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TECHNICAL ARTICLE

Biological Removal of High Loads of Thiosulfate Using a Trickling Filter Under Alkaline Conditions

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Abstract Uncontrolled release of thiosulfate can cause high oxygen demand, or generate toxic compounds under anaerobic scenarios. Biooxidation of thiosulfate in a biotrickling filter (BTF) colonized by an alkaliphilic sulfide-oxidizing bacterial consortium was studied at pH ≈ 10. Inlet thiosulfate concentrations were varied from 3.5 to 21.3 g L^{-1}, with a residence time of 216 s, emulating conditions encountered in wastewater from mining processes. Sulfate production, oxygen concentration, and biomass in both packing and effluent were periodically analyzed to characterize bioreactor performance. Removal efficiencies near 100 % were obtained during the entire experimental period, with a maximum elimination capacity of 242 g thiosulfate m^{-3} h^{-1}. Although the BTF was able to transfer large amounts of oxygen to biooxidize thiosulfate to sulfate, under high initial thiosulfate loads, thiosulfate was not completely oxidized to sulfate, since biooxidation was conditioned to oxygen supply. Respirometric tests performed to investigate biomass adaptation and activity revealed oxygen consumption values of 0.5 mmol O_2 (g protein)$^{-1}$ min^{-1} for the period with the highest thiosulfate inlet load.

Keywords Alkalinity · Alkaliphilic bacteria · Bioreactor · Thiosulfate elimination

Introduction

Reduced sulfur compounds (RSC) in wastewater or gaseous streams can have adverse effects on human health and the environment. RSC are frequently found in effluents from mining operations, petroleum refineries, tanning, the paper industry, and coal processing (Janssen et al. 1997; Sahinkaya et al. 2011). The undesirable emission of wastewater containing thiosulfate is mostly related to the leaching of metal ores. Indeed, the most promising alternative reagent to cyanide for gold extraction is thiosulfate, normally in ammonia solutions and catalyzed by Cu(II) (Abbruzzese et al. 1995; Grosse et al. 2003; Muir and Aylmore 2004). A wide range of conditions have been reported for the leaching of copper-silver-gold bearing materials with thiosulfate, ranging from 10 to 220 g of thiosulfate L^{-1}, temperatures between 20 and 70 °C, and alkaline pH values up to 11 (Aylmore and Muir 2001; O'Malley 2002).

Thiosulfate is metastable; it can be readily decomposed either to polythionates and sulfate under oxygen consuming conditions, or to toxic sulfide ion under anaerobic or reducing conditions (Dhawale 1993; González-Sánchez and Revah 2007; Tykodi 1990). As a consequence, uncontrolled thiosulfate disposal can cause de-oxygenation of waterways and potential formation of toxic compounds.

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Both chemical and biological technologies have been applied to remove thiosulfate from respective wastewater. For example, Vohra et al. (2011) investigated the oxidation of thiosulfate using photo-catalysis under different pH conditions. Removal efficiencies up to 98 % were obtained, starting from concentrations of 200 mg of thiosulfate L^{-1} at pH 4, but efficiency decreased at higher pH. Similar removal trends were found for binary systems (i.e. thiosulfate and *p*-cresol, thiosulfate and thiocyanate).

On the other hand, biological systems have been demonstrated to be a feasible and efficient way to treat RSC, avoiding the disadvantages associated with chemical processes, such as operational costs and the use of hazardous chemicals (González-Sánchez et al. 2008a; Liljeqvist et al. 2011; Tang et al. 2009). Similarly, biotrickling filters (BTFs) have been successfully employed to remove several RSCs from polluted air streams, generally under neutral or acidic conditions (Hort et al. 2009; Ramírez et al. 2011).

Biooxidation is normally performed by autotrophic aerobic sulfide-oxidizing microorganisms where elemental sulfur and/or sulfate are generated depending on the availability of dissolved oxygen (DO) (González-Sánchez et al. 2005; Janssen et al. 1997).

$$S_2O_3^{2-} + 0.5O_2 \rightarrow S^0 + SO_4^{2-} \tag{1}$$

$$S_2O_3^{2-} + 2O_2 + H_2O \rightarrow 2SO_4^{2-} + 2H^+ \tag{2}$$

Few studies have focused on biofiltration of RSC under alkaline conditions (Arellano-Garcia et al. 2010; de Graaff et al. 2012; González-Sánchez et al. 2008a, b; van Den Bosch et al. 2009). González-Sánchez et al. (2008b) reported the alkaline biofiltration of gaseous H_2S and found that the absorption rate of H_2S was appreciably enhanced with an alkaline solution (close to pH 10), but with the disadvantage that high pH could induce higher concentrations of dissolved sulfide and thus increase the inhibition of the growth and activity of alkaliphilic sulfide-oxidizing microorganisms (González-Sánchez and Revah 2007). The detoxification of sulfide to thiosulfate under alkaline conditions, deploying first an abiotic oxygen-consuming step process, has been reported by González-Sánchez and Revah (2007) and de Graaff et al. (2012). Sulfide oxidation rates of around 100 mol S^{2-} h^{-1} m^{-3} (130 g S^{2-} h^{-1} m^{-3}) with efficiencies close to 100 % was reported under alkaline conditions (pH $\approx$10) by González-Sánchez and Revah (2009) in a packed recycling reactor. Improved performance was limited by the intrinsic rates of the alkaline cultures and oxygen transfer.

Literature related to biooxidation of thiosulfate is limited to biological systems operating at neutral or acid conditions. This study evaluated the treatment of high thiosulfate loads in an alkaline BTF, emulating conditions encountered in mine water processes.

Materials and Methods

Biotrickling Filter System

The pilot-scale BTF shown in Fig. 1 was used to treat an aqueous thiosulfate solution. The rectangular acrylic reactor had cross-section dimensions of 9 cm × 14.5 cm and a bed height of 42 cm, resulting in a volume bed (V_{bed}) of 5.48 L. The BTF was packed with open pore polyurethane foam (EDT, Germany) cubes (4 cm on a side) with a specific area of 600 m^2 m^{-3}, a density of 35 kg m^{-3}, and a porosity of 0.97. Some cubes were cut to allow a tight fit of the support. This packed reactor configuration promotes both biofilm formation and oxygen transfer.

A pH 10 alkaline mineral medium (AMM; Sorokin et al. 2001) containing various thiosulfate concentrations (from 3.5 to 21.3 g L^{-1}) was added continuously at a flow rate (F) of 1.5 L day^{-1}; the effluent drained from an overflow in the reactor. Liquid was internally recirculated from the reactor reservoir at flow rates of either 0.89 or 1.91 L min^{-1}. An air stream was continuously fed to the reactor at a flow rate of 0.105 L min^{-1} (an empty bed residence time of 3.6 min). Air and trickling liquid flowed in opposite directions. The pH of the system was maintained at 10 by automatic additions of 2 N NaOH or 2 N HCl aqueous solutions. All experiments were performed at room temperature (25 °C).

The thiosulfate loading rate ($Q_{S_2O_3^-}$), percent thiosulfate removal efficiency (%RE), and thiosulfate elimination capacity (EC), are defined by:

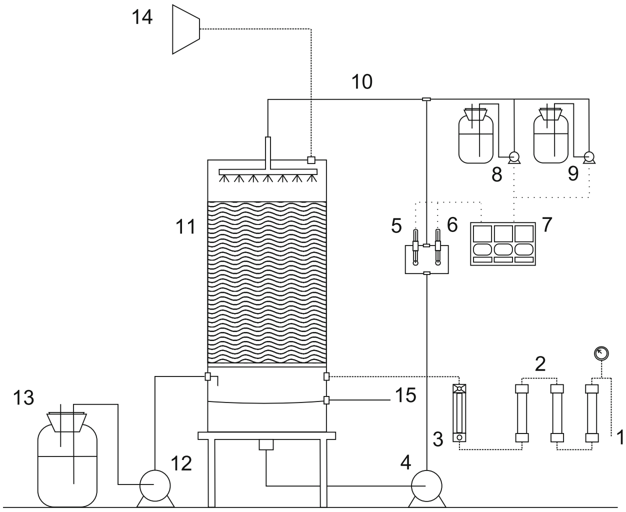

Fig. 1 Pilot scale alkaline biotrickling filter for the thiosulfate removal. *1* Air from compressor, *2* air filters, *3* rotameter, *4* recirculation pump, *5* pH probe, *6* dissolved oxygen probe, *7* pH controller and dissolved oxygen monitor, *8* NaOH solution pump, *9* HCl solution pump, *10* recirculation stream, *11* BTF inoculated with ASBC, *12* liquid medium pump, *13* alkaline mineral medium vessel, *14* air extractor, *15* effluent purge

$$Q_{S_2O_3^{2-}} = \frac{F}{V_{bed}}\left[S_2O_3^{2-}\right]_{in} \tag{3}$$

$$\%RE = 100 \times \left(\frac{\left[S_2O_3^{2-}\right]_{in} - \left[S_2O_3^{2-}\right]_{out}}{\left[S_2O_3^{2-}\right]_{in}}\right) \tag{4}$$

$$EC = \frac{F}{V_{bed}} \times \left[\left[S_2O_3^{2-}\right]_{in} - \left[S_2O_3^{2-}\right]_{out}\right] \tag{5}$$

where $[S_2O_3^{2-}]_{in}$ and $[S_2O_3^{2-}]_{out}$ are the inlet and outlet thiosulfate concentrations (g thiosulfate L^{-1}).

From Eqs. 1 and 2, it can be seen that a maximum of two moles of sulfate can be produced by the complete oxidation of one mole of thiosulfate. The sulfate molar yield can be defined as the moles of sulfate obtained from one mole of oxidized thiosulfate. As one of the sulfur atoms in the thiosulfate molecule is already fully oxidized, the sulfate molar yield is bound between 0.5 and 1.0 and is defined as:

$$Yield\ SO_4^{2-} = \frac{0.583\left[SO_4^{2-}\right]}{\left(\left[S_2O_3^{2-}\right]_{in} - \left[S_2O_3^{2-}\right]_{out}\right)} \tag{6}$$

where the constant 0.583 corresponds to a conversion factor from mass to molar units for sulfate and thiosulfate.

Microorganisms

The BTF was inoculated with an adapted alkalophilic sulfide-oxidizing bacteria consortium (ASBC) able to grow under high salinity and alkalinity, previously described by González-Sánchez and Revah (2007). The BTF was operated for more than a year with a mixture of sulfides at relatively low inlet concentrations (Arellano-Garcia et al. 2010). After this period, a continuous aqueous stream containing dissolved thiosulfate was fed, as described above.

Oxygen Transfer Rate

According to the stoichiometric coefficients in Eqs. (1) and (2), DO is a key factor regulating the oxidation of thiosulfate. In this sense, the extent of thiosulfate oxidation can be analyzed by examining the molar relation between the oxygen consumption and the thiosulfate oxidation rates, as follows:

$$\begin{aligned}\frac{\text{Oxygen consumption}}{\text{Thiosulfate oxidation}} &= \frac{3.5K_La([DO]^* - [DO])}{F\left(\left[S_2O_3^{2-}\right]_{in} - \left[S_2O_3^{2-}\right]_{out}\right)} \\ &= \frac{3.5K_La([O_2]/H - [DO])}{F\left(\left[S_2O_3^{2-}\right]_{in} - \left[S_2O_3^{2-}\right]_{out}\right)}\end{aligned} \tag{7}$$

where 3.5 corresponds to the molar conversion factor for the oxygen and thiosulfate ratio (112.2/32), K_La is the overall volumetric mass transfer coefficient of oxygen (h^{-1}), which is a function of the trickling liquid rate (11.2 and 23.3 h^{-1}), $[DO]^*$ is the DO concentration at the gas–liquid interface (g L^{-1}), $[DO]$ is the DO concentration measured in the trickled liquid (g L^{-1}), $[O_2]$ is the oxygen concentration at the bulk gas phase (g L^{-1}), and H is the dimensional Henry coefficient, calculated at the operation temperature and corrected by AMM salinity.

Analysis

Daily samples were taken from the influent and effluent (points 13 and 15 in Fig. 1). The concentrations of thiosulfate were measured following a titrimetric method proposed by Rodier (1998). Sulfate was determined turbidimetrically (Clescerl et al. 1989). Biomass content in both packing material and the effluent was analyzed by the Lowry method. From previous experiments (not presented here), it was determined that 25 % of the dry cell weight corresponds to protein. Intermittently and at the end of the experimental period, some cubes (4 × 4 × 4 cm) from the colonized packing were removed and the biomass detached and determined. The removed packing, about 2 % of total volume, was replaced with clean cubes. The DO concentration in the trickled liquid was measured with a polarographic probe (Applisens, model Z01005902020; Applikon, Schiedam, the Netherlands).

Respirometric Test

The oxidation activity of the ASBC was evaluated using respirometric techniques at the beginning and end of the experimental period. The attached ASBC biomass was removed mechanically from the packing material and then centrifuged (at 3,600×*g*), washed, and re-suspended in AMM without thiosulfate. The respirometric experiments were performed in a jacketed glass chamber (2.2 mL) connected to a polarographic oxygen probe (YSI 5331; YSI Co., Yellow Springs, OH, USA). Thiosulfate was used as a substrate at concentrations ranging from 1 to 10 mmol L^{-1} (González-Sánchez and Revah 2007). The tests were performed as follows: the chamber was maintained at 30 °C and filled with air-saturated AMM (30 °C, 78 kPa) and a cell suspension containing around 0.8 g protein L^{-1}. The endogenous respiration was monitored for few minutes, and then a concentrated thiosulfate solution was added to measure oxygen consumption.

Results and Discussion

Thiosulfate Removal

The BTF reactor was operated under transient conditions for 40 days. Figure 2a, b show the overall performance results obtained during the entire experimental period. Since the packing material was already inoculated with an alkaliphilic sulfide-oxidizing bacterial consortium, the

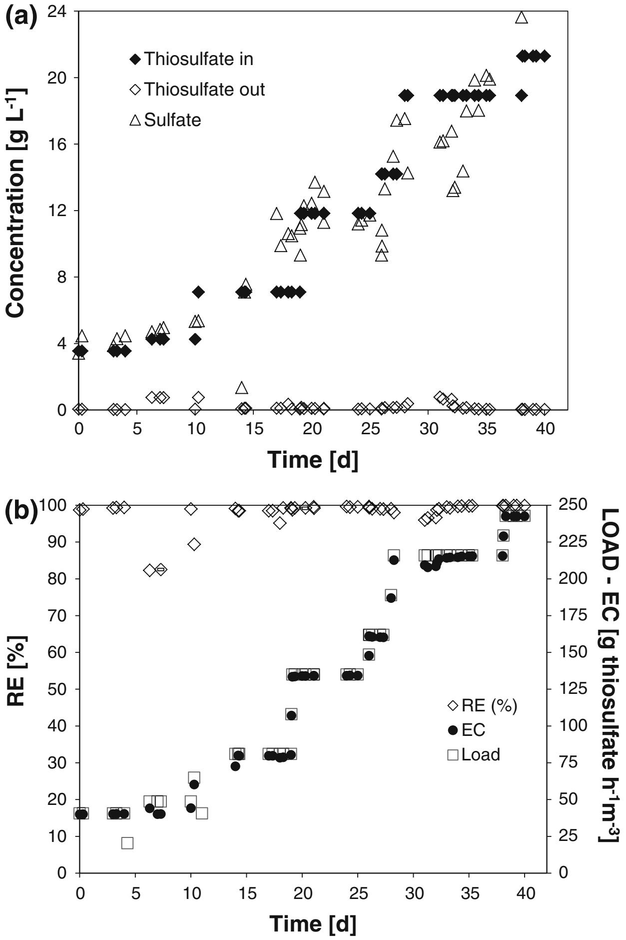

Fig. 2 Performance of thiosulfate biooxidation in the BTF. **a** Inlet and outlet thiosulfate concentrations, **b** thiosulfate removal efficiency, thiosulfate load, and elimination capacity

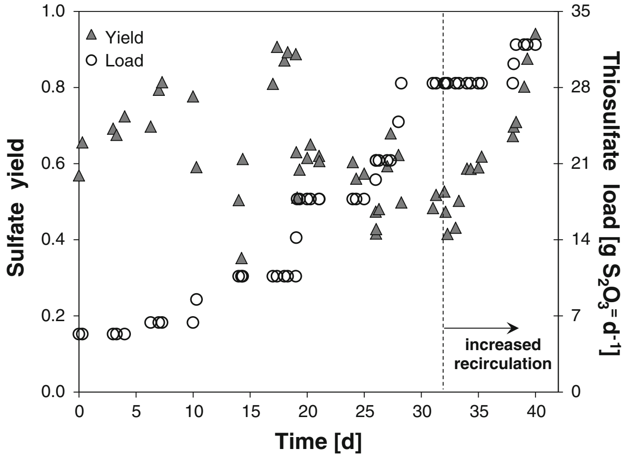

Fig. 3 Evolution of sulfate yield for the complete experimental period

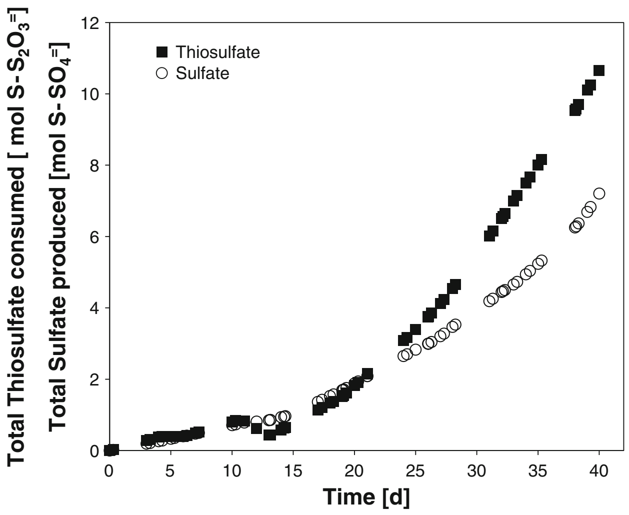

Fig. 4 Accumulated molar balances for oxidized thiosulfate and sulfate produced and recovered in the effluent

biofilter was operated under a variable increasing inlet concentration starting from 3.55 up to 21.30 g thiosulfate L^{-1}, similar to those encountered in mine wastewater. By setting the inlet flow rate at 1.5 L day^{-1}, the maximum thiosulfate load was 243 g thiosulfate m^{-3} h^{-1}.

Almost complete thiosulfate removal efficiency (an average of 98 %) was attained for the experimental period. Thus, the total EC overlapped the load (Fig. 2b), with a maximum value of 242 g of thiosulfate m^{-3} h^{-1}. This is greater than the $\approx$160 g of thiosulfate m^{-3} h^{-1} reported previously for reactors operating with suspended biomass at pH 7 or bioreactors packed with activated carbon as biofilm carrier working under acidic conditions and low temperatures (González-Sánchez et al. 2005; Liljeqvist et al. 2011).

Sulfate production was monitored to describe the extent of thiosulfate oxidation. The evolution profile of the sulfate yield, calculated according to Eq. (6), is depicted in Fig. 3. Two dynamic experimental periods can be identified: the liquid recirculation was increased from 0.89 to 1.91 L min^{-1} at day 32 to avoid oxygen limitation under high thiosulfate loads. The sulfate yield showed values of around 0.8 for several days during the first 20 days of operation, indicating that Eq. (2) predominated.

A slight decrease in the sulfate yield was observed from day 25, when inlet thiosulfate exceeded 20 g of thiosulfate day^{-1}. High yield values (up to 0.95) were once more attained after the trickled flow rate was augmented. Values lower than 0.5 for sulfate yield were seldom found when both the thiosulfate inlet load and the trickled liquid stream were increased, and can be attributed to the adaptation to transient conditions.

Solid elemental sulfur was not observed during the study and it is has been shown that it is not thermodynamically stable under alkaline conditions; it is disproportionated to other stable RSC species (González-Sánchez and Revah 2007; Liljeqvist et al. 2011). Accumulated mass balances in terms of moles of sulfur can be estimated from thiosulfate consumption and sulfate recovery in the effluent (Fig. 4). Until day 20, when inlet load was increased above 14 g of thiosulfate day^{-1}, practically all thiosulfate was recovered as sulfate. Although sulfate yield reached higher values (i.e. 0.95) at day 40 (Fig. 3), integral mass balances for the same experimental period involving inlet load rise indicated that thiosulfate was oxidized to other RSC species.

The evolution of pH in the system was determined by the relative rates of reactions 1 and 2; the natural tendency is for acidification. This may have a favorable effect for streams having pH values >10, as the acid produced allows the system to be maintained between pH 9 and 10, where the alkaliphilic bacteria have their optimum values. Furthermore, less acidification is promoted by favoring reaction 1 through the residence time and oxygen transfer control, as discussed later.

Oxygen Transfer

Figure 5 shows the relationship between oxygen consumption and thiosulfate oxidation, following Eq. (7). According to Eqs. (1) and (2), the stoichiometric molar ratio can vary between 0.5 and 2.0. A high dispersion in molar ratio rates values can be observed, revealing that thiosulfate oxidation in BTF is performed by the two aforementioned pathways. Under relative low inlet load, molar rates near 2 were observed, showing that thiosulfate is mainly oxidized to sulfate, confirming results depicted if Figs. 3 and 4. Decreasing molar ratio rates were detected as thiosulfate was increased (day 25). Molar ratio rates values recovered with higher oxygen availability when the recirculation rate was increased.

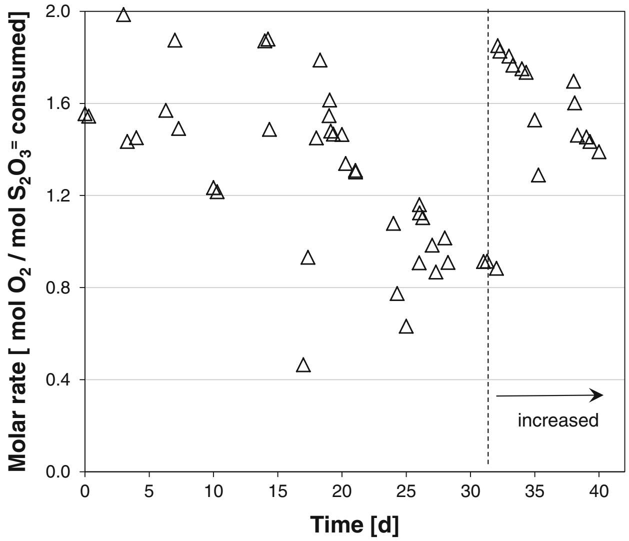

Fig. 5 Evolution of molar rate: moles of oxygen consumed by moles of thiosulfate oxidized

Oxygen transfer capacity of the system was examined in terms of thiosulfate EC (Fig. 6). Results showed that system was able to supply oxygen as the thiosulfate load was raised. This indicates that the system has the potential to reach higher elimination capacities since oxygen transfer rate can be controlled to lead the thiosulfate oxidation to total or partial conversion to sulfate. Also, the calculatied experimental slope for the entire experiment was 1.37 ± 0.35 mol of oxygen consumed/mole of thiosulfate oxidized, indicating again that the system has the capacity to oxide thiosulfate to mainly sulfate.

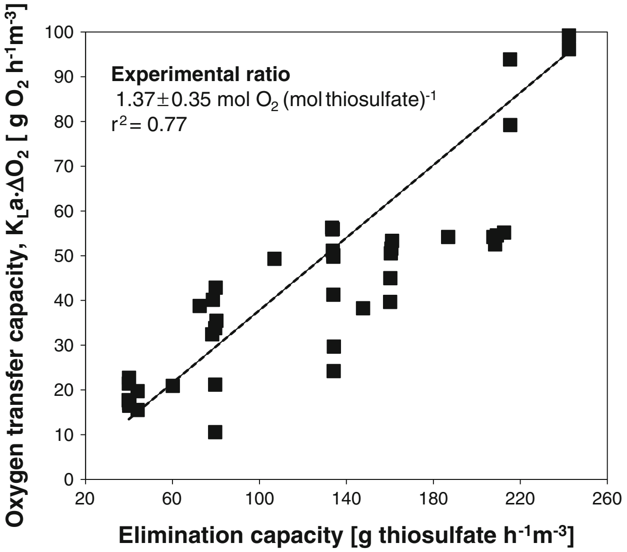

Fig. 6 Thiosulfate elimination capacity versus oxygen transfer capacity for total BTF

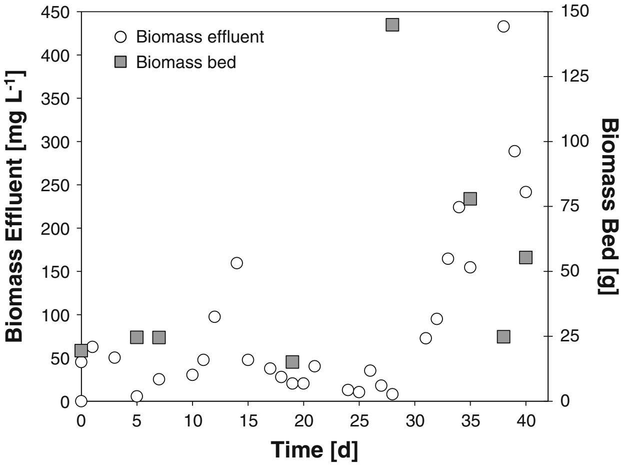

Fig. 7 Biomass concentration in the effluent and in the reactor

Table 1 Oxygen consumption rates for endogenous respiration and thiosulfate degradation at the beginning and end of the experimental period

Days	Endogenous respiration (mg O_2 L^{-1} min^{-1})	Oxygen consumption rate[a] (mg O_2 L^{-1} min^{-1})	Maximum specific oxygen consumption rate [mmol O_2 (g protein)$^{-1}$ min^{-1}]
7	0.1540	0.1773	0.0277
40	0.1783	0.8823	0.5046

[a] Oxygen consumption rate measured after thiosulfate pulse addition

Biomass Growth and Activity

Biomass growth and activity were monitored in both the packing material and the effluent. A slight biomass concentration increment in the effluent was detected after day 32 (Fig. 7), which is ascribed to high biomass growth resulting from the thiosulfate load and detachment caused by the increased recirculation flow. Biomass formation on packing increased conspicuously as thiosulfate inlet load reached values above 28 g day^{-1} and, despite the reduction on day 32, biomass activity increased markedly during the entire operation period. The experimental data for the microbial oxidation activity obtained from respirometric tests is shown in Table 1. As can be seen, the alkaliphilic sulfide-oxidizing bacterial consortium increased its volumetric oxygen consumption, due to both an increase in the specific rate and biomass content. Table 1 also shows that the biomass adapted to thiosulfate reached maximum values of specific oxygen consumption of 0.5 mmol O_2 (g protein)$^{-1}$ min^{-1}.

Conclusions

Transient performance of a BTF removing high thiosulfate loads under alkaline conditions was studied. The biological system was able to remove thiosulfate loads that emulated conditions encountered in wastewater from mining processes at greater rates than reported previously. Analysis of species generated revealed that most of the thiosulfate was oxidized to sulfate, and that the rate of thiosulfate biooxidation can be controlled by modifying the oxygen transfer rate. The treatment of thiosulfate contained in wastewater from mining processes using alkaline BTF with immobilized biomass appears to be a promising alternative in terms of elimination capacities and cheaper operating costs.

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