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Developing a Biofilm of Sulfur Oxidizing Bacteria, Starting-up and Operating a Bioscrubber Treating H_2S

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Abstract: Development of an acclimatized SOB biofilm, startup and performance of a fixed bed bioscrubber packed with corrugated tube parts as a media having high specific surface area was investigated. Bioscrubber was a cylindrical Plexiglas air-and water-tight column with 10 L in working bed volume. Sludge from a tannery wastewater treatment plant was used as a seed for SOB separation, acclimation and enrichment. Enriched acclimatized SOB were applied as inoculum for biofilm development, which was carried out by recirculating the prepared microbial suspension through the bed. Thickness of the developed biofilm was 56 μm in which active acidophilic autotrophic H_2S oxidizing bacteria were completely predominated. Activity measurements showed highest biodegradation rate of biofilm at liquid pH around 3. Due to employing an efficient specialized biofilm, startup period of the reactor was quite short and H_2S removal efficiency just 12 h after starting up reached above of 92% and increased to 96% at day 3 of starting up while inlet H_2S concentration gradually was increased to around 30 ppm. At the end of start up pH of the recycle liquid was modified to the optimal value of 3±0.5 in which biofilm demonstrated the highest activity in terms of OUR after which removal efficiency increased around 3% while other operating conditions were consistent. Furthermore, performance of the bioscrubber was evaluated at various inlet H_2S concentrations ranging from 30 to 150 ppm_v. It was indicated that the inlet H_2S concentrations in studied range did not affect the performance of the bioscrubber so that the removal efficiency of H_2S was greater than 99.4% at all concentrations. These observations suggested that the development of an efficient specialized SOB biofilm on a media with high specific surface area will decrease the startup course and achieve high removal efficiency in the bioscrubber treating H_2S. In addition, operation in acidic recycle liquid will overcome use of alkaline to adjust the pH, which reduce the operation cost of the control system.

Key words: Biotechnology, bioscrubber, biofilm, sulfur-oxidizing bacteria, hydrogen sulfide

INTRODUCTION

Over the past few decades, the impact of air pollution on human health and the environment has received an increasing amount of attention from the public, the government and industry. As a result of increasingly stringent regulations governing releases of different pollutants such as hydrogen sulfide (H_2S) research into developing new air pollution control technology and improving existing technologies has grown dramatically (Shareefdeen and Singh, 2005). Biological treatment of the contaminated air is an emerging and environmentally friendly technology for air pollution control that represents a major development towards reducing industrial air pollution (Zuber *et al*., 1997; Deshusses and Cox, 1998).

Biotechniques for treating polluted air streams include biofilter, bioscrubber, biotickling filter, activated sludge diffusion, membrane bioreactor, fluidized bed reactor and miscellaneous processes (Smet *et al*., 1998; Burgess *et al*., 2001; Chung *et al*., 2001; Moussavi *et al*., 2005a). Among them, bioscrubbers because of existing a free liquid phase have proven superior for removing such acid-producing pollutants as H_2S (Smet *et al*., 1998).

Different Sulfur Oxidizing Bacteria (SOB) under a wide variety of conditions can accomplish biodegradation of H_2S (Smet *et al*., 1998). Most attention has been paid to *Thiobacillus* spp. that is authotrophic and can oxidize H_2S as a sole energy source and fix CO for the carbon source to sulfate (Shinabe *et al*., 1995). Due to using the synthetic media, inoculation of the bioscrubbers with an appropriate culture is always required. On the other hand, since microorganisms play a vital role in a bioreactor, development of a biofilm containing enough active SOB can achieve rapid start-up and high removal efficiency in a bioscrubber treating H_2S. Rapid starting up a treatment

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processes will minimize releasing the pollutant(s) to the environment.

Bioscrubber application can be more attractive than the use of the conventional biofilter or biotrickling filter at relatively high pollutant concentration. A potential disadvantage is the longer start up time and this may be overcome by using an inoculum of an acclimated culture (Shareefdeen and Singh, 2005).

In this study acclimation and enrichment of SOB and development of an efficient SOB biofilin on surface of media with the aim of optimization of bioscrubber to achieve in high performance in H_2S removal have been presented.

MATERIALS AND METHODS

Bioscrubber setup and operation: The schematic experimental setup of the bioscrubber investigated is given in Fig. 1. Bioscrubber was a Plexiglas cylindrical column packed with a high specific surface area media, which were 15 mm parts of corrugated tube with 625 m^{-2} m^{-3} in specific surface area. Main dimensions of the investigated bioscrubber were as follows: inner diameter, 13.5 cm; total height, 120 cm; bed height, 70 cm; bed volume, 10 L. Bioscrubber was air-and water-tight and operated in countercurrent flow direction. To reduce the channeling of the fluids in the reactor, a perforated plate was installed in the middle of the bed which was served for redistribution of air and recycle liquid across the bed.

Mixing the compressed oil-free air with a concentrated H_2S flow from a costume-made H_2S generator produced air stream containing H_2S. Concentration of H_2S in the inlet air was regulated by adjusting the concentrated H_2S flow rate. Inlet airflow was also controlled at the desirable rate by a flow meter provided on the inlet line. The synthetic waste air stream was introduced to the bioscrubber at the bottom of the column in where flowed upward while liquid phase moved downward (countercurrent flow). Two air sampling ports were provided on the inlet and outlet lines to monitor inlet and outlet H_2S concentrations and to determine the bioscrubber performance in terms of H_2S removal efficiency. Liquid solution was recirculated trough the bed by a centrifuge pump. A dosing pump injected the nutrient solution in rate of 2.5 L day^{-1} from a solution container to the recirculation line to supply the required microorganisms' nutrients. Nutrient solution was prepared by dissolving 0.5 g KH_2PO_4, 1 g K_2HPO_4, 0.5 g NH_4Cl, 0.25 g $MgCl_2.6H_2O$ and 1 mL trace element solution in 1 L tap water. Liquid phase pH was controlled at the desired value by alkalinity and bufferic capacity of the injected liquid.

After development of the SOB biofilm on the surface of media, bioscrubber was started up with introduction of air containing H_2S to the reactor. Inlet concentration during start up was progressively increased from 3 to 30 ppm. When removal efficiency did not change significantly versus time, start up was considered to be done. Then the reactor was switched to the next step of the experiment. During the entire period of the study, bioscrubber was operated at room temperature (20-25°C). H_2S was the sole SOB energy source and there was no external carbon source except CO_2 from the air and carbonates from the nutrient solution. Main characteristics and operation parameters of the bioscrubber and values are presented in Table 1.

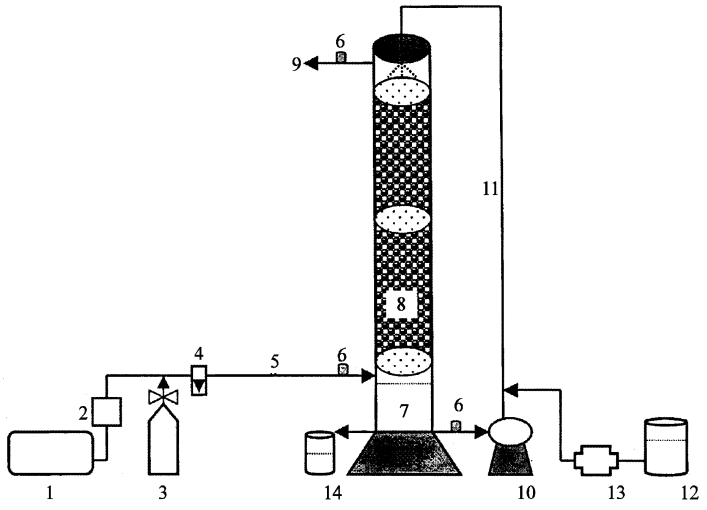

Fig. 1: schematic drawing of bioscrubber experimental setup (1-compressor, 2-GAC column, 3-H generator, 4-flow meter, 5-inlet line, 6-sampling port, 7-liquid sump, 8-bed, 9-outlet line, 10-recirculation pump, 11-recirculation line, 12-nutrient solution container, 13-dosing pump, 14-waste liquid container)

Table 1: Characteristics and operation parameters of bioscrubber

Characteristic/parameter	Unit	Value
Air flow rate	L min^{-1}	30
Inlet H_2S concentration	ppm_v	3-50
EBRT	sec	30
Volunetric loading	$m^3 m^{-2} h$	84
H_2S loading	g H_2S $m^{-3} h$	8.3-25
Recycle liquid volume	L	5
Recycle liquid flow rate	L min^{-1}	0.5-1
Nutrient solution injection	L day^{-1}	2.5
Liquid retention time	day	2
Recycle liquid pH	-	2.5-4.5
Gas/liquid flow	-	Countercurrent

Preparation of acclimated SOB biomass: The purpose of this stage was separation, acclimation and enrichment of SOB to make sure that there are sufficient H_2S oxidizing bacteria in the bioscrubber inoculum. Since there is likely to be many SOB in the tannery wastewater treatment system, sludge from a local tannery wastewater treatment plant was taken and used as a starter culture to prepare SOB biomass. SOB acclimation and enrichment was accomplished in a bench scale activated sludge process. Procedure employed has been described in detail elsewhere (Moussavi *et al*., 2005b).

Inoculation and Development of SOB biofilm in bioscrubber: After acclimation of SOB and production of an active suspension containing SOB as the predominant microbial group, bioscrubber was inoculated with enriched SOB suspension. To do this, microbial biomass was thickened and then resuspend in 5 L nutrient solution giving 3500 mg L^{-1} MLSS concentration into which sulfide solution was added to supply energy source of H_2S oxidizing bacteria. Microbial suspension was then poured into the sump of bioscrubber and was recirculated across the media by a centrifuge pump with a flow rate of 0.5 L min^{-1}. Inlet airflow rate to bioscrubber during inoculation and the biofilm development was adjusted at 20 L min^{-1}. To determine how much biomass has been immobilized onto the media, MLSS concentration of recycle liquid was daily monitored by sampling from recirculation line. Inoculation was considered to be completed when MLSS of recycle liquid dropped off under 450 mg L^{-1}. After that the bioscrubber was started up with injection of air containing H_2S.

Biofilm thickness: Total biofilm thickness was determined according to Horn *et al.* (2003) based on the total biomass attached to the entire media in the bioscrubber with assumption evenly distributed biomass on the surface. One month after inoculation and biofilm development, top of the bioscrubber was opened and 100 parts of media were taken out, drained, weighted and the wet biomass attached to them were calculated from the difference of the wet mass of the taken media and the dray mass of an equal number of clean media parts. Total biomass in the bed was derived from product of wet biomass on one part of media and total number of media parts packed in the bioscrubber. Volume of the wet biomass was then calculated from the mass of the wet biofilm assuming a wet biofilm density of 1 g (Alonso *et al*., 1999; Horn *et al*., 2003). Finally, biofilm thickness was obtained by dividing the wet biofilm volume in the bed by the total surface area of the media in the bioscrubber.

Microbial count: The diversity of the microbial groups in the developed biofilm and the recycle liquid was investigated by plating technique based on colony forming unit, with the aim of demonstrating the predominate group of bacteria in the biofilm. In this step, two microbial groups of heterotrophics and autotrophic sulfur-oxidizing bacteria were quantified. For recycle liquid, 1 mL aliquots from 10 times dilution series solution of recycle liquid in 9 g L^{-1} NaCl solution taken from recirculation line was used to culture. For microbial tests of the biofilm, media taken out from the bed for biofilm thickness measurement were detached and then suspended in 1 L of the nutrient solution. Samples for microbial cultures were prepared by serial 10 fold diluting of this biofilm suspension and aliquots of 0.5 mL were used for microbial count. Measurement of microbial count was carried out in triplicate.

Biofilm activity test: Biofilm activity measurements was cried out to confirm being active of immobilized SOB on the surface of the bioscrubber media and find out the optimum pH of recycle liquid in which microorganisms and as a result biofilm can achieve the highest rate of H_2S biodegradation. Activity of the microorganisms in the biofilm were measured in different pHs ranging from 2 to 7 in which Oxygen Uptake Rate (OUR) was employed as the activity criterion. Sample for OUR tests were taken from the biofilm suspension prepared for microbial analysis and count. OUR tests were carried out in an Erlenmeyer equipped with an oxygen probe. For each test 100 mL of biofilm suspension were poured into the vessel, pH was adjusted at the desired value by NaOH or HCl solutions and suspension was saturated from oxygen. Then the same volume of stock 2 solutions was added to the sample and immediately residual dissolved oxygen concentration was monitored over the time. Test was ended when DO dropped to around 1 mg L^{-1}. Total OUR was then calculated according to the consumed oxygen, elapsed time and sample volume and recorded as mg O_2 L^{-1} min^{-1}.

ANALYSIS

Samples for H_2S measurement in the inlet and outlet air of the bioscrubber were taken by purging a measured volume of the air trough an alkaline suspension of cadmium hydroxide (Lodge, 1990). Sampling set up was consisted of two impingers, an air flow meter, an air vacuum pump and tubing and valves. For H sampling, 50 mL of alkali absorption solution were pipeted into each impinger. To protect them from light effect, impingers were wrapped up entirely in aluminum foil. Sampling airflow rate was 1 L min^{-1} and sampling time was in range of 5 to 30 min depending on the H_2S concentration. After sampling, prepared impinger solutions were analyzed for absorption fraction at wavelength of 670 nm using Perkin Elmer Lambda 25 spectrophotometer. A calibration curve was prepared using sulfide standard solutions. Then, H_2S concentration in the air sampled was computed according to the sample absorption value and calibration curve. Recycle liquid samples taken from recirculation line sampling port were analyzed for total sulfide, sulfate, MLSS, ammonia nitrogen, phosphorus and pH. Liquid pH was measured by a calibrated pH electrode. All other parameters were measured according to Standard Methods (APHA *et al*., 1998). Live heterotrophic bacteria and authotophic sulfur oxidizing bacteria counting were carried out by 10 times dilution series of biofilm suspension and recycle liquid in 9 g L^{-1} NaCl and plating on to two different media using pour plate method (APHA *et al*., 1998). SOB were cultured on thiosulfate agar and Heterotrophic bacteria were counted on nutrient agar media. Sulfide solution was added into the thiosulfate agar media to supply energy source for SOB. Colonies formed on selected plates were counted after incubation and results were reported as CFU per milliliter of sample.

RESULTS AND DISCUSSION

Acclimation and enrichment of SOB: Three liters thickened active sludge taken from a local tannery wastewater treatment plant was used as a SOB seed for acclimation and enrichment stage, which were accomplished in a 10 L bench scale activated sludge process operated in a batch mode. After adding the seed to the reactor and filling it up with nutrient solution into which Na salt was dissolved to supply source of energy for SOB, microbial suspension was started to aerate. Aeration provided oxygen demand and mixing. Then pH, total sulfide and sulfate of the mixed liquor were daily monitored over aeration time. Figure 2 indicates the results of pH and sulfate variations of the suspension

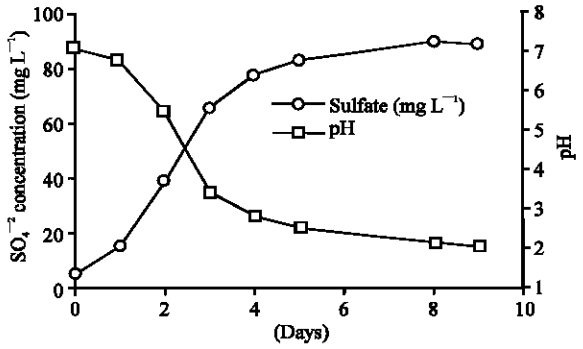

Fig. 2: Variation of pH and sulfate versus time during acclimation of sulfur oxidizing bacteria

versus the aeration time. As it can be seen in Fig. 2, sulfate concentration increased from initially 5 to 88 mg L^{-1} while aeration was preceded to day 9, although the highest microbial activity was at first 4 days so that after that concentration of sulfate did not change significantly. While sulfate was increasing, pH dropped of from 7.1 at the beginning of the aeration to 2 after 9 days aeration. pH reduction is due to 11 production from H biodegradation. When sulfate concentration of the microbial suspension did not change significantly, the acclimation stage was ended.

The increase of sulfate and as a result the decrease of pH of microbial mixed liquor with the increase of aeration time shows that the seed used has had SOB and they have quickly acclimated to metabolize sulfide in high concentration as a sole electron donor and oxidize that to the H_2S as a biodegradation end products. Microbial examinations on thiosulfate and nutrient agar media confirmed the presence of SOB in the suspension as a predominated group of microorganisms.

Since conversion of sulfide to sulfate in biodegradation pathway yield more energy than the formation of other products like sulfate and elemental sulfur, SOB prefer this biodegradation route (Janssen *et al*., 1999). From production of sulfate and reduction of pH it can be concluded that the biodegradation of sulfide in the suspension has been completed meaning the high activity of the acclimated SOB. Pinjing *et al*. (2001) reported a 4-5 days acclimation period for the H_2S metabolizing microorganisms.

To growth and increase the SOB biomass to be used as an inoculum in bioscrubber, after acclimation mixed liquor was allowed to settle, supernatant was poured out and reactor re-filed up with nutrient solution while sulfide solution was added into that as sole source of energy for SOB and the aerated. This procedure was repeated three times for 3 days each. After enrichment total mass of SOB was mg based on dry mass.

Biofilm development: When the acclimated SOB were enriched, the produced biomass was thickened and then resuspend in 5 L nutrient solution. MLSS of the resulted SOB suspension was 3200 mg L^{-1}. SOB energy source was supply by adding the sulfide solution into the inoculum suspension. Microbial suspension then was started to recirculate in 0.5 L mm^{-1} flow rate through the bed in a batch mode which bacteria started to immobilize on the media. During recirculation sulfide solution including the required nutrient was added to the reactor sump to supply nutritional requirement of SOB while MLSS concentration as the SOB biomass criteria was monitored daily based in liquid samples taken from recycle line. After 30 days recirculation, MLSS was dropped off to the about 450 mg L^{-1}. By calculation and ignoring the synthesis of SOB, around 4500 mg microbial cells in terms of dry mass had been immobilized on the bioscrubber media. Because the media before packing had passed a soaking stage in a SOB suspension, total biomass on attached to the media was more than this amount (see below). Monitoring the microbial suspension during biofilm development process indicated high sulfide removal efficiency that could be related to sufficient active SOB in the bioscrubber, which gradually attached to the bed. It can be concluded from above that SOB biofilm has been established on the media and it is active as well.

Bioscrubber startup: After development of the biofilm bioscrubber was started up with introducing air containing 3 ppm_v H_2S as a sole elector donor for SOB which was gradually increased to 30 ppm on average and flow rate of 20 L min^{-1} that is corresponding to EBRT of 30 sec. During start up period flow rate of recycle liquid was set at 1 L min^{-1} (corresponding to velocity of 4.2 m^3 m^{-2} h^{-1}), pH of the liquid phase was adjusted at 4-4.5 and nutrient flow rate was maintained at 2.5 L min^{-1}. Figure 3 shows the results of H_2S removal efficiency during the startup stage. At it has been indicated, H_2S was removed more than 92% at 12 h after introducing air and startup. Removal efficiency increased to 96% at day 3 of starting up while inlet H_2S concentration gradually was increased to around 30 ppm. After third day of startup performance of the bioscarubber did not change significantly and considered to be steady. Average of H_2S removal efficiency in the steady state condition in the startup stage was 96.4%, resulting an elimination capacity of g H_2S m^{-3} h^{-1} in the bioscrubber bed. Mass balance calculations showed a complete oxidation of H_2S removed to the sulfate. High removal efficiency of H_2S immediately after start up can be related to be an acclimatized and active SOB biofilm on a high specific surface area media.

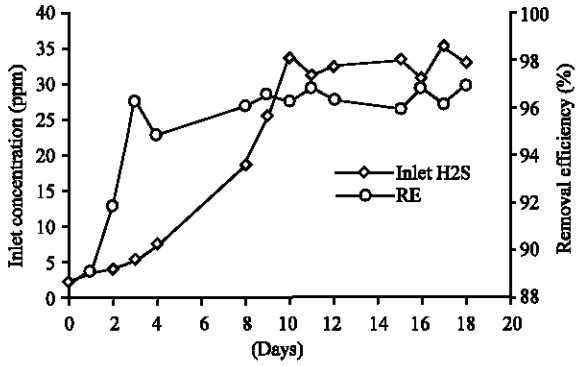

Fig. 3: H removal efficiency variation during the start up period of the bioscrubber active SOB biofilm on a high specific surface area media

A wide range of startup condition and time has been reported for H removal in bioreactors in which a moving liquid phase exists. Li *et al.* (1998) reported a 30-40 days for stabilization of H_2S removal efficiency in a fibrous bed bioreactor (Li *et al.*, 1998). Sercu *et al.*, 2005) investigated a two-stage biotrickling filter removing H_2S and dimethyl sulfide and resulted that H_2S was immediately removed below 3 ppm at when inlet concentration between 255 and 420 ppm was introduced to the reactor on day 22 of startup. A study was done on a pilot biotrickling filter by Wu *et al.* (2001) for treatment of odorous sewage air inoculated with *Thiobacillus thiooxidans*.

They considered the acclimation and immobilization stage completed after 80 days when H_2S removal efficiency reached greater than 90% at a gas retention time gradually decreased from 30 to 5 sec (Wu *et al.*, 2001). Cox and Deshusses (2002) studied cotreatment of H_2S and toluene in the biotrickling filter at two different pH (natural and acidic). H_2S introduced to the reactors at the average concentration of 7.7 ppm 22 days after operation of reactors with toluene. H_2S removal efficiency was close to 100% around 5 days after H_2S introduction. Although they concluded that the inoculation was not required, the start up of 5 days showed that the adaptation of H_2S oxidizing bacteria was needed. A retrofitted chemical scrubber treating H_2S to biotrikling filter was inoculated by activated sludge in which startup stage lasted around 10 days to achieve removal efficiency 99% for inlet concentration ranging from 5 to 25 ppm (Gabriel and Deshusses, 2003a).

According to results of this study and some example of the cited literature and owing to using a synthetic media in the fixed bed bioreactors with a flowing liquid phase treating H it is advisable from engineering and operational viewpoint to develop a biofilm by a pre-acclimated enriched SOB inoculum to rapid start up.

Thickness: After rapid and successful startup, top of the bioscrubber was opened to characterize the developed biofilm and measure its activity. The most important characteristic of bifilm from diffusion of pollutant(s) and nutrients viewpoint is thickness. The thickness of biofilm formed on the media was determined using total wet biomass immobilized in the bed. Mean wet biofilm mass attached to one part of the media was calculated as the difference of the drained wet mass of 100 parts of media taken out from top of the bioscrubber and the dry mass of same number of clean media divided by the number of weighted parts (n = l00). Then, total biofilm mass was calculated from mean wet biomass attached to one part of media times total number of parts in the bed which was 350 g. With take into account of 6.25 m^2 total surface area in the bioscrubber and assuming wet biomass density of 1 g cm^{-3} and its uniformity of biofilm along the bed was 56 μm. This value is less than biofilm in the bioreactors treating organic pollutants such as VOCs (Bitton, 1999). This can be explained by this fact that cell yield of autotrophic SOB which are predominate microorganisms in this reactor is much lower than that in heterotophics (Gabriel and Deshusses, 2003b). Owing to the high removal efficiency with fairly thin biofilm it can be concluded that the developed biofilm is in range of active thickness above which the diffusion of compounds becomes a limiting factor to biodegradation (Bitton, 1999). As biofilm activity is not proportional to the quantity of the attached biomass and as a result thickness of biofilm (Gabriel and Deshusses, 2003a), having a specified active biofilm containing SOB as predominate microorganisms is showed to be very effective to achieve high performance in bioscrubber treating H_2S.

Activity: Activity of the biofilm was measured using OUR test at various liquid pH to find the best pH in which highest rate of biodegradation happened. Sulfide was added to all samples just before running the test at the same amount. Figure 4 represents the results of OUR measurement of biofilm suspension at pH ranging 2 to 7. The highest activity with 1.4 mg O was measured at pH 3 and the lowest one happened at pH <5 which was 50% lower than the activity at pH 3. These results show that the sulfide oxidizing bacteria are acidophil and the optimum pH of recycle liquid to enjoy the highest capacity of SOB is 3.

Our results contrast with results of Cox and Deshusses (2002) who reported the lowest biofilm activity at acidic pH (<4). This difference might probably be because of type of employed microorganisms. Although low pH is not favorable for H absorption, it provides favorable conditions for SOB, which are predominated in the developed biofilm. The higher the activity of SOM, the more the removal efficiency would be.

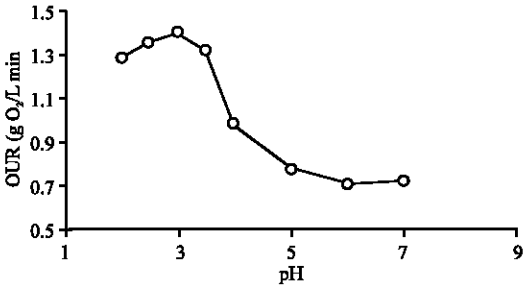

Fig. 4: OUR in biofilm suspension at various pH Biofilm characteristics

Table 2: Mean of microbial count in biofilm and recycle liquid

	Biofilm	Recycle liquid
SOB*	2.48×10^9	5.21×10^6
HPC	3.22×10^6	1.30×10^5

* Units are in cfu mL^{-1}

Microbial count and diversity: Diversity of microorganisms of the biofilm and recycle liquid was determined by pour plate technique to indicate the predominate bacteria in the biofil developed. Two different groups of bacteria were investigated which were heterotrophic and aototrophic SOB. Figure 5 shows the results of the microbial counts for both recycle liquid and biofilm suspension. HPC and SOB were counted 3.22×10^6 and 2.48×10^6 cfu mL^{-1} in the biofilm on average, respectively whereas mean of HPC and SOB in recycle liquid were $5.2l\times10^6$ and $1.3\times l0^6$ cfu mL^{-1}. According to Table 2 total SOB population was 3 log greater than HPC in the reactor meaning the acclimated SOB were completely predominate microorganisms in the bioscrubber studied. Also, as it can be seen the population of total microorganisms is 3 orders of magnitude more than them in the recycle liquid, which revealed that the microbial density in biofilm has been higher than, that in recycle liquid. Since the H_2S oxidation in the bioscrubber is done by bacteria and by take into account microbial density, it can be inferred that the most part of H_2S biodegradation is accomplished in the biofilm. Furthermore, high performance of bioscrubber even during the start up phase in H_2S removal efficiency can be related to the presence of active biofilm containing the high density of acclimated SOB.

Colonies formed on the thiosulfate media was further analyzed by gram staining to identify of the species of SOB. A spread layer was prepared from the SOB colonies and was stained. It was found that the SOB were bacillus gram negative bacteria. As a result, SOB in the bioscrubber were authotrophic, sulfide oxidizer, acidophile, gram negative, single cell and able to growth in temperature under 55°C (incubation temperature 35°C).

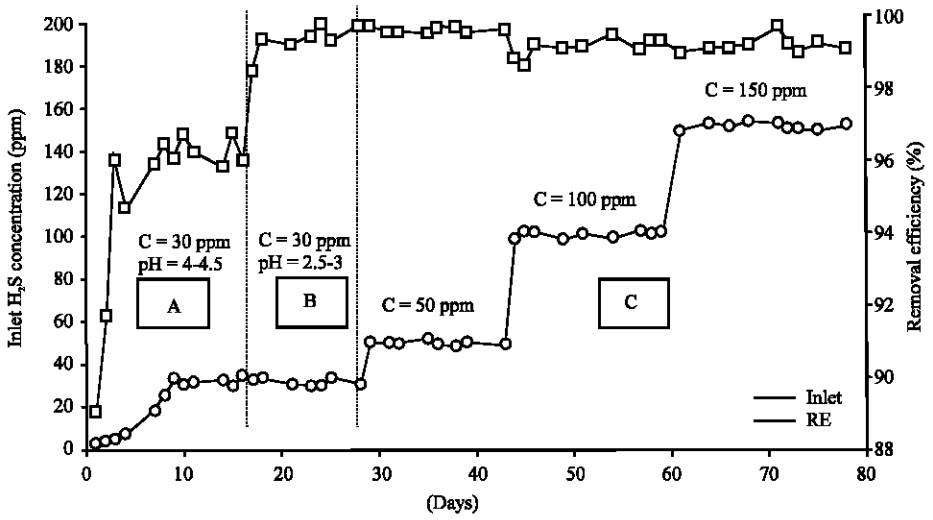

Fig. 5: Removal efficiency of H_2S in the bioscrubber versus time including startup and normal operation (operating conditions are: Air flow rate = 20 L min^{-1}, liquid flow rate = 1 L min^{-1}, EBCT = 30 sec and inlet H_2S concentration = 30 to 150 ppm)

As these characteristics represent the *Thiobacillus* genus (APHA, AWWA, WEF, 1998), it can be concluded that the SOB were probably belong to this genus of bacteria. Although, in order to exactly specify type of the SOB, further examination with the modem techniques such as Denaturing Gradient Gel Electrophoresis (DGGE) is needed.

Effect of liquid pH on H_2S removal efficiency: As it was explained, H_2S biodegradation in biofilm suspension was the highest at pH 3. It was also demonstrated that product of the H biodegradation was sulfuric acid which reduced the pH of the recycle liquid and so need to regulate the pH at a desirable value. pH of the liquid was then adjusted at 3±0.5 which was the pH in which the maximum SOB activity happened. Buffering capacity of the injected nutrient capacity was enough to neutralize the acid produced from H_2S biodegradation. After pH modification the performance of the bioscrubber was monitored. Other operating parameters were constant. Figure 5 (section B) shows the results of H_2S removal efficiency after pH renewing. Figure 5 indicates that the removal efficiency increased from 96.4 to 99.6%; that is around 3% increase. That was expectable due to the pH of the SOB environment was modified to the optimal value and their activity consequently the biodegradation rate has increased. Although SOB can oxidize H_2S in a wide pH ranging 2-8 (Pinjing *et al*., 2001), in our experiment pH 3 of the liquid phase achieved the higher removal efficiency.

Although a few researchers have reported high H_2S removal efficiency at pH 3 or lower (Gabriel and Deshusses, 2003a, b), most of publications about H_2S removal in bioscrubbers and biotrickling filters show the highest removal efficiency at pH greater, close to neutral (Wu *et al*., 2001; Cox and Deshusses, 2002; Jin *et al*., 2003; Shareefdeen and Singh, 2005). Contrary of our results with some of literatures can be explained by type of predominate microorganisms on the media of the reactor and probably the characteristics of the packing media.

In the present study, acidophilic SOB were predominate which preferred the low pH. However the low pH of the liquid phase is not favorable for H_2S absorption, high surface area of the media had created a high surface contact between gas and liquid phase resulting in somehow compensating the effect of low pH on H_2S absorption. In the experiments that higher H_2S removal efficiency were demonstrated at pH 2 of the recycle liquid of the biotrickling filters (Wu *et al*., 2001; Gabriel and Deshusses, 2003b), packing had a high specific surface area. Further examinations are needed to prove the reciprocal effect of the liquid pH and media characteristics on H_2S removal efficiency in the bio-scrubbers.

Effect of H_2S inlet concentration on its removal efficiency: To investigate the effect of inlet H_2S concentration of performance of the bioscrubber, air containing H_2S concentrations of 50, 100 and 150 ppm was introduced to the reactor. Performance of the bioscrubber in terms of removal efficiency in each concentration was monitored to get the steady-state conditions. Other operating parameters were kept constant as the same as

the previous stage. The results are presented in Fig. 5 (sections C). As it has been shown H_2S removal efficiency did not change after increasing the inlet concentration to 50, mean H_2S removal efficiency at steady-state condition for inlet concentrations of 50, 100 and 150 ppm which are corresponding to inlet loading of 8.3, 16.7 and 25 g H_2S m^{-3} h^{-1} was in turn 99.7, 99.4 and 99.4%. Elimination capacity of the bioscrubber was also 8.3, 16.6 and 24.9 g H_2S m^{-3} h^{-1} for the tested concentration, respectively. These results show that the developed bioscrubber could effectively handle the moderate to high inlet H_2S concentrations without any undesirable effect on its removal efficiency. Also, by comparing H_2S elimination capacity with loading and removal efficiency reveal that the reactor has not reached to the maximum capacity meaning the loading is quite lower than the critical loading and the reactor can accept higher loading (higher air flow rates or inlet concentration) while it works in high performance. According to above, removal efficiency of H_2S in the investigated conditions might be limited by H_2S mass transfer phenomenon. Further detailed experiments are needed to confirm this hypothesis.

Literature data on H_2S removal efficiency in the investigated bioscrubbers and biotrickling filters shows a vast range of removal efficiency and as a result elimination capacities ranging from 8 to 140 gH_2S m^{-3} h^{-1} (Smet *et al*., 1998). Data on H_2S removal efficiency varied because of the tested operating conditions by different researchers. For instance, Li *et al*. (1998) reported the removal efficiency around 95% for H_2S at inlet concentration of 27 ppm and retention time of 30 sec in a bioscrubber. In operating conditions including retention time of 36 sec in a biotrickling filter studied by Cox and Deshusses (2002), H_2S removal efficiency declined from around 100 to 70-80% when inlet concentration was increased from 50 ppm to 170 ppm.

Jin *et al*. (2005) reported H_2S removal efficiency of 94 and 87% at inlet concentration of 100 ppm and liquid pH of 3 and 2, respectively. Although, there is a few published paper showing higher removal efficiency for H_2S especially in acidic recycle liquid in biotrickling filter (Burgess *et al*., 2001; Gabriel and Deshusses, 2003a).

CONCLUSIONS

By comparison with the literature, we can conclude that the bioscrubber studied in the current investigation was more efficient than most of reported at almost the same operating conditions. This can be related to applying a media with high specific surface area and creating an active specific biofilm on it and also providing the optimal growth conditions (pH and nutrients) for biofilm microorganisms.

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