The effect of nutrients on extracellular polymeric substance production and its influence on sludge properties

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Abstract

The effect of nutrients on extracellular polymeric substance (EPS) production and its impact on sludge properties and removal efficiencies were investigated in an in-depth field survey of wastewater treatment plants. Thereafter, laboratory studies were performed to evaluate the effect of a combination of nutrients - nitrogen and phosphorus and operational conditions on EPS production, and sludge settling and dewatering characteristics.

Multiple regression analysis was performed to assess the effect of variables in nutrient operational conditions on the EPS production and sludge properties. The field survey revealed that although filamentous micro-organisms were found in most of the sludge samples, they did not always cause sludge bulking. Further, it was observed that EPS production was lower in anaerobic than in aerobic processes. An evaluation of the effect of the deficiency and excess of nitrogen and phosphorus was conducted in batch experiments on synthetic wastewater with glucose as the carbon source. The study revealed that the EPS components, namely proteins and carbohydrates had a more profound effect on sludge properties compared to total EPS, with protein being more significant than carbohydrate. Both nitrogen deficiency (COD: N < 100:2) and nitrogen excess (COD: N > 100:10) improved the sludge properties. The optimum phosphorus ratio determined was COD:P, ranging from 100:3 to 100:5, at which sludge properties in terms of settling, dewatering and the final clarification improved.

Keywords: extracellular polymeric substance, activated sludge process, sludge properties, bio-flocculation

Introduction

In wastewater treatment systems, the biological treatment process is one of the most important and popular systems used for domestic and industrial wastewater treatment. Among the numerous available methods, the activated sludge process is one of the major biological wastewater treatment techniques. This process consists of two units: a bioreactor where organic waste is digested by micro-organisms, and a sedimentation basin where activated sludge is separated from the treated effluent. In the first phase, the active mass of micro-organisms in the aerated bioreactor convert the suspended and colloidal organic material to end-products such as carbon-dioxide, water and inert material. This is the carbon source utilisation phase. The second phase is the flocculation of the micro-organisms and other suspended or colloidal components into rapidly settleable biomass. Thus a clear, low biochemical oxygen demand (BOD) effluent can be obtained, with this phase playing an important role in the production of high-quality effluent. Biological aggregation provides a convenient and effective method for separation biological flocs from the mixed liquor medium, after they have fulfilled their metabolic role. Flocculation of biomass is responsible for changes in supernatant turbidity and variation in settling and dewatering properties. Therefore, the overall function of the activated sludge process depends largely on good flocculation and on the sedimentation behaviour of the sludge.

A detailed field survey conducted by Urbain et al. (1993) reveals that about 25% of the activated sludge treatment units have settling problems with the Sludge Volume Index - SVI > 150 or 200 mℓ/g. Poor settling of activated sludge results in the discharge of suspended solids into the receiving water, which is due to operational problems caused by the deflocculating biomass in the sedimentation basin. This deflocculation is due to lack of natural extracellular polymeric substance (EPS) flocculants within the biomass (Sheintuch et al., 1986).

EPSs produced by bacteria play an important role in controlling the flocculation and floc properties, including settling and dewatering (Bura et al., 1998). EPSs are macromolecular compounds that are found in the intercellular spaces of microbial aggregates. They originate from micro-organisms (excretion and lysis) and wastewater (biosorption). EPSs were identified as the major components of the activated sludge floc matrix. The mechanism of the biological flocculation is interpreted as a result of the interaction of those polymers that have sufficiently accumulated at the microbial surface during endogenous growth. The EPSs present a dominant bridging mechanism between the floc components, namely cellular, bio-organic, and inorganic compounds.

By controlling EPS production, the settling and dewatering of biomass can be improved. Various operational conditions can affect EPS production, such as nutrient concentration, sludge retention time, pH, the ratio of food/micro-organism (F/M) and hydraulic retention time. Controlling the nutrients of the feed wastewater has been identified as one of the methods of controlling EPS. The nature and concentration of nutrients affect the biodegradation of organic waste (Bura et al., 1998). Nutrients are necessary components for the growth of bacteria as well as to stimulate the production of surface biopolymer EPSs, which play a part in settling sludge. Until now, there have been only a few studies on the effects of nutrient balance (COD: N: P) on EPS production and composition, and settling and dewatering characteristics of sludge.

By optimising the nutrient ratio, EPS can be controlled. Thus, the efficiency of the secondary treatment process can be improved. This study focuses on the identification of the nutrient effect on EPS production and its significance on sludge properties in activated sludge processes.

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TABLE 1
Feed wastewater characteristics of the WWTP in the field survey

Industry	Wastewater treatment process	COD (mg/ℓ)	BOD (mg/ℓ)	COD/N	COD/P	BOD/COD
Domestic 1	Activated sludge	192.0	82	17.1	14.6	0.43
Domestic 2	Activated sludge	110.8	46	6.8	16.5	0.42
Shopping centre	Activated sludge	452.3	280	18.2	23.2	0.62
Hotel	Activated sludge	573.1	254	32.0	42.7	0.44
Hospital waste	Oxidation ditch	71.6	26	8.5	6.9	0.36
Abattoir	Activated sludge	1550.8	760	55.4	51.0	0.49
Electronic	Activated sludge	125.0	62	111.6	8.5	0.50
Pulp and paper 1	Activated sludge	1447.1	525	344.5	87.7	0.36
Pulp and paper 2	Activated sludge	1376.5	475	204.8	15.1	0.35
Brewery 1	Activated sludge	143	64	5.8	3.5	0.45
Brewery 1	UASB	716.4	387	21.3	17.5	0.54
Brewery 2	Activated sludge	1288.0	465	460.0	6.8	0.36
Soft drink 1	Activated sludge	1058.8	475	∞	56.6	0.45
Soft drink 1	UASB	411.8	46	6.8	16.5	0.42
Soft drink 2	Activated sludge	400.0	152	95.2	6.2	0.38

Materials and method

The study was undertaken in two phases: A field survey and laboratory investigations.

Phase I: Field survey

In the field survey phase, properties of biological sludge and wastewater characteristics from 15 wastewater treatment plants (WWTP) were sampled and analysed. In order to avoid any possible climatic influences, all the samplings were carried-out in a two week period. A correlation between EPS, its contributing factors and sludge properties (Capillary Suction Time - CST, SVI) was evaluated by statistical analysis. Grab samples were obtained from the influent to the aeration tank, mixed liquor of activated sludge and effluent after the aeration tank. Samples were also collected from two anaerobic wastewater treatment processes to compare the EPS properties with aerobic processes. The characteristics of the feed wastewater of the field-survey plants are represented in Table 1.

TABLE 2
Variations of COD:N:P ratio in the experimental study

COD:N / COD:P	100:1	100:2	100:3	100:5	100:7	100:10	100:12	100:15
100:0.5	*	*	*	*	*	*	*	*
100:1	*	*	*	*	*	*	*	*
100:2			*			*	*	*
100:3			*					
100:5			*					
100:7			*					
100:10			*					

Phase II: Laboratory-scale investigations

The effect of nutrients on EPS production was evaluated in sequencing batch reactors (SBR) using a synthetic wastewater (Chao and Keinath, 1979) comprising glucose as the carbon source, with NH_4Cl and K_2HPO_4 as nitrogen and phosphorus source, respectively. The pH of the wastewater was maintained between 6.8 and 7.5 (using either H_2SO_4 - 0.1 N or NaOH - 0.1 N), which is optimum for bacterial growth.

Based on the settling and dewatering characteristics of sludge, the optimum COD:N:P value was investigated in a parametric study conducted in 2-l batch reactors. Twenty-six reactors were operated with a sludge retention time (SRT) of 10 d, HRT of 6 h and COD influent of 800 mg/ℓ at different nutrient ratios (COD:N:P). DO concentration of the mixed liquor was maintained at a minimum of 2 mg O_2/ℓ. Sludge concentrations within the reactor were maintained at 6 to 7 g/ℓ and a F/M ratio of 0.4 to 0.6 g COD/g MLSS·d. All reactors were operated for the full three retention periods (30 d) necessary for sludge to be fully equilibrated (Forster and Newton, 1980). Table 2 summarises the variation of COD:N:P ratio used in the parametric study. Here, at each run nitrogen and phosphorous were fed with concentrations corresponding to COD:N:P ratio as presented in this table.

The operation sequence of the sequencing batch reactors consists of 5 min fill, 6 h reaction, 5 min withdrawal, 5 h settling, and finally a 5 min effluent withdrawal, resulting in a total cycle time of 11 h and 15 min.

Sludge characterisation study

The sludge was sampled when each batch reached steady-state (COD removal was above 80% while MLSS remained stable). The sludge was examined for EPS content, dewatering property in terms of capillary suction time (CST) and sludge settleability (SVI).

Statistical analysis

Statistics SPSS software was used to analyse the correlation between different variables in the feed wastewater by means of multiple regression analysis (Box et al., 1978 as cited in Urbain et al., 1993; Sponza, 2002). The linear correlation was assessed with R^2 Durbin-Watson statistics. R^2 represents the regression coeffi-

TABLE 3
Qualitative analysis of sludge characteristics and the presence of filamentous micro-organisms

Industry	Wastewater treatment process	EPS (mg/g)	SVI (mℓ/g)	CST (s/g SS)	MLSS (mg/ℓ)	SS (mg/ℓ)	Filament index[1]	pH
Domestic 1	Activated sludge	12.9	75.0	0.8	11 960	10	1	6.8
Domestic 2	Activated sludge	69.7	382.8	7.4	2 090	20	3	7.4
Shopping centre	Activated sludge	57.0	190.8	12.5	3 250	30	2	7.1
Hotel	Activated sludge	70.1	192.0	8.3	2 060	50	3	6.2
Hospital waste	Oxidation ditch	30.0	51.3	1.8	3 900	40	4	7.3
Abattoir	Activated sludge	18.5	79.1	3.6	9 200	20	2	7.0
Electronic	Activated sludge	32.8	173.6	4.7	2 420	10	3	7.1
Pulp and paper 1	Activated sludge	27.0	88.5	18.7	520	70	4	5.9
Pulp and paper 2	Activated sludge	7.0	339.6	16.1	2 120	50	2	6.5
Brewery 1	Activated sludge	11.2	163.6	4.1	4 340	50	2	7.1
Brewery 1	UASB	3.7	-	2.7	27 540	60	2	5.9
Brewery 2	Activated sludge	52.9	192.3	2.6	4 420	10	5	6.5
Soft drink 1	Activated sludge	93.9	455.6	6.3	1 800	40	3	7.0
Soft drink 1	UASB	5.2	277.0	4.5	2 744	10	2	6.3
Soft drink 2	Activated sludge	60.0	291.7	2.9	2 400	60	4	7.1

[1] Filament index in the range of 0 to 5 (Not present to proliferation).

cient and reflects statistical significance between dependent (SVI, CST, effluent turbidity, EPS) and independent (protein, carbohydrate, nitrogen, phosphorus) variables by an analysis of variance (F-test) statistics (a = 0.05). With the exception of data from settling (mℓ/g SS) and dewatering tests (s/g SS), care had been taken to use absolute units, i.e. mass per litre effluent, mass per g SS of sludge, to avoid autocorrelations.

Analytical methods

All analyses for both effluent and sludge were conducted as prescribed in *Standard Methods* (1995). EPS was extracted by two methods: glutaraldehyde (Azeredo et al., 1998) and thermal extraction (Morgan et al., 1990). The EPS composition in terms of carbohydrates and proteins was measured by the phenol sulphuric method with glucose as a standard (Dubois et al., 1956) and Biuret method (Rodney, 1993), respectively.

Results and discussion

Phase I: Field Survey

EPS in bio-flocculation and dewatering: An analytical approach

Based on the field survey, the composition of the wastewater (Table 1) extracted from various sources exhibited a large variation in BOD_5/COD ratio ranging from 0.35 to 0.62. COD/N was in the range from 5.8 to 460 indicating that wastewater was both deficient and rich in nitrogen. In all cases, phosphorus was in excess, with COD/P ranging from 3.5 to 87.7. Thus, from the field survey, a nitrogen deficiency and excess could be evaluated whilst only phosphorus excess could be interpreted.

An evaluation of the sludge properties of the field-survey as represented in Table 3 revealed that filamentous bacteria were present in all surveyed samples. From the study, sludge with a filamentous index of 1 displayed good settling as represented by a SVI of 75 mℓ/g. Here, it was also noted that some treatment plant sludge with a filamentous index of 2, which is an indication of poor proliferation of filamentous bacteria, displayed poor settling characteristics with SVI > 100 mℓ/g. These field results demonstrate that a direct correlation between the filamentous organisms and sludge bulking does not always exist. This indicates the need for a in-depth biological analysis in terms of EPS, carbohydrates and proteins.

Comparison of EPS in aerobic and anaerobic process

A comparison of aerobic and anaerobic systems in Table 3 indicated that EPS from the same wastewater source differed and was found to be lower in anaerobic than aerobic processes. The anaerobic process of the brewery and soft drink industries yielded EPSs of 3.7 and 5.2 mg/g, while the corresponding EPSs for the aerobic processes were 11.2 and 93.9 mg/g, respectively. A possible explanation for this as proposed by Morgan et al. (1990) is that anaerobic sludge is dominated by methanogenic bacteria that do not possess peptidoglycan, an essential component of which undecaprenyl phosphate is the lipid carrier. It is believed that this lipid carrier is instrumental in the release of exo-polysaccharides; hence limited in anaerobic processes. Further, the difference in the yield of the anaerobic process (Yield coefficient -Y = 0.05) is significantly lower than that of the aerobic process (Y = 0.5), therefore EPS produced in anaerobic processes can be degraded and thus serve as an energy source.

Multi-correlation among EPS, sludge properties and plant operation conditions

The effects of EPS on sludge properties were investigated by multi-correlation, taking into consideration the effects of operational conditions and wastewater characteristics. Table 4 summarises the results of the statistical analysis which revealed that sludge samples varied from normal sludge to bulking and difficult-to-dewater sludge. SVI and CST varied in the range of 51.3 to 455.6 mℓ/g and 0.8 to 18.7 s/g SS, respectively.

A positive correlation between EPS and SVI was evident and in agreement with Urbain et al. (1993), Forster (1971), and Chao

TABLE 4
Linear coefficients of the correlation statistically significant at a 0.95 probability level (α = 5 %), i.e. $r \geq 0.5$

Variables	SVI	CST	SS	EPS	MLSS	COD/N	COD/P	BOD/COD	pH	FI[†]
SVI	1.00	-	-	-	-	-	-	-	-	-
CST	0.22	1.000	-	-	-	-	-	-	-	-
SS	0.327	0.450	1.00	-	-	-	-	-	-	-
EPS	0.580*	0.051	0.022	1.000	-	-	-	-	-	-
MLSS	-0.618*	-0.421	0.017	-0.475	1.000	-	-	-	-	-
$^{COD}/_{N}$	-0.004	0.356	0.115	-0.009	-0.259	1.000	-	-	-	-
$^{COD}/_{P}$	-0.016	0.533*	0.298	-0.155	-0.159	0.326	1.000	-	-	-
$^{BOD}/_{COD}$	0.128	-0.437	-0.194	0.132	0.349	-0.559*	-0.238	1.000	-	-
pH	0-.060	-0.346	-0.398	0.340	-0.319	-0.483	-0.598*	0.244	1.000	-
FI	0.032	0.036	0.176	0.479	-0.400	0.611	-0.021	-0.275	0.005	1.000

* Correlation is significant at the 0.05 level (2-tailed).

[†] FI: Filamentous Index

and Keinath (1979). However, total EPS could not be related to CST and SS.

The result showed that all independent variables did not have correlation with EPS, except for MLSS and filamentous index (FI). While the filamentous index had a reasonably good correlation with EPS, it did not have a strong correlation with sludge property parameters such as SVI, CST and SS. There was some correlation between EPS, BOD/COD, MLSS, pH and COD; however this was not strong.

Thereafter, multiple-regression analysis (a step-wise method) was used to investigate the effects of these factors on EPS. An analysis of the results did not provide any further inter-relationships between these complex operational parameters to the microbial environment.

Effect of nutrient on EPS production and sludge properties

From the field survey of the wastewater plants no direct correlation between the nutrients-nitrogen and phosphorus, EPS production and sludge properties could be obtained. It was realised that the results obtained were inconclusive since various wastewaters under different operating conditions were evaluated and EPS alone could not explain the difference in sludge properties. A possible explanation for this could be the absence of an appropriate analytical technique for measuring EPS. When the glutaraldehyde method was used for extraction, it accounted for less than 8% of the sludge dry mass. Another limitation of this extraction method was that the protein and carbohydrate components of EPS in glutaraldehyde solvent could not be measured directly due to the contamination of the glutaraldehyde solvent. In addition, precipitated EPS could not be dissolved completely in distilled water. Beccari et al. (1980) and Forster and Clark (1983) reported that even with a drastic extraction method procedure such as heat treatment, EPS accounted for less than 14% of the sludge dry mass. Thus, there is a need to establish a standardised EPS measurement technique to evaluate the effect of EPS components on the other parameters.

The analytical limitations in measuring EPS and the absence of correlation between EPS and sludge properties prompted the need to evaluate the EPS carbohydrate and protein components and the effect of nutrients on these. This study required a controlled operational environment and was thus undertaken as a parametric study in laboratory-scale reactors.

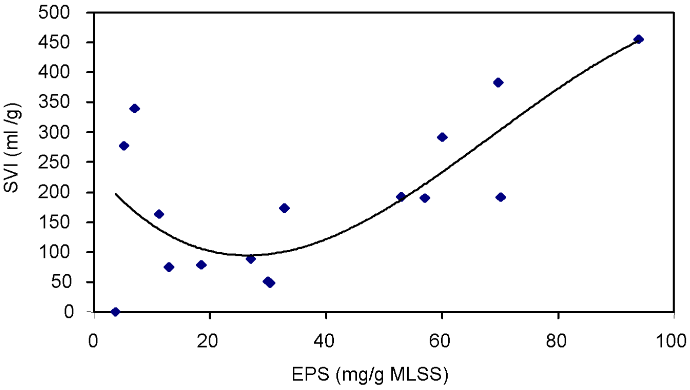

Figure1
Effect of EPS production on SVI value

Phase II: Laboratory Investigations

Influence of nutrients on EPS composition

The effect of EPS production and its components was evaluated in a parametric study by varying nutrient ratios of nitrogen and phosphorus. The results are presented in Table 5. From the results, total EPS varied between 24.4 and 89.9 mg/g SS with protein contributing between 2 to 15%, while the carbohydrate component comprised 16 to 94% of total EPS.

The difference between EPS and its components with different nitrogen and phosphorus concentrations was correlated by F-test statistical analysis. The results revealed that protein, carbohydrate and total EPS differ significantly with a variation in nitrogen concentration (EPS: protein: carbohydrate F = 5.747:14.142:4.912; df = 7). However, this ratio was unaffected by different phosphorus content (EPS: protein: carbohydrate F = 1.797:0.697:0.785; df = 2). When using the step-wise method, it was found that protein was inversely proportional to nitrogen, while unaffected by phosphorus. Whilst both nitrogen and phosphorus were inversely proportional to carbohydrate content, phosphorus had a more pronounced affect on carbohydrate than nitrogen. Protein and carbohydrate content was found to be directly proportional to the EPS content.

TABLE 5
EPS production, its components and sludge properties under different nutrient conditions

COD:N:P	N*(mg/ℓ)	P*(mg/ℓ)	EPS (mg/g SS)	Protein (mg/g SS)	Carbo-hydrate (mg/g SS)	SVI (mℓ/g)	CST (s/g SS)	Turbidity (NTU)
100:0:0	0	0	38.44	1.47	9.42	57.3	16.02	25.0
100:1:0.5	8	4	59.70	8.56	43.80	39.1	1.24	8.8
100:2:0.5	16	4	38.70	5.65	44.10	46.1	1.38	13.0
100:3:0.5	24	4	47.40	5.41	29.50	75.9	5.66	27.5
100:5:0.5	40	4	54.90	5.48	32.30	66.2	5.76	35.0
100:7:0.5	56	4	67.40	9.40	47.70	50.2	10.33	27.0
100:10:0.5	80	4	24.40	1.67	15.70	32.8	1.51	15.0
100:12:0.5	96	4	39.20	1.77	18.20	25.3	1.38	10.0
100:15:0.5	120	4	52.20	1.25	20.20	27.1	1.41	10.0
100:1:1	8	8	70.20	4.56	37.90	115.8	1.93	8.5
100:2:1	16	8	70.00	6.03	34.50	56.9	1.91	15.5
100:3:1	24	8	75.90	6.29	33.20	53.3	2.04	25.0
100:5:1	40	8	84.40	6.4	33.10	69.8	4.29	32.0
100:7:1	56	8	77.10	6.98	37.10	38.9	1.31	40.0
100:10:1	80	8	34.10	2.77	23.20	39.8	1.83	17.0
100:12:1	96	8	33.30	1.75	21.90	30.1	1.50	15.0
100:15:1	120	8	36.50	1.07	19.40	27.5	1.04	16.0
100:10:2	80	16	33.40	0.85	11.20	29.5	1.03	21.0
100:12:2	96	16	33.30	1.93	22.90	22.9	0.66	17.0
100:15:2	120	16	25.60	1.65	24.20	24.2	0.94	19.0
100:3:0	24	0	68.56	3.81	24.87	90.3	4.03	15.0
100:3:2	24	16	68.80	7.23	36.30	54.3	1.94	23.0
100:3:3	24	24	57.90	6.36	24.60	65.6	1.54	7.0
100:3:5	24	40	81.40	4.24	13.50	36	1.81	24.0
100:3:7	24	56	89.90	5.91	16.10	44.6	3.22	22.0
100:3:10	24	80	71.20	3.65	14.00	43.5	2.46	18.0

* Nitrogen source: NH_4Cl; Phosphorus source: K_2HPO_4

Effect of EPS and its components on SVI and CST

Using the step-wise method for multiple regression analysis, protein displayed a more pronounced effect on SVI and CST than carbohydrate, while total EPS had no correlation with SVI or CST. The increase in protein concentration contributed to the increase of hydrophobicity (24 % of amino acids) of the sludge surface (Urbain et al. 1993) and improved sludge settling due to the bonding ability of proteins to cations. Protein was a key component in electrostatic bonds with multivalent cations due to the negatively charged amino acids (25%), which are major components of the protein in EPS.

Effect of nutrients on sludge properties

For varying nitrogen concentrations, SVI varied between 22.9 and 115.8 mℓ/g, CST ranged from 0.7 to 10.3 s/g SS, and turbidity from 7 to 40 NTU. Figures 2 and 3 present the effect of nitrogen for varying phosphorus concentrations on sludge dewatering and effluent turbidity, respectively. Nitrogen effect on SVI, CST and effluent turbidity followed similar trends with sludge properties improved at 100:2 ≤ COD: N ≥ 100:10. At carbon:nitrogen ratios below 2 and above 10, the CST averaged at 2 s/g for the phosphorus ratios evaluated. The corresponding effluent turbidity was between 10 to 25 NTU.

Nitrogen deficient conditions result in an increase in protein concentrations in EPS by lowering the SVI and CST from those obtained under balanced nutrient conditions (COD:N:P = 100:5:1). The increase in protein caused the increase of hydrophobicity of sludge surface that led to the improvement of sludge properties (Higgins and Novak, 1997; Jorand et al., 1998). At high nitrogen concentrations (COD: N ≥ 100:10), the structure or the molecular mass of EPS components produced changes leading to the improvement of sludge properties. Increasing EPS molecular mass negatively affects the sludge settling (Forster, 1985) and results in elevated nitrogen concentrations in the effluent.

For varying phosphorus concentrations, SVI varied from 36 to 75 mℓ/g, CST from 1.5 to 5.7 s/g SS, and effluent turbidity from 7 to 27.5 NTU. The range of SVI, CST and turbidity is narrow and in the typical range for good settling and dewatering. The increasing ratio of COD: P from 100:0.5 to 100:5 improved settling, dewatering and clarifying or lowering SVI, CST and effluent turbidity. When COD: P increased more than 100:5, sludge properties worsened. However, the effect of phosphorus on sludge properties was not as profound as that of nitrogen.

Phosphorus depletion caused an increase in carbohydrate levels in EPS, which resulted in a worsening of sludge settling and dewatering or increasing of SVI and CST. Similar observations were reported by Shin et al. (2001) and Morgan et al. (1990). Thus, high concentrations of anionic surface biopolymers could consequently be correlated with deteriorating sludge settling characteristics as a result of the influence of floc repulsion due to a decrease in surface charge.

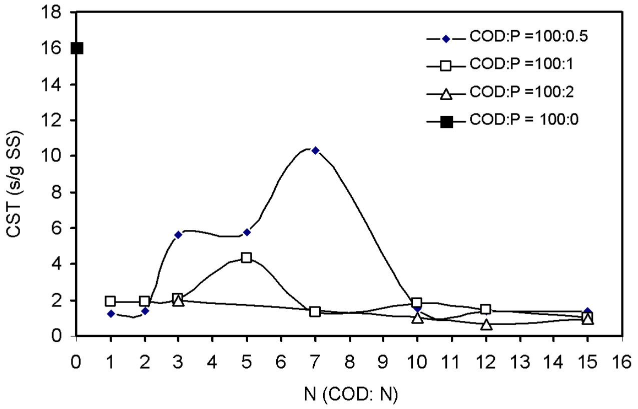

Figure 2
Effects of nitrogen on sludge dewatering or CST value at different COD:P ratios

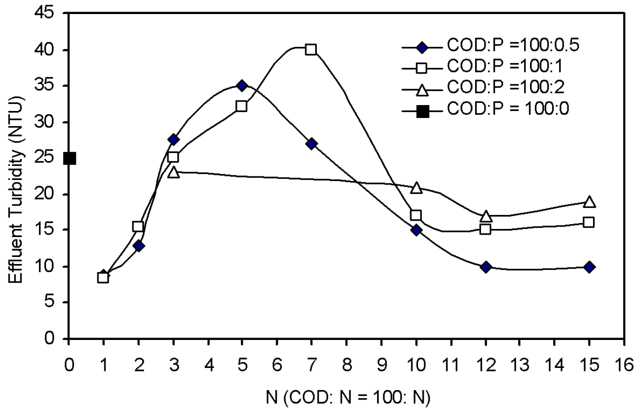

Figure 3
Effects of nitrogen on sludge clarifying or effluent turbidity at different COD:P ratios

Conclusions

From the field survey it was evident that the simultaneous effects of many factors on EPSs directly or indirectly affect sludge properties. EPS was lower in anaerobic than in aerobic processes, possibly due to the absence of lipid carriers in anaerobic sludge or the utilisation of EPS as an energy source in anaerobic processes. The results indicated that total EPSs seemed to have positive correlation with sludge settling but no correlation with sludge dewatering and clarifying.

The laboratory investigations were undertaken at different nutrient ratios of phosphorus and nitrogen to evaluate the effect of total EPS and its protein and carbohydrate component on sludge properties. EPS components were found to strongly affect sludge properties, whilst total EPS had no effect. Protein was identified as having a more profound effect on sludge properties than carbohydrate, with an improvement in both CST and SVI. Both nutrients namely phosphorus and nitrogen affected sludge properties. The optimum COD: P ratio ranged from 100:3 to 100:5, while the optimum COD: N ranged from 100:2 ≤ COD: N ≥ 100:10. Although an excess of nitrogen improves sludge properties, a negative consequence of this is increased effluent nitrogen levels.

This study has indicated that total EPS is insufficient in predicting sludge properties due to uncertainty in analytical procedures and the combined effects of operational parameters. It is therefore necessary to evaluate the effect of EPS on sludge properties in terms of the EPS protein and carbohydrate components. This approach revealed the effect of nutrients present in the wastewater on EPS production and better correlation between EPSs and sludge properties.

References

AZEREDO J, OLIVEIRA R and LAZAROVA V (1998) A new method for extraction of exopolymers from activated sludges. *Water Sci. and Technol.* **37** (4-5) 367-370.

BECCARI M, MAPPELLI P and TANDOI V (1980) Relationship between bulking and physicochemical-biological properties of activated sludges. *Biotechnol. and Bioeng.* **22** 969-979.

BURA R, CHEUNG M, LIAO B, FINLAYSON J, LEE BC, DROPPO IG, LEPPARD GG and LISS SN (1998) Composition of exocellular polymeric substance in the activated sludge floc matrix. *Water Sci. and Technol.* **37** (4-5) 325-333.

CHAO AC and KEINATH TM (1979) Influence of process loading intensity on sludge clarification and thickening characteristics. *Water Res.* **13** 1213-1223.

DUBOIS M, GILLES KA, HAMILTON JK, REBERS PA and SMITH F (1956) Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28** (3) 350-356.

FORSTER CF (1971) Activated sludge surfaces in relation to the sludge volume index. *Water Res.* **5** 861-870.

FORSTER CF (1985) Factors involved in the settlements of activated sludge: nutrients and surface polymers. *Water Res.* **19** (10) 1259-1264.

FORSTER CF and CLARK AR (1983) The production of polymer from activated sludge by ethanolic extraction and its relation to treatment plant operation. *Water Pollut. Contr.* 430-433.

FORSTER CF and NEWTON JD (1980) Activated sludge settlement – Some suppositions and suggestions. *Water Pollut. Control* **52** 338-351.

HIGGINS M and NOVAK J (1997) The effect of cations on the settling and dewatering of activated sludges: Laboratory results. *Water Environ. Res.* **69** (2) 215-223.

JORAND F, BOUE-BIGNE F, BLOCK, JC and URBAIN V (1998) Hydrophobicity/hydrophilic properties of activated sludge exopolymeric substances. *Water Sci. and Technol.* **37** (4-5) 307-315.

MORGAN JW, FORSTER CF and EVISON L (1990) A comparative study of the nature of biopolymers extracted from anaerobic and activated sludge. *Water Res.* **24** (6) 743-750.

RODNEY FB (1993) *Modern Experimental Biochemistry* (2nd edn.) The Benjamin/Cumming Publishing Co. Inc.

SHEINTUCH M, LEV O, EINAV P and RUBIN E (1986) Role of extracellular polymer in the design of activated sludge. *Biotechnol. and Bioeng.* **28** 1564-1576.

SHIN HS, KANG ST and NAM SY (2001) Effect of carbohydrate and protein in the EPS on sludge characteristics. *Water Sci. & Technol.* **43** (6) 193-196.

SPONZA DT (2002) Extracellular polymer substances and physico-chemical properties of flocs in steady and unsteady-state activated sludge systems. *Process Biochem.* **37** 983-998.

STANDARD METHODS (1995) *Standard Methods for the Examination of Water and Wastewater (*19th edn.), APHA, AWWA, WEF, Washington DC., USA.

URBAIN V, BLOCK JC and MANEM J (1993) Biofloculation in activated sludge: An analytical approach. *Water Res.* **27** (5) 829-838.