A biofilter integrated with gas membrane separation unit for the treatment of fluctuating styrene loads

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ABSTRACT

Biofiltration for volatile organic compound control in waste gas streams is best operated at steady contaminant loadings. To provide long-term stable operation of a biofilter under adverse contaminant feeding conditions, an integrated bioreactor system with a gas separation membrane module installed after a biofilter was proposed for styrene treatment. Styrene was treated effectively, with average styrene effluent concentrations maintained at less than 50 mg m^{-3} and a total removal efficiency of over 96% achieved when the biofiltration column faced fluctuating loads. The maximum elimination capacity of the integrated bioreactor system was $93.8 \text{ g m}^{-3} \text{ h}^{-1}$, which was higher than that obtained with the biofiltration column alone. The combination of these two processes (microbial and chemical) led to more efficient elimination of styrene and buffering of the fluctuating loads. The factors on gas membrane separation, microbial characteristics in the integrated bioreactor and membrane fouling were also investigated in this study.

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1. Introduction

Biofilters, bioscrubbers, and biotrickling filters are more cost-effective than conventional physical–chemical technologies for treating air streams contaminated with volatile organic compounds (VOCs), particularly for high volume with low concentration emissions (Ottengraf et al., 1986; van Groenestijn and Hesselink, 1993). Control of VOCs through biofiltration systems has gained increasing attention and research focus in recent years (Chang et al., 2001; Gallastegui et al., 2011; Hassan and Sorial, 2009; Maestre et al., 2007; Rene et al., 2010). Biological processes rely on specific microbial species, with the biodegradation of organic contaminants to mineral products occurring over numerous steps and producing intermediate compounds. For these reasons, bioreactors are sensitive to surges in VOC loadings. If a biofilter is overloaded, acetic acid accumulation and high effluent concentration of untreated gas may occur.

While biofiltration for VOC control in waste gas streams is best operated at steady contaminant loading, variations are common in real-life applications. One approach to reduce outlet containment concentration is by installing several biofilters in series to enlarge the effective reactor volume and prolong the empty bed retention time (Barona et al., 2004). Although studies have verified the effectiveness of this technology during transient contaminant loadings,

large land area requirement is a significant disadvantage and limits its feasibility in areas where land acquisition costs are relatively high. Integrated reactors, consisting of a biofiltration unit followed by a catalytic oxidation unit or an adsorption unit, have been used to improve the removal efficiency of waste gases containing relatively high VOC concentrations (Li et al., 2007, 2008). However, catalytic oxidation of VOCs requires high operating temperatures and adsorbents need to be regenerated after saturated adsorption, processes that are both costly and energy intensive.

Attenuation of input fluctuation to biological processes provides an effective solution, especially for sources where contaminant loading fluctuations are commonly encountered. Single and double activated carbon adsorption columns have been used as buffer units before a biofilter for VOC emission control with unstable pollutant loads (Cai and Sorial, 2009; Li and Moe, 2005; Kim et al., 2007; Weber and Hartmans, 1995). However, applications of this technology are restrictive in regards to desired contaminant concentration. Since contaminants are mostly captured and accumulate in the adsorption column, the biofilter is exposed to substrate starvation until breakthrough occurs in the adsorption column. Substrate starvation significantly influences biological activity within the biofilter and longer starvation causes longer biofilter performance acclimation (Cox and Deshusses, 2002; Kim et al., 2007). In addition, the buffer capacity of the adsorption column is quickly exhausted when large contaminant loading fluctuations are encountered.

Separation of organic vapor from air streams to control VOC emissions has been widely investigated in recent years. Gas

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membranes used for organic vapor separation have gained interest and acceptance as they selectively permeate organics over air (Kim et al., 2002; Liu et al., 2006, 2009). Advantages of the gas separation membrane include quick, low energy consumption and little volume. In the removal and recovery of VOCs from air streams at a certain pressure by membrane separation, a large volume of air flows on one side of the membrane at the feed pressure. When a vacuum is applied to the other side to create a partial pressure driving force, the VOCs selectively permeate through the membrane in preference to air, and the concentration of VOC in the treated air stream is then low enough for venting into the atmosphere. Vapor permeation through membranes offers significant energy savings and VOC recycling opportunities compared to conventional control processes, particularly if the VOC concentration is high (Yeom et al., 2002a). Systems are compact and mobile and can be configured to treat a wide range of feed stream flow rates and compositions. The separation of aromatic VOCs has been investigated over various gas membranes (Dingemans et al., 2008; Obuskovic et al., 2003; Sohn et al., 2000; Wang et al., 2005). However, there is scarce studies on utilization of gas membrane separation in maintain biofilter performance during transient VOC loadings.

This study aimed to maintain long-term consistent performance of biological processes using a gas membrane separation module installed after a biofilter during transient VOC loadings. Separation occurs because different compounds transport across the membrane at different rates. In the gas membrane module, permeation of VOC through membranes was much faster than the other components in the off-gas. Therefore, the permeated stream becomes enriched in VOC, while the retentate stream turns to a VOC depleted air. The enriched-VOC in the permeated stream was recharged back to the biofilter and the retentate stream, depending on the extent of the purification, can be emitted into the atmosphere. The combination of gas separation and microbial processes resulted in increased removal efficiency, less sensitivity to inlet load surge and minimized the volume of the bioreactor. This paper outlines the observations on the performance of the biofiltration column and gas membrane module under different conditions. Characteristics of microbes in the biofiltration column and membrane fouling were also investigated.

2. Methods

Styrene monomer, a colorless and aromatic oily liquid used predominately in the petrochemical and polymer-processing industries, contributes to natural resource pollution via the release of styrene-contaminated effluents and off-gases. This compound is listed in the 129 priority pollutants by the Environmental Protection Agency in the USA, and was used as the target compound in this study.

2.1. Integrated bioreactor system

Continuous experiments were conducted in a bench-scale biofilter followed by a gas separation membrane module for controlling styrene as a single contaminant (Fig. 1). This bioreactor system included a biofilter unit and a gas membrane unit. Biological degradation of styrene was carried out in a plastic column biofilter measuring 30 cm long, 15 cm wide, and 100 cm high, with an effective volume of 30 L. Polyurethane foam cubes (1 cm^3) with the specific surface area of $1.3\text{--}1.6 \times 10^5 \text{ m}^2 \text{ m}^{-3}$ were used as packing material for attached microorganism growth. The separation and condensation of styrene from off-gas were carried out over a gas membrane module. The membranes used were configured in a 0.05 m diameter spiral wound module containing 0.5 m^2 of gas membrane. The module provided by Tianbang National Engineering

Fig. 1. The schematic diagram of the integrated bioreactor. 1, Air pump; 2, flowmeter; 3, styrene container; 4, mix chamber; 5, gas membrane module; 6, biofiltration column; 7, inlet stream sampling point; 8 and 9, sampling points; 10, spray pipe; 11, effluent of the biofiltration column sampling point; 12, retentate side sampling point; 13, permeate side sampling point; 14, pressure gauge; 15, vacuum pump.

Research Center of Membrane Technology Company, Ltd. (TBM) consisted of layers of membrane wrapped around a porous collection pipe. The individual membrane layers were separated by spacers. Gas feed passed parallel to the membrane surface, and the permeated stream penetrated through the membrane perpendicular to the flow. The polydimethylsiloxane (PDMS) membranes used for this process were in composite form, wherein a thin layer of non-porous silicone rubber was coated on an appropriate porous polymeric substrate. In most cases, organophilic rubber membranes are preferred in the separation of VOCs from air because they are much permeable to VOCs. Among the rubbery membranes, PDMS had high permeability and high selectivity for VOCs (Kimmerle et al., 1988; Strathmann et al., 1986), and exhibited excellent membrane performance for the removal of VOCs from other non-permeable gas components (Kim et al., 2002; Lahiere et al., 1993). The separation can be operated at ambient temperature and the lifetime of the PDMS membranes is 3 years. Depending on separation system design, over 90% of the organic vapor can be recycled from the feed stream.

Off-gases containing styrene firstly entered the biofiltration column. Styrene was absorbed and subsequently biodegraded into carbon dioxide and water by the microorganisms attached to the packing material. Gases with residual styrene were then fed into the gas membrane module. The styrene-rich stream permeated through the membrane into a collection pipe and was recharged back to the biofiltration column by a vacuum pump from the permeate side. The styrene depleted residues were then discharged from the retentate side. Valves were installed on the permeate line to prevent the permeate pressure from exceeding the feed pressure. The flow rate of the permeated stream was $0.35 \text{ m}^3 \text{ h}^{-1}$ and the circulating rate of the air in the system was 30.4% (v:v).

Two air compressors supplied the airflow. A small stream of air was bubbled through the vessel containing pure styrene solvent, and was then combined with a larger air stream in the mix chamber. This resulted in a synthetic gas with a concentration of styrene between 339 and 1585 mg m^{-3} . The desired concentration of styrene in the influent air stream was obtained by varying the two air flow amounts. Calibrated flow meters were provided to measure flow rates.

Sampling ports were located at the inlet and outlet of the biofilter and gas membrane module to determine the styrene concentrations in the untreated and treated stream, respectively. The experiment was performed in a laboratory, with seasonal temperature changes

from 23 to 33 °C. The oxygen concentrations in the system monitored by an oxygen detector (GasAlertMaxXT, BW Technologies Ltd. Company, Canada) were in the range of 18–20%. The pH and relative humidity (RH) in the biofiltration column were measured regularly to ensure they stayed at optimal range.

Initially, the biofiltration column was seeded with aerobic microbial cultures obtained from a previous styrene-degrading bioreactor in our laboratory for a fast start up. The cultures on packing material in bioreactor mentioned above served as an inoculum. Ten grams of packing material were cut into pieces and soaked in 1000 mL of nutrient solution which contained K_2HPO_4 0.5 g L⁻¹, KH_2PO_4 9.0 g L⁻¹, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1 g L⁻¹, NH_4Cl 2.0 g L⁻¹ (pH 5.5–6.0). The mixture was shaken for 30 min. Then, the liquid with suspended cells was cultured in 3 L nutrient solution with 10 µg L⁻¹ styrene as the sole carbon source at 30 °C, 160 rpm for 3 days, and then transferred into the same nutrient solution three times for enrichment of microorganisms capable of degrading styrene. The microbial cultures were harvested by centrifugation at 4000 rpm for 20 min (Biofuge Stratos, Heraeus, Germany). The concentrated cells were re-suspended in 5 L nutrient solution and inoculated onto the packing material (polyurethane foam cubes) of the biofiltration column. The initial inlet concentrations of styrene were in the range of 359.0–401.9 mg m⁻³. Substantial improvement in biofilter performance was achieved by intermittent nutrient solution irrigation providing necessary nutrients to the microorganisms and by proper moisture content of the packing media in the biofiltration column.

2.2. Analytical methods

The surface properties of new and used membranes were observed through a scanning electron microscope (HITACHI S-3000N/EDAX Inc., Japan).

Temperature, pressure, and feed flow rates of permeate and residual streams were recorded. The performance of the combined system was detected by measuring the concentrations of experimental compounds in the inlet and outlet gases, in addition to the pH and RH in the biofiltration column. Styrene concentrations in the air stream were measured using a Gas Chromatograph (Agilent 6890N, USA) with flame ionization detector (FID). A HP-5 column Ø 0.32 mm × 30 m in size was used at 120 °C, with nitrogen at 25 psi used as the carrier gas. The GC/FID was operated at an injection temperature of 250 °C and detector temperature of 250 °C. The pH value was measured by a pH meter (PH-3C, Shanghai). A Dewpoint Thermohygrometer (WD-35612, OAKTON, Germany) was used to measure RH and temperature.

Polyurethane foam cubes (packing material) were collected periodically from the bioreactor for microbial analysis. The 1 g cubes were cut into pieces, soaked in 10 mL of sterile distilled water, and then homogenized for 15 min using a magnetic stirrer. Serial dilutions of the homogenized sample were prepared and 0.1 mL liquid was spread onto culture mediums (BR, Aoboxing Biotech, Co., China). Bacteria were enumerated on nutrient agar and LB agar. Fungi were enumerated on Czapek agar, potato dextrose agar, and Gause's synthetic medium. Malt extract agar, corn meal agar, and glucose yeast extract agar were used for yeast growth, while JCM agar was used for streptomyces growth. The distinctive individual colonies were subcultured and purified by streaking on fresh agar plates after 1 week of incubation at 28 °C. Culture purity was verified by an absence of growth on medium plates and by microscopic examination.

Identification of the individual genera and strains was performed using morphology, Gram-staining and biochemical tests. Preliminary taxonomic classification of the bacterial isolates was performed using classical biochemical properties to distinguish

those strains that were distinct of colony morphology. Each bacterial isolate was characterized by Gram staining and determining their biochemical properties (Buchanan and Gibbons, 1984). The streptomyces isolates were grown on JCM agar at 28 °C for 7–10 days to determine spore chain morphology and their ability to produce soluble pigments (Yan, 1975). The colors of aerial and substrate mycelium were also observed. Their morphological characteristics were examined with microscope objectives at scanning electron microscopy (SEM, HITACHI S-3000N/EDAX Inc., Japan). The TLC (Hasegawa T.) of the whole cell hydrolysates was applied to ascertain the appearance of LL-A2 pm (diaminopimelic acid). Fungal cultures were identified in routine morphological test (Dai, 1987). The isolation and identification methods in detail have been described in our previous report (Li and Liu, 2009).

For analysis of the styrene degradation characteristics of the isolate, the strain was inoculated onto inorganic nutrients agar containing 6.0 g L⁻¹ KH_2PO_4 , 6.0 g L⁻¹ $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 3.0 g L⁻¹ $(\text{NH}_4)_2\text{SO}_4$, 0.02 g L⁻¹ $\text{CaCl}_2 \cdot 12\text{H}_2\text{O}$, 0.05 g L⁻¹ MgSO_4 , 20 g L⁻¹ agar and 20 µL L⁻¹ styrene as sole carbon source and incubated for 3 days at 35 °C. Fungi strains were inoculated onto the same medium with additional 100 mL (1:3000) of Rose Bengal solution for bacteria inhibition and incubated for 7 days at 28 °C. The plates were observed daily.

A Six-stage Andersen Sampler (228–9530K, SKC Gulf Coast Inc., USA) was used to capture microorganisms emitted from the biofiltration column. Six glass Petri dishes containing suitable culture medium were kept under sieves arranged in gradually decreasing order of pore size. Air was drawn through the sampler sieves at a constant flow of 28.3 L min⁻¹ using a pump (Andersen, 1958; Wüst et al., 2003). All inside surfaces were maintained in a sterile condition until sampling. Airborne microorganisms were enriched from the collected air samples in 90 mm Petri dishes containing different agar media and were cultivated. The bacteria were incubated in Nutrient agar (BR, Aoboxing Biotech, Co., China) at 30 °C for 48 h. Fungi were cultivated in Rose Bengal Medium (BR, Aoboxing Biotech, Co., China) at 30 °C for 7 days. Positive-hole correction method was used to determine colony count concentrations (Andersen, 1958; Macher, 1989). The results were calculated as the geometric mean of the replicates and were expressed as colony forming units per cubic meter of air (CFU m⁻³).

3. Results and discussion

3.1. Performance of the integrated bioreactor system

To test the styrene elimination capacity of the integrated bioreactor system, different experiments were performed in which the inlet styrene concentration was varied over a period of 4 months. The continuous conversion experiments were divided into three stages according to the operation state of the biofilter unit. Stage I (day 1 to day 7) was the period of acclimation. Stage II (day 8 to day 59) was the steady state period of the biofiltration unit. Stage III (day 60 to day 118) presented the overload occurrences in the biofilter. The concentration of styrene in the inlet gases was 339–1585 mg m⁻³. Total gas flow rate in the biofilter was 1.5 m³ h⁻¹.

In this study, biodegradation of styrene in the biofilter unit was carried out continuously, and the gas membrane module was operated only when the biofiltration column underwent fluctuating loads. Fig. 2 demonstrates the changes in the concentrations of styrene in the inlet stream (C_{in}), the effluent stream of the biofiltration column (C_e), and the retentate stream of gas membrane module (C_r). As the membrane module was installed following the biofilter, the effluent stream of the biofiltration column was also the feed stream of the gas membrane module. Removal efficiencies of the

Fig. 2. The changes of styrene concentration in the integrated bioreactor. C_{in} , concentrations of styrene in inlet stream; C_e , concentrations of styrene in effluent stream of the biofiltration column; C_p , concentrations of styrene in permeate stream; C_r , concentrations of styrene in retentate stream of gas membrane module.

biofiltration column ($R_{(B)}$) and total removal efficiencies ($R_{(T)}$) are shown in Fig. 3. As seen in Figs. 2 and 3, the outlet concentrations of styrene changed with inlet concentrations. As the biofiltration column was seeded with aerobic microbial cultures obtained from styrene-degrading bioreactor, removal efficiency of styrene increased gradually from 67.7% to 89.3% within 1 week. Inoculation quickened acclimation to styrene for the biofilter unit. Removal efficiency of styrene then remained at a stable level for the next 2 months, that is, the biofilter unit was in a steady state (Stage II). During this state, the mean concentration of styrene in the effluent stream was 45.5 mg m^{-3} when the inlet concentration of styrene was just below 700 mg m^{-3} , and mean removal efficiency was 90.9%.

When the inlet concentration of styrene was suddenly increased from 611.8 to 1243.3 mg m^{-3} at day 60, the concentration of styrene in the effluent stream of the biofilter unit increased to 251.2 mg m^{-3} and average removal efficiency dropped from 92.0% to 79.8%, which indicated that overloading the biofilter had overwhelmed its capacity. In Stage III, the average inlet and outlet styrene concentrations were 1432.3 and 259.5 mg m^{-3} , respectively. Mean removal efficiency of the biofilter unit was only 79.6%. While enlarging the volume of the biofiltration column would be very effective in dealing with this problem, land area requirements and inconvenience in operation make this a problematic option. To ensure elimination efficiency, the gas membrane module was used to reduce styrene concentration. Results in Figs.

Fig. 3. The removal efficiencies of biofiltration column ($R_{(B)}$) and total removal efficiencies ($R_{(T)}$).

2 and 3 reveal that the average concentration of styrene from the gas membrane module was as low as 49.9 mg m^{-3} , and total styrene conversion efficiency was over 96% in the integrated bioreactor system. In the gas membrane module, residual styrene was not only separated but also condensed. Most styrene in the residual gas could be recharged back to the biofiltration column, resulting in extended retention time. Integrated bioreactors with a biofilter and gas membrane are, therefore, a feasible option for treating fluctuating styrene loads without enlarging the biofilter volume. Such a system would significantly reduce land requirements if these experimental conditions were applied to a scaled-up commercial process.

Removal capacity of the biofilter was presented in Fig. 4, where the elimination capacity of styrene was plotted versus the styrene load at different inlet concentrations. Results show that removal capacity increased consistently with load. During Stage II, a gradual and linear increase in elimination capacity occurred up to a styrene load of approximately $44.2 \text{ g m}^{-3} \text{ h}^{-1}$. Such behavior indicated that a linear relationship existed between removal rate and inlet load at low loading rates, and that the pollutants were nearly completely removed. With further load increases during Stage III, the elimination rate increased more slowly up to a critical load and then remained constant, which indicated that maximum elimination capacity of the biofiltration column had been achieved. The biofilter unit itself had a maximum styrene elimination capacity of $77.7 \text{ g m}^{-3} \text{ h}^{-1}$. This threshold was obtained at an inlet concentration of 1430.6 mg m^{-3} and at a superficial gas velocity of $1.5 \text{ m}^3 \text{ h}^{-1}$. However, total elimination capacity increased steadily with inlet load, and a maximum value of $93.8 \text{ g m}^{-3} \text{ h}^{-1}$ was obtained in the integrated bioreactor system due to the separation of the gas membrane unit. Combining biofiltration and gas membrane separation significantly improved elimination performance, particularly with high contaminant loadings. Such results are potentially valuable for the industrial application of integrated systems where gas membrane modules followed by a biofilter can be installed and operated when required. This, in turn, makes whole treatment much more economically feasible.

3.2. Factors on gas membrane separation

The performance of styrene separation in the membrane module was investigated by changing styrene concentration in the feed stream (C_f) and pressure differences between the feed and permeate sides (P_d). The concentration of styrene varied from 86.2 to 714.3 mg m^{-3} . The feed gas flow (Q_f), P_d and temperature were $1.5 \text{ m}^3 \text{ h}^{-1}$, 5.0 kPa and 31°C , respectively. As shown Fig. 5a, C_f

Fig. 4. Elimination capacity vs. styrene inlet load for various gas flow rates (∇ , elimination capacity of the biofilter; $\blacktriangle$, total elimination capacity).

was maintained below 75 mg m^{-3} , when C_f was less than 300 mg m^{-3} . The removal efficiency of the gas membrane module (R_G) reached over 75%. Then, C_r increased dramatically with the increase in C_f . When C_f was over 700 mg m^{-3} , C_r exceeded 320 mg m^{-3} , and R_G was reduced to 55%. This is probably because of the styrene permeability through the PDMS membrane. In this study, with the increasing of C_f , constant increase of the styrene concentration in permeated stream (C_p) could be observed under the same experiment condition (Fig. S1 in the Supplementary materials available on-line). Similar results were obtained by previous research (Lahiere et al., 1993; Paul et al., 1988; Yeom et al., 2002b). The gas permeability through a membrane is determined by solubility and diffusivity of the gas. High sorption uptakes of styrene will occur on organophilic polymers, such as PDMS, which resulted in membranes swelling. The enlarged interstitial spaces between polymer chains in the membrane due to membrane swelling not only favor the diffusion but also allow high solubility for styrene on membranes. Thus, an increase styrene concentration in the feed stream will enhance the styrene permeability through the membrane. Due to large amount of styrene permeated through the membrane, C_r could maintain less than 75 mg m^{-3} . However, the increasing speed of C_p tended to retard under high feed concentration (e.g. 700 mg m^{-3}) (Fig. S1 in the Supplementary materials available on-line). A large amount of styrene could be detected from the retentate stream as a result. Enlarging the membrane area by installing more modules would be required to reduce the C_r

when large amounts of styrene are presented in membrane module feed streams.

Membrane air separation is a pressure driven process, as transport through the membrane is induced by pressure differentials across the membrane. Continuous separation is achieved only when a continuous driving force is applied (Lahiere et al., 1993; Rao et al., 2011). In the present study, this was directly proportional to the pressure difference in the feed and permeate stream (P_d). Fig. 5b shows the changes of C_r versus P_d at different C_f , i.e. 367.5 and 526 mg m^{-3} . At C_f of 367.5 mg m^{-3} , C_r was higher than C_f when P_d was below 1.5 kPa. It decreased swiftly from 340.6 to 221.6 mg m^{-3} with P_d ranging from 2.0 to 4.0 kPa. The C_r decreased to 211.3 mg m^{-3} with further increasing P_d . Similarly, at C_f of 526 mg m^{-3} , C_r was higher than C_f when P_d was below 1.0 kPa. The C_r decreased from 512.9 to 364.2 mg m^{-3} as P_d increased from 1.0 to 4.0 kPa. Further increases in P_d slowly reduced C_r to 349.7 mg m^{-3} before it stabilized. The P_d had the greatest impact on the performance of the membrane separator. At lower C_f , C_r was apparently more influenced by P_d . Air containing styrene was separated into one phase of enriched-styrene in the permeate stream and one phase of poor-styrene in the retentate side stream. As permeation rates depended on P_d , high P_d induced high permeate concentration of the styrene. Most styrene transported through the membrane under high P_d resulted in low C_r and high R_G in the gas membrane module.

Styrene separation in the membrane module depended on styrene content in the feed stream and the pressure differences between the feed-side and permeate-side. Increasing membrane area or adding more membrane modules would benefit the treatment of off gas emissions containing high concentrations of styrene. The optimum number of membrane modules and pressure differences could be selected according to the concentration of styrene from the biofilter in actual cases.

The moisture will affect the styrene permeation through the membrane. A contrast test was conducted to investigate the influence of moisture on the membrane separation efficiency. The concentration of styrene in feed stream was 226.8 mg m^{-3} and the flow rate was $1.5 \text{ m}^3 \text{ h}^{-1}$. The concentration of styrene in retentate stream would increase from 45.7 to 82.3 mg m^{-3} when the relative humidity of feed stream increased from 30% to 76%. High moisture content in the feed stream inhibited styrene passing through the membrane which brought about the increasing styrene present in retentate stream.

3.3. Microbial characteristics

Gas-phase bioreactors utilize microbial metabolic reactions to remove contaminants from air, in which microbial communities use the contaminants as food or substrate. Consequently, treatment of pollution relies on developing better microbial communities. To investigate the microbial population formed in the biofiltration column, a basic characterization of the process culture was attempted using plate culture techniques. Samples were collected from the biofiltration column on day 1, day 50, and day 107 during Stage I, Stage II and Stage III, respectively. They were inoculated onto media for isolation and identification. Bacteria, fungi, yeast, and streptomyces were isolated from the integrated bioreactor. Isolates were tested for their ability to biodegrade styrene. These microbial analyses and degradation tests showed that *Candida tropicalis*, *Pseudomonas* genera, *Aspergillus niger* and *Penicillium frequentans* were able to grow using styrene as a sole carbon and energy source. *Pseudomonas* strains have often been detected from the biofilters used for styrene removal (Alonso et al., 2003; Okamoto et al., 2003). *Aspergillus oryzae* and *Penicillium* sp. have been identified from eukaryotic strains isolated from the biofilters during the treatment of styrene (Cox et al., 1997; Paca et al., 2001).

Fig. 5. (a) Influence of styrene concentration in feed stream; (b) influence of the pressure difference between feed and permeate side. $\blacktriangle$, $C_f = 367.5 \text{ mg m}^{-3}$; ∇ , $C_f = 526 \text{ mg m}^{-3}$; C_r , concentrations of styrene in feed stream; C_r , concentrations of styrene in retentate stream; Q_f , gas flow rate of feed stream; P_d , pressure difference between feed side and retentate side.

Among yeasts, *C. tropicalis* has the highest hydrocarbon oxidation ability. They can grow using hydrocarbon as a source of carbon, resulting in the production of peroxisome and enzymes of the fatty acid beta-oxidation. These four species were considered as styrene-degraders.

On the first day of operation, the biofiltration column was inoculated with biomass from a styrene-degrading reactor in laboratory and styrene as a sole pollutant was treated in the bioreactor. The 3.77×10^6 CFU g⁻¹ of total species was detected from the packing media, with 72.64% determined as styrene-degraders (Table 1). The total species counts in Stage II were almost the same as that in Stage I. However, the percentage of styrene-degraders in Stage II was greater (86.22%), indicating that the amount of styrene-degraders increased significantly within 2 months of operation. Competition may occur when different species of microorganisms present within one bioreactor. They will compete with each other for active adsorption sites that allow them to contact the contaminants. Large amounts of styrene may serve as an energy source or building material. Styrene degrading species strive to grow and reproduce, and they will grow vigorously in an environment where food is abundant. When the biofiltration column was overloaded (Stage III), the average concentration of styrene in the inlet stream reached 1432.3 mg m⁻³. Total species increased significantly, while styrene-degraders declined slightly. The gas membrane module was used to reduce styrene in the effluent stream of the biofiltration column in Stage III. The styrene in the gas membrane module not only separated from other components, but also became more concentrated. As the enriched styrene was recharged back into the biofiltration column, the concentration of untreated styrene was maintained in a certain range, which supported a certain amount of styrene-degraders. Acetic acid and other intermediate products may have accumulated in the biofilter, especially when it was overloaded (Berger and Peters, 1999; Devinny and Hodge, 1995; Devinny et al., 1999a). When intermediate degrading species increased, the total species were obviously enhanced.

Multiple metabolic steps are required to transform styrene to carbon dioxide and water, and different species may specialize in different parts of the process. *C. tropicalis*, *Pseudomonas* genera, *A. niger*, and *P. frequentans* were the four dominant styrene-degraders found in the biofiltration column. However, their relative abundances were different in individual stages. Large numbers of eubacteria, yeast and fungi were detected after inoculation, with *Pseudomonas* genera and *C. tropicalis* dominating *P. frequentans*. *A. niger* was not observed in Stage I, but did appear in Stage II. *Pseudomonas* genera and *C. tropicalis* were dominant when overload presented in biofiltration column, but eukaryotic organisms such as *A. niger* and *P. frequentans* were also noted. If a substrate is present in high concentrations and provides abundant energy, microor-

ganisms may compete vigorously for space and nutrients and prevent microorganisms that use other substrates from growing. Those microorganisms that degrade the substrate will grow rapidly and become abundant when the contaminant in the air becomes a dominant substrate. The average concentration of styrene in Stage III (1432.3 mg m⁻³) was much greater than that in Stage II (488.8 mg m⁻³). The microorganisms in Stage II degraded relatively low concentrations of styrene, while species in Stage III survived on higher concentrations. The styrene was degraded mainly by *Pseudomonas* genera under lower concentrations, and by *C. tropicalis*, *Pseudomonas* genera, and *P. frequentans* under high concentrations.

The pH value in the biofiltration column was maintained between 5.0 and 5.5 in Stage I and Stage II, which was suitable for bacterial growth. Under these conditions, *Pseudomonas* was found to dominate. The pH reduced gradually to 4.0 in Stage III, which was likely from acetic acid accumulation due to the overloaded biofilter. *C. tropicalis*, which can survive in acidic conditions, grew rapidly and became abundant. In most cases, fungal species will dominate bacterial species under acidic and dry conditions. The relative humidity in the biofiltration column was 75–85%. Prevailing mildew (e.g. *A. niger* and *P. frequentans*), which can suffer from dry ambience, emerged in Stage III.

The microbial populations differed in amount, species and distribution characteristics due to the properties of the compounds being treated, their concentrations and the microenvironment in the biofiltration column (Devinny et al., 1999b). The correlation of integrated bioreactor removal efficiency and bacterial community composition in the biofilter under different styrene's load indicated that the separation of gas membrane remedied biofilter defects and strengthened the biofilter by prolonging retention time and maintaining enough styrene for the growth of styrene-degrading species when overloading occurred. It is gas membrane separation unit make contribution for the relative stable total removal efficiency.

3.4. Gas membrane fouling

Porous media were packed into the bioreactor to support the microorganisms and provide access to the contaminants in the air-flow. Some of the attached microorganisms may have been released from the bioreactor with the gas stream. Emission of microorganisms from the biofiltration column was determined by collecting samples with an Andersen sampler. Results revealed that 1897 ± 164 CFUs m⁻³ of bacteria and 577 ± 65 CFUs m⁻³ of fungi with different particle sizes were released from the biofiltration column (Table 2). They were unable to pass through the gas membrane and most were recharged back into the biofiltration column, which prevented air-borne microorganisms from being released into the atmosphere. However, some air-borne microorganisms remained in the gas membrane module and led to the membrane fouling. Scanning electron photomicrographs (SEM) provided detailed images of the surface structures of the new

Table 1
Counts of microbial population in the biofiltration column.

Population		Stage I	Stage II	Stage III
Styrene-degraders (log counts/g)	<i>Pseudomonas</i> genera	6.28	6.33	7.22
	<i>Candida tyopicalis</i>	6.01	6.12	7.57
	<i>Aspergillus niger</i>	ND	5.27	6.76
	<i>Penicillium</i>	5.31	5.54	6.82
	<i>frequentans</i>			
Other species (log counts/g)		6.01	5.77	7.16
Total (log counts/g)		6.58	6.63	7.91
$P_{sd} (\%) = M_{sd}/PT \times 100$		72.64	86.22	81.96
$P_{os} (\%) = M_{os}/PT \times 100$		27.36	13.78	18.04

ND, not detected; M_{sd} , population of styrene-degraders; M_{os} , population of other species; P_T , total population; P_{sd} , percentage of styrene-degraders; P_{os} , percentage of other species.

Table 2
Concentration and particle size distribution of airborne bacteria and fungi in effluent stream from biofilter.

Particle size (μm)	Bacteria (CFU m ⁻³)	Fungi (CFU m ⁻³)
>7.0	336 ± 21	63 ± 7
4.7–7.0	126 ± 16	110 ± 9
3.3–4.7	317 ± 25	95 ± 11
2.1–3.3	932 ± 83	152 ± 23
1.1–2.1	138 ± 12	85 ± 10
0.65–1.1	48 ± 7	24 ± 5
Total	1897 ± 164	577 ± 65

and used gas membrane. The new membrane had a clean and smooth surface (Fig. S2a in the Supplementary materials available on-line), while bacillus and salts particles were observed on the used membrane surface (Fig. S2b and d in the Supplementary materials available on-line). The microphotographs demonstrate that most microorganisms readily colonized on the membrane surface and accumulated in flexures to form biofilm (Fig. S2c in the Supplementary materials available on-line).

Biological technologies are based on using microorganisms to biodegrade gaseous contaminants and produce innocuous end products. Packing media in biofilters often contain 60–80% water to maintain microorganism growth. The relative humidity in the gas out of the biofiltration column was usually over 75%. Although most moisture was discharged from the retentate side or recycled from the permeate side, a small amount condensed and formed a water film on the membrane surface. When the air-borne microorganisms emitted from the biofilter column remained in the gas membrane module, the moisture in the stream not only decreased the separation capacity of the membrane module (Javaid, 2005; Koros and Fleming, 1993), but also provided suitable microenvironment for airborne microorganisms' growth. As such, the abundance of microorganisms resulted in membrane fouling and clogging, which had significant impact on gas membrane module performance.

Moisture and microorganisms released from the biofiltration column led to gas membrane fouling. Special design desiccation tubes were used to dry the gas and capture the microorganisms before they entered the gas membrane module, so that the gas membrane was undisturbed by moisture and microorganisms, and consequently had a much longer life. For easy replacement, two desiccation tubes were installed on the biofiltration column, which could be operated in turn.

4. Conclusions

Compared to a stand-alone biofilter, the integrated treatment system reliably treated fluctuating styrene loading with high removal efficiency. The separation of the gas membrane remedied the biofilter defects and strengthened the biofilter by prolonging retention time and maintaining enough styrene for the growth of styrene-degrading species when overloading occurred. Gas membrane modules can be installed in series following the biofilter, and therefore the optimum number of membrane modules and pressure differences could be selected according to the concentration of styrene from the biofilter. This novel approach to biofiltration lays a foundation for integrated control technology.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2012.02.025.

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