Isolates identification and characteristics of microorganisms in biotrickling filter and biofilter system treating H$_2$S and NH$_3$

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Abstract

A combination system of biotrickling filter (BTF) and biofilter (BF), adopting surfactant-modified clinoptilolite and surfactant-modified wood chip as the media respectively, was applied to treat H$_2$S and NH$_3$ simultaneously. The identification and sole carbon sources utilization patterns of isolates in the combination system were studied by Biolog system. The isolates were identified as Bacillus sphaericus, Geobacillus thermoglucosidasius (55°C) and Micrococcus luteus (ATCC 9341) in BTF, and Aspergillus sydowii (Bainier & Sartory) Thom & Church in BF. Among 95 substrate classes supplied by Biolog system, the carboxylic acids and methyl esters had the highest utilization extent for the four species, followed by the amino acids and peptides. The descending sequence of carbon sources utilization capability of isolates was A. sydowii (52.6%), M. luteus (39.5%), B. sphaericus (21.6%), and G. thermoglucosidasius (17.7%).

Key words: Biolog system; hydrogen sulfide; ammonia; isolates identification; BTF/BF system

Introduction

H$_2$S and NH$_3$ are irritating, smelly substances with very low odor thresholds: 0.14 mg/m$^3$ for H$_2$S and 32.1 mg/m$^3$ for NH$_3$, and different water solubility: 4.12×10$^6$ mg/m$^3$ for H$_2$S and 5.11×10$^5$ mg/m$^3$ for NH$_3$. They are usually simultaneously emitted from various industries or processes, including food processing, rubber processing, fish processing, animal husbandry, compost plants, and wastewater treatment plants, which explain the interest devoted to optimizing their removal (Devinin et al., 1999; Chung et al., 2005). Excess amounts of H$_2$S and NH$_3$ have to be removed for the sake of safety, health and reduction of environmental impacts, such as greenhouse effect, acid rain, and eutrophication.

The biotrickling filter (BTF) and biofilter (BF) have recently been regarded as the best available control technology for odor treatment, if the treatment concentration, the treatment cost of pollutant, and the advantage of completely degrading the contaminants into innocuous or less-contaminating products are considered (Leson and Winer, 1991; Chung et al., 2005). Accordingly, some papers have been published recently in the removal of H$_2$S and NH$_3$ simultaneously by BTF (Chung et al., 2005), BF (Chung et al., 2000, 2001; Malhautier et al., 2003; Chen et al., 2004; Jones et al., 2004; Lee et al., 2005) or a combination of BTF and BF (Yu et al., 2007a, b).

Li and Liu (2004) underlined that bacteria and fungi had a synergistic function so that the hydrophilic and hydrophobic compounds could be efficiently removed by the combined-bioreactor. Therefore, it could be supposed that the combination of BTF and BF should be a good system for the removal of hydrophilic and hydrophobic compounds simultaneously. Yu et al. (2007a, b) validated this surmise and applied a BTF/BF system to treat the mixture of H$_2$S and NH$_3$ successfully. The results indicated that the acclimation period was shortened 14–18 d compared to the literature (Chung et al., 2005), the optimum process conditions were 120 cm for the total height of BTF/BF system and 4.56 L/h for the recycle liquid rate, and the removal mechanisms could be the bio-chain principle and the adsorption-bioregeneration of clinoptilolite (Yu et al., 2007a, b). However, there is dearth of the supported information in the biological aspects. Therefore, further studies are still necessary about the predominant isolates identification.

As to biological processes, the predominant isolates were one of the key factors for achieving high removal efficiencies in controlling odors pollution, indicating that the predominant isolates identification is necessary (Chung et al., 2005). Many highly efficient deodorizing sulfide bacteria had been isolated and identified, such as Thiobacillus thiooxidans (Malhautier et al., 2003), Thiobacillus thioparus CH11 (Chung et al., 2000), Pseudomonas putida CH11 (Chung et al., 2001), Arthrobacter thiooxidans (Lee et al.,...
2005) etc. Similarly, some deodorizing ammonia bacteria had also been found, such as *Nitrosomonas* (Malhau-tier et al., 2003), *Nitromonas europaea* (Chung et al., 2000), *Arthrobacter oxidans* CH8 (Chung et al., 2001), *Arthrobacter thiooxidans* (Lee et al., 2005) etc. Biolog system is highly reproducible and widely applied in the identification of isolates based on the ability of the isolates to oxidize 95 different carbon sources (Calbrix et al., 2005; Haack et al., 1995).

In this study, the BTF/BF system was applied to treat the mixture of H$_2$S and NH$_3$. The specific objectives of this research were to identify the predominant isolates, and to analyze microbial sole carbon sources utilization patterns.

1 Materials and methods

1.1 Medium preparation and microbial cultivation

A clinoptilolite was obtained from Tianjingshan Mountain, Henan Province, China. Its characteristics are as follows: specific gravity, 2.16 g/cm$^3$; rigidity, 3–4; specific surface, 230–320 m$^2$/g; diameter, 8–10 mm. A kind of wood chip was obtained from Qingdao City, Shandong Province, China, and its characteristics was as follows: length, 2–4 cm; diameter, 0.3–0.5 cm. The clinoptilolite and wood chip were surfactant-modified using hexadecyltrimethylammonium (HDTMA) and epichlorohydrin in length, 2–4 cm; diameter, 0.3–0.5 cm. The clinoptilolite and wood chip were surfactant-modified using hexadecyltrimethylammonium (HDTMA) and epichlorohydrin by the methods described by Li et al. (2000) and Zhang and Liu (2004), respectively.

Prior to the experiment, the surfactant-modified clinoptilolite and surfactant-modified wood chip were packed into BTF and BF, respectively. The detailed results of microbial acclimation can be seen in Yu et al. (2007b).

1.2 BTF/BF system and operation

A schematic BTF/BF system used in this study is shown in Fig.1. Both BTF and BF columns with an internal diameter of 20 cm, were 100 cm height and packed with surfactant-modified clinoptilolite and surfactant-modified wood chip to a depth of 80 cm respectively.

The BTF/BF system was operated in a counter-current mode, i.e., with the gas flowing upward and the recirculation liquid flowing downward. The column wall contained 3 sampling ports (40 cm, 60 cm, 80 cm from the bottom, respectively) for measuring H$_2$S and NH$_3$ concentrations and analyzing microbial groups. H$_2$S and NH$_3$ (synthetic gas generated from Na$_2$S and H$_2$SO$_4$, NH$_3$·H$_2$O and NaOH, respectively), supplied from separate reactors, were introduced into BTF from the bottom by an air compressor and then flowed to the bottom of BF. The H$_2$S and NH$_3$ concentrations were controlled by adjusting the concentration of Na$_2$S and NH$_3$·H$_2$O solution and then following a flow meter and a three-way valve (Fig.1). Finally, the purified gas discharged into the environment. A recirculation liquid was pumped from a low level tank with temperature control at 30°C to a high level tank, and uniformly sprayed onto the top of BTF through a nozzle.

One third of the recirculation liquid was replaced every week, and the composition of recirculation liquid as follows (units in mg/L): glucose, 300; CO(NH$_2$)$_2$, 50; K$_2$HPO$_4$, 150; MgSO$_4$, 225; CaCl$_2$, 275; FeSO$_4$·7H$_2$O, 25.

1.3 Sampling and identification methods

Samples used for the isolates identification were taken from sampling ports at 40 cm of BTF and BF respectively, after the operation reached steady state for 45 d. The isolates used for identification were achieved from the plate streaking method.

Lawns of each isolate were streaked on TSA plates and incubated at 28°C for 24 h. Gram stain results showed the isolates in BTF were Gram positive (GP) bacteria. And they were predominant in quantity. The GP-ROD isolates used for identification were achieved from the plate streaking method.

The detailed identification procedures could be seen in the manufacturer’s manual (Biolog Inc., Hayward, CA, USA). The incubation results were put into Microlog computer program (Biolog system), and a database search was conducted for the closest match.

2 Results and discussion

2.1 Identification and cluster analysis of isolates

The acclimation period and the optimum process conditions of BTF/BF system have been studied (Yu et al., 2007a, b). The results indicated that the period of packing biofilm was 10–14 d, which shortened 14–18 d compared to the activated carbon used in the literature (Chung et al., 2005), and the removal efficiency for H$_2$S and NH$_3$ attained 76.9% and 78.6%, respectively. The optimum height and recycle liquid rate of composite biofilter were 120 cm and 4.56 L/h, respectively (Yu et al., 2007a).
The predominant organism groups were a complicated ecosystem including bacteria, protozoa and algae in the biotrickling filter while was only fungi in the biofilter (Yu et al., 2007b). Therefore, it is very important to know the structure and metabolic function of the microbial community in a bioreactor to improve its performance. In this experiment, three predominant bacteria were isolated from BTF and one predominant fungi was isolated from BF. By careful observation under a microscope (Olympus 800, Olympus Optical Co Ltd, Japan), two isolates were rod-shaped and straight, \((1.0–1.5) \mu m \times (2.0–3.0) \mu m\), and one isolate was spherical, \(1.0–1.5 \mu m\) in diameter. FF had spores which was similar spherical, \((6.5–7.0) \mu m \times (9.5–10.0) \mu m\). The isolates were identified as *Bacillus sphaericus*, *Geobacillus themoglucosidasius (55°C)*, *Micrococcus luteus* (ATCC 9341) in BTF and *Aspergillus sydowii* (Bainier & Sartory) Thom & Church in BF by using Biolog system, which showed a 100% similarity of the identified isolates to their database. Biolog identifications were accepted as correct if the similarity index of the genus and species name was 75% or greater at 4 h or 50% or greater at 24 h (Klingler et al., 1992), indicating that the identification results in this study were credible. While the identification results are different from the literature (Malhautier et al., 2003; Chung et al., 2000; Lee et al., 2005), this might contribute to the isolates diversity of degrading H$_2$S and NH$_3$ or environmental differences of reactors.

The cluster analysis used a mathematical approach to display the relative similarities and differences between isolates based on pattern matching, offering a visual method for examining groups using the linear cluster analysis. The cluster analysis of isolates could be seen in Fig.2, indicating that these identification isolates had a smaller DIST (distance) than other nine microcosms.

Long et al. (2000) indicated that *Bacillus sphaerium* played a strong role in nitrogen fixation. Monica et al. (2000) discovered that *Micrococcaceae* could degrade effectively nitrite, nitrate and nitrogen oxides. Zhang et al. (2005) found that *Aspergillus* could remove nitrogen oxides in the aerobic conditions. Therefore, we could refer that ammonia is removed by the nitrification and denitrification.

### 2.2 Analysis of sole carbon sources utilization patterns of isolates

On the basis of the database from Biolog system, the sole carbon sources utilization patterns of isolates were formed (Tables 1 and 2). The results indicate that *M. luteus* (ATCC 9341) has the highest utilization extent (52.6%), corresponding to 50 types of different carbon sources, followed by *A. sydowii* (Bainier & Sartory) Thom & Church (39.5%), *G. themoglucosidasius (55°C)* (21.9%) and *B. sphaericus* (17.7%), corresponding to 38, 21 and 17 types of different carbon sources, respectively. Among the substrate classes represented by the highest number of compounds, the carboxylic acids and methyl esters had the highest utilization extent for the four species, followed by the amino acids, peptides. A lower percentage of sugar phosphates than the other substrate classes was utilized, especially by *G. themoglucosidasius (55°C)*.

*A. sydowii* has a relative high carbon sources utilization extent (39.5%), indicating that the isolate is easy to incubate. Zhang et al. (2005) observed that *Aspergillus* has a strong degradability to cellulose and lignin. Based

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**Fig. 2 Cluster analysis of isolates.**

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on the result, we can infer that wood chip could provide Aspergillus with the carbon sources. Therefore, the BF system is easy to operate, since BF does not need the external carbon sources.
Due to a general lack of understanding of the physiological characteristics of the microorganisms, it is important to analyze the sole carbon sources utilization patterns to change the culture conditions. First, replacing glucose by the carboxylic acids and methyl esters which have a higher utilization extent for the predominant isolates in the recirculation liquid, could shorten the acclimation or start-up period of BTF/BF system. Secondly, the enrichment efficiency could be enhanced by designing a selective medium which makes the predominant isolates reproduced rapidly. Furthermore, the pyruvic acid methyl esters which belong to an available but low solubility carbon source, could be added to an enrichment medium in order to enhance the isolation efficiency by producing a transparency circle.

3 Conclusions

In this study, four species, *Bacillus sphaericus*, *Geobacillus thermoglucosidasius* (55°C), *Micrococcus luteus* (ATCC 9341) in the BTF and *Aspergillus sydowii* (Bainier & Sartory) Thom & Church in the BF, were found to be able to degrade H2S and NH3 (Yu et al., 2007a, b). The descending sequence of carbon sources utilization capability of isolates was *A. sydowii* (52.6%), *M. luteus* (39.5%), *B. sphaericus* (21.6%), and *G. thermoglucosidasius* (17.7%). Among the substrate classes represented by the highest number of compounds, the carboxylic acids and methyl esters had the highest utilization extent for the four species, followed by the amino acids and peptides.

References


