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Factors Affecting the Removal of Metals During Activated Sludge Wastewater Treatment I. The Role of Soluble Ligands

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Abstract. Samples of mixed liquor were withdrawn from a laboratory-scale activated sludge simulation operated at a range of sludge ages from 3 to 12 days in order to examine the difference in metal uptake by the mixed liquor biomass in the absence and presence of soluble ligands. One half of the samples were centrifuged, washed, and resuspended in physiological saline solution, and the rest were left unchanged. In another experiment, aliquots of synthetic sewage were added to mixed liquor samples to investigate the effect of increased soluble influent sewage ligands on metal uptake.

It was found that at low sludge ages, uptake of metals by biomass was lower in the presence of soluble ligands than in their absence, but as the sludge age increased uptake in the presence of soluble ligands also increased. It is suggested that at low sludge ages, ligands predominantly prevented metal uptake by the biomass by chelating the metals and stabilizing them in solution and, at longer sludge ages, ligands predominantly enhanced uptake. Increasing the concentration of synthetic sewage caused a reduction in metal removal and it is suggested that soluble ligands in the synthetic sewage were responsible for preventing metal uptake.

Toxic metals can exist as complexes with inorganic and organic ligands in the aquatic environment (Stumm and Morgan 1970; Schitzer *et al.* 1971). The effect that such ligands have on the form and toxicity of metals has been widely recognized in recent years (Jeffries and Butler 1975; Loveless and Painter 1968; Sprague 1968; Johnston 1964). Considerable work has been undertaken on the speciation of metals in natural waters and the effect that chelation has on the distribution of metals in the aqueous environment and this has recently been reviewed (Florence 1982). Ligands can greatly affect the sorption of metals to particulates in water by promoting (Davis and Leckie 1978; Elliot and Huang 1979; Bourg *et al.* 1979) and preventing (MacNaughton and James 1974; Bourg and Schindler 1978; Inoue and Munemori 1979) sorption. This is important when considering the sorption of metals to sediments in natural waters.

The presence of anthropurgic ligands in industrial and municipal wastewater and the consequential effect of these substances on the removal of metals in wastewater treatment processes has received limited attention (Cheng *et al.* 1975; Neufeld and Herman 1975). They demonstrated that the uptake of metals by the activated sludge biomass is mainly a sorption process and the presence of such ligands may, therefore, have an effect on metal removal by the mixed liquor.

In this study, the uptake of metal by mixed liquor solids in the presence and absence of ligands has been examined in order to assess the importance of metal-ligand interactions on the removal of metals from solution by the biomass.

Materials and Methods

Activated Sludge Simulation

A laboratory-scale activated sludge simulation was used, as described by Stoveland and Lester (1980). Synthetic sewage pre-

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Table 1. Operational parameters of a laboratory-scale activated sludge simulation operated at a range of sludge ages between 3 and 12 days

Sludge age (days)	MLSS[a] mg/L	ESS[b] mg/L	SVI[c] mL/g	COD inf.[d] mg/L	COD eff.[e] mg/L
3	1,401	13.8	276.5	544.0	96.3
4.5	2,358	13.0	275.7	433.3	188.0
6	4,502	19.0	272.3	410.4	98.7
9	2,527	86.3	467.3	521.25	169.5
12	2,809	26.25	281.6	437.7	215.6

[a] Mixed liquor suspended solids
[b] Effluent suspended solids
[c] Sludge volume index
[d] Influent chemical oxygen demand
[e] Effluent chemical oxygen demand

pared according to Sterritt and Lester (1981) was supplied at a nominal influent chemical oxygen demand (COD) of 400 mg/L. The synthetic sewage contained 234 mg/L neutralized Bacterial Peptone (L34, Oxoid Ltd.), 156 mg/L Lab Lemco Meat Extract (L29, Oxoid Ltd.) plus mineral salts. It was prepared as a concentrated stock solution and sterilized at 121°C for 1 hr in 10 L batches. The simulations were operated at sludge ages of 3 to 12 days; a stabilization period was allowed according to criteria suggested by Bisogne and Lawrence (1971). Influent and effluent COD, mixed liquor suspended solids (MLSS) and effluent suspended solids (ESS), were determined according to methods recommended by the Government of Great Britain (1972). Sludge volume index (SVI) was measured by a modified method described by Stoveland and Lester (1980). Dissolved oxygen and pH were consistent and therefore only determined every two to five days. Operational parameters for the simulations are presented in Table 1. Values for MLSS varied between 1,401 and 2,809 mg/L increasing to 4,502 mg/L at $\theta_c = 6$ d. ESS was generally good; SVI values were about 270 mL/g and COD removal was generally good.

Effect of Ligands on Metal Uptake by Biomass

At each sludge age, the following experiments were conducted. A range of five concentrations of metals were added directly to 35 mL samples of mixed liquor at pH 7.4. The mixed liquor was subsequently incubated in a shaking incubator at 17.5 ± 1°C and 100 oscillations per min, for three hr and filtered through 0.2 μm microporous filters (Amicon Ltd., Stonehouse, Gloucestershire) to separate soluble metal prior to determination of soluble and total metal concentrations. Additionally, samples of mixed liquor were washed and resuspended in 0.8% (w/v) NaCl prior to metal additions, to remove soluble organic ligands. Metals were added and the samples incubated as before, in order to assess the uptake of metals in the absence of these ligands.

Metal concentrations were determined by flameless atomic adsorption spectrophotometric techniques (Sterritt and Lester 1980; Kempton *et al.* 1982) using Perkin Elmer models 5000 and 603 atomic absorption spectrophotometers in conjunction with HGA 500 and HGA 400 heated graphite atomizers, respectively.

The potential effect of soluble ligands present in the influent in metal uptake by the mixed liquor solids was examined by adding metals directly to samples of mixed liquor amended with synthetic sewage, to give soluble COD of between 50 and 400 mg/L.

These experiments are presented schematically in Figure 1. The concentrations of added metals are presented in Table 2. Cadmium, Co, Cu, Ni and Tl were added as the nitrate salts and Mn as the sulphate. These metals were chosen for study because they are generally the most soluble in wastewater and would therefore depend more on uptake by the biomass for their removal than on the formation of insoluble salts (Lawson *et al.* 1983; Rossin *et al.* 1982). As the metal solutions were slightly acidic, Tris buffer, pH 7.8 (Perrin and Dempsey 1974) was added to the flasks containing the metal-mixed liquor mixtures so that the final pH throughout the experiment was pH 7.4.

Results

The values of COD for the study on the effect of increased influent ligand concentration is presented in Table 3. The amount of metal associated with the mixed liquor solids (M_B) is obtained from

$$M_B = M_T - M_S \quad (1)$$

where M_T was the total and M_S the soluble metal concentration in the mixed liquor. In order to normalize the concentration to allow comparison of results at different metal concentrations and different sludge ages the metal associated with the biomass was expressed as M_B' or:

$$M_B' = (M_T - M_s)/(M_T \cdot \mathrm{MLSS}) \quad (2)$$

To compare removal of metals by the biomass in the presence and absence of ligands the ratio of M_B' for experiments conducted in the presence and absence of soluble ligands, M_L, was calculated:

$$M_L = (M_B')_L/(M_B')_O \quad (3)$$

where L and O represent the value for M_B' in the presence and absence of ligands respectively.

Also, in order to compare M_B' for metals at increasing COD (*i.e.*, increasing influent ligand concentration), similar ratios, M_I, were calculated at each COD:

$$M_I = (M_B')_c/(M_B')_O \quad (4)$$

were $(M_B')_c$ was the value for uptake of metals at different COD values. M_L and M_I values are presented in Table 4 and Figures 2 to 7, respectively.

If values for either M_L or M_I were >1 the removal of metals from solution by the biomass was greater in the presence of ligands than in their absence; the converse was true if the values were <1. For M_L, there was a general trend for the values for all metals to increase with increasing sludge age. For Cd, values of M_L increased with increasing Cd concentration and most values were <1. At 3 and 6 day

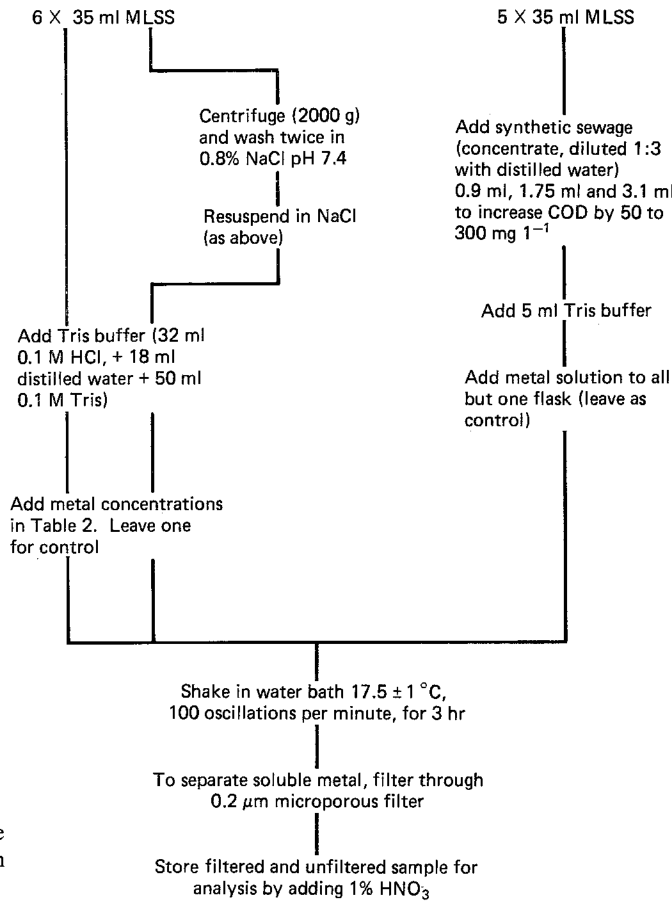

Fig. 1. Schematic representation of experiments on the uptake of metals in the presence and absence of soluble ligands and in the presence of increasing concentrations of influent ligands

Table 2. Metal concentrations added to flasks of mixed liquor in studies on the uptake of metals by the mixed liquor biomass in the presence and absence of ligands

Metal concentration mg/L	Cd	Co	Cu	Mn	Ni	Tl
	0.005	0.005	0.01	0.005	0.005	0.005
	0.01	0.01	0.05	0.01	0.01	0.01
	0.05	0.05	0.1	0.05	0.1	0.05
	0.1	0.1	0.15	0.1	0.15	0.1
	0.15	0.15	0.5	0.15	0.25	0.15

Table 3. Spiked Chemical Oxygen Demand values for the study of the effect of increased COD on the uptake of metals

Sludge age (days)	Chemical oxygen demand mg/L			
3	53	139.3	263.5	488
4.5	120	206.3	330.5	555
6	53	139.5	263.5	488
9	200	286.3	410.5	635
12	178	264.3	388.5	613

sludge ages only, uptake of Cd in the presence of ligands was up to 20% lower than in their absence, but most values, particularly at longer sludge ages showed that Cd uptake was increased by between 10 and 60% of the uptake in the absence of ligands. Values of M_L for Ni also exhibited a tendency to increase with increasing metal concentration, but were only >1 for higher Ni concentration at longer sludge ages; all other values were <1. The results varied widely for Ni, but on the whole uptake decreased in the presence of ligands by approximately 40%; at the higher concentrations at longer sludge ages uptake increased from 4 to 55% in the presence of ligands. For Tl, the values decreased with increasing Tl concentration and were >1 at most sludge ages. The overall apparent trend was that uptake of Tl increased in the presence of ligands by values ranging over 100%, but the results varied widely and in some cases uptakes by the mixed liquor biomass decreased as much as 40%. Manganese M_L values also decreased with increasing metal concentration, but the values were >1 only at longer sludge ages. At short sludge ages (3 and 4.5 days), the presence of soluble ligands decreased Mn uptake by 35 to 55% and at 6 days and higher

Table 4. Ratio of the uptake of metals by the mixed liquor biomass in the presence and absence of ligands (M_L) at five sludge ages

Metal sludge (days)	Cd M_L values	Co	Cu	Mn	Ni	Tl
3	1.19	0.68	0.71	0.45	0.00	0.61
	1.31	0.45	0.73	0.91	1.76	2.19
	0.81	0.63	0.76	0.64	0.696	0.42
	0.82	0.74	0.76	0.57	0.62	0.75
	0.89	0.56	0.80	0.58	0.76	2.06
4.5	1.51	3.56	1.01	1.01	1.01	13.13
	1.02	1.52	1.01	1.01	0.91	1.09
	0.12	0.84	1.01	0.96	2.45	1.40
	1.31	0.74	1.01	0.73	2.75	0.88
	1.54	0.80	1.01	0.85	0.699	0.93
6	0.51	2.96	1.00	0.73	0.99	0.62
	0.99	1.86	1.00	0.99	0.85	0.91
	1.13	0.82	1.00	0.99	0.94	1.51
	1.06	0.82	1.04	0.84	1.13	1.61
	1.29	0.62	1.03	0.85	1.40	0.68
9	1.69	1.20	1.17	1.20	0.00	1.81
	2.13	1.39	0.68	1.20	0.62	0.67
	1.21	0.97	0.60	0.74	1.27	0.87
	1.14	0.97	1.19	0.74	0.95	0.85
	1.30	0.98	1.29	0.62	1.04	1.01
12	1.44	3.08	1.52	1.54	0.42	0.00
	1.51	2.10	1.49	1.54	0.35	1.23
	1.49	1.21	1.33	1.43	0.86	1.56
	2.36	1.22	1.22	1.19	1.13	5.70
	1.80	0.97	1.52	0.997	1.13	3.92

concentrations at 9 days by up to 27%. At 12 days, sludge age uptake of Mn increased in the presence of ligands by up to 55% at low concentrations but declined sharply with increasing Mn concentration. Cobalt values also decreased with increasing metal concentration and nearly all values were <1. The uptake of Cd decreased in the presence of ligands by up to 55%; only at low concentrations of Co at 9 and 12 days sludge ages was Co uptake increased in the presence of ligands (by up to 20%). Copper M_L values did not change with increasing Cu concentrations; at sludge ages of 4.5 days and longer the values were >1 and uptake of Cu increased in the presence of ligands by up to 55%, with lower increases at short sludge ages, rising sharply with sludge age.

In summary, all metals exhibited increasing values for M_L as the sludge age increased. Cadmium, Cu, and Tl had most values >1 (*i.e.*, uptake of these metals by the mixed liquor biomass increased in the presence of ligands) while for Mn and Ni values only increased above 1 at longer sludge

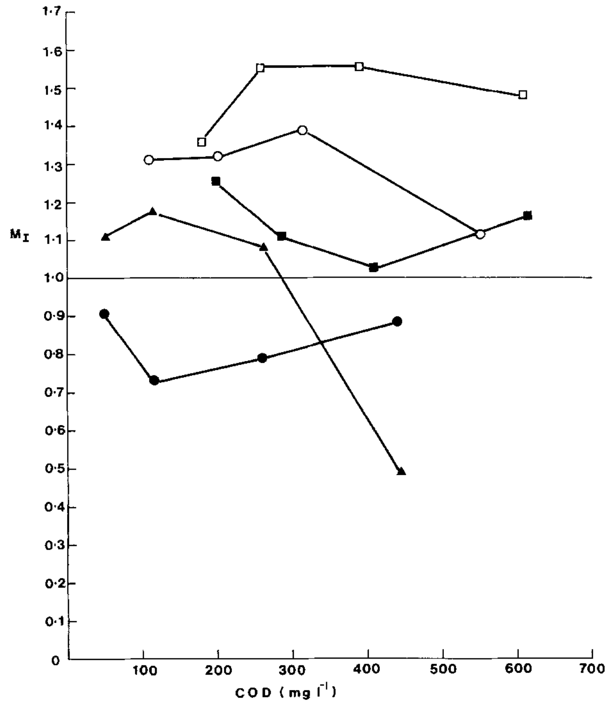

Fig. 2. Ratio of the uptake of Cd by the mixed liquor biomass in the presence and absence of ligands (M_I) against increasing COD at a range of sludge ages: θ_c = 3 d ●, θ_c = 4.5 d ○, θ_c = 6 d ▲, θ_c = 9 d ■, θ_c = 12 d □

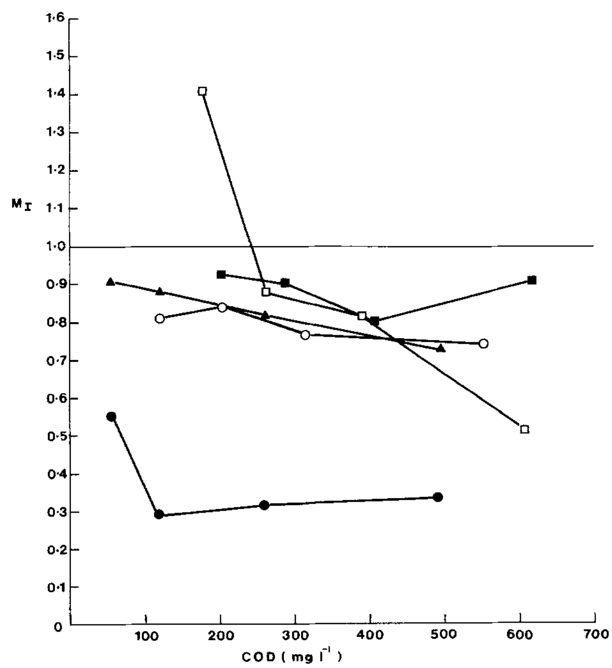

Fig. 3. Ratio of the uptake of Co by the mixed liquor biomass in the presence and absence of ligands (M_I) against increasing COD at a range of sludge ages: θ_c = 3 d ●, θ_c = 4.5 d ○, θ_c = 6 d ▲, θ_c = 9 d ■, θ_c = 12 d □

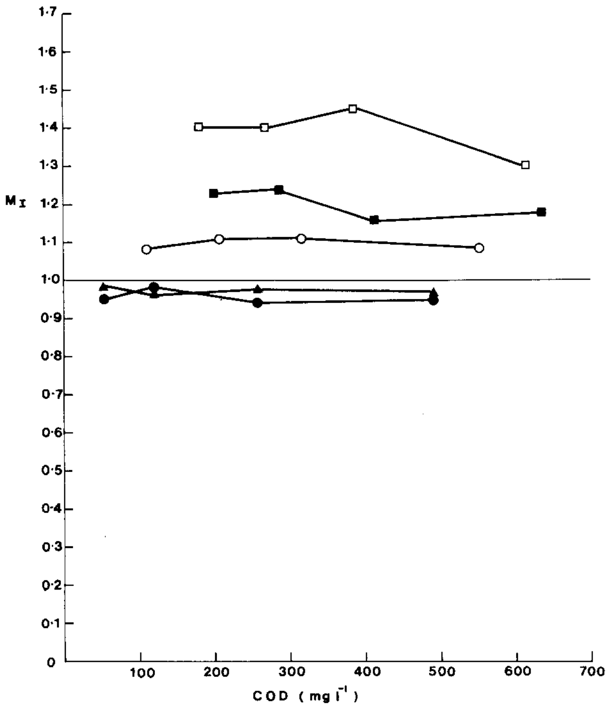

Fig. 4. Ratio of the uptake of Cu by the mixed liquor biomass in the presence and absence of ligands (M_I) aginst increasing COD at a range of sludge ages: θ_c = 3 d ●, θ_c = 4.6 d ○, θ_c = 6 d ▲, θ_c = 9 d ■, θ_c = 12 d □

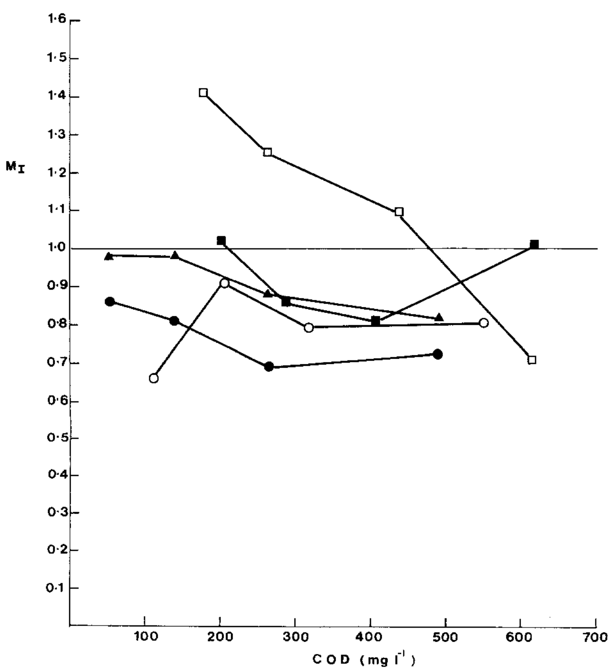

Fig. 5. Ratio of the uptake of Mn by the mixed liquor biomass in the presence and absence of ligands (M_I) against increasing COD at a range of sludge ages: θ_c = 3 d ●, θ_c = 4.5 d ○, θ_c = 6 d ▲, θ_c = 9 d ■, θ_c = 12 d □

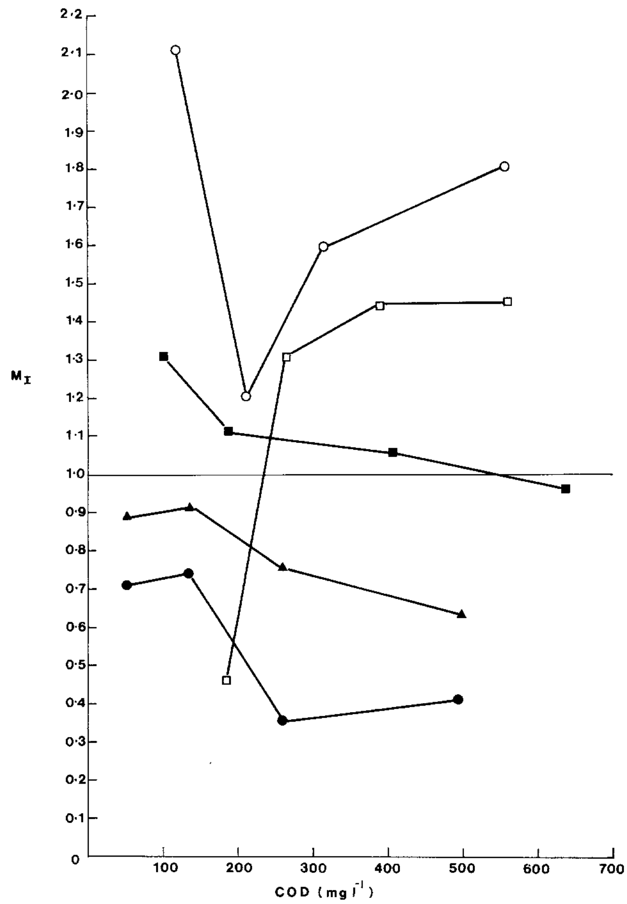

Fig. 6. Ratio of the uptake of Ni by the mixed liquor biomass in the presence and absence of ligands (M_I) against increasing COD at a range of sludge ages: θ_c = 3 d ●, θ_c = 4.5 d ○, θ_c = 6 d ▲, θ_c = 9 d ■, θ_c = 12 d □

ages. On the whole, at lower sludge ages uptake decreased in the presence of ligands. For Co, the majority of values of M_L were <1 (*i.e.,* uptake of Co by the mixed liquor biomass decreased in the presence of ligands).

During the experiments to determine the effect of increasing COD on metal uptake, the added metal concentrations were kept constant at 0.05 mg/L for Cd, Co, Mn and T1 and 0.1 mg/L for Cu and Ni.

From the values for M_I, indicating the effect of increased soluble COD, presented in Figures 2 to 7, two general trends are apparent. The values for M_I generally increased with increasing sludge age and, except for Cu, they decreased with inceasing COD (or concentration of influent ligands); Cu values showed a linear relationship with COD, indicating that influent ligands may not have influenced Cu significantly. Values for Co were generally less than 1; values for Mn and Ni were <1 at low sludge ages only; Cd values were <1 at low sludge

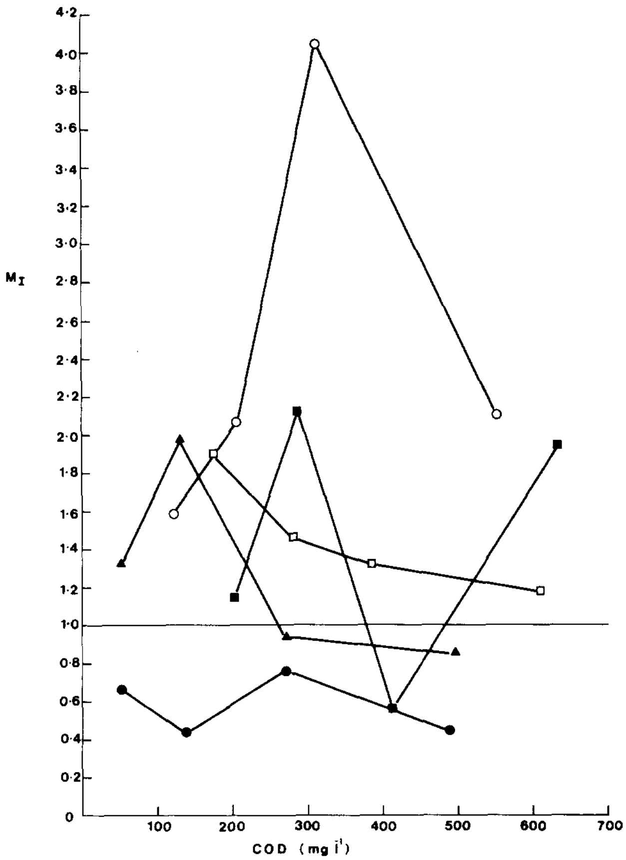

Fig. 7. Ratio of the uptake of Tl by the mixed liquor biomass in the presence and absence of ligands (M_I) against increasing COD at a range of sludge ages: θ_c = 3 d ●, θ_c = 4.5 d ○, θ_c = 6 d ▲, θ_c = 9 d ■, θ_c = 12 d □

ages and high CODs only and Cu and Tl values were <1 at the 3 days sludge age only.

A bulking sludge developed at a sludge age of 7.5 days and experiments were carried out on this sludge to examine the effect of sludge dispersion on M_L and M_I. The MLSS was 1,751 mg/L, the ESS 112.5 mg/L, the SVI 724 mL/g and percentage COD removal efficiency only 22.6%. Values for M_L and M_I for this sludge are presented in Table 5. M_L showed no similarity with non-bulking sludge for Cd, Co, Ni and Tl. Copper and Mn values were higher than would be expected for a nonbulking sludge at 7.5 days, *i.e.*, uptake increased by more than would be expected at a 7.5 days sludge age. For Co, M_L values did not change with increasing Co concentration. For Tl, there was no trend with increasing Co concentration, but for all other metals, the values for M_L decreased with increasing metal concentration. This differed from trends in non-bulking sludges where only values of M_L for Co, Mn and Tl decreased with increasing concentration. Cobalt and Tl values were predominantly <1; all other metals demonstrated values >1, which

Table 5. Ratio of the uptake of metals by mixed liquor biomass in the presence and absence of ligands (M_L) and at increasing chemical oxygen demands (M_I) for bulking sludge

	Cd	Cu	Co	Mn	Ni	Tl
	2.06	1.14	0.79	1.19	1.30	1.81
	2.19	1.17	0.79	1.19	1.50	0.67
M_L	1.14	1.23	0.75	1.16	0.62	0.87
	1.05	1.18	0.78	1.04	3.30	0.85
	1.18	1.08	0.996	1.06	0.69	1.01
	0.19	1.05	0.72	1.07	0.72	0.68
	0.97	1.08	0.73	1.02	0.56	1.17
M_I	1.06	1.08	0.75	1.03	0.52	0.84
	0.79	1.05	0.69	0.81	0.32	0.88

would be typical of a non-bulking sludge at 7.5 d sludge age. M_I values were typically low in comparison to non-bulking sludges. Nickel and Mn values decreased with increasing COD, in common with values for non-bulking sludge, but no trends were apparent for the other metals. M_I was >1 for Cu and Mn only, which contrasts with non-bulking sludge where all metals except Co would be expected to show values >1 at a 7.5 days sludge age; in this case all other metals demonstrated values <1.

Values for M_L and M_I were lower than would be expected for a nonbulking sludge at 7.5 days sludge age. These results suggest that a more dispersed sludge removed less metal than a well-settled sludge in the presence of ligands.

Discussion

The trend for both M_L and M_I values to increase with sludge age indicated that at longer cell retention times an increasing quantity of metal was removed from solution in the presence of soluble ligands. This suggests that the ligands were in some way promoting the removal of metals from solution by the biomass, perhaps due to the degradation of compounds originally present in the influent which could have prevented uptake, or to products of the biomass which would have been present in greater quantity at longer sludge ages, due to the longer cell retention times. These microbial products may have affected metal removal efficiency by complexing metals and then becoming associated with the mixed liquor solids. At longer cell retention times, the concentration of such ligands may have increased to levels high enough to compete with ligands present in the influent and thus have an effect on the overall metal removal properties of the biomass. From values for M_I, it would appear that metal uptake by the mixed liquor solids decreased in the presence of increasing influent ligands concentration, which suggests that ligands originating in the synthetic sewage prevented removal of metals from solution by the biomass. The soluble ligands present in synthetic sewage may have complexed metals, stabilizing them in solution so that they were unavailable for uptake by the mixed liquor solids. The complexation of metals to soluble high molecular weight organics has been demonstrated in the influent and effluent of the activated sludge process (Rossin *et al.* 1982; Sterritt and Lester 1982; Lawson *et al.* 1983).

There was an overall trend for metal removal by the biomass to decrease with increased metal concentration. This indicated a tendency towards saturation of the available binding sites. However, both Cd and Ni are exceptions to this trend, demonstrating increased uptake as concentration increased at most sludge ages. For these metals, an unsaturated ligand-metal complex system may have existed, which enhanced uptake of the metals by the biomass. M_I values for both Cd and Ni were <1 and, therefore, soluble ligands in the influent were probably able to compete successfully for the two metals when they were present in sufficient quantity.

Apparently, the system under study was very complex. A number of soluble ligands competed for the available metals forming complexes which affected the removal of metals from solution by the biomass in different ways. From studies on metal speciation in aqueous solution (Neubecker and Allen 1983; Van den Berg and Kramer 1979), it has been shown that metal complexation is dependent on the stability constants of the metal-ligand complexes concerned. Ligands compete for metals in solution and those which form the most stable complex will preferentially complex, until their binding sites are saturated, at which point other weaker ligands will complex the metal. As there were no sharp changes in the values of M_L and M_I, each metal may not have preferentially formed complexes with one ligand suggesting that the matrix was very complex with respect to its metal binding characteristics.

Generally, it has been considered that the toxic form of a metal is the free ionic form (Florence 1982; Allen *et al.* 1980). The results presented here, however, indicate that soluble ligands may enhance or prevent uptake of metals by the mixed liquor. Although soluble ligands prevent uptake of metals by the biomass in many studies (Pickett and Dean 1979; Jeffries and Butler 1975; Loveless and Painter 1968; Tynecka *et al.* 1975), few authors have indicated that ligands increase uptake. Sterritt and Lester (1980) found that Cd uptake by a *Pseudomonas* species were enhanced by the presence of citrate in the medium, but not acetate or glucose. Gadd and Mowill (1983) demonstrated that Cd uptake by *Saccharomyces cerevisiae* was dependent on the presence of glucose. Guthrie *et al.* (1977) suggested that bacteria which concentrate metals from their environment may form complexes or adsorb metals onto polymers synthesized by the cell, to detoxify the metals and withstand elevated concentrations in their environment.

Apparently, from the results of this study, many ligands present in the influent synthetic sewage probably complexed metal, preventing their uptake by the biomass. However, it is also apparent that a proportion of soluble ligands, increasing in concentration as the cell retention time lengthened, actually aided uptake by the biomass. These ligands may have been products of bacterial metabolism, released into solution by lysis, or ligands which were originally synthesized by the cells to complex metals and acted as a detoxification mechanism. As the dispersed mixed liquor of the bulking sludge exhibited less uptake in the presence of ligands than a well-settled mixed liquor, the ligands which aid uptake may be exocelluar polymers produced by the cells which aid in the formation of activated sludge flocs characteristic of a well-compacted sludge (Friedman and Dugan 1968). These polymers may have been released from the cells in sufficient quantity at longer sludge ages and affected the uptake of metals by the biomass.

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