

Microbiological and biochemical effects of activated sludge diffusion for sulphide biooxidation in wastewater treatment plants

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ABSTRACT. Sulphide was selected as a model odorous compound and its removal efficiencies were experimentally evaluated along with its effects on the biomass and the biological processes in an AS diffusion test. Two bench scale sequencing batch reactors (SBRs) were fed in parallel on real primary sewage and monitored after adding increasing concentrations of sulphide to one of them. In this reactor, an average sulphide removal of 94% was measured. Microbial composition did not show relevant variations after the addition of sulphide, and the good features of activated sludge flocs were maintained also in terms of sludge settleability.

1 INTRODUCTION

Many reduced sulphur compounds can be used as electron donors by a variety of bacteria having relevance in wastewater treatment. The most common sulphur compound used as energy source is hydrogen sulphide (H_2S) that is oxidized by microorganisms to elemental sulphur, up to sulphate, mainly in presence of molecular oxygen (Brook and Madigan, 1988). The biological oxidation of sulphide is used in the process called "activated sludge diffusion" (AS diffusion) to treat odorous air at wastewater treatment plants, since odours associated with sewage and wastewater treatment are mainly generated by H_2S (Barbosa *et al.*, 2003; Stuetz and Frenchen, 2001). In biological reactors equipped for AS diffusion, the gaseous sulphur compounds diffuse into the liquid phase where microorganisms are able to oxidize them.

Among these oxidizing bacteria, there are some filamentous microorganisms that are of critical importance in wastewater treatment, because they are responsible for bulking of the activated sludge. These were morphologically characterized as *Beggiatoa* spp., *Thiothrix* spp., type 021N and type 0914 (Jenkins *et al.*, 1993). Hydrogen sulphide, especially when present in relatively high concentrations, can selectively favour the growth of these microorganisms with consequent solid separation problems (Jenkins *et al.*, 1993). High concentrations of sulphide can also cause inhibition of ammonia nitrification, although the specific mechanisms of this inhibition are still unclear (Barbosa *et al.*, 2002).

Aim of this work was to evaluate the impact of H₂S on activated sludge biomass with special reference to microbial composition and biochemical activities.

2 MATERIALS AND METHODS

Two bench-scale sequencing batch reactors (SBRs), with a working volume of 3.0 L, were inoculated with identical mixed liquor and fed in parallel on domestic clarified sewage. The two reactors were continuously operated for 243 days in cycles of 8 hours according to the operating phases shown in Table 1.

Anoxic conditions were kept during Mixed fill and Mixed react steps to achieve denitrification. During the Draw step of each cycle, 1.8 L of treated effluent were discharged and replaced during the following Fill step with an equal volume of feed (stored at 4°C), resulting in hydraulic retention time (HRT) of 13 h 20 min and a volumetric exchange ratio (VER) of 0.6. A sludge retention time (SRT) of 18 days was achieved with a manual removal of exceed sludge finalized to maintain a constant concentration of mixed liquor suspended solids (MLSS) of 3 g L⁻¹.

The organic loading rate was variable depending on the characteristics of influent sewage with an average value of 0.2 kgCOD kgMLSS⁻¹ d⁻¹.

Aerobic conditions were attained during Aerated react step with a residual dissolved oxygen (DO) concentration of 1-3 mg L⁻¹ during the *feast* period (Wilderer *et al.*, 2001). Settling characteristics of the sludge were expressed as sludge volume index (SVI) (IRSA, 1984) and temperature, dissolved oxygen (DO) and pH values were daily measured to monitor physical-chemical parameters of the process.

One of the two biomasses was kept under selective pressure of hydrogen sulphide, in order to study the biochemical and microbiological effects of AS Diffusion. For this purpose, one of the two reactors (SBR1) was fed with an addition of a liquid solution of sodium sulphide (Na₂S) during the first 4 hours of the Aerated react step, with a volumetric loading rate of 17.03 mgS L⁻¹_{reactor} d⁻¹. This loading rate corresponds to a gas-phase concentration of 240 mgS (Nm³)⁻¹, i.e. about twice the maximum value found in the literature about municipal wastewaters (Bowker, 2000; Hansen *et al.*, 2000).

The two reactors were identical except for this additional feeding. The headspace of each reactor (0.5 L) was connected to a trap containing a concentrated solution of zinc chloride (ZnCl₂), so that the sulphide escaping the reactor as a gas precipitated in the trap as zinc sulphide (ZnS).

2.1 Chemical analysis

Chemical oxygen demand (COD), metabolic balances of nitrogen and sulphur were monitored by chemical characterization of the reactors' influent and effluent.

COD, ammonia, nitrite and sulphate concentrations were determined according to Italian standard methods (APAT and IRSA-CNR, 2003). Nitrate, sulphide and total sulphur concentrations were determined according to standard methods (Eaton *et al.*, 1995) as well mixed liquor suspended solids and volatile suspended solids (VSS).

2.2 Microscopic analysis

The microbial composition of the mixed biomasses was investigated through microscopic observations with special focus on the characterization of filamentous organisms.

Characterization of the filamentous microorganisms and description of sludge microscopically properties was performed according to Jenkins' manual (Jenkins *et al.*,

1993). The method of subjective scoring of filament abundance, proposed by Jenkins (Jenkins *et al.*, 1993), was applied to value the amount of filamentous bacteria. Activated sludge samples were periodically (once every SRT) collected from both reactors during the whole experimentation period up to a total number of 14 samples.

Table 1. Operating sequence of SBR system during one complete cycle (8h).

Phase	Mode	Time (hours)	Operations
Fill	Mixed fill	0.06	Stirring, feeding
React	Mixed react	2.24	Stirring
	Aerated react	4.30	Stirring, aeration
Settle	-	0.56	-
Draw	-	0.04	Discharge

Stains (Gram and Neisser stains) (Jenkins *et al.*, 1993) were performed as well the test for sulphur oxidation (S Test) (Jenkins *et al.*, 1993).

Microscopic observations were made with an Olympus BX 50 phase contrast microscope equipped with a digital camera (JVC TK-C1380).

3 RESULTS AND DISCUSSION

After a start-up period of 60 days, the two SBRs were operated in steady state under identical conditions until 147th day (Period 1) when the Na₂S dosing system was turned on to feed the reactor SBR1 with a sulphide addition (Period 2) and the other one reactor (SBR2) operated as a control reactor (blank system).

In Table 2 are reported the main parameters, representative of biochemical activities in the system, during the two experimental periods (before and after the external addition of Na₂S) and shows the performance of the two reactors in terms of COD and nitrogen removal efficiencies.

The average COD concentration of the influent wastewater during the two periods was about 390 and 350 mgCOD L⁻¹ respectively, and the removal efficiencies of the two parallel systems were about 90% in the first period. During the second period, when SBR1 was added with Na₂S, the COD removal efficiency decreased for both reactors, and this was attributed to a lower quality of the influent wastewater. Therefore, COD and so the oxidative activity of heterotrophic population does not seem to be affected by sulphide addition.

No relevant differences were observed with respect to nitrification and denitrification, either between the two periods for reactor SBR1, or between the two reactors. Therefore, both autotrophic nitrifiers and heterotrophic denitrifiers would not seem to be affected by the sulphide externally fed to SBR1. This is in contrast with the findings of other researchers, who noted inhibition of nitrification possibly due to the presence of sulphide (Barbosa *et al.*, 2002).

As regard to sulphide oxidation, the efficiency of its removal was about 93% in SBR1 and 77% in SBR2 (average values) and since no significant differences between the effluent sulphide concentrations were observed in the two reactors, a complete depletion of the external dose provided to SBR1 is suggested.

The amount of effluent gaseous sulphur accumulated in the traps was 0.07 mgS d⁻¹ for reactor SBR1 and 0.08 mgS d⁻¹ for reactor SBR2 (average values). Also in this case the two reactors behaved in similar ways, confirming that the external sulphur addition did not cause detectable differences in the liquid and gaseous effluents.

Table 2. Average concentrations and removal efficiencies of the main process parameters.

		Average		Efficiency	
		Period 1	Period 2	Period 1	Period 2
		mg/L	mg/L	%	%
COD	Influent	387	349 (+19) ^(a)		
	Effluent SBR1	33	91	91.4	73.8 (75.1) ^(b)
	Effluent SBR2	43	83	89.0	76.3
NH ₃ -N	Influent	37.7	41.1		
	Effluent SBR1	2.0	0.9	94.8	97.7
	Effluent SBR2	n.d. ^(c)	n.d. ^(c)	> 99	> 99
NO ₃ ⁻ -N	Influent	0.0	0.0		
	Effluent SBR1	19.1	19.1		
	Effluent SBR2	22.1	22.1		
NO ₂ ⁻ -N	Influent	0.0	0.0		
	Effluent SBR1	0.1	0.1		
	Effluent SBR2	0.5	0.0		
Sulphide	Influent		4.3 (+9.5) ^(d)		
	Effluent SBR1		1.0		93.4
	Effluent SBR2		1.1		76.6

^a average COD contribution due to the addition of Na₂S is reported in brackets;

^b adjusted COD removal efficiency calculated considering the contribution of Na₂S in terms of influent COD is reported in brackets;

^c n.d. = below the method's detection limit;

^d sulphide concentration externally supplied to SBR1.

Figure 1 shows the mass balance of sulphur compounds and suggest no relevant differences between the two reactors. Most of the influent sulphide was oxidised to sulphate and left the plant in the effluent. The remaining sulphide was partly found in the effluent and a very limited amount was stripped. A certain amount of sulphur accumulated in the mixed liquor and was removed from the plant with the waste sludge, as pointed out by other authors (Barbosa *et al.*, 2003).

Microscopic examination of the samples indicated the presence of the filamentous microorganisms in a decreasing abundance as shown in Table 3 (filaments rated as *few* category of abundance, are not listed).

Filament abundance never reached values of high density (corresponding to *abundant* and *excessive* categories of abundance) and only occasionally values of medium density (*very common*). Generally, filament abundance was rated as *common* (filaments observed in all flocs but at low density in a range of 1-5 filaments per floc) that their overall presence was below levels regarded as pathologic (bulking).

Some well-known filamentous microorganisms such as *Thiothrix* spp., type 021N, and type 0014 were detected in both reactors during the two experimental periods (i.e. before and after dosing sulphide to reactor SBR1). The filamentous organism *Beggiatoa* spp. only appeared during the second experimental period (Period 2), although it was present in both reactors and rather sporadically. Thus, the presence of this bacterium was not linked to the external dose of sulphide provided to reactor SBR1, but rather to the presence of sulphide in the sewage or to other factors not related to sulphur compounds. In addition, when some filamentous species (*Thiothrix* spp., type 021N) became prevalent in the bulk, they never reached the threshold that would determine sludge pathologies (bulking).

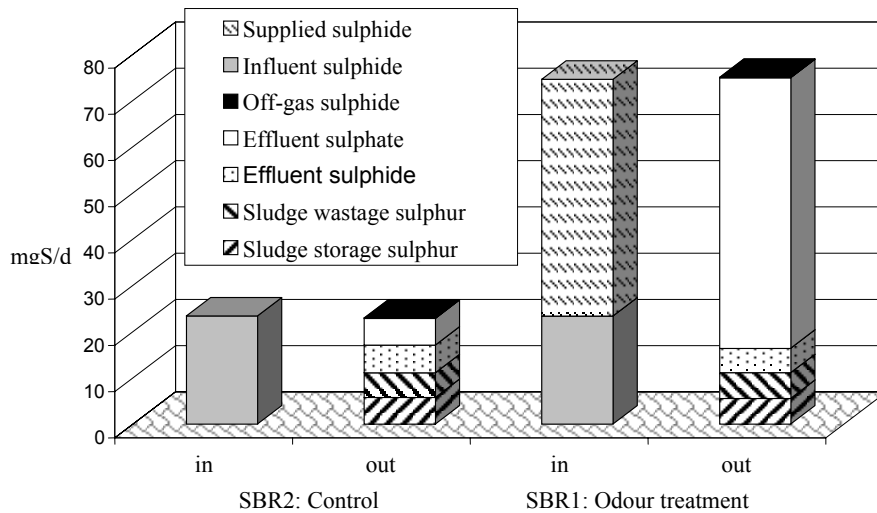


Figure 1. Mass balance of main sulphur compounds during the second period.

Microscopic observation of the activated sludge sampled in both reactors showed good quality features also during the second experimental period (Figure 2). In terms of the protocol proposed by Jenkins, the flocs had good morphological structure, good compactness, their shape was irregular, and their size was 150-500 μm (Jenkins *et al.*, 1993). The filamentous bacteria detected, were mainly located within the floc structure. Their influence upon the structure of flocs was judged irrelevant.

The sludge volume index (SVI) was mostly below 150 mL gMLSS^{-1} for both reactors, with no substantial differences between SBR1 and SBR2 (Figure 3). During the last 20 days of the experimental period the SVI had a sensible increase which appears related to the rapid increase of temperature (Krishna and van Loosdrecht, 1999) in the reactors (warm season), as shown in Figure 3.

Therefore, microscopic observation showed no relevant differences in both composition and structure of the biomass in the two mixed sludges, during all the period of the experimentation. In particular, the reactor kept under selective pressure of H_2S showed no tendency of the biomass to develop specific filamentous organisms as consequence of the addition of hydrogen sulphide.

Table 3. Abundance of filamentous organisms.

Sample	Reactor	Overall abundance	Individual abundance			
			very common	common	Some	
Start-up	1	SBR1	very common	type 0041/0675, MP	type 021N, 0092, NOC, THI, HH	
		SBR2	common	NO, MP	type 021N, 0041/0675, 0092, THI, HH	
	2	SBR1	common		type 021N, 0041/0675, MP	
		SBR2	common		type 021N, 0041/0675, NOC	
Period 1	3	SBR1	some		HH	
		SBR2	some		HH	
	4	SBR1	common	THI	HH	
		SBR2	common	THI	HH	
	5	SBR1	common	THI	type 021N, HH	
		SBR2	common	THI	type 021N, MP	
	6	SBR1	very common	THI	type 021N, HH	type 0041/0675
		SBR2	common	THI	type 021N, 0041/0675, HH	
	7	SBR1	common		type 0041/0675, THI, HH	
		SBR2	common	HH	type 021N, 0041/0675, THI	
	8	SBR1	common	THI	type 0041/0675, HH	
		SBR2	common	THI	type 0041/0675, HH	
	9	SBR1	common		type 0041/0675	type 021N, THI, HH
		SBR2	common		type 021N	type 0041/0675, THI, HH
10	SBR1	very common	THI	type 021N, 0041/0675, HH, MP		
Period 2		SBR2	common	HH	type 0041/0675, THI	
	11	SBR1	common	type 0041/0675	THI, HH	
		SBR2	common		type 0041/0675, THI, HH	
	12	SBR1	common	type 021N	type 0041/0675, THI, HH	
		SBR2	common	MP	type 021N	type 0041/0675, THI
	13	SBR1	common	MP	type 021N	
		SBR2	very common	MP	type 021N	
	14	SBR1	common		HH, MP	
		SBR2	common	MP	type 021N	

NOC = *Nocardia* spp.

SN = *Sphaerotilus natans*

THI = *Thiothrix* spp.

HH = *Haliscomenobacter hydrossis*

NL = *Nostocoida limicola*

MP = *Microthrix parvicella*

BEG = *Beggiatoa* spp.

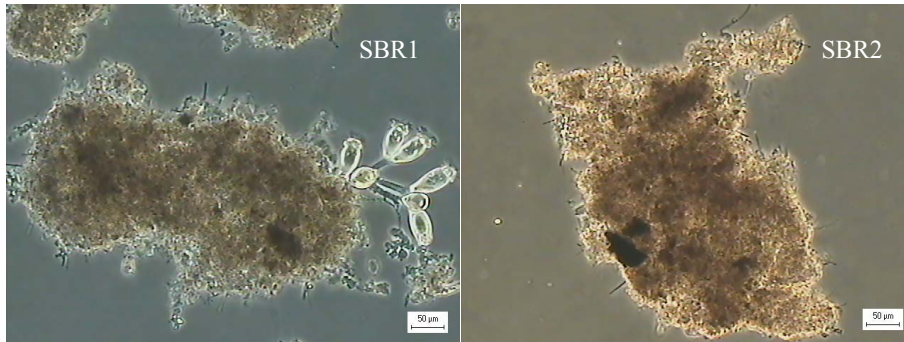


Figure 2. Floc structure during the second experimental period in the two reactors (100x).

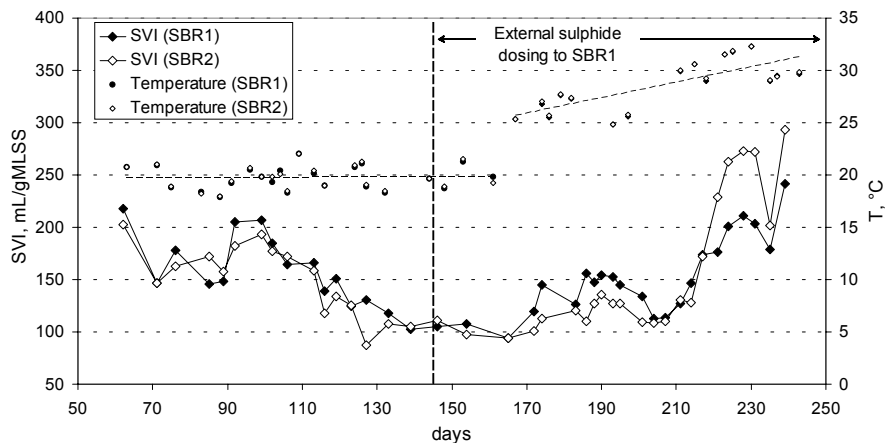


Figure 3. Sludge volume index and temperature trend in the reactors.

4 CONCLUSIONS

An experimental test was performed to evaluate possible effects of H_2S on activated sludge biomass and biodegradation processes in the *Activated Sludge Diffusion* system. Under the operating conditions adopted, the following observations were made:

- No significant effects of sulphide on the oxidation ability of the heterotrophs were detected, therefore no effects on COD removal efficiencies.
- No evident effects on nitrification and denitrification due to the presence of sulphide were shown by heterotrophic and autotrophic populations.
- The external sulphide addition to SBR1 did not cause significant differences between the liquid and gaseous effluent sulphide concentrations observed in the two reactors, suggesting that the external dose provided to SBR1 was completely depleted.
- No tendency of the biomass to develop specific organisms was observed as a consequence of the addition of sulphide. Thus no relevant effects of sulphur

compounds were observed with respect to sludge pathologies that could affect process efficiencies also in terms of settleability. Therefore, “activated sludge diffusion” was confirmed as an interesting system among the strategies for odour reduction that can be implemented at wastewater treatment plants.

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6 REFERENCES

- APAT and IRSA-CNR (eds.) (2003) Metodi analitici per le acque. *Manuali e linee guida 29*, Agenzia per la Protezione dell’Ambiente e per i servizi Tecnologici, Istituto di Ricerca sulle Acque del Consiglio Nazionale delle Ricerche, Roma.
- Barbosa, V.L., Burgess, J.E., Darke, K. and Stuetz, R.M. (2002) Activated sludge biotreatment of sulphurous waste emissions. *Reviews in Environmental Science and Bio/technology*. 1: 345-362.
- Barbosa, V.L., Dufol D., Callan J.L., Sneath R. and Stuetz R.M. (2003) Hydrogen sulphide removal by activated sludge diffusion. In: *Proc. of the 2nd IWA International Workshop & Conference on Odours and VOCs. Measurement Regulation and Control Techniques*. 14-17 September 2003, Singapore.
- Bowker R.P.G. (2000) Biological odour control by diffusion into activated sludge basins. In: *Selected Proc. of the CIWEM-IWQ Internat. Conf. on the Control and Prevention of Odours in the Water Industry*, 22 - 24 September 1999, London. Stuetz and Boon (eds.), IWA Publishing, London, pp. 127-132.
- Brock, T.D. and Madigan, M.T. (1988) *Biology of Microorganism*, 5th Edition, Prentice-Hall, New Jersey.
- Eaton, A.D., Clesceri, L.S. and Greenberg, A.E. (1995) *Standard Methods for the Examination of Water and Wastewater*, 19th Edition, American Public Health Association, Washington, DC.
- Hansen, N.G. and Rindel, K. (2000) Bioscrubbing, an effective and economic solution to odour control at wastewater treatment plants. *Water Sci. Technol.* 41:155-164.
- IRSA (ed.) (1984) Metodi analitici per i fanghi. Parametri tecnologici. *Quaderni 64*, Istituto di Ricerca sulle Acque del Consiglio Nazionale delle Ricerche, Roma.
- IRSA (ed.) (1999) Il problema del bulking filamentoso e delle schiume biologiche negli impianti a fanghi attivati. *Quaderni 110*, Istituto di Ricerca sulle Acque del Consiglio Nazionale delle Ricerche, Roma.
- Jenkins, D., Richard, M.G. and Daigger, G.T. (1993) *Manual on the Causes and Control of Activated Sludge Bulking and Foaming*, 2nd Edition, Lewis Publishers, New York.
- Krishna, C. and van Loosdrecht, M.C.M. (1999) Effect of temperature on storage polymers and settleability of activated sludge. *Water Res.* 33:2374-2382.
- Stuetz, R. and Frechen, F.B. (eds.) (2001) *Odours in Wastewater Treatment. Measurements, Modelling and Control*, IWA Publishing, London.
- Wilderer, P.A., Irvine, R.L. and Goronszy, M.C. (2001) Sequencing batch reactor technology. Scientific and technical report n. 10. IWA publishing.