Process sustenance in an organic carbon exhausted chemo-bioreactor by sweetmeat waste dosing

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Abstract External organic carbon was added into nutrient exhausted spent mushroom compost (SMC) substrate in a reactor designed for sulfate reduction. Sweetmeat waste (SMW) supplementary was used as organic carbon dose. Tracer study established moderate dispersion in the reactor. Pulse dosing experiment showed that consistent 60 % sulfate reduction is possible even in a deficient matrix. Maintaining pH by $NaHCO_3$ in the reactor was found effective than using quick lime.

Keywords Compost reactor, nutrient exhaustion, external organic carbon dosing, sweetmeat waste

Introduction

Treatment of sulfate-rich waste water is a common practice in mining industries where sulfate, dissolved metals and low pH are a major concern. Passive remediation strategies have been adopted by scientists and researchers for low sludge production and cost effectiveness. Along with other microbial species, sulfate reducing bacteria (SRB) play a major role by reducing sulfate to sulfide with simultaneous alkalinity generation. Successive alkalinity producing systems (SAPS) have been remodelled as a chemo-bioreactor with a view to field application. Selection of the reactor matrix in these systems is related to the biodegradability of the material and longevity of the system. Mainly cellulosic and lingo-cellulosic waste materials have been used for their microstructure of microbial attachment and availability of readily exploitable nutrients. Plant-based waste materials have been characterized and successfully utilized (Coetser *et al.* 2006). These materials contain abundant lignin (10 to 28 %) with easily biodegradable substrates for biological sulfate reduction. Lignin is one of the recalcitrant substances, and its slow biodegradation by limited microbial species makes it suitable for preparation of a skeleton for biofilm development. In the reactor process, lignin present in the matrix acts as the long-term attachment site for microbes (Das *et al.* 2012), which reside where they can collect nutrients from their surroundings.

One of the limitations of using these plant waste based matrices in treatment systems is the depletion of available nutrients, especially organic carbon, after a limited period of use. Depending on the nature of waste used, available organic carbon was exhausted from these matrices after certain period of use (Coetser *et al.* 2006; Das *et al.* 2012). However, the lignin portion, which serves as the attachment site for active biofilm, remains unaltered. During operation, it is not feasible to replace the exhausted organic-carbon matrix due to the length of time required to establish an active biofilm. Introduction of an external supply of organic carbon into the reactor is a preferable engineering option to sustain the reactor.

Selection criteria for an external organic carbon source for the reactor include the biodegradability, availability and cost effectiveness of the material. Pure and synthetic materials are reported to be readily utilized by SRB (Das *et al.* 2013). However, use of pure or synthetic materials at the large scale of the reactor would be cost prohibitive. Recently, food waste has been tested as a possible alternative because it is readily available at low cost in most areas (Das *et al.* 2013).

Sweetmeat waste (SMW) is produced in high amount in the Indian subcontinent. These materials contain high sucrose along with readily SRB degradable organic acids (Das *et al.* 2013). Successful application of SMW has been tested in batch mode for biological sulfate reduction with suitable nitrogen supplementation. Sulfate reduction is reported to occur in a continuous reactor with sucrose as the sole source of organic carbon (Lopes *et al.* 2010; Bertolino *et al.* 2012). Sulfidogenesis and acidogenesis can take place simultaneously in sucrose fed reactor without any observed interference (Mizuno *et al.* 1998). However, a pH balance is always preferable to prevent the process shifting towards acidogenesis.

The objective of the study was to investigate the possibility of performance continuation in an organic carbon exhausted spent mushroom compost reactor using an external supply of sweetmeat waste as the organic carbon source. Efficacy of quick lime and $NaHCO_3$ as the neutralizing agent was also tested during the dosing process.

Methods

The tubular reactor (58 × 3.85 cm) used in this study was made of an acrylic sheet with steel adaptors at both ends (Fig. 1). The working volume in the acrylic sheet column was 674.868 mL. A stainless steel mesh (1 mm pore diameter) was placed in the junction for better flow dispersion. Butyl rubber tubing was used over the mesh to minimize the oxygen flux. All joints of the reactor were sealed with Teflon tape. Two ports, one for dosing and another for sampling, were situated in the adapters, closed with a removable rubber cork. Flow was regulated with a peristaltic pump. Silicone tubing with stoppers was used to connect the reservoir, column and effluent container.

The column was packed with 80 g of autoclaved spent mushroom compost, collected from a household farm after mushroom cultivation. Porosity of the reactor bed was calculated as 0.6347, leaving 428 mL of void volume with a mass density of 0.1185 g/cm^3.

Tracer pulse input was used to obtain the residence time distribution (RTD) characteristics of the SMC packed column. A similar column, packed with same amount of SMC, had a porosity of 0.6293. It was washed with 50 bed volumes of Milli-Q water (conductivity of 0.33 µS/cm) with a hydraulic retention time (HRT) of 18 h. A conductivity tracer test was performed using a NaCl solution (3.5 g/L) injected at the bottom dosing port using a Dispovan syringe. The flow rate during tracer test was 41 mL/min, resulting in a theoretical HRT of 626.25 seconds (0.17395 h). The conductivity at the outlet was measured online using a conductivity probe in a multi-parameter water analyzer.

The inoculum was previously acclimatized with sweetmeat waste (SMW) containing media for 6 months. Inoculum used for the reactor was maintained in anaerobic water (100 mL/L reducing agent supplement and 2.1 g/L NaCl) with 1 mM bromoethane sulfonic acid containing a total cell count of 2.26×10^{11} ($\pm 8.256 \times 10^{9}$)/mL, with SRB of 6.89×10^{8} MPN/mL and fermentative bacteria of 1.64×10^{8} MPN/mL, respectively. Reducing agent supplement (RAS) was prepared with

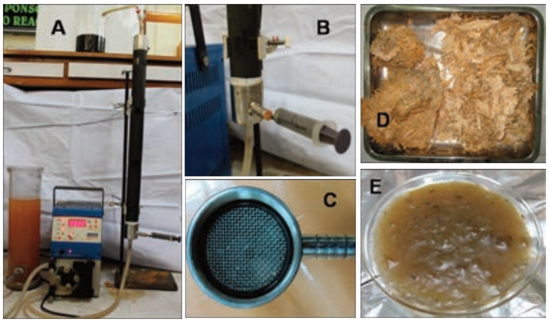

Fig. 1 *Column reactor used in the experiment. (A) The reactor during continuous operation with dosing; (B) Dosing port; (C) Adapter with steel mesh and butyl rubber tubing; (D) Spent mushroom compost; and (E) Sweetmeat waste.*

7.5 g/L of both Na-thioglycollate and ascorbic acid.

After packing, the column was purged with N_2 (99.99 %) Three bed volumes of RAS (pH 7.5) containing 7.5 g/L each of ascorbic acid and Na-thioglycollate were passed slowly through the column, with HRT maintained at 36 hours. Inoculation of the reactor was done by passing two bed volumes of inocula through the colum. To facilitate biofilm development, the reactor was covered with black paper to prevent light penetration, and maintained at batch mode for 3 weeks.

Continuous operation of the reactor was started after the static stage with an initial HRT of 36 hours, and was gradually decreased to a HRT of 18 hours after four days. Feed solution was composed of (g/L) Na_2SO_4, 2.1; $FeSO_4.7H_2O$, 0.025; $MgCl_2.6H_2O$, 0.125, and pH 7.2±0.1 was maintained by using a NaOH solution. The operation continued for 3 months, after which the operation was ended due to lack of sulfate reduction.

Dosing of SMW as an external organic carbon was initiated at that time. The reservoir solution was prepared as follows (g/L): Na_2SO_4, 2.1; $FeSO_4.7H_2O$, 0.025; $MgCl_2.6H_2O$, 0.125; trace-element solution, 10 mL; pH was maintained at 7.4 by adding 1.4 g/L $NaHCO_3$. This experiment continued for 30 days.

For the evaluation of an improved alkaline supplement, another 35 day experiment was performed replacing $NaHCO_3$ with quick lime in the reservoir sample. Exactly 0.48 g/L quick lime was required to provide the desired pH (7.4). However, a slight change in the quick lime concentration was found to result in a drastic change in solution pH; as a result, the reactor was operated with a $NaHCO_3$ neutralized reservoir solution for 15 days.

HRT was kept at 18 h throughout the dosing experiment, resulting in a superficial flow velocity of 1.84 cm/h. The mass SO_4^{-2} loading rate was 816±4 mg/d. In every 24 h interval, 10 mL of SMW solution was dosed into the reactor through the dosing port using a sterile Dispovan Syringe. The dosing solution was prepared with filtered 1:10 (w/v) SMW solution comprised of (per litre): NH_4Cl, 17.549 g; K_2HPO_4, 1.679 g and vitamin solution, 15 mL. The dosing solution had chemical oxygen demand (COD) of 81600 mg/L, and hence, the ratio of mass COD dosing/d to mass SO_4^{-2} loading/d was maintained at 1.

Filtered (0.22 µm cellulose nitrate membrane) samples were used for analytical purposes except for pH and conductivity. Sulfate concentrations, acidity (to pH 8.3 by NaOH), alkalinity (to pH 4.5 by HCl), chemical oxygen demand (COD; spectrophotometric), dissolved sulfide (titrimetric) and total organic carbon (TOC) were the parameters analyzed in the experiment.

Results and Discussion

Hydraulic characteristics of the reactor were studied throughout the pulse input tracer test. The test was carried out in triplicate, mean values were taken and tracer response curves were prepared. Each curve was analyzed for mean residence time and variance, and the dispersion number (d) was calculated (Metcalf and Eddy 2003) to be 0.07583, indicating moderate dispersion in the reactor. Lack of dispersion might allow maximum time for substrate conversion; however, it can also inhibit bacterial activities due to high substrate concentration. On the other hand, high dispersion might result in short-circuiting. Hence, an intermediate level of mixing could be preferable.

Continuous flow was started after 3 weeks of static period. At the end of the static mode there was high alkalinity, pH, dissolved organic carbon (DOC), and dissolved sulfide present in the effluent with almost all sulfate reduced (Fig. 2).

Released DOC facilitated the biofilm development and sulfate reduction. By SRB metabolism, bicarbonate was produced that subsequently increased the alkalinity of the water. After initial inequilibrium due to flow continuation, the reactor produced enhanced performance when it was able to reduce 85 to 96 % of influent sulfate. Adequate DOC and dissolved sulfide was also observed in the effluent during this period. However, after 11th week, sulfate reduction process slowed down resembling lesser DOC content. Exhaustion of SRB degradable DOC, halted the process after some instances is a common phenomenon in SMC packed reactor reported earlier (Das *et al.* 2012).

Earlier we proposed external addition of organic carbon into an exhausted SMC reactor would sustain the process. SMW was characterized for this purpose. It contained high sucrose along with easily SRB utilizable organic acids. Adequate sulfate reduction was reported with SMW as organic carbon source in batch study (Das *et al.* 2013).

SMW media was added as pulse dose, initially into the reactor at that point of time. Theoretical HRT was set for 18 h and the dosing interval was maintained at 24 h. Sulfate loading rate was kept constant. Sulfate concentration in the effluent started to decrease gradually after the dosing experiment started (Fig. 3).

For the first 7 days of the dosing experiment, sampling was performed as follows: in each 2 h interval up to 16th hour after dosing, each 30 min interval for 16th to 21st hour and each 1 h interval from 22nd to 24th hour. Maximum sulfate reduction was achieved between 17.5 h to 20 h after dosing, peaking at the 19th hour. The sample taken during the 18.5 h to19.5 h exhibited maximum sulfate reduction.After each dosing, effluent samples were continuously taken for 24 h till the next dosing. The grab samples were taken from time to time account for other analyses. Throughout the experiment, when reservoir pH was maintained by NaHCO$_3$, maximum sulfate reduction was more than 60 % and overall sulfate reduction was near 60 % (Fig.3A). pH of the effluent was maintained consistently near 8.0, with high alkalinity ranging from 800 to 1100 mg/L as CaCO$_3$ and little acidity of 10 to 30 mg/L as CaCO$_3$ (Fig.4).

Sulfate reduction of 350 to 470 mg/d in average was achieved against 550 to 650 mg/d COD and 150 mg/d TOC consumption during this experiment. Dissolved sulfide in the effluent was 90 mg/L in average (Fig. 3B).

After the acceptable sulfate reduction with external SMW dosing, the reservoir solution composition was changed to observe the effect of the neutralizing compound, and NaHCO$_3$ was replaced with quick lime. However, during the course of the experiment, sulfate reduction did not reach the desired level, with the maximum sulfate reduction value hardly reaching 20 %, and overall sulfate reduction was around 10 % (Fig. 5A).

This observation can be explained by the buffering difference of the two reservoir sam-

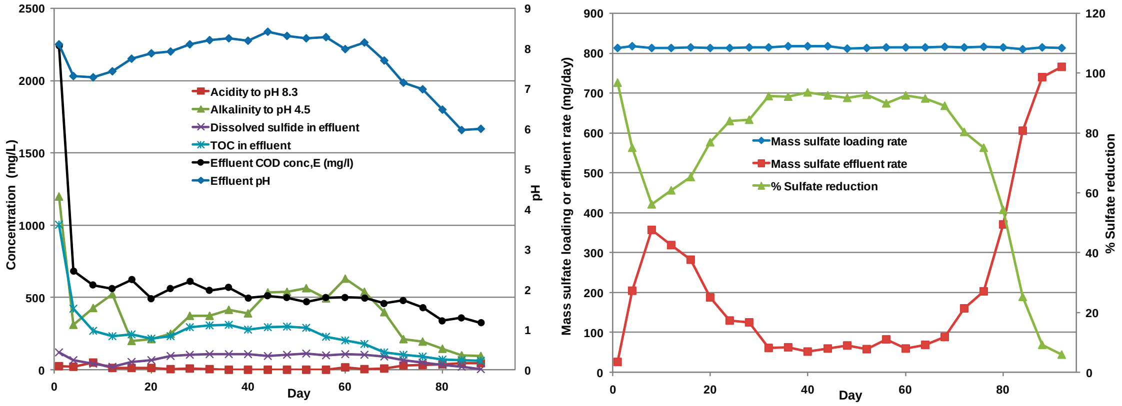

Fig. 2 *Effluent characteristics of the reactor without external organic carbon dosing (A) pH, acidity, alkalinity, chemical oxygen demand (COD), dissolved organic carbon (DOC) and dissolved sulfide; (B) Mass loading and effluent rate of sulfate and sulfate reduction(%).*

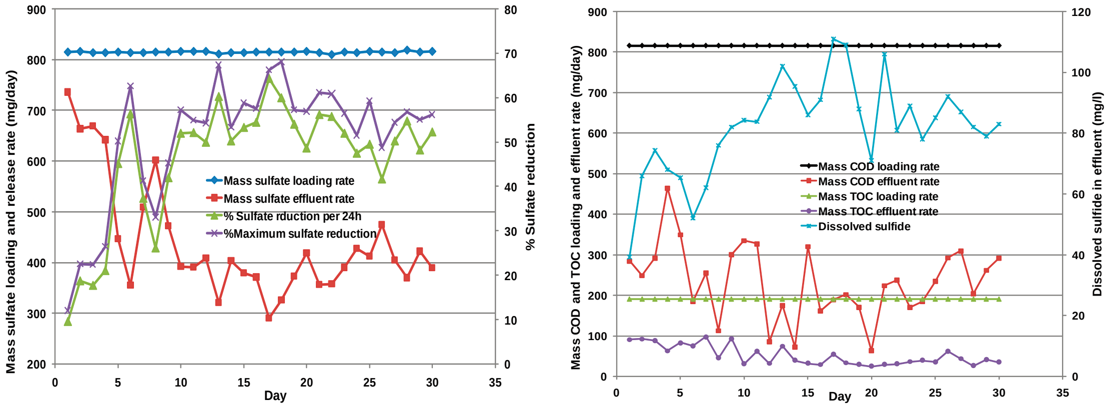

Fig. 3 *Parameters during 1–30 day of dosing experiment (pH in the reservoir maintained with NaHCO₃). (A) Mass sulfate loading and effluent rate with sulfate reduction (%) (B) Mass COD and TOC loading and effluent rate with effluent dissolved sulfide concentration*

ples. In $NaHCO_3$ supplemented reservoir solution, acidity and alkalinity content were 29.375 ±0.625 and 665.625± 5.413 mg/L as $CaCO_3$. These values were 1.146±0.1 and 13.958±0.21 mg/L as $CaCO_3$ respectively when the solution pH was maintained with quick lime. Also, bicarbonate is a better buffer in the pH range of the experiment. In sucrose and sulfate rich media, acidogenesis and sulfidogenesis occurred simultaneously. Fermentative bacteria target sucrose and produce volatile fatty acids (VFA), thereby reducing the solution pH. Sulfidogenesis on the other hand neutralizes the pH by oxidizing these VFA and producing bicarbonate alkalinity (Bertolino *et al.* 2012). In the dosing experiment, initial acidity produced by fermentative bacteria was neutralized by alkalinity in form of $NaHCO_3$. That effectively allowed SRB to perform. However, quick lime could not counter the initial acidity production due to its low alkalinity, hence could not in any case maintain the pH near neutral (Fig. 4). SRB are reported to work in the pH range of 5 to 9 (Mizuno *et al.* 1998). As the pH decreased due to increasing acidity, sulfate reduction also declined gradually (Fig. 5A). Most of the consumed COD (around 600 mg/d) and TOC (around 150 mg/d) were not utilized in sulfidogenesis. Therefore, low dissolved sulfide was produced (Fig. 5B).

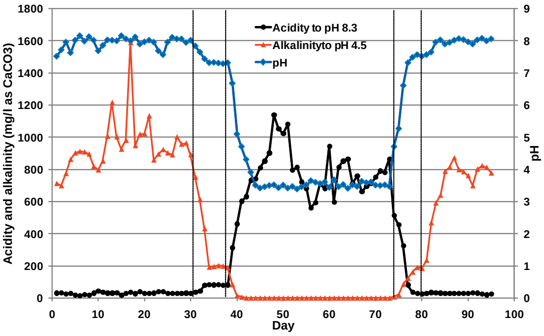

Fig. 4 *Acidity, alkalinity and pH of the effluent during dosing experiment. Day 1- 30 and 81- 95, reservoir pH maintained with NaHCO₃ day 39-73, reservoir pH maintained with quick lime; day 31–38 and 74–80, reservoir filled with only 2.1 g/L Na₂SO₄ without any external dosing.*

Confirmation of the mentioned phenomenon was tested by reintroduction of $NaHCO_3$ into the reservoir. When quick lime in the reservoir solution was replaced with $NaHCO_3$, sulfate reduction started steadily again and reached maximum. Dissolved sulfide in the effluent also increased gradually (Fig. 6).

Conclusions

SMW was tried as external organic carbon dose into a nutrient exhausted reactor treating sulfate-rich wastewater. The study revealed that

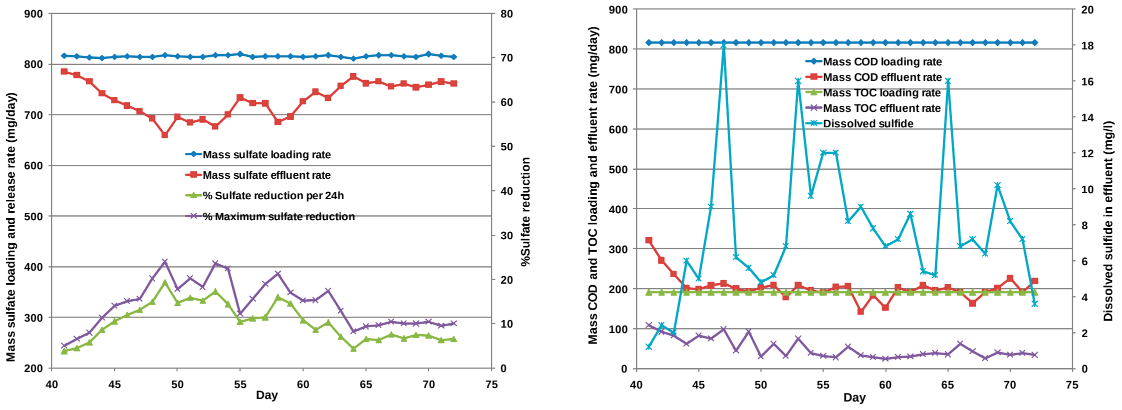

Fig. 5 *Parameters during 39–73 day of dosing experiment (pH in the reservoir maintained with quick lime). (A) Mass sulfate loading and effluent rate with sulfate reduction (%) (B) Mass COD and TOC loading and effluent rate with effluent dissolved sulfide concentration*

filtered SMW can be used with buffering for desired results. As a pH buffer, $NaHCO_3$ was found more effective than quick lime. Around 60 % sulfate reduction was observed when mass loading ratio of COD to SO_4^{-2} was set as 1. The study shows rejuvenation of ineffective SAPS is possible with external supplementary dose.

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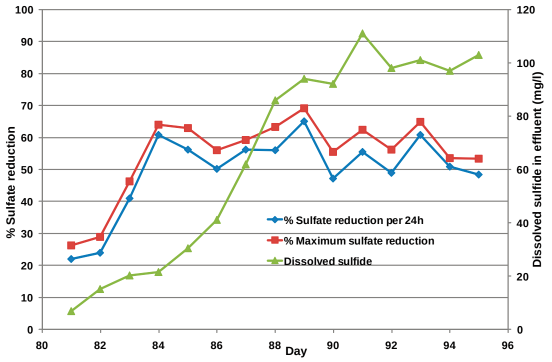

Fig. 6 *Sulfate reduction (%) and dissolved sulfide concentration in the effluent when reintroduced into the reactor with* $NaHCO_3$ *buffered influent (81–93 day of dosing experiment).*

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