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Hydrogen Sulfide Removal in Biofilter: Evaluation of a New filter Material by Immobilization of *Thiobacillus* sp.

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Abstract

Different agricultural residue was evaluated as a biofilter material for the removal of Hydrogen sulfide (H_2S) using *Thiobacillus* sp. A Combination of four different agricultural residues, viz., sugarcane bagasse, coconut coir pith, rice husk and saw dust were evaluated as biofilter material. From results, it was observed that filter material having composition of 2:4:2:2, 4:4:2:2 and 4:2:2:4 has resulted in retaining 70% moisture content at the end of 10 days. The biofilter was operated for 128 days in three phases by varying inlet H_2S concentration from 321 to 2020 ppmv. Biofilter exhibited 100% removal efficiency (RE) at an inlet concentration of 570 ppmv, 99% RE at an inlet concentration of 1416 ppmv and 66% RE at a maximum inlet concentration of 2020 ppmv. The filter performance in terms of RE, dropped to 48% when the air was not humidified before sending to the filter, which has direct relation with the MC of the filter material. The RE recovered to 66% with recovery of moisture content (MC) to 57% upon subsequent re-introduction of the humidifier in to the circuit. The pH has dropped from 7.8 to 4.8 during the course of operation of the filter. H_2S was effectively removed under different operating conditions using mixed agricultural residue as filter material. However humidification was most essential to maintain the required moisture content in the biofilter. SEM analysis has shown the good growth of *Thiobacillus* sp. in the filter bed and hence the new material proved to be good support for the immobilization of *Thiobacillus* sp.

KEYWORDS: hydrogen sulfide, biofilter, humidifier, experimental design, kinetics

1. Introduction

Hydrogen sulfide (H_2S) is released in to the atmosphere by various industrial activities and processes viz waster water treatment plant, paper and pulp, petroleum and petrochemicals [1]. H_2S is an odorous, toxic gas and shows negative effects on the environment [2]. The removal of H_2S from gases is required for the reasons of health and safety [2,3]. Conventionally different chemical and physical removal methods like adsorption, absorption, condensation, oxidation etc. have been used for H_2S from industrial gaseous emissions [4,5]. These conventional processes are expensive and energy intensive hence, in the past few decades biological control of air pollution has gained interest because of its obvious advantages besides being competitive and also environmentally benign [6-10].

Among available biological methods for waste gas treatment, biofiltration is a viable and potential cost-effective alternative technology when compared with conventional technologies [1, 11]. Higher pollutant removal efficiencies could be achieved under proper operating conditions. As the end products are harmless hence the processes are environmental friendly [10, 12]. Various researchers have worked using variety of filter media including natural and synthetic and also mixtures of both [6, 13-18]. Biofilter bed material should have high specific area for better microbial attachment, good water retaining capacity, low pressure drop to get better contaminant removal at cheaper cost.

Chemoautotrophic bacteria are known for hydrogen sulfide removal especially from the genus *Thiobacillus* seems to be better because of simple nutritional requirements. In particular *Thiobacillus thioparus* and *thioxidans* [19] *T. thioparus* strains CH11 [20] and DW 44 [21] have the ability to grow using H_2S as energy source.

In our earlier studies we have isolated an autotrophic sulfide oxidizing bacteria, *Thiobacillus* sp. [22] and tested for oxidation of dissolved sulfides (S^{2-}) under different operating conditions in fluidized bed reactors [23-25]. The classical method of experimental optimization involves changing one variable at a time, keeping the others constant. In addition, it is not practical to carry out experiments with every possible factorial combination of the test variables, because of the large number of experiments required [26]. Besides this, it is a tedious, cumbersome, and time-consuming process especially when a large number of parameters are taken into account. The optimum composition of four biofilter materials used in the biofiltration process was determined by means of a Central Composite Design (CCD) [26]. The typical CCD consists of three parts viz a factorial design with number of factors to be studied with each having two levels, secondly a set of centre point experiments with each factor at the median values used in the factorial part and finally a set of axial points, with experiments

runs under identical conditions portion which will take on values both below and above the median of the two factorial levels, and typically both outside their range.

The filter material used in a biofilter should be a good platform for undergoing metabolic activities. The material should give a good surface area for the microorganisms to intermingle on it and to undergo its activity. The whole efficiency and the amount of H_2S degraded depends on the physical properties of the bed material. The selection of bed material is also specific because certain bed material combination would reach the required efficiency during contaminant removal. Four different agricultural waste materials viz sugar cane bagasse, coconut coir pith, rice husk and saw dust were selected to test as filter material in the biofilter for H_2S removal. All the above four materials are waste products of their respective processing industries and has different properties. Their availability in the local area is abundant and whose disposal also a nuisance for the industries. Also the reason for the selection of these materials was their water retaining capacity under different conditions and particle size distribution. Rice husk is high lignin containing material and good insulating material because they are difficult to burn and hydrophobic hence less likely to allow moisture to propagate mold or fungi. Other three materials, coconut coir pith, sugarcane bagasse and saw dust are hydrophilic in nature and have a considerable water retaining capacities. Hence these materials were selected to test as biofiltration media for H_2S removal using immobilized *Thiobacillus* sp. As moisture plays a vital role in biofilter operation, it is important to maintain required moisture content in the filtration media during its operational period. If a biofilter bed retains excess moisture it is difficult to operate biofilter as it affects the pressure drop and supports for the anaerobic growth in the core of the bed. Hence, rice husk was added to control and maintain the moisture content. Appropriate combination of these materials could maintain the moisture content during the biofilter operation.

In the present studies we tested the isolated *Thiobacillus* sp. for the gaseous sulfides (H_2S) in biofiltration using mixed agricultural residue, sawdust, bagasse, rice husk and coconut coir pith, as filter material. The 'heart' of a biofiltration system is the filter bed or packing material and this material should have the following characteristics: (1) high moisture retention capacity, (2) high porosity and high buffer capacity, (3) high available nutrient content, (4) a diverse and adaptable microbial population, (5) low cost, and (6) long life (mechanically resistant and chemically inert and stable). Hence, four agricultural materials, sawdust, bagasse, rice husk and coconut coir pith, tested for moisture retaining capacity under different combinations and further the best combination was used for the immobilization of *Thiobacillus* sp. and subsequent H_2S in biofilter have been studied.

2. Materials and Methods

2.1 Biofilter bed material

Four different agricultural waste materials viz sugar cane bagasse, coconut coir pith, rice husk and saw dust were selected to test as filter material in the biofilter for H_2S removal. All the above four materials are waste products of their respective processing industries and has different properties. Their availability in the local area is abundant and whose disposal also a nuisance for the industries. The filter material was characterized for various parameters like, particle size, moisture, bulk density and pH.

2.1.1 Optimum combination of filter material by Central Composite Design

The optimum composition of four biofilter materials used in the biofiltration process was determined by means of a Central Composite Design (CCD) [26]. The four variables viz. coconut coir pith, (x1); sawdust (x2); rice husk, (x3); and sugarcane bagasse (x4) were studied at five levels –2, –1, 0, +1, and +2 as shown in Table 1. A 2^4, full factorial central composite design with eight star points and six replicates at the central points were employed, which indicated that 30 experiments were required by this procedure as shown in Table 2. In order to search for the optimum combination of four materials for the H_2S oxidation, experiments were performed according to the CCD experimental plan as shown in Table 2. The optimum composition of rice husk, sawdust, sugarcane bagasse and coconut coir pith was determined by doing moisture content test under different combinations. Approximately 200 gm of filter material was prepared according to the combination given in table 2 and moisture content was increased to 85-89% by adding water. Continuous humidified air was supplied to the wet material at a flow rate of 0.18 m^3/hr for a period of 10 days. During this period, filter material was analyzed for moisture content at a frequency of 24 hr. The results are recorded and tabulated.

Table 1: Range and Levels of the Variables for CCD Studies

Variables/levels	-2	-1	0	1	2	Δx
Coconut coir pith, (parts) x1	1	2	3	4	5	1
Sawdust, (parts), x2	1	2	3	4	5	1
Rice Husk (parts) x3	1	2	3	4	5	1
Sugarcane bagasse, (parts) x4	1	2	3	4	5	1

Where Δx is interval

Table 2: Design table for the selection of best composition of four different materials studied and Moisture content data of filter material at different combinations of four materials according to CCD

Expt no					Days									
	x1	x2	x3	x4	1	2	3	4	5	6	7	8	9	10
1	2	2	2	2	75	73	73	69	67	61	58	56	53	53
2	4	2	2	2	70	68	63	62	61	60	60	58	58	58
3	2	4	2	2	**75**	**75**	**74**	**74**	**73**	**73**	**71**	**70**	**70**	**70**
4	4	4	2	2	**75**	**74**	**74**	**73**	**71**	**70**	**70**	**70**	**70**	**70**
5	2	2	4	2	68	67	66	65	65	63	62	61	61	61
6	4	2	4	2	72	69	62	58	55	50	50	45	43	43
7	2	4	4	2	75	72	72	70	70	69	68	66	65	65
8	4	4	4	2	75	73	70	68	67	65	65	65	64	64
9	2	2	2	4	70	68	68	67	66	65	64	61	61	60
10	4	2	2	4	**75**	**74**	**74**	**73**	**73**	**73**	**72**	**70**	**70**	**70**
11	2	4	2	4	65	65	64	63	59	58	56	56	55	55
12	4	4	2	4	75	74	73	69	63	59	58	56	56	56
13	2	2	4	4	73	69	68	67	66	66	65	65	65	65
14	4	2	4	4	72	69	63	60	58	56	55	49	44	42
15	2	4	4	4	70	65	65	65	70	70	65	65	65	65
16	4	4	4	4	69	65	65	63	63	61	58	56	55	55
17	1	3	3	3	71	70	70	67	67	66	65	65	64	64
18	5	3	3	3	74	70	70	64	64	63	62	62	60	60
19	3	1	3	3	75	70	70	68	68	66	66	65	65	65
20	3	5	3	3	69	66	66	65	64	59	55	54	52	52
21	3	3	1	3	72	70	69	69	64	64	61	61	60	60
22	3	3	5	3	70	68	68	63	62	59	54	50	46	45
23	3	3	3	1	70	68	67	67	64	64	58	58	52	52
24	3	3	3	5	75	74	74	71	68	66	62	61	59	59
25	3	3	3	3	75	73	69	68	62	58	57	56	56	56
3	3	3	3	3	75	74	72	67	63	57	56	56	55	55
27	3	3	3	3	75	73	71	69	64	61	59	57	57	56
28	3	3	3	3	75	72	69	67	67	63	58	57	56	56
29	3	3	3	3	75	74	68	65	64	64	63	59	58	58
30	3	3	3	3	75	74	71	67	66	62	59	57	57	56

2.1.2 Biofiltration process for H_2S removal

Biofilter: The biofilter column in the present study was made up of glass. The height and diameter of the column was 1.2 m and 0.08 m respectively. The height of the filter media was 0.77 m. Four equidistant sampling ports were provided along the length of the filter media. **Humidifier:** The humidifier column is made up of glass having internal diameter of 0.06 m and height of 1m. Water was sprayed at the top through fine nozzles with 1/16 HP self- priming centrifugal pump (Suguna India) and the air was supplied counter currently from the bottom of the humidifier. The water collected at the bottom of the humidifier was continuously recycled to the top of the humidifier. **H_2S generation unit:** H_2S gas was generated by the addition of sodium sulfide (Na_2S) and hydrochloric acid (HCl) solutions in a glass column. The solutions (1% Na_2S and 0.1 N HCl) were taken separately in two glass reservoirs (having stoppers for flow control) and trickled at desired flow rate to a glass jar. The H_2S gas formed in the glass jar was collected by passing air from the cylinder to the bottom of the jar. **Air supply:** Air was supplied by using pure air cylinders (79%N; 21%O_2; Pioneer Gases ltd., India). Airflow rate was monitored with the help of a calibrated rotameter (Instrumentation engineers India) in the range of 0.792 m^3/hr to 0.128 m^3/hr. **Inoculum preparation:** Isolated *Thiobacillus* sp culture was used as inoculum in the biofiltration process for the H_2S removal. Culture was grown in 250 ml Erlenmeyer flasks with 100 ml of *Thiobacillus* sp growth medium having composition (g/l) [22] NH_4Cl, 1.0; K_2HPO_4, 0.6; $CaCl_2.2H_2O$, 0.2; $FeCl_3.H_2O$; 0.02, $Na_2S_2O_3.5H_2O$, 10; $CaCO_3$ 3. Flasks were sterilized at 121^oC temperature, 15 lb pressure for 20 minutes and inoculated with one loop full of *Thiobacillus* sp. All the flaks were incubated in a shaker at 30^oC temperature and 150 rpm for 6 days. At the end of incubation period cells were harvested by centrifuging at 5000 rpm for 5 minutes. The supernatant was discarded and the biomass pellet was washed twice with sterile distilled water to remove any trace media components present. The biomass was weighed and collected in a conical flask under aseptic conditions. **Filter bed material preparation:** The filter material (mixture of four material) was mixed with isolated *Thiobacillus* sp (1 g per every 10 g of filter material) and $CaCO_3$ (500 mg per every 10 gm of filter material). The above material was packed in the filter in layers of 0.25 m height and upon each layer coconut coir was distributed evenly to avoid the compaction of the bed. Initial moisture content of the filter material was increased by adding water mixed with (g/l) NH_4Cl, 5.0; K_2HPO_4, 1.0; $CaCl_2.2H_2O$, 0.8; $FeCl_3.H_2O$, 0.1.

2.1.3 Experimentation

Figure 1 shows the biofiltration setup used for H_2S removal in the present studies under different operating conditions.

Phase I: In the first phase, experiments were carried out with the humidifier in the circuit. H_2S concentration was varied from 321 ppmv to 2020 ppmv in a stepwise manner and biofilter was operated for 88 days. The desired concentration of H_2S gas in the air mixture was obtained by manipulating the air flow and flow rates of 1% Na_2S and 1 N HCl. **Phase II:** In the second phase, experiments were carried out without humidifier. H_2S concentration was kept constant at 1416 ppmv and biofilter was operated for 25 days. **Phase III:** In the third phase, experiments were carried out again by introducing humidifier in the circuit. However, in this phase H_2S concentration was kept constant at concentration of 1416 ppmv and biofilter was operated for 15 days.

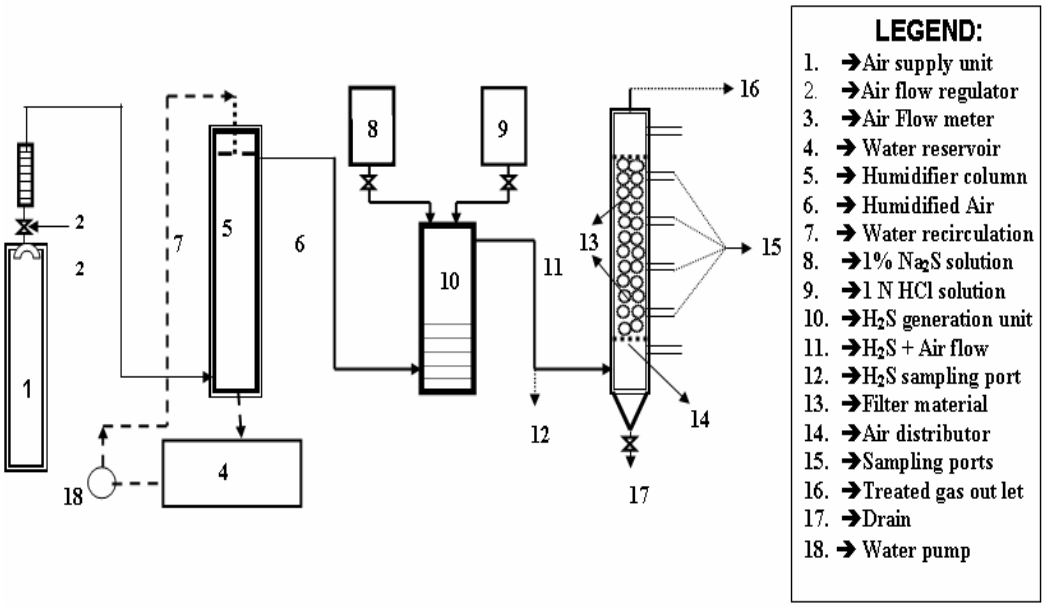

Figure 1: Schematic flow diagram of Biofiltration system with Humidifier

2.1.4 Analytical methods

Operational parameters estimation: The inlet and outlet gas to the biofilter was analyzed for H_2S by the Tutweiler's apparatus [27]. Filter media samples were collected periodically from the sampling ports and analyzed for pH, particle size, moisture content, sulfate, sulfide and sulfur analysis as per standard methods [28, 29]. The pH of the filter material was analyzed by soaking one part of filter

material in ten parts of double distilled water for 30 minutes. The solution was allowed to cool down to room temperature, filtered using Whatman 40 filter paper and the pH was recorded using a digital pH meter [29]. **Pressure drop measurement:** The pressure drop was measured by connecting a U-tube manometer across the biofilter. **Microbial count:** Cell numbers were measured at the start and end of the experiment. About 5 gm of filter material was sampled and mixed thoroughly in sterilized water, kept in the shake flask for 8 hours. Serially diluted suspension was streaked onto the thiosulfate mineral media for *Thiobacillus* sp. The flasks were then incubated at 30 ± 2^0C for a period of 7 days. The cell numbers were expressed in colony forming units (cfu). **Scanning electron microscope (SEM):** SEM was used to study the growth of *Thiobacillus* sp. on the filter material. Two samples were analyzed by SEM, (1) sample collected from top part and (2) sample collected from bottom part of the biofilter of the biofilter. Both the samples were collected at the end of 128 days of biofilter operation. The filter material samples were taken out from their respective ports and were fixed with 2% glutaraldehyde and then dehydrated in gradient ethanol. Finally, the samples were dried, coated with gold and observed with a (HITACHIS3000N, Singapore) microscope.

2.1.5 Biofilter performance evaluation

Operational and performance parameters include, Removal Efficiency (RE), Empty-Bed Residence Time (EBRT), Inlet Mass Loading Rates (IMLR) and Elimination Capacity (EC).
The biofiltration performance was discussed in terms of the
Removal Efficiency, $RE = [(C_i - C_e)/ C_i] \times 100$ (%),
Elimination Capacity, $EC = Q \times (C_i - C_e) \times \beta / V$ (g/m^3/hr) and
Empty Bed Residence Time, $EBRT = V \times 3600 / Q$, (Sec)
H_2S Inlet mass Loading Rate, $IMLR = (Q^* C_i^* \beta)/V$, (g/m^3/hr),

2.1.6 Kinetics and Modeling

The H_2S removal rate of biofilter using mixed packing material inoculated with *Thiobacillus* sp was evaluated by following kinetic analysis. Assuming plug flow of H_2S into biofilter and homogeneous distribution of *Thiobacillus* sp, following equation was applied. In order to conduct kinetic analysis of biological removal of H_2S and Michaelis-Menten type equation was assumed [30-32].

Two processes, mass transfer and microbial utilization of contaminants simultaneously occur in biofiltration. It is assumed that, this is first order substrate kinetics and oxygen concentration required for aerobic respiration of microorganism in biofilm is not limiting, under these conditions, substrate

utilization rate of a compound by microbial flora as well as enzymatic reactions can be expressed by the Michelis-Menton equation.

Gas flow through the biofilter can be characterized as pseudo plug flow with minimal back mixing. For an ideal plug flow reactor without dispersion at steady state can be modeled by the following equation.

$$\frac{dC}{dt} = \frac{Q}{A} x \frac{dC}{dH} + R \quad \text{--------(1)}$$

At steady state dC/dt = 0 and the reaction rate is defined as

$$R = \left[\frac{V_{\max} C}{K_m + C} \right] \text{-------(2)}$$

The elimination capacity is defined as $EC = (C_i - C_e) x Q x \beta / V$, gm H_2S/ m^3 hr

Where C is the pollutant concentration (ppmv)

H is height of the filter bed, m

Integrating equation 1 under conditions $C = C_i$ at H=0 and $C=C_e$ at $H=H_o$, we get

$$\frac{\left(V/Q\right)}{\left(C_i - C_e\right)} = \frac{K_m}{\left(V_{\max} x C_{\ln}\right)} + \frac{1}{V_{\max}} \quad \text{---------(3)}$$

Where V=AxH and $C_{\ln} = \dfrac{(C_o - C_e)}{\ln\left(\dfrac{C_o}{C_e}\right)}$

From above equations we can modify the M-M equation in to three equations

$$\frac{1}{EC} = \frac{1}{V_{\max}} + \frac{K_m}{(V_{\max} x C_{\ln})} \text{----------(4)}$$

$$\frac{C_{\ln}}{EC} = \frac{K_m}{V_{\max}} + \frac{C_{\ln}}{V_{Max}} \text{----------(5)}$$

$$EC = V_{\max} - K_m x \frac{EC}{C_{\ln}} \text{----------(6)}$$

where V_{max} is the maximum elimination capacity biodegradation rate (g-$H_2S/m^3/hr$) and K_m is the saturation (Michaelis–Menten) constant (ppmv). Depending on the concentration of substrate, the rate can be first-order ($C_{ln} << K_m$) or zero-order reaction ($C_{ln} >> K_m$).

RE is the fraction of pollutant degraded by biofilter and EC is the mass of the contaminant removed per unit volume of filter material per unit time. As RE varies with inlet contaminant concentration airflow and biofilter size it is an incomplete descriptor of biofilter performance.

Kinetic analysis during 128 days of operation, the data for the kinetic analysis were obtained from the transient increase in H_2S concentrations, which was realized by increasing the flow rate from 0.792 to 0.18 m^3/hr. The kinetic data

was analyzed separately for three phases of biofiltration process. During phase I, biofilter was operated with humidifier in the process and the inlet H_2S concentration was varied in the range of 321-2020 ppmv. The kinetics was analyzed at individual inlet H_2S concentrations and also at stable conditions. In Phase II, humidifier was bypassed and the filter was fed with 1416 ppmv of inlet H_2S and the kinetics were analyzed for entire phase. Similarly kinetics for Phase III was calculated when the humidifier was reintroduced back into the process. Estimation of kinetics under different conditions will help in proper understanding of biofiltration process for the removal of H_2S using *Thiobacillus* sp.

The M-M equation constants are evaluated using equations 4, 5 and 6. Equation 4, 5 and 6 are different forms of equation 2 and are useful for the estimation of V_{max} and K_m. Equation 4 has a form of double-reciprocal method or Lineweaver-Burk, Equation 5 is Hanse-wool method and equation 6 is Eadie-Hofstee method for the estimation of MM constants. Estimation of these parameters is useful for comparing the performance of biofilter under different conditions.

2.2 Results and Discussions

2.2.1 Packing Material: Determination of best composition of filter material by Moisture Content (MC) analysis using Central Composite Design (CCD)

The results of CCD experiments for studying the effect of combinations of four independent variables on MC are presented in Table 2. This was done by doing moisture content estimation at different combinations of materials for 10 days. Two grams of sample was dried in an oven for 1 hour at 100^{o}C temperature and weighed. The MC was estimated till a stable MC observed. Hence the experiments were terminated at the end of 10 days.

From the above available data for the reduction in moisture content during 10 days, it was observed that experiments 3, 4 and 10 has resulted in retaining around 70% moisture content at the end of 10 days. The three samples did not loose their moisture content gradually. The most common in these three samples is the hydrophobic material i.e rice husk is in fewer ratios, which made the mixture to maintain its moisture. Among the three best experiments, the combination of experiment 10 (4:2:2:4) was used further in biofilter material preparation and inoculated with *Thiobacillus* sp. The MC variation in experiment 10 (Table 2) was more gradual when compared with 3 and 4 hence experiment 10 was selected. The filter materials were analyzed for physical parameters like pH, moisture content, bulk density and particle size before the experiment. Table 3 shows the individual and composite characteristics of filter material. It could be observed from the table that the filter material was having 0.128 g/cc of bulk

density, 28% MC at atmospheric conditions and pH of 7.2. The preliminary property, MC, of the filter indicated that this material could be used as bed material for the immobilization of microbes in the biofilter for the removal of H_2S.

Table 3: Biofilter operating Conditions and properties of the filter material

Parameter	**Quantity/**		
Moisture Content (Wet) Initial	~89%		
Operating Temperature, ^{0} C	30±5		
Height of the filter bed, m	0.77		
Volume of the filter bed, m^3	3.87×10^{-3}		
Weight of the Filter Material used, gm	490		
Particle size distribution in combined filter material			
> 2.8 mm	11.50		
< 2.8 to > 2 mm	09.25		
< 2 to >1.4 mm	21.35		
< 1.4 to >1 mm	16.79		
< 1 mm	41.11		
> 2.8 mm	11.50		
Type of material	**Bulk density, gm/cc**	**Moisture content, %**	**pH**
A) Rice husk	0.124,	31	7.4
B) Bagasse	0.076,	33	6.4
C) Saw dust	0.408,	29	5.8
D) Coconut coir pith	0.132,	26	7.5
E) Composite fibrous material (A,B,C and D) at 4:2:2:4 parts	0.128,	28	7.2

* Obtained by sieve analysis

2.2.2 Biofilter performance with mixed agriculture residue as filter material

a. Pressure drop

During the operation of biofilter, superficial gas velocities were changed in the range of 18 to 185 h^{-1} and temperature was maintained at 30°C. The pressure drop at each superficial gas velocity was measured. When operation reached steady

state (about 3 days), a new superficial gas velocity was selected. Influence of superficial gas velocity on pressure drop is shown in Figure 2. The figure shows, pressure drop was only in the range of 24 to 78mm H_2O/ m of the column. It indicates pressure drop of the biofilter increases with increasing superficial gas velocity and in a good linear behavior. The possible reason is due to no significant biomass accumulation in the filter material. Consequently, the biofilter exhibited excellent dispersion characteristics. This indicated that fibrous material used in the biofilter, as inert support material was good in terms of maintaining less compaction characteristics.

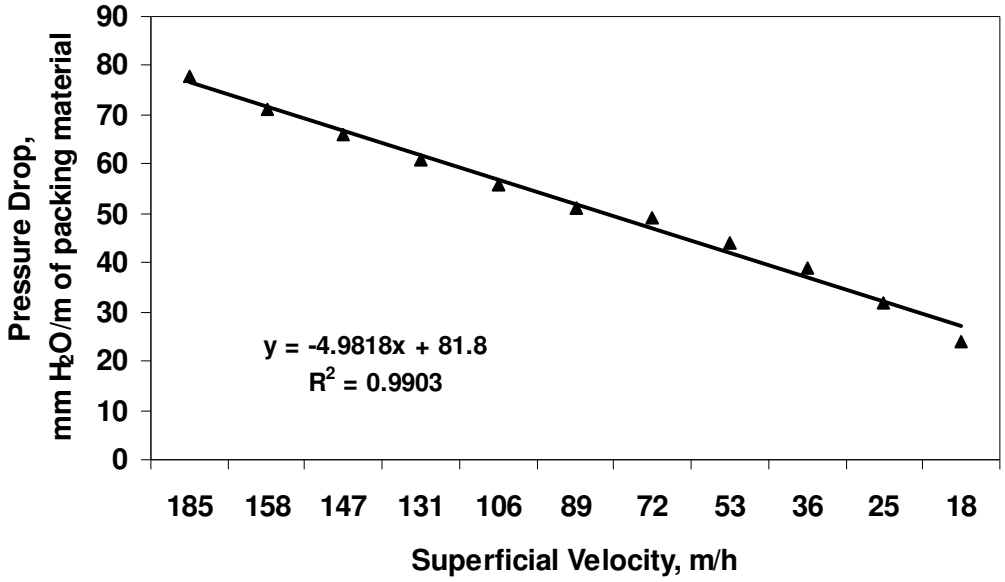

Figure 2: Effect of pressure drop with superficial velocity

b. Removal efficiency (RE)

Variation of inlet and outlet concentration of H_2S and RE during the course of operation of filter for 128 days is shown in Figure 3. Initially filter was started with 321 ppmv of H_2S and operated for 18 days. During this period RE was in the range of 37-100%. The RE was initially 37% and increased steadily as operational period increased. In the first 7 days of filter operation, the RE was in the range of 37-90%. The efficiency increased to 100% after 16 days of filter operation. This 16 days period can be considered as stabilization period for microbial population to adjust to the native conditions with respect to the filter. Further the filter was operated continuously by enhancing inlet H_2S concentration in a stepwise manner. At each concentration the filter was operated to get stable RE.

Figure 3 also indicates, the inlet concentration was 570 ppmv on 41st day of filter operation, the RE of H_2S was 100%. The inlet concentration of H_2S was increased from 955 ppmv to 1416 ppmv from 42nd to 67th day of operation. During this period of operation, the RE was 99.16%. The exit concentration of H_2S in this period was varying between 8 to 12 ppmv. In the subsequent phase of 10 days, at inlet concentration of 2020 ppmv, RE dropped to 66.14% and outlet concentration rose to 684 ppmv. The outlet concentration of 12 ppmv was in the acceptable range of H_2S discharge standards as per Central Pollution Control Board [33] compared to the 684 ppmv, which was obtained at 2020 ppmv inlet concentration. Therefore, the inlet concentration was brought back to the previous value of 1416 ppmv and filter was operated from 78th to 88th (11 days). In this period RE of 99.22% and outlet concentration of 11 ppmv was achieved reproducing similar performance at 1416 ppmv inlet H_2S concentration at which the filter was operated previously. The performance also explained that the filter can be operated at desired inlet concentration in the range of 0-1416 ppmv as per exit gas concentration requirements. The performance of biofilter indicated that RE was strongly dependent on the H_2S inlet concentration and in the present case of biofilter configuration a maximum inlet H_2S concentration of 1416 ppmv was only possible for steady state effective performance of the filter within the acceptable range of outlet H_2S concentration.

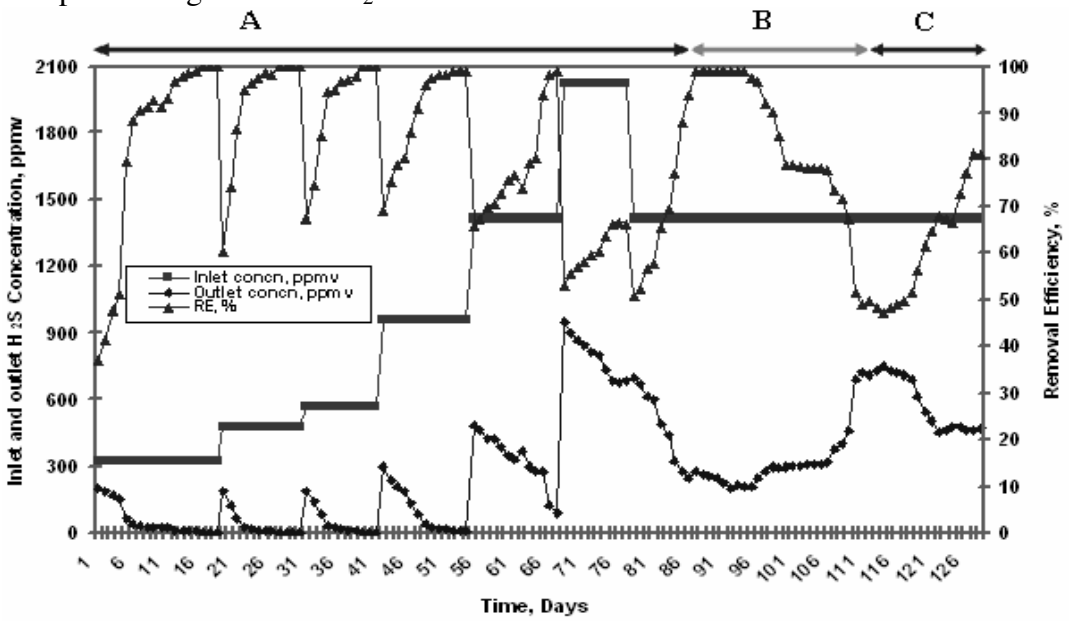

Figure 3: Variation of inlet & outlet concentration of H_2S and RE during the course of operation of filter for 128 day

c. Empty Bed Residence Time (EBRT)

The inlet concentration was varied from 321 – 2020 ppmv by gradually decreasing the airflow rate. By this the EBRT increased from 17 to 108 seconds with the increase in inlet concentration of H_2S. This approach was intended to provide higher time of contact period at higher inlet contaminant concentration. Figure 4 shows the effect of EBRT on RE and EC. The filter RE was 66.14% when the EBRT was 108 seconds at the inlet concentration of 2020 ppmv. In order to operate the filter at 99-100 % RE around 77 seconds of EBRT was required at an inlet concentration of 1416 ppmv.

The degradation mechanism of H_2S in a biofilter was very complex as it involves physical limitations, such as the pollutant mass transfer rate from gas phase to the aqueous phase (biofilm) and chemical changes depending on the microbiological activity. Upon establishment of phase transfer activity in the filter, the microbial population may require higher EBRT at higher inlet concentration of H_2S as the equivalent aqueous phase concentration of sulfide was higher. The EBRT for removing 99.22 % H_2S having inlet concentration of 1416 ppmv was 77 seconds, which was comparable to the existing literature data [34]. However, at inlet concentration of 2020 ppmv, the RE dropped to 66.14% even though 108 seconds of contact time was provided which suggested that filter RE was largely dependent on inlet concentration of the H_2S.

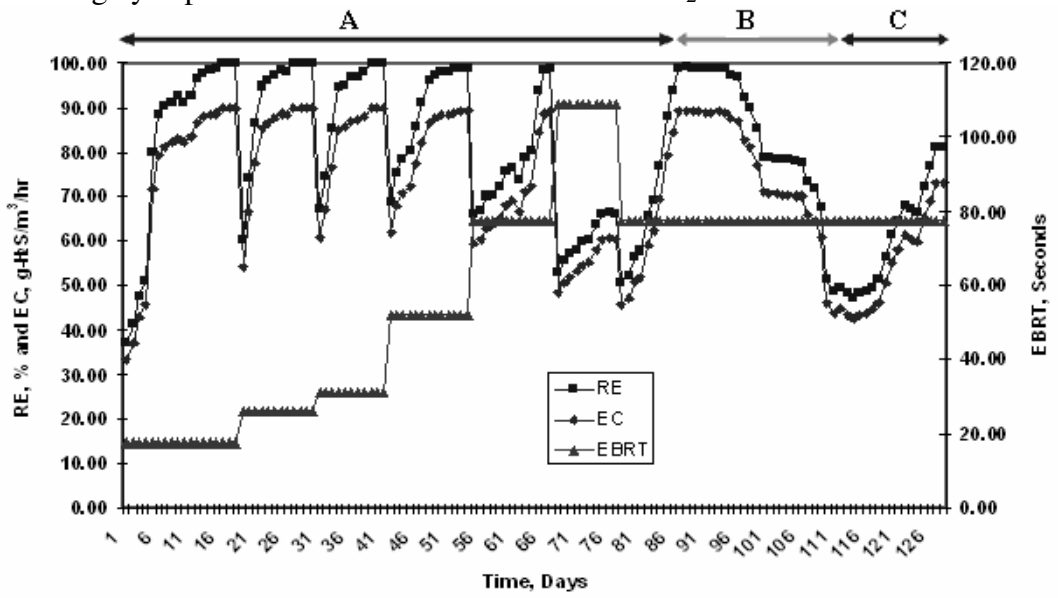

Figure 4: Effect of EBRT on RE and EC during biofilter operation for 128 days

d. Microbial count

Initially the microbial count was $1.8x10^3$ cfu/gm of sample and at the end of biofilter operation, the *Thiobacillus sp*. count increased to $16x10^5$ cfu/gm of sample. Even though the filter was operated under acidic pH, the increase in colony count of *Thiobacillus sp*. indicated that this class of bacteria could survive under acidic pH. The RE of H_2S in the range of 90-95% at acidic pH also confirmed this observation. Higher RE at acidic pH was reported earlier [35] with frequent washing of filter bed with water. But, frequent washing is not practicable in full-scale biofilter plants. Therefore, in the present study washing of filter bed was avoided in order to observe the drop in pH and RE of the biofilter. RE in the range of 90-95% was achieved in the present study even though the filter bed was not washed.

2.2.3 Effect of humidification and inlet H_2S concentration on the performance of biofilter

a. Effect of humidification and inlet H_2S concentration on RE

In order to understand the effect of MC on the filter performance, after stabilization of the biofilter at 90 g-H_2S/m/hr IMLR, 46.53 hr^{-1} VLR and 1416 inlet H_2S concentration, the humidifier was bypassed and contaminated gas was directly sent to the biofilter (Table 4). It can be observed from Figure 5 and 6 that the effect of humidifier on the biofiltration was very significant. Until 88^{th} day of operation with the humidifier in the circuit, the RE was more than 99% and MC was 64%. The RE dropped to 66.14 % only when the inlet H_2S concentration was increased beyond 1416 ppmv despite maintaining the MC at 68%. The RE was dropped to 51% with the drop in MC due to the bypassing of humidifier. However, for the same inlet concentration of H_2S at 1416 ppmv, the filter RE maintained in the range of 99-48%, when the MC was in the range of 68-42%. This clearly indicated that maintenance of MC around 55-65% was highly essential in order to obtain the highest possible RE at the optimum IMLR & VLR. In the biofiltration process, if the gas to be treated was unsaturated (dry), then there was a requirement of humidification of gas before entering the filter in order to operate the biofiltration process at highest possible RE. The humidifier was bypassed from the circuit and air having approximately 1416 ppmv of H_2S was passed through the biofilter. The variation in MC of the biofilter (without humidifier) and H_2S RE during the course of operation of the filter was shown in Figure 5. The RE of 90 to 99 % was achieved during the first 10 days of biofilter operation. During this period MC was in the range of 64 to 58 %. Subsequently, the MC was gradually decreased to a minimum of 42 %. It was evident that from

the Figure 5, RE of the biofilter came down to 48.31% from a maximum RE of 100 % due to drop in MC of the filter. This clearly indicated that appropriate maintenance of MC in the filter for stable performance was highly important. Results of the earlier research work [36, 37] have indicated that MC in the range of 55 to 65% was optimum for the removal of pollutants from waste air streams.

In the present study, at the end of 24 days of biofilter operation the MC of the filter material had come down to 42 % from 68 % and the RE of the filter also dropped to 48.31 % from 100 %. Therefore, it was evident from the above result that a minimum MC of 50-65% was very much essential in the biofilter, so that sufficient aqueous layer on the inert material to the extent of H_2S gas phase transformation and microbial activity was available in the biofilter. Even though MC was an important parameter for the activity of the microbes present in the biofilter, some times excess MC leads to the formation of anaerobic zones and high-pressure drop across the filter bed. At the same time low MC leads to drying out the filter bed at the gas inlet thus possibility of cracking out of filter bed and channeling of gas flow may occur [34]. Hence it is very important to maintain the moisture content in the optimum range of 50 to 65% for the efficient and long-term performance at industrial scale.

Table 4: Operational data of biofiltration of H_2S at stable conditions

S. No	Q, m^3/hr	C_i, ppmv	C_e, ppmv	EBRT, Sec	Operational period, days	RE, %	EC, g-H_2S /m^3/hr	IMLR, g-H_2S /m/hr	MC, %
1	0.792	321	0	17.58	**18* (88$^{\$}$)**	100	~90	~90	82
2	0.534	477	0	26.08	**12***	100	~90	~90	79
3	0.447	570	0	31.16	**11***	100	~90	~90	78
4	0.267	955	8	52.16	**13***	99.16	~90	~90	74
5	0.18	1416	89	77.37	**13***	93.71	~84	~90	70
6	0.128	2020	684	31	**10***	66.14	~60	~90	68
7	0.18	1416	265	77.37	**11***	81.29	~73	~90	64
8	0.18	1416	732	77.37	**25^{+}**	48.31	~44	~90	42
9	0.18	1416	471	77.37	**15***	51.1	~60	~90	45

*** With humidifier, $^{+}$ Without humidifier, $^{\$}$ Initially 88 days operated with humidifier**

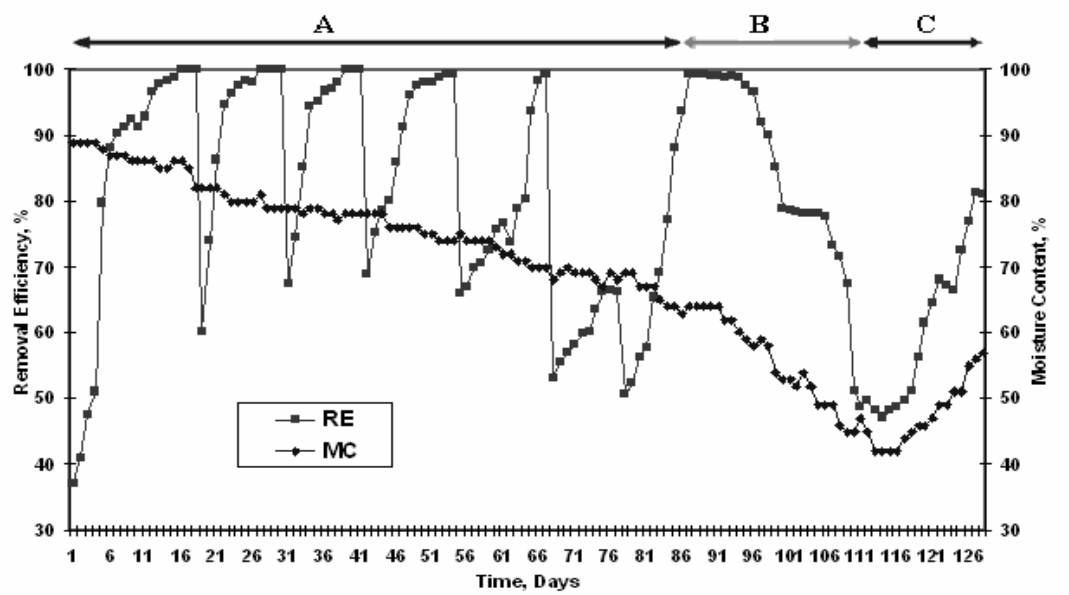

Figure 5: Effect of MC on RE, EC during biofilter operation for 128 days

b. Effect of inlet H_2S concentration and humidification on Elimination capacity (EC) of the filter bed

The EC of the biofilter quantifies the removal rate of contaminant per unit volume of the biofilter. The effect of inlet H_2S concentration and MC on the biofilter performance is shown in Figure 6. During the steady state operation of the biofilter when the initial concentration of H_2S was maintained in the range of 321-955 ppmv, EC of 89.33 gm/m^3/hr was obtained with 99.16 % RE. EC of 89.35 gm/m^3 of filter/hr could also be obtained when the initial concentration of H_2S was 1416 ppmv but at reduced RE of 98.31 % and outlet concentration of 12 ppmv of H_2S. However, when the initial concentration was increased to 2020 ppmv, the EC dropped to 60.42 gm/ m^3 of filter/hr. It can be deduced from this result that EC was dependent on inlet concentration of H_2S. This clearly indicated that, when the filter was under steady state operation, EC of 89 gm/ m^3 of filter /hr could be obtained with mixed agricultural residue as supporting material within the pressure drop of 24-78 mm of H_2O/m of bed and MC of 65% (Table 4). EC dropped to 43.50 gm/ m^3 of filter /hr due to the drop in MC of the filter, when the filter was operated without the humidifier at 1416 ppmv of initial concentration of H_2S. In subsequent operation when the filter was operated at the same inlet concentration by bringing back the humidifier into the circuit, EC of only 73.01 g-H_2S/m^3 of filter/hr could be obtained. In this phase of filter operation MC was in the range of 42-57 %. It clearly showed that EC was not only dependent on the

inlet H_2S concentration but also on MC of the filter bed. Many times in regular operation, it is expected that filter needs to be operated at the designed EC. In order to satisfy this condition the operator of the filter needs to check that the MC of the filter material remains at around 65%.

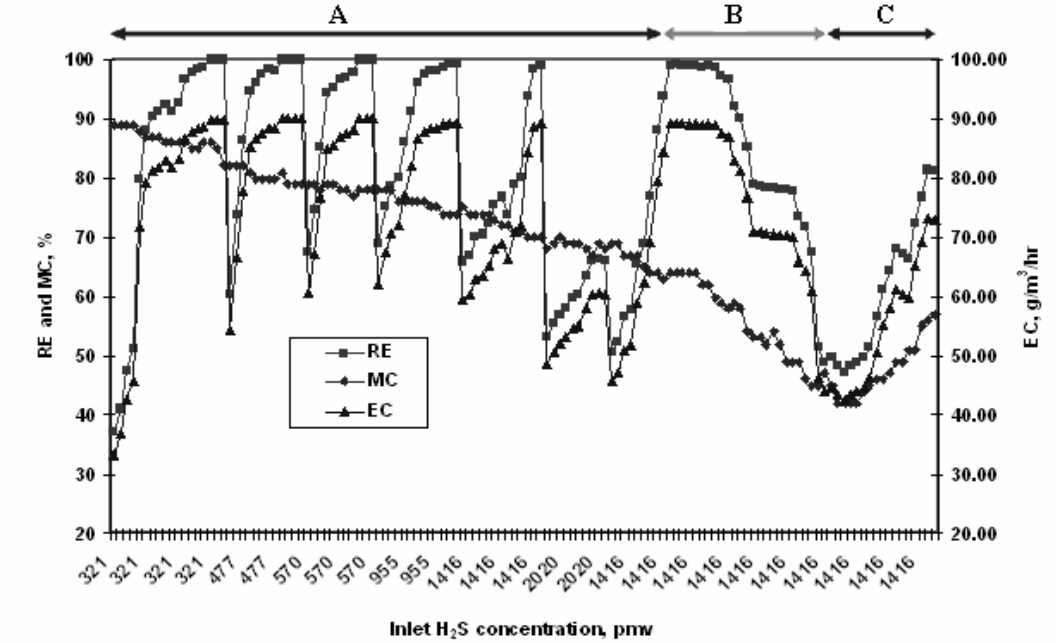

Figure 6: Effect of inlet H_2S concentration on RE, MC and EC during biofilter operation for 128 days

c. Effect of pH

The microorganism present in the inoculum, which was added to the filter material, oxidizes H_2S to sulfite, sulfate, sulfur and H_2SO_4 may form as the terminal or end products and accumulation of the H_2SO_4 in the filter bed causes the pH to fall [Joanna et al., 2001]. Figure 7 shows the variation of pH (pH of the port 1 filter material sample) and RE during the course of operation of the biofilter in all the phases (under steady state conditions). It could be observed from Figure 8 that during 128 days of biofilter operation the pH came down from 7.8 to 4.8. It can also be observed from the same figure that the pH drop could be divided into three phases. In the initial phase of 30 days (0-30), the pH dropped from 8.9 to 7.6, in the second phase of 40 days (30-70), the pH dropped from 7.2-6.2 and in the last phase of 70 days, the pH dropped from 6.2 to 4.8. The rate of pH drop was highest in the last phase whereas it was the lowest in the first phase. The lower rate of pH drop in the first phase can be attributed to the $CaCO_3$ present in the

filter material which was added initially and lower inlet concentration of H_2S (477 ppmv) in the gas mixture during that period.

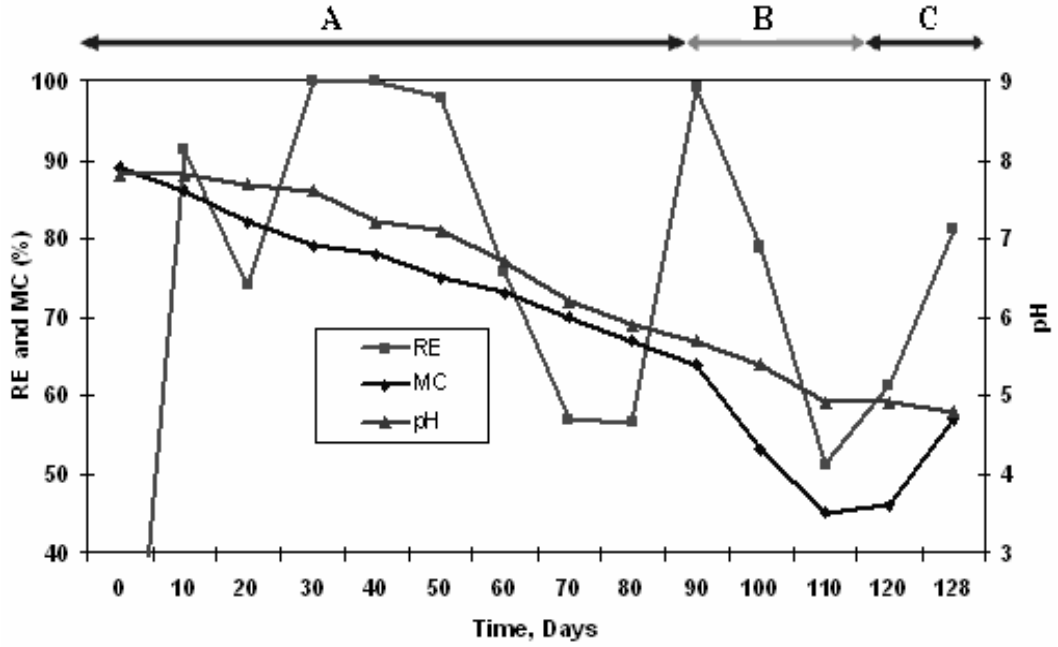

Figure 7: Variation of pH (pH of the port 1 filter material sample), MC and H_2S RE during biofilter operation for 128 days (under steady state conditions)

It can also be observed from the Figure 3 that the RE was more or less uniform during the entire period of operation, except in the initial period of 12 days. The initial 12 days period could be considered as a start up period. During this start up period, stabilization of the phase transfer of H_2S gas to liquid and acclimatization of the microbial culture would be taking place as reported previously [38]. Stable performance despite the pH drop indicated that microbial population present in the filter belongs to the generic class of *Thiobacilli*, which can oxidize sulfurous compounds in acidic phase also. This finding was of paramount importance since neutralizing chemicals that were being used during midcourse of operation of the biofilter can be avoided.

It was also evident from Figure 7 that even though the pH had dropped to 5.7 till the 88^{th} day of operation of the filter with humidifier in the circuit, the RE was more than 99% at the inlet H_2S concentration of 1416 ppmv. In the next phase of operation of the filter till 128^{th} day, the pH had dropped further to 4.8 from 5.9. Even though the inlet concentration of H_2S was reduced again to 1416 ppmv from 2020 ppmv, the pH continued to fall due to the accumulation of acidic end products. By the incorporation of humidifier in the circuit during the last

phase of filter operation, even though MC and RE were recovered to some extent, the pH continues to fall. This showed that the filter performance was highly dependent on MC and inlet H_2S concentration rather than pH. The MC was in the range of 89-46 % during entire period of operation of 128 days. It clearly indicates that variation in RE was dependent on MC and not on pH of the system.
After the termination of the experiments (i.e. after 128 days), the pH of filter material in all the ports were analyzed. Figure 8 shows the pH variation along the length of the filter bed at the end of experimentation. It can be observed form the figure that pH was increasing along the length of the bed and pH was lower in lower ports (4.8) of the bed in comparison with the upper ports (6.1) of the bed. This showed that most of the H_2S removal was taking place below the third port of the bed at pH of lower than 6, indicating that 80-90% of the reaction had took place in the lower part of the bed.

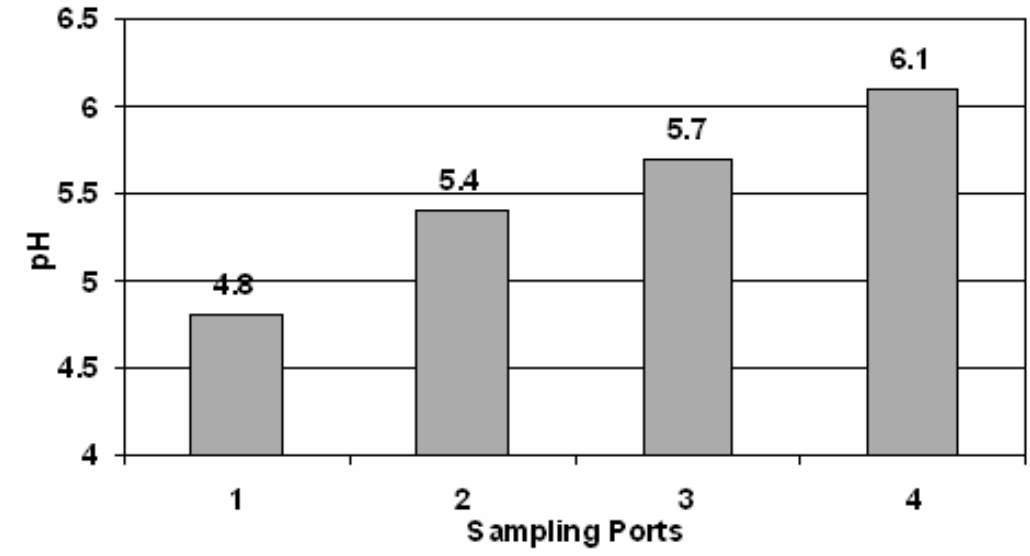

Figure 8: pH of filter material in all the ports after termination of the experiments (i.e. after 128 days)

2.2.4 Metabolic product accumulation in the biofilter

The metabolic end products generated and their conversion ratios are likely to remain nearly the same irrespective of changes in the concentration or loading rates [39]. Hence at the end of 128 days of biofiltration operation, samples of filter material was collected and analyzed for various oxidative products of biological sulfide oxidation system by *Thiobacillus* sp. from the oxidation of H_2S in the immobilized cell biofilter. Approximately 5 gm of filter material was collected from each sampling port located along the length of the biofilter column. The

material was mixed with 50 ml of sterile distilled water and allowed to soak overnight at the temperature of 30±5°C in an incubator. Under aerobic conditions, oxidation of H_2S by chemotrophs occurs with O_2 as an electron acceptor, while the electron donors could be S^0, H_2S or $S_2O_3^{2-}$ [40]. However under oxygen limiting conditions, sulfur is the major end product, while sulfate is formed when sulfide is a limiting factor. This can be represented by the following reactions:

$$2HS^- + O_2 \rightarrow 2S^o + 2OH^-$$
$$2S^o + 3O_2 + 2OH^- \rightarrow 2SO_4^{2-} + 2H^+$$
$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+$$

In aerobic autotrophic oxidation of sulfide following reaction would occur:

$$H_2S + CO_2 + \text{nutrients} + O_2 \rightarrow \text{Cells} + S \text{ and/or } SO_4^{2-} + H_2O$$

The incomplete oxidation of H_2S is generally reflected by high values of sulfites, sulfates and thiosulfates. The sulfate and sulfide at individual ports are estimated and tabulated. From the values it was observed that the sulfate and sulfide content increased by the end of experimentation.

The amount of SO_4^{2-} formed (Table 5) through out the biofilter was more than the amounts of $S_2O_3^{2-}$ and S^{2-}, while the remaining portion was attributed to elemental sulfur and other end products. The terminal product in the biological H_2S by aerobic *Thiobacillus* sp is sulfuric acid. From the pH results during 128 days of biofilter operation, it can be inferred that most of the H_2S has oxidized biologically into sulfuric acid by turning down the bed pH into acidic zone. However in biofilters packed with wood chips and granular activated carbon treating H_2S as single compounds, the accumulation of elemental sulfur or ammonium sulfate has been shown to reduce the performance from 99% to 75% and 30%, respectively [41].

Table 5: Metabolic products accumulation in the biofilter material at the end of 128 days of operation.

Port	Sulfate, mg/l	Thiosulfate, mg/l	Sulfide, mg/l
1	5.1	2.4	0.0145
2	4.5	3.45	0.011
3	14.5	9.85	0.012
4	9.6	6.91	0.006

2.2.5 Kinetic analysis

The apparent kinetic parameters of the maximum elimination capacity (V_{max}) and half-saturation (K_m) constant to degrade H_2S are calculated by the Lineweaver-

Burk (LB), Hanse-Woolf (HW) and Eadie-Hofstee (EH) methods. The kinetic constants were estimated under different conditions according to the operational phases of biofilter during 128 days. The $V_{max\ and}$ K_m values are calculated and tabulated in Table 6. From table it is clear that maximum elimination capacity and saturation constants were significantly affected by the biofilter operation parameters. The maximum elimination capacity, g-H_2S/m^3/hr, (LB) was more in the case of phase I, was 72.11 and gradually decreased in phase II (48.306) and phase III operations (24.545). Similar trend was also observed in the case of data analysis by HW plot for V_{max} in phase I, II and III were, 60.984 40.360 and 23.443 g-H_2S/m^3/hr respectively and by EH plot for V_{max} in phase I, II and III, were 81.229, 50.867, 25.157 g-H_2S/m^3/hr respectively. The regression coefficients in all the cases showed good fit of the data in all the three phases of operation with three types of equations.

Table 6: Maximum removal rate (V_{max}) and saturation constant (K_m) of H_2S removal of biofilter during all the phase of biofilter operation

	L-B		H-W		E-H	
	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m
Phase I*	77.211	-7.805	60.984	238.279	81.229	-5.03
Phase II	48.306	-157.407	40.360	-228.945	50.867	-146.256
Phase III	24.545	-481.163	23.443	-499.208	25.157	-472.711

*** data used was of stable conditions**

The stable conditions data was used for the kinetics constants estimation in the phase I. Inlet H_2S concentrations were varied seven times in a period of 88 days. At each inlet concentration, the biofilter was allowed to reach stable conditions in terms of RE and again inlet H_2S concentration was varied. In order to understand the exact kinetics of H_2S removal it is appropriate to estimate the V_{max} and K_m at each inlet H_2S concentration. The kinetic constants were estimated using all the three, LB, HW and EH plots. Table 7 shows the V_{max} and K_m at each inlet H_2S concentrations using three plots. From the data it is clear that the maximum elimination capacity of biofilter 70.7667 was observed in the case of 570 ppmv of inlet concentration. Though the required outlet H_2S was achievable at 1416 ppmv of inlet H_2S concentration the maximum elimination capacity was only 55.119. Similar trend was observed for V_{max} and K_m when estimated by EH and HW plots. It indicates that the biofilter maximum elimination capacity is at 570 ppmv. However more experiments are required in pilot scale system for a longer duration, before scaling up the process at industrial levels. Kinetic parameters obtained in the present studies are important design criteria for developing the pilot scale operation for efficient operation. This limitation is due to the effect of high concentrations on the Monod kinetics of biodegradation [8].

In some cases, it is known that very high concentration of substrate can become inhibitory [9].

Table 7: Maximum removal rate (V_{max}) and saturation constant (K_m) of H_2S removal of biofilter in Phase I at individual inlet H_2S concentrations

	L-B		H-W		E-H	
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
321	-24.240	49.153	-52.413	32.971	-19.983	56.408
477	-15.947	67.947	-35.284	55.308	-14.331	70.266
570	-16.418	70.7667	-37.697	59.881	-15.298	72.178
955	-71.860	60.385	-85.178	57.1308	-70.509	60.922
1416	-127.548	55.119	-155.925	52.172	-126.268	55.439
2020	-792.952	21.717	-795.947	21.591	-791.044	21.799
1416	-161.199	45.551	-222.12	40.108	-155.387	47.034

2.2.6 Scanning electron microscope (SEM) studies of filter material for the growth of *Thiobacillus* sp

At the end of biofilter operation the filter material samples were collected from sampling ports along the length of the biofilter, one from the top port of the biofilter and the second one from the bottom port of the biofilter. The filter material samples were analyzed by SEM for *Thiobacillus* sp growth. The SEM micrographs shown in Figure 9 clearly depicted the growth of *Thiobacillus* cells on the surface of filter material throughout the biofilter. Further, characteristic rod shaped morphology of the microorganism was distinctly apparent on the surface of the biofilter material. It was observed from the analysis that the growth of *Thiobacillus sp* cells on the biofilter material is good and which in turn responsible for the H_2S oxidation and reduction in the pH of the filter bed. The growth of the *Thiobacillus* sp cells in the bottom port (Figure 9 A) was more in comparison with the top port (Figure 9 B) of the biofilter. It also correlates the difference in pH of the filter bed, which was 4.8 at bottom port and 6.1 at top port, indicates that reduction in pH of the filter material did not affect the growth, hence no reduction in H_2S removal efficiencies. Microscopic observation of filter material showed that the combination of filter material used for H_2S oxidation using *Thiobacillus* sp is supportive. Also it can be concluded that only *Thiobacillus* sp was growing in the filter material. The surface of the filter material is supportive for the growth of *Thiobacillus* sp and also to retain the required moisture content for the microbial growth.

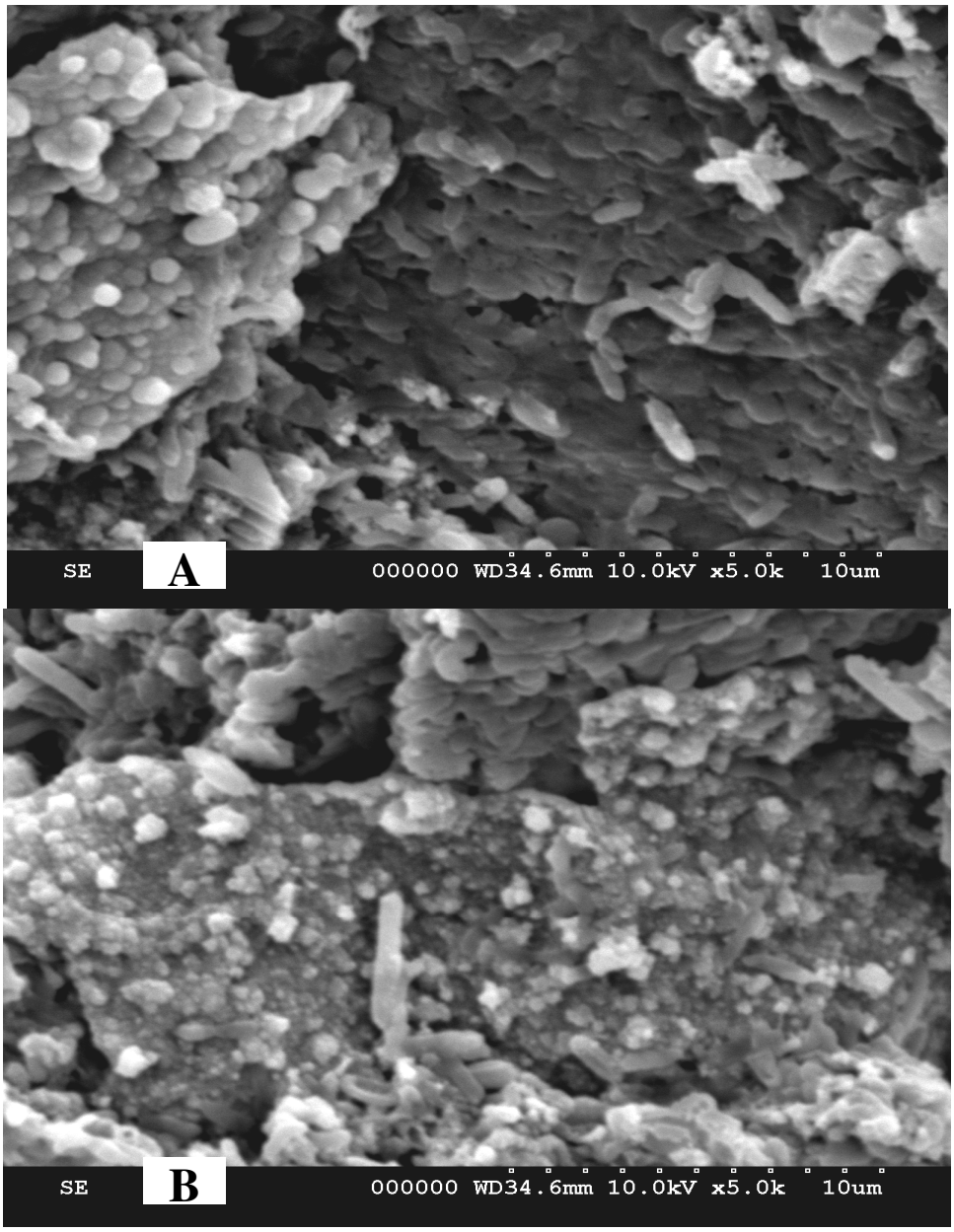

Figure 9: Scanning electron microscope (SEM) micrographs of *Thiobacillus* sp. growing on biofilter material collected from port 1 (bottom) and port 4 (top) of filter bed. Image (A) sample collected from bottom port of the biofilter. Image (b) sample collected from top port of the biofilter. Both the samples were collected at the end of 128 days of biofilter operation.

3. Conclusions

Microbial removal of H_2S, by using biofilter is gaining interest because of its obvious advantages besides being competitive. However, most of the installed biofilters were failing, as the operators were not aware of process parameters, which are important for appropriate operation of the biofilter. Furthermore, biofilters packed with natural materials were subjected to operating difficulties such as compacting or channeling. Therefore there is a need to identify new natural filter material which can overcome the aforesaid difficulties.

In the present work, a new filter material from mixed agricultural residue was studied for the removal of H_2S. This material is a combination of sawdust, bagasse, rice husk and coconut coir pith inoculated with *Thiobacillus* sp. This material was studied for various process parameters that are important for performance evaluation; specifically, for the effects of humidification and inlet concentration of H_2S on the performance of biofilter. The study showed that mixed agricultural residue could be one of the best alternate biofilter bed materials for biofiltration of H_2S. A suitable combination of four materials was worked out in order to retain the maximum moisture content.

It was concluded that the material has less compaction characteristics as it offered low-pressure drop in the range of 24-78 mm H_2O per meter of the packing height throughout the filter operation allowing gas to disperse efficiently for maximizing the RE. The pressure drop data recorded in the present studies was comparable to the pressure obtained with synthetic material as filter bed material in the previous studies [34]. Besides this, no nutrients were added externally during the entire period of operation of the filter, suggesting that agricultural residue used in the present studies could be a cheaper alternative. The material has 28% MC and it was maintained at 42 % even when the filter was operated without humidifier. It can be derived from this result that water retaining capacity of the newly defined combination of agricultural residue is good, which is one of the prerequisites for bed material in biofiltration process.

The microbial count increased from $1.8x10^3$ to $16x10^5$ cfu/gm during the period of 128 days of biofilter operation indicating that the *Thiobacillus* sp responded positively to the process parameter changes made during the operation of the filter. The growth of microbial cultures occurred to the extent of requirement without clogging the filter. In the present study, filter exhibited 100% RE at an inlet concentration of 570 ppmv, 99% RE at an inlet concentration of 1416 ppmv and 66% RE at a maximum inlet concentration of 2020 ppmv. The inlet concentration of H_2S affected the performance of the biofilter and above the inlet concentration of 1416 ppmv, the exit concentration increased beyond the concentration levels suggested by statutory bodies.

Therefore, it can be recognized that the present configuration of the filter with agricultural residue as bed material can remove H_2S to the required level up to inlet concentration of 1416 ppmv only. In the present study, a maximum IMLR of ~90 g-H_2S /m^3 filter bed /hr and EC of ~90 g-H_2S /m^3 filter bed /hr was obtained when the EBRT was in the range of 17-77 seconds. The filter performance in terms of RE, dropped to 48% when the air was not humidified before sending to the filter, which has direct relation with the MC of the filter material. However, the RE recovered to 66% with recovery of MC to 57% upon subsequent re-introduction of the humidifier in to the circuit. But, original MC and RE could not be obtained, which strongly recommends the humidification of waste air before the biofiltration process.

The pH had dropped from 7.8 to 4.8 during the course of operation of the filter. Despite drop in pH, the RE remained steady and varied with MC and inlet H_2S concentration, which suggested that *Thiobacillus* sp grown in the filter can perform even in the acidic phase without intermittent washing of the filter bed. This result suggested that addition of neutralizing chemicals which is normally made may not be required in this case.

Earlier workers studied that [42] when bed mixing was carried out; the removal capacity remained constant, close to 100%. In the present study, the RE was around 99% at 1416 ppmv of inlet H_2S concentration without mixing the filter media. It indicates that the new filter material and their combination played a crucial role in retaining the MC during the operation and also lower pressure drop with good growth of *Thiobacillus* sp across the filter bed. In another study [34], increasing the inlet H_2S concentration beyond 355 ppmv has resulted in reduced RE of the filter bed using *Thiobacillus thioparus*. In the present study, the biofilter has exhibited good RE around 99% even at 1416 ppmv of inlet H_2S concentration. Estimation of kinetic parameters is important and valuable tool in analyzing the biofilter process for H_2S removal. In the present study, kinetic constants were estimated using three different plots i.e LB, EH and HW. The SEM analysis has shown the good growth of *Thiobacillus* sp in the filter bed and hence the new material is good support for the immobilization.

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