g L^{-1} 3,5-dichlorophenol, a known inhibitor, as a reference substance was used for checking the sensitivity of AS. The accepted range for the EC_{50} after 3 h incubation is 5-30 mg L^{-1}, and an EC_{50} below this range is an indication of abnormally sensitive AS and renders test results invalid (OECD, 1984).

Acclimatization Studies. The acclimatization studies were performed using a bench-scale, sequencing batch reactor (SBR) system, comprising four 4 L HDPE containers operated at constant temperature (20 ± 2 °C) on a fill and draw system, made up of four phases: fill (10 min), react (22.5 h with aeration), settle (1 h) and decant (20 min). Porous ceramic air diffusers were used to aerate and mix the contents of the reactors. There was no sludge recirculation. AS from a municipal, nitrifying WWTP was used to seed the reactors at start-up. The reactors were operated with a food-to-microorganism (f/m) ratio of 0.2 kg BOD kg^{-1} d^{-1} using the synthetic wastewater described above, with sodium bicarbonate ($NaHCO_3$) added to final concentration of 0.3 g L^{-1} synthetic wastewater to prevent a drop in pH due to nitrification (Christofi et al., 2003). Distilled, deionized water was used to prepare the synthetic wastewater and for dilution to the desired BOD. Sludge age (10 d) was controlled by manual sludge wasting.

After the reactors reached steady state conditions, with MLSS concentration as the primary indicator, the acclimatization process began. Beginning on Day 12, synthetic AMD, simulating Avoca AMD (Table 1), was added to Reactors B, C, and D at loading rates of 5%, 10%, and 25%, respectively, as a percentage of the volume of influent wastewater. Reactor A was the control and received no AMD. The pH in all reactors was maintained between pH 6.5 and 8.0 by the addition of $NaHCO_3$ as needed. For microscopic analysis, two replicate samples, each of 25 μL volume, were extracted with a micropipette. Floc morphology, filamentous growth measured by filament index values (Jenkins et al., 2004), and the presence of microorganisms (i.e. protozoa) were evaluated. Sludge volume index (SVI) was measured in a 1 L graduated cylinder according to standard methods (APHA, 1995). Finally, periodic AS OUR inhibition tests on samples from all reactors were used to examine changes in response of acclimatized AS to spiked additions of synthetic AMD.

Metal Removal Studies

The removal of Al, Fe, Cu, Mn, and Zn, by settled MWW, AS, and RAS was studied in laboratory batch experiments, which were carried out at room temperature to determine the capacity of each material to treat synthetic AMD. Synthetic AMD was prepared fresh daily at a range of metal concentrations (Table 2) from stock metal solutions of 1000 mg L^{-1} made using $Fe(SO_4)\bullet 7H_2O$, $Al_2(SO_4)_3\bullet 18H_2O$, $Cu(SO_4)\bullet 5H_2O$, $Zn(SO_4)\bullet 7H_2O$, and $Mn(SO_4)\bullet H_2O$ and distilled, deionized water. The pH was adjusted as required with sulphuric acid (H_2SO_4, 1 M). Aliquots (250 mL) of settled MWW, AS, or RAS (obtained from WWTP M-1) were placed in glass beakers and spiked with 250 mL synthetic AMD at each of the three dilutions. The pH was not controlled after mixing.

Table 2. Physicochemical characteristics of synthetic AMD used in metal removal studies.

Parameter (units)	Dilution 1	Dilution 2	Dilution 3
pH (s.u.)	2.7	2.7	2.7
Fe (mg L^{-1})	162	216	270
Al (mg L^{-1})	60	80	100
Cu (mg L^{-1})	9	12	15
Zn (mg L^{-1})	18	24	30
Mn (mg L^{-1})	9	12	15

The mixture was thoroughly mixed at 20 ± 2 °C with the use of porous ceramic air diffusers situated at the bottom of the beakers. At four time intervals (5, 30, 60, and

120 min), a 50 mL aliquot of the mixture was sub-sampled from each beaker. For settled MWW tests, samples were immediately filtered through a 0.45 µm cellulose nitrate filter, using a syringe. For AS and RAS tests, the sample was immediately centrifuged at 2000 rpm for four min, after which the supernatant was filtered through a 0.45 µm cellulose nitrate filter, using a syringe. The filtrate was acidified with 2-3 drops of 1 N nitric acid (reagent grade, Sigma-Aldrich) and stored in acid-washed polyethylene bottles at 4 °C prior to metal analysis. The filtrand and remaining solids were discarded. Control metal concentrations of the settled MWW, AS, and RAS were determined by performing the above procedure using distilled water in place of synthetic AMD. Three replicates were performed for each test. Blanks were also prepared using distilled water.

Sample preparation and metal analyses were performed according to standard methods (APHA, 1995). All samples were microwave digested (Ethos EZ microwave digestion labstation, Milestone) prior to metal analysis using inductively coupled plasma atomic emission spectrophotometry (ICP-AES) (Sequential ICP-AES, Liberty AX). Duplicates and method blanks were analyzed for quality control, and a mixed metal solution of Fe, Al, Cu, Zn, and Mn was prepared (CertiPUR®, Merck) and tested for quality control immediately following calibration, after every ten samples, and at the end of each sample run.

For each metal, the removed fraction was calculated as follows:

$$C_{removed} = C_{initial\ treatment} - C_{sample} - C_{control} \qquad (2)$$

Percentage metal removal was then calculated as follows:

$$\text{Metal removal (\%)} = (C_{removed} / C_{initial\ treatment}) * 100 \qquad (3)$$

Results and Discussion

Toxicity Studies

AS from four different WWTPs was used to evaluate the toxicity of high-strength AMD. In the case of WWTP M-1, AS was sampled on five different dates (spanning two months) to investigate how the response of AS from the same WWTP varies in time. EC_{50} results for all sludges ranged from approximately 19% to 52% AMD (Table 3, Fig. 1).

Table 3. Results of OUR inhibition tests using high-strength synthetic AMD (sd: standard deviation).

WWTP Code	AMD	Replicates (n)	EC_{50} (%)	sd
M-1-b	Severe	4	51.6	9.9
M-1-c	Severe	6	41.4	1.8
M-1-d	Severe	6	34.8	11.3
M-1-e	Severe	6	44.5	6.5
M-1-f	Severe	6	20.3	6.4
M-4-a	Severe	4	39.5	7.9
I-3-a	Severe	6	29.5	10.6
I-3-b	Severe	6	19.1	7.1
M-2-b	Severe	4	33.0	3.6
M-2-c	Severe	3	35.3	3.9

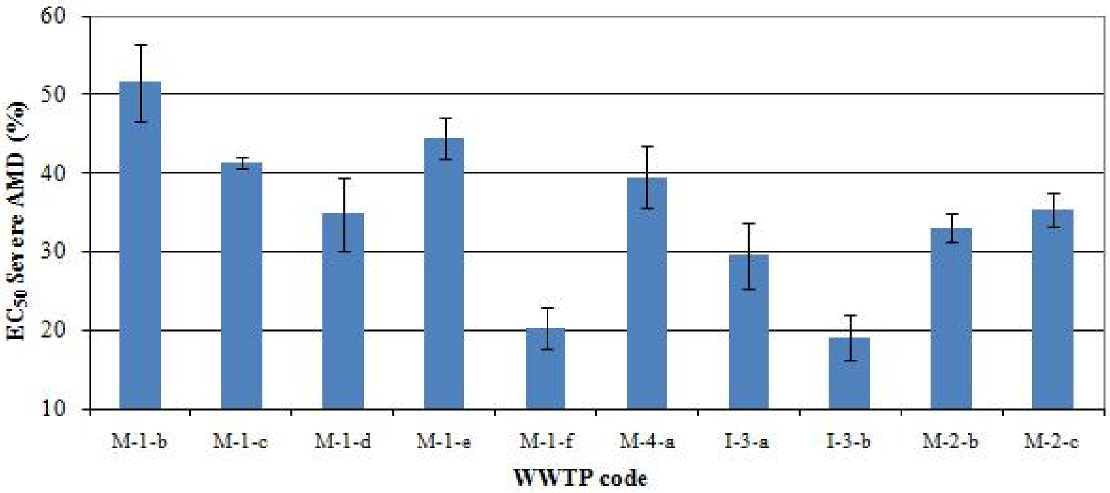

Figure 1. EC_{50} values for severe AMD using AS from different WWTPs (bars indicate standard error).

One-way ANOVA on the entire dataset indicated statistically significant differences among means ($p<0.001$); Tukey multiple comparison tests indicated that the EC_{50} for AS from industrial WWTP I-3-a was significantly less than the EC_{50} for M-1-b and M-1-e. The EC_{50} for AS I-3-b was significantly less than all sludges except M-1-f and I-3-a. Thus, rather than demonstrating a higher tolerance to shock loads of heavy metals, the AS from the industrial WWTP was more inhibited in the presence of high-strength synthetic AMD.

As well as comparing inhibition of sludges from different WWTPs, this data may be used to compare sludges from the same WWTP but sampled on different dates. Five batches of AS from WWTP M-1 were compared (batches b, c, d, e, and f). Significant differences were observed among results, with the EC_{50} for M-1-d significantly less than M-1-b and the EC_{50} for M-1-f significantly less than M-1-b, M-1-c, and M-1-e. The EC_{50} results indicate that the toxic effect of synthetic AMD to AS sampled from a single WWTP can change significantly over time.

Acclimatization Studies

Effect of AMD Acclimatization on Floc Morphology and SVI. Results are summarized in Table 4. For optimum settling and suspended solids removal, compact AS floc structure is most desirable. Flocs with diffuse structure and/or very small "pin" flocs develop when flocculation is poor, leading to poor settling and loss of biomass in treated effluent. Floc morphology is also an important parameter controlling metal removal in WW treatment (Leppard et al., 2003). In Reactors A and B the floc morphology disimproved throughout the acclimatization period, changing from compact to diffuse structure, with pin flocs eventually developing. In Reactors C and D, pin flocs were observed earlier, but subsequently disappeared; however, overall floc morphology changed from compact to diffuse in these reactors as well. The SVI of AS from all four reactors followed identical trends (Fig. 2). After a brief period of exceedingly high SVI values (Day 11- Day 14), the SVI dropped to normal values, remaining between 80-120 mg g^{-1}. Reactor D had the lowest SVI during the last 10 days of the acclimatization period. Although the SVI was normal, the sludge supernatant in all reactors became turbid and remained so after Day 24. Filament index values also remained generally constant at 4, with only a slight decrease observed in Reactors A and B on Day 33.

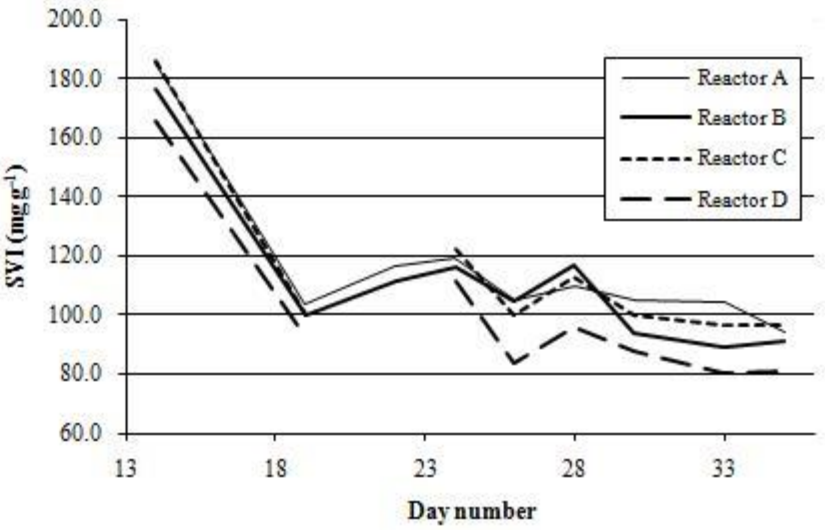

Figure 2. Sludge volume index (SVI) values of AS during acclimatization study.

Table 4. Sludge volume index (SVI) (mL g^{-1}), cone test observation of supernatant quality, floc morphology, and filament index value (FI) for Reactors A, B, C, and D during acclimatization to continuous loading of AMD.

	Reactor A				Reactor B				Reactor C				Reactor D			
Day number	SVI	Cone test	Floc morphology	FI	SVI	Cone test	Floc morphology	FI	SVI	Cone test	Floc morphology	FI	SVI	Cone test	Floc morphology	FI
14	185.7	Clear	Irregular, compact	4	176.4	Clear	Irregular, compact	4	186.1	Clear	Irregular, compact	4	165.3	Clear	Irregular, compact	4
19	103.8	-	-	-	100.0	-	-	-	100.0	-	-	-	92.6	-	Irregular, compact, pin flocs	4
22	116.7	Clear	-	-	111.5	Clear	-	-	-	Clear	-	-	-	Clear (white haze)	-	-
24	119.2	Turbid	Irregular, diffuse	4	116.0	Turbid	Irregular, diffuse	4	122.2	Turbid	-	-	111.1	Turbid	-	-
26	104.8	Turbid	-	-	104.8	-	-	-	100.0	-	-	-	84.0	-	-	-
28	109.5	-	-	-	116.7	-	-	-	112.5	-	-	-	96.0	-	-	-
30	105.0	Turbid	Irregular, diffuse	4	93.8	Turbid	Irregular, compact	4	100.0	Turbid	Irregular, diffuse, pin flocs	4	88.0	Turbid	Irregular, diffuse	4
33	104.0	Turbid	Irregular, diffuse, pin flocs	2	88.9	Turbid	Irregular, diffuse, pin flocs	3	96.4	Turbid	Irregular, diffuse	4	80.0	Turbid	Irregular, compact	4
35	94.4	Turbid	-	-	91.4	Turbid	-	-	96.6	Turbid	-	-	80.6	-	-	-

Effect of AMD Acclimatization on Toxicity. OUR inhibition tests were conducted using spiked additions of the same synthetic AMD used during acclimatization (Table 1). Results for Reactors B, C, and D indicate that acute toxicity, as indicated by OUR inhibition, was approximately the same for all reactors at Day 15 (Table 5) All reactors then experienced increased inhibition at Day 24 (most evident for Reactor D, the reactor receiving the highest influent AMD concentration). Finally, on Day 36, all reactors were less inhibited by AMD than on Day 15. Adding synthetic AMD to Reactor D on Day 36 made no discernible difference in OUR, indicating that this AS was the most acclimatized to synthetic AMD. Interestingly, the OUR of the control reactor was also less inhibited by AMD on Day 36 than on Day 15. The reason for this is not known.

Table 5. OUR inhibition of acclimatized activated sludge (AS).

Reactor influent load	**OUR_{max} Day 15**	**EC_{50} Day 15**	**OUR_{max} Day 24**	**EC_{50} Day 24**	**OUR_{max} Day 36**	**EC_{50} Day 36**
A: Control	66.3	69.5%	74.5	104.5%	68.8	93.0%
B: 5%	61.2	87.6%	67.7	74.7%	62.0	104.3%
C: 10%	56.9	100.7%	69.9	88.9%	57.7	108.1%
D: 25%	61.7	86.7%	69.6	66.0%	42.2	No inhibition

Metal Removal Studies

Metal removal was assessed after adding spikes of high strength synthetic AMD at three concentrations to settled MWW, AS, and RAS and then removing the suspended solids from solution. Metal removal in a WWTP occurs via different mechanisms in different stages of the treatment process (Buzier et al., 2006; Goldstone et al., 1990a; Oliver and Cosgrove, 1974). During primary sedimentation, insoluble metals, precipitated metals (e.g. hydroxides, carbonates, and orthophosphates), and metals adsorbed onto particulate matter settle and are subsequently removed in the primary sludge. During the biological treatment stage, dissolved metals and metals associated with fine particulate matter are taken up by adsorption or entrapment in the matrix of activated sludge flocs and removed (along with any residual insoluble metals) by settling in the secondary clarifiers. Precipitation and adsorption processes are not treated separately in this discussion; the key result is removal, and the mechanism is not examined further in this report.

For all metals, it was determined by ANOVA that there were no significant differences ($p>0.05$) among removal rates (mg L^{-1}) at 5, 30, 60, and 120 min for settled MWW, AS, or RAS. Removal was observed to be complete within 5 min, and metals were not observed to go back into solution during the 120 min contact period. Rapid (3-10 min) metal uptake by AS has been described previously (Chang et al., 2007; Cheng et al., 1975), and Yuncu et al. (2006) reported that uptake of high concentrations of Cd, Cu, Ni and Zn was almost 85% complete within 5 min of mixing and almost 100% complete within 90 min (initial metal concentration 200-400 mg L^{-1}). Because there were no significant differences among removal rates at different times, the three replicate samples for each of the four time intervals were analyzed together (as a set of 12 replicates) to determine the mean and sd of removal rates (Table 6).

Table 6. Removal efficiency (mg L^{-1}) of settled wastewater (MWW), activated sludge (AS), and return activated sludge (RAS) upon mixing with synthetic AMD (sd: standard deviation).

Metal	Concentration (mg L^{-1})	Removal efficiency (mg L^{-1}) (sd)			
		Settled MWW	AS (MLSS 2 mg L^{-1})	AS (MLSS 4 mg L^{-1})	RAS (MLSS 6 mg L^{-1})
Al	60	40.5 (4.0)	12.4 (4.8)	22.1 (4.6)	28.9 (5.2)
Cu	9	4.1 (0.7)	2.2 (0.7)	3.5 (0.5)	4.2 (0.7)
Fe	162	54.5 (14.8)	11.1 (15.8)	17.2 (15.1)	26.1 (16.7)
Mn	9	4.1 (0.9)	1.4 (0.9)	1.1 (0.8)	2.3 (1.0)
Zn	18	6.8 (1.6)	2.5 (1.5)	2.7 (1.5)	3.8 (1.7)
Al	80	37.8 (3.2)	12.7 (4.7)	21.7 (7.7)	27.1 (4.9)
Cu	12	4.5 (0.6)	2.7 (0.4)	4.0 (0.9)	4.7 (0.3)
Fe	216	57.1 (8.5)	16.8 (12.4)	19.3 (17.9)	20.8 (13.4)
Mn	12	4.4 (0.6)	2.4 (0.5)	1.5 (1.0)	2.3 (0.6)
Zn	24	7.6 (1.1)	3.7 (1.0)	3.5 (2.0)	3.9 (0.9)
Al	100	35.3 (13.5)	16.8 (5.0)	26.9 (6.3)	31.3 (8.2)
Cu	15	5.2 (0.7)	3.5 (0.3)	5.0 (0.6)	5.7 (0.4)
Fe	270	56.8 (32.6)	27.2 (18.8)	23.0 (22.8)	26.9 (18.2)
Mn	15	4.2 (1.6)	3.6 (0.8)	2.1 (0.8)	3.2 (0.9)
Zn	30	7.9 (2.3)	5.8 (1.1)	5.2 (1.3)	4.6 (1.1)

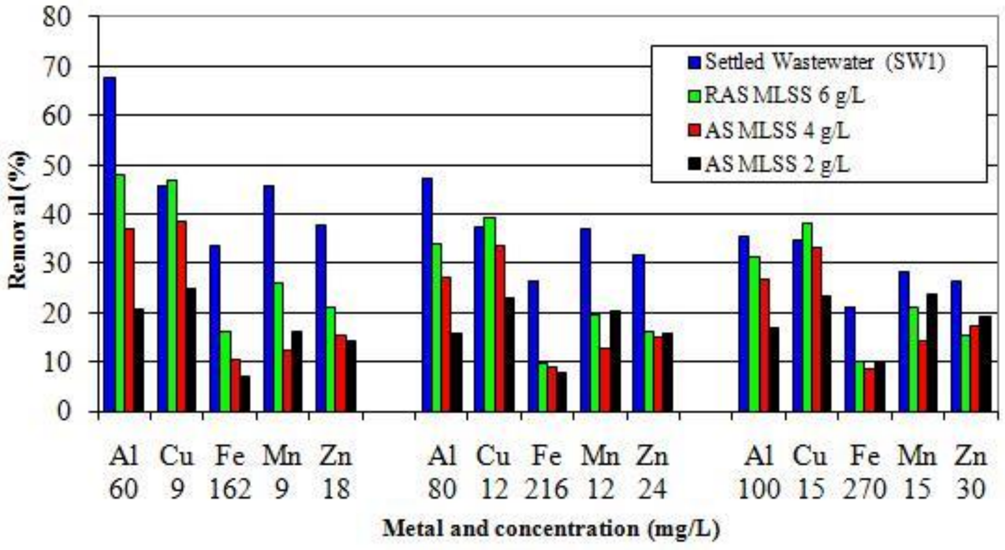

Figure 3. Removal efficiency (%) of settled wastewater (MWW), activated sludge (AS), and return activated sludge (RAS) upon mixing with synthetic AMD.

In most cases, maximum removal was achieved by mixing with settled MWW (SW1) (Table 6, Fig. 3). SW1 had significantly higher removal (mg L^{-1}) than all sludges under all treatments for Al (with the exceptions of AS MLSS 4 g L^{-1} and RAS MLSS 6 g L^{-1} for Dilution 3), Fe (with the exception of AS MLSS 2 g L^{-1} for Dilution 3), Mn (with the exceptions of AS MLSS 2 g L^{-1} and RAS MLSS 6 g L^{-1} for Dilution 3), and Zn (with the exception of AS MLSS 2 g L^{-1} for Dilution 3). For Cu, SW1 had significantly higher removal than AS MLSS 2 g L^{-1} for all three dilutions.

In removal tests using sludge, it was observed that removal (mg L^{-1}) of Al and Cu increased with MLSS. No clear relationship between MLSS and removal was observed for Fe, Mn, or Zn. Metal removal by biomass is to some extent controlled by the availability and affinity of binding sites (Brown and Lester, 1979). Therefore, because an increase in the biomass concentration leads to an increase in the number of available sites for ion exchange, adsorption capacity and overall metal removal are expected to increase with biomass concentration (e.g. Cheng et al., 1975; Özbelge et al., 2005; Stoveland and Lester, 1980; Yang et al., 2010). Increased adsorption occurs up to an optimum point, but at higher concentrations there may be a screening effect between cells, limiting the number of available binding sites, which effectively decreases adsorption capacity (Hammaini et al., 2007).

For Al, Fe, Mn, and Zn, removal (mg L^{-1}) by settled MWW did not change significantly with metal concentration. For Cu, removal by settled MWW increased significantly with metal concentration. For Al and Fe, increasing the metal concentration had no discernible effect on removal by sludge; removal rates did not change significantly for any sludge. Similarly, Santos and Judd (2010) observed no evidence of trends linking influent metal concentration to metal removal in AS systems. In contrast, removal did increase significantly with metal concentration for all sludges for Cu, Mn, and Zn (with the exception of RAS MLSS 6 g L^{-1}).

The differences in removal trends among metals, e.g. variation in removal efficiency with influent metal concentration or biomass concentration, are potential indicators of ways to improve removal efficiency for different metals, and require further investigation. In practice, the removal of metals from WW during treatment using the AS process is primarily controlled by entrapment and settlement of particulate non-settleable metal-containing solids (Brown and Lester, 1979; Santos et al., 2010), and suspended solids removal is therefore of utmost importance (Goldstone et al., 1990a). Particularly at high influent concentrations, it would be expected that significant fractions of metals would be removed from solution by precipitation (Chang et al., 2007; Cheng et al., 1975), in addition to removal via other means. Regardless of the mechanism(s), removal ultimately occurs when the metals settle out of suspension and accumulate in the sludges; therefore, optimization of sludge settling will enhance metal removal efficiency.

Conclusions

As indicated by the toxicity test results, the ability of AS to withstand elevated metal concentrations, such as those found in AMD, is affected by the source of the AS and its history of exposure to different toxic substances. In acute toxicity tests, the OUR inhibition caused by severe AMD was significantly different for municipal and industrial AS, with municipal AS less inhibited. Use of AS from WWTPs treating MWW can ensure that the population of microorganisms has not become adapted to a specific industrial wastewater and is therefore more robust to changes in metal concentrations or pH shocks caused by adding AMD.

Municipal AS was used in an acclimatization study with AMD simulating the major adit drainages at Avoca, SE Ireland. OUR inhibition tests on the acclimatized AS indicated that over a relatively short timescale (approximately 3-4 weeks), the AS microbial community can adapt to AMD sufficiently that shock loads of metals and acidity do not significantly inhibit OUR. SVI values improved in all reactors, and were lowest for Reactor D, the reactor receiving the highest loading of synthetic AMD, at the end of the acclimatization period. This result is significant; the SVI is a measure of sludge settling properties, which are primarily controlled by flocculation and are extremely important to MWW treatment as well as for metal removal (Jin et al., 2003). Turbidity of sludge supernatant became a problem in all reactors after 3 weeks of acclimatization. Turbidity was observed in the control reactor as well as the reactors receiving AMD; thus AMD cannot be assumed to be the sole cause of this change. Filament index values remained constant throughout the acclimatization study, but floc morphology disimproved. To evaluate the impacts of these changes, the effect of AMD loading on removal of BOD, COD, suspended solids and nutrients requires further investigation.

Removal of metals from solution can reduce the bioavailability of metals sufficiently to prevent significant acute toxic effects. In short-term batch tests, significant removal of Fe, Al, Zn, Cu, and Mn by AS and settled MWW was observed, with particularly high removal efficiency for Al and Cu. Further work to minimize soluble species concentrations and to elucidate the removal efficiency achieved using standard operating practices, i.e. sedimentation and settling, is required. Where high-strength AMD is mixed with alkaline material such as MWW and AS the key removal mechanism is most likely to be precipitation and subsequent settling, with adsorption onto organic matter and biological flocs playing an important, albeit secondary role. The importance of precipitation as a metal removal mechanism was demonstrated by the high metal removal rates achieved on mixing AMD with settled MWW.

Another important aspect of co-treatment is the fate of metals in sludge, and the impacts of increased concentrations of metals in the AS in terms of sludge digestion or disposal by spreading on land. Sludge reuse and disposal and the potential for metal recovery from sludge will be considered during laboratory-scale process evaluation studies to compare co-treatment performance under different system configurations.

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