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Research on Simultaneous Removal of Cyclohexane and Methyl Acetate in Biotrickling Filters

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Abstract **-** In order to further purify two kinds of organic waste gases, methyl acetate and cyclohexane, which were difficult to be biodegraded in refinery wastewater treatment process, a new scheme for purifying mixed waste gas by bio-film construction with dominant bacteria was put forward. Because of the packing has a great influence on the performance of the bio-trickling filter (BTF) for purifying exhaust gas, the effect of two kinds of packing, namely ceramsite and volcanic rock, on cyclohexane purification was investigated. The results indicated that the filter efficiencies with two different packing were equivalent under the same conditions, but ceramsite was finally chosen as the next experimental packing because of its low weight. With activated sludge in secondary sedimentation tank from wastewater treatment plant as the original strain, the dominant degrading bacteria of methyl acetate and cyclohexane were obtained. After initial identification, a vertical BTF was startup with membrane of the dominant cyclohexane degradation bacteria *Ochrobactrum intermedium*. The tower can be startup quickly in 10 days, and the removal efficiency can reach over 95%. Moreover, the maximum eliminate capacity of cyclohexane was achieve 58.59 $g\cdot m^{-3}\cdot h^{-1}$. When the reactor entered in stable phase, two kinds of methyl acetate predominant degrading bacteria, *Bacillus velezensis* and *Ochrobactrum anthropi*, were added and the methyl acetate gas was injected into the BTF. The performance of purify the mixture of cyclohexane and methyl acetate in the BTF was investigated in different operating conditions. The results showed that when the empty bed residence time was 176.6 s and 83.3 s, for cyclohexane concentration in the inlet air ranging from 50 $mg\cdot m^{-3}$ to 300 $mg\cdot m^{-3}$, the removal efficiency was above 90%. Meanwhile, for methyl acetate concentration ranging from 300 $mg\cdot m^{-3}$ to 800 $mg\cdot m^{-3}$, the removal efficiency was achieve 100%. The maximum eliminate capacity of the two kinds of pollutions was attain 72.99 g·m-3·h-1. In addition, the BTF was strongly resistant to impact load, and the pressure drop was remained at 5~100 pa over the entire operation time, no blockage appeared in the tower.

Keywords**:** Bio-Trickling Filter, Cyclohexane, Methyl Acetate, Packing, Bacteria.

1. Introduction

Volatile organic compounds (VOCs) are the general term of a class of compounds including aliphatic and aromatic compounds, generally referred to as organic chemical substances with high vapor pressure and volatile at normal temperature and pressure[1]. In recent years, the emission of VOCs from industrial sources in China has increased year by year[2], and the relevant statistics show that the total VOCs emitted by petrochemical industry are about 25% of the total emission of the whole country, while the source management, technological improvement, and end treatment are all very important for the petrochemical industry[3].

Hydrocarbons such as gasoline or diesel produced in crude oil and petroleum products include aliphatic hydrocarbons, aromatic hydrocarbons and cycloaliphatic moieties where the cycloaliphatic moiety and the polycyclic aromatic hydrocarbons and tar species are the most biodegradable class of substances. Cyclohexane and its derivatives are the main substances in the alicyclic petroleum fractions and have been listed in volatile organic compounds (VOCs)[4]. It has mild irritation to the eye and upper respiratory tract, and continuous inhalation affects the central nervous system[5] and the product is extremely flammable, thus having a higher risk. Methyl acetate is a colorless transparent liquid with fragrance. As an organic solvent, it is widely used in resin, coating, ink, bio-diesel[6], and paint, adhesive and leather production. It has a certain influence on the residents living around and can cause skin cancer. The research of the pre-laboratory project shows that the waste gas produced by the chemical fiber wastewater treatment plant of a petrochemical plant contains both cyclohexane and methyl acetate gas, and the output concentration is not high, but it cannot achieve good degradation effect in the pre-treatment stage of the vertical and horizontal BTFs. Therefore, it has important industrial reference significance to explore the simultaneous removal performance of BTF tower in the laboratory.

At present, there are few researches on biodegradation of cyclohexane in biological process and its properties, which are mainly focused on screening of bacteria, selection of filler, metabolic pathway and final product. Dallinger et al.[4] found that Candida and trichospore can degrade cyclohexane first to cyclohexanol before conversion to cyclohexanone and confirm cyclohexanone as its final degradation product. Liu[7] mixed organic gas with low concentration of acetic acid, n-hexane and styrene to simulate organic waste gas, using a laboratory scale BTF treatment of organic waste gas, and compared the performance of 4 kinds of fillers which include sponge, coral stone, ceramic and plastic hollow ball. The results showed that the sponge and ceramsite were suitable as packing for BTF. Arriaga et al.[8] added fungi to the bacterial bio-filter with perlite as filler to enhance the removal of cyclohexane. The results showed that fungal mycelium was more likely to catch contaminants and the reactor had higher degradation of cyclohexane under acidic conditions. Lee et al.[9], used hexane as sole carbon source, domestication and selection a bacterium from the soil which contaminated by petroleum, and identification results for Rhodococcus sp. EH831, genera Rhodococcus, further study found that the bacteria can degrade a variety of organic compounds which include alcohol, chlorinated hydrocarbon, cycloparaffin, ethers, ketone, aromatic hydrocarbons, PAHs and petroleum hydrocarbons, the degradation of hexane metabolites were studied by gas chromatography-mass spectrometry and solid phase microextraction techniques, eventually found that after a series of changes, hexane has been degradation for carbon dioxide, strict aerobic metabolic pathways. At present, there were few researches on the treatment of methyl acetate with BTF, and there were no clear relevant emission standards. But as a kind of VOCs, the research and development of the control methods of VOCs is urgent to meet the requirements of the National Key Industry Volatile Organic Compounds Reduction Action Plan[10].

Based on the difficult degradation of methyl acetate and cyclohexane produced in a petrochemical refinery wastewater treatment process, this paper selected activated sludge of the secondary sedimentation tank of WWTP as original strains to select predominant strains which can degrade methyl acetate and cyclohexane, and then the predominance strains were preliminarily identified and preserved. The membrane formation of the BTF was investigated by means of the rapid start-up method, and the start-up time and the effect of the bio-film formation and the short-term operation efficiency were investigated, and the related process operation of the BTF tower was also explored.

2. Materials and Methods

2.1. Screening and Domestication of Predominant Degrading Strains

2.1.1 Source of Strain

The original strain was the activated sludge from the secondary sedimentation tank of Beijing Gaobeidian wastewater treatment plant.

2.1.2. Composition of Culture Medium

Bacteria: Using inorganic culture medium A (g/L): K_2HPO_4, $3H_2O$ 2.3, $FeSO_4$, $7H_2O$ 0.03, $MgSO_4$ 0.1, $CaCl_2$ 0.02, NH_4NO_3 1.00, $MnSO_4$ 0.03, KH_2PO_4 0.7, and regulating pH at 7.

Fungi: Using the fungal inorganic salt medium B (g/L): 2.00 ammonium tartrate, Na_2HPO_4 0.47, KH_2PO_4 0.45, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4$ 0.001, anhydrous $CaCl_2$ 0.01, $ZnSO_4 \cdot 7H_2O$ 0.001, $CuSO_4 \cdot 5H_2O$ 0.001, bacteria inhibitor 0.05, and regulating pH at 5.6.

Isolation and purification: Using solid medium LA (g/L): beef extract 3, peptone 5, NaCl 5, agar 15.

The above medium was sterilized at 121°C for 20 minutes. The component of medium which could not be sterilized was filtrated by bacterial filtration membrane and then added to the medium after individual sterilization.

2.1.3 Domestication, Screening and Identification of Strains

The domestication and screening of the toluene degradation strain was proceeded in aerobic oscillation bottles (volume 350ml, containing medium A 70 mL), and a certain amount of cyclohexane and methyl acetate was added after inoculation, and sealed the bottles (in order to prevent the volatilization of the target pollutant). Afterwards, the bottles were cultured at 30℃ and 120 $r \cdot min^{-1}$ for 3 days, then transferred to fresh A (B) medium every 3 days with 3% amount of the inoculation and continued to be acclimated for 3 times. The final acclimated culture medium was diluted and coated on the LA plate, and the single colony was obtained after cultured at constant temperature for 24 h, and then the single strain was inoculated and purified. The purified strains were initially screened and re-screened, and finally the predominant degrading strains of cyclohexane and methyl acetate were obtained.

The predominant biodegradable strains were identified by Boyoushun biotechnology co., LTD.

2.2. Construction of Reactor System

Experimental device for purifying toluene by vertical BTF was shown in Fig. 1. The BTF was made of an organic glass tube with an outer diameter of 120 mm and a wall thickness of 5 mm, the total height of 1000 mm. The height of the packing layer is 500 mm, which is divided into three sections with 170 mm, 170 mm, 160 mm, so the sectional area was 9.50×10^{-3} m^2, and the total packing volume was 4.75×10^{-3} m^3. The preparation of cyclohexane was made by gas dynamic volumetric method[11].

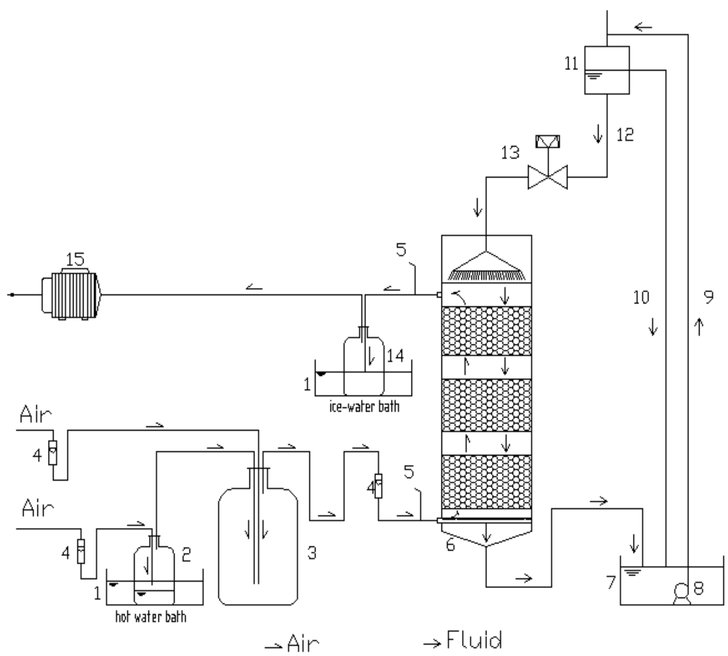


Fig. 1: Schematic diagram of the BTF. 1.thermostatic water bath; 2.toulene solution; 3.air mixed bottle; 4. rotameter; 5. sampling port; 6.BTF; 7. recirculation tank; 8. immersible pump; 9. solution up flow tube; 10.overflow tube; 11.collecting tank; 12.downflow tube; 13.electromagnetism valve; 14.air condensate bottle; 15.air compressor.

2.3. Determination and Analysis Method

The concentration of gas phase cyclohexane and methyl acetate was determined by Agilent 7890A gas chromatograph, FID detector, and 19091J-413 capillary column. Test conditions: column temperature 60℃, detector 300℃, injection port 100℃, and carrier gas N_2. PH value is determined by precise pH meter; the pressure loss is determined by U-type pressure gauge.

The surface biofacies were observed by SEM. The preparation method of the ceramic scanning electron microscopy sample was referenced [12].

2.4. Selection of Packing

In this study, volcanic rock and ceramsite were selected as packing of two BTFs respectively. The performance of two packing was compared, and the effect of the two different packing on the same pollutants in BTF was monitored.

3. Results and Discussion

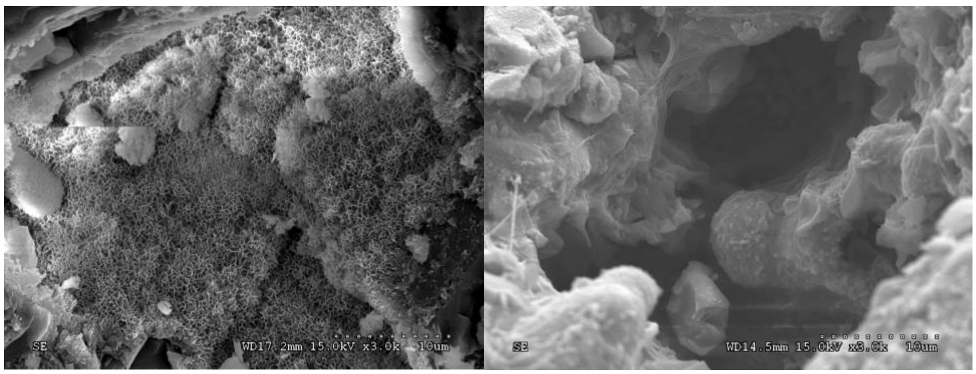
3.1 Degradation of Cyclohexane by BTF with Activated Sludge

3.1.1 Selection of Packing

Ceramsite And Volcanic Rock Were Selected As The Packings For Bet Test At The Same Time, And The Results Were Shown In Table 1.

Table 1: Two kinds of packing performance.

Sample	Aspect	BET （$m^2 \cdot g^{-1}$）	Pore volume （$cm^3 \cdot g^{-1}$）	Pore diameter （nm）
ceramic	Ball and micropore	1.08	0.0005	1.85
volcanic	Irregular and micropore	3.24	0.0078	9.62



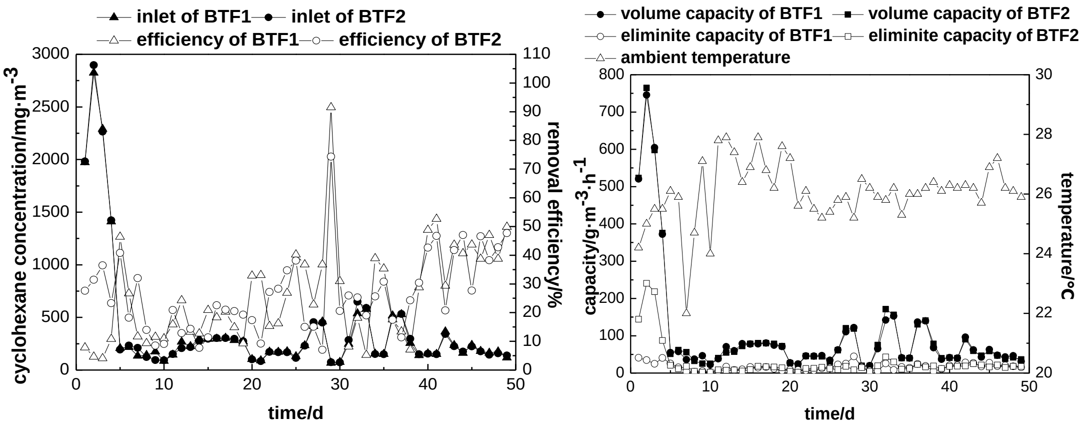
(a) (b)

Fig. 2: SEM picture of blank packing ((a) volcanic; (b) ceramic.

The table 1 shows that the surface area, entrance and the aperture value of volcanic rock are bigger than those of ceramsite. As can be seen in Fig. 2, the surface microporous on volcanic rock are more numerous and fine-textured than those on the ceramsite. Two BTFs were constructed to compare the performance between the two packings in the actual application, one was filled with ceramist (BTF1) and the other was filled with volcanic rock (BTF2).

3.1.2. Bio-Film Formation and Start-Up of BTF

The activated sludge was used for the start-up of the two BTFs. The intake is 0.18 $m^3 \cdot h^{-1}$, and the concentration of gas phase cyclohexane was 1500-3000 $mg \cdot m^{-3}$ initially, and then adopted to 500 $mg \cdot m^{-3}$ after the activated sludge acclimated high concentration cyclohexane. The empty bed residence time (EBRT) was 95 s, and the room temperature was 22℃~28℃.



(a) (b)

Fig. 3: the change of degradation efficiency for cyclohexane during the start-up period in the activated sludge biofilm BTF ((a) the curve of purification efficiency and the inlet concentration; (b) the curve of removal capacity, volume capacity and ambient temperature)

Fig. 3 shows that after the inlet concentration of cyclohexane change from high to low, the removal efficiency (RE) of two BTFs were tend to stable state, but in terms of the elimination capacity, the cyclohexane degradation amount was low. The performance of BTF which start up with activated sludge was inferior during long-term domestication, and there was little difference between two different packing. Therefore, the selected predominant strain was applied to start up the BTF in the next experiment. In addition, considering the high weight of the volcanic rock, which will increase the load of the filter in practical application, the ceramic was selected as the filler in the next experiment.

3.2. Degradation of Cyclohexane in BTF with Predominant Degradable Bacteria
3.2.1. Strain Selection

Table 2: Screening process and results of strain.

Sludge of Gaobeidian	Initial cyclohexane addition（μL/70mL）			Initial methyl acetate addition（μL/70mL）		
	Solution A	Solution B	Degradation state during 48 h	Solution A	Solution B	Degradation state during 48 h
The first time	3	3	Incomplete degradation	3	3	Complete degradation during 48h
The second time	3	3	Incomplete degradation	5	5	Complete degradation during 48h
The third time	2	2	Incomplete degradation	10	10	Complete degradation during 48h

It can be seen from table 2 that the initially screened bacteria from the activated sludge were poor at the degradation of cyclohexane, while good at the degradation of methyl acetate. And then the selected bacterial species of cyclohexane were further cultured, with the initial concentration of cyclohexane was about 30,000 $mg \cdot m^{-3}$, the degradation efficiencies of bacteria and fungi were determined respectively.

Table 3: Further domestication of cyclohexane degrading bacteria.

	The degradation efficiency of gas phase cyclohexane/%		
Incubation time	24h	48h	72h
fungus	49.3	55.9	66.0
bacteria	57.6	94.3	98.0

The table 3 shows that the relatively refractory target pollutants cyclohexane, which though not can be entirely degraded within 72 h, the bacteria degradation efficiency reached more than 95% after many bacterial-screening experiments. During the screening of degradation strains of cyclohexane, it was found that the effect of the degradation of bacteria was better than that of fungi, so the bacteria were selected to apply in the next start-up of the BTF.

3.2.2. Strain Identification

To identification species, the selected bacteria were taken to Boyoushun biotechnology co., and the identification results were shown in table 4.

Table 4: strain identification results.

Cyclohexane	fungus	*Trichosporon dermatis*
	bacteria	*Ochrobactrum intermedium*
Methyl acetate	bacteria 1	*Bacillus velezensis*
	bacteria 2	*Ochrobactrum anthropi ATCC 49188*

The two strains of this experiment were also used in wastewater treatment, malodorous treatment and bioremediation. *Trichosporon dermatis*, fermented hydrolysate waste liquid yeast, were used in sewage treatment[13, 14]. *Ochrobactrum intermedium*, which belongs to the same genus as *Ochrobactrum anthropi*, the surfactants it produced can be used to repair sludge in refinery wastewater tank[15]. *Ochrobactrum anthropi*, which is special aerobic, strict respiratory metabolism, can make use of various amino acids, organic acids and carbohydrates as carbon sources, and are studied on wastewater treatment[16]. *Bacillus velezensis*, a bacterium, which can decompose organic substances, organic sulphides and organic nitrogen that produce malodorous gases[17].

The results show that, the screened cyclohexane and methyl acetate degrading bacteria are commonly used in sewage or waste gas treatment, which can be used for the start-up of BTF after further culture.

3.2.3. Start-Up of BTF with Predominant Bacteria

According to the experimental results in the previous stage, the ceramsite was selected as fillers, and the cyclohexane predominant degrading bacteria were used as the original bacteria to start up the BTF. The air input of this stage is 0.09 $m^3 \cdot h^{-1}$, and the inlet concentration of the cyclohexane was 300-1300 $mg \cdot m^{-3}$. The EBRT was 200 s, and the room temperature is 19℃~21℃. Fig. 4 (a) and (b) illustrated the change of the removal efficiency (RE) and elimination capacity of the BTF in the start-up stage.

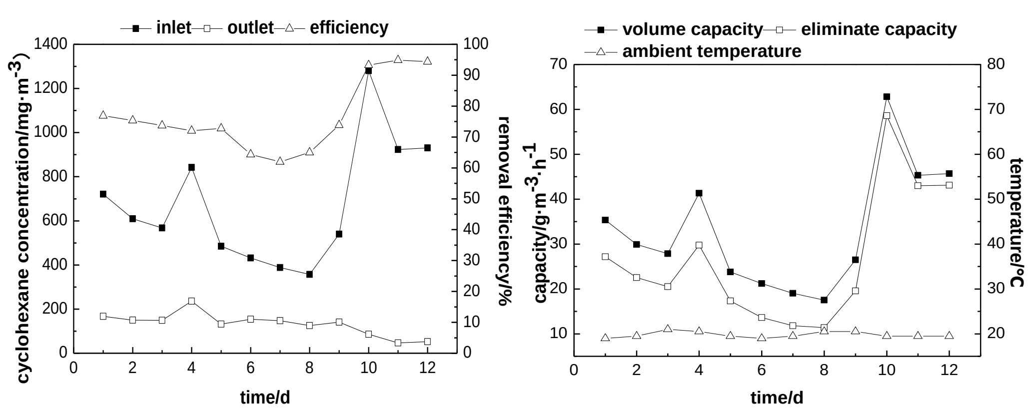


Fig. 4: The curve of REs of cyclohexane during the start-up stage in superiority strain biofilm BTF ((a) the curve of RE and the inlet concentration; (b) the curve of RE, volume capacity and ambient temperature).

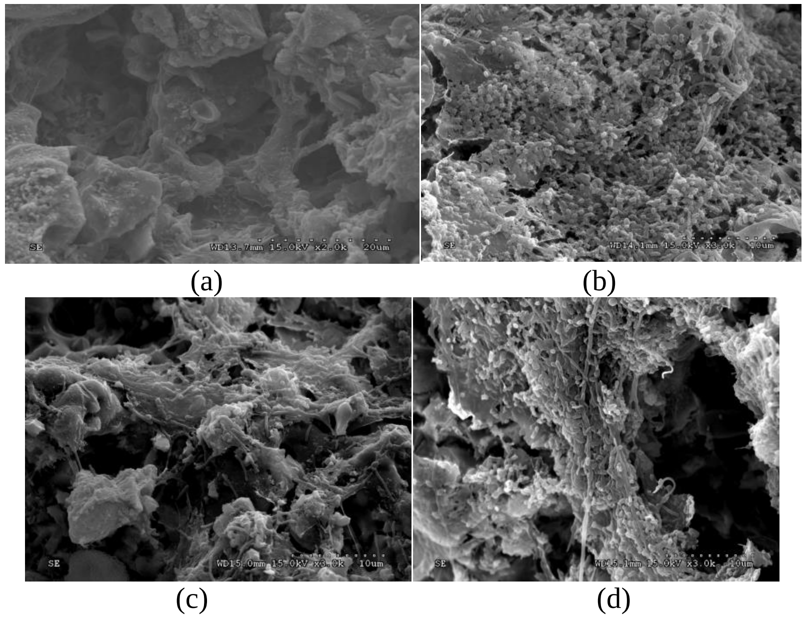


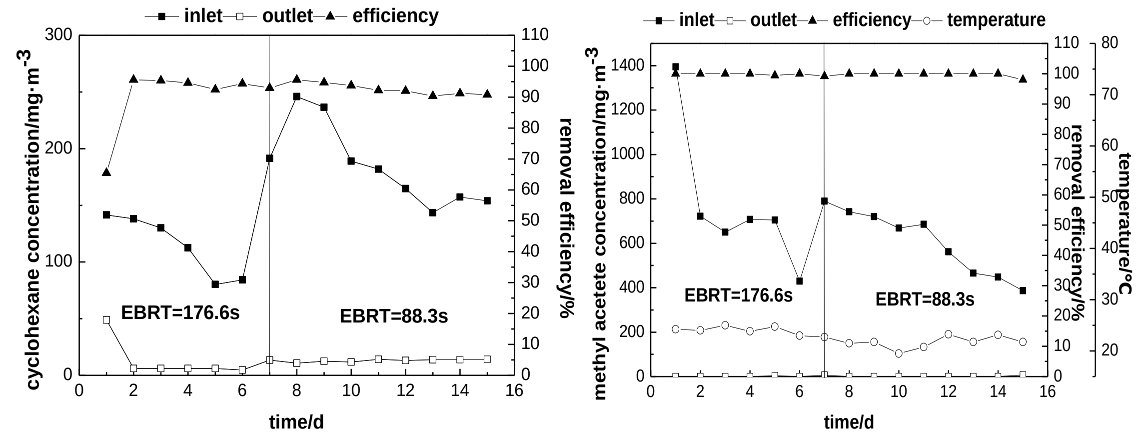
Fig. 5: SEM image of microbial growth on the surface of ceramic before and after film success ((a) blank ceramic, (b) the upper layer, (c) the middle layer and (d) the lower layer).

It can be concluded from Fig. 5 that at the EBRT of 200 s, the RE increasing with the inlet concentration of cyclohexane which changing slowly from high to low firstly between 300-1300 mg·m^{-3} and then increased significantly, eventually remains at around 95%. The elimination capacity was gradually close to the volume capacity, and the maximum elimination capacity reached at 58.59 g·m^{-3}·h^{-1}, and finally remains in a high level. As presented in figure 6, after biofilm formation, the microorganisms on ceramsite surface in the three layers of the BTF gradually grew, which were attached and adhesive with each other. The microorganisms gradually occupy the holes on the surface of the ceramsite, and build holes again, which was forming a huge pollutants capture network. Thus, the biofilm formation was successfully completed.

3.2.4. Study on Degradation of Methyl Acetate and Cyclohexane Mixture at Stable Operation Stage

In order to further explore the coordination treatment effect of the same BTF degrading the two different pollutants together in stable stage, a certain amount of predominant methyl acetate degrading bacteria was added to the BTF, and methyl acetate was input at the same time. Because the two kinds of predominant bacteria are derived from the same source, the results show that the BTF operated well.

The intake gas was 0.10 m^{3}·h^{-1}, the inlet cyclohexane concentration was ranged at 50-300 mg·m^{-3}, the inlet methyl acetate concentration was basically controlled in 300-800 mg·m^{-3}, the EBRT was 176.6 s, and room temperature is 19℃~21℃.



(a) (b)

Fig. 6: The change of removal efficiency, inlet concentration and ambient temperature with different EBRT in stable-stage ((a) the curve of cyclohexane RE and the inlet concentration; (b) the curve of methyl acetate RE, inlet concentration and ambient temperature).

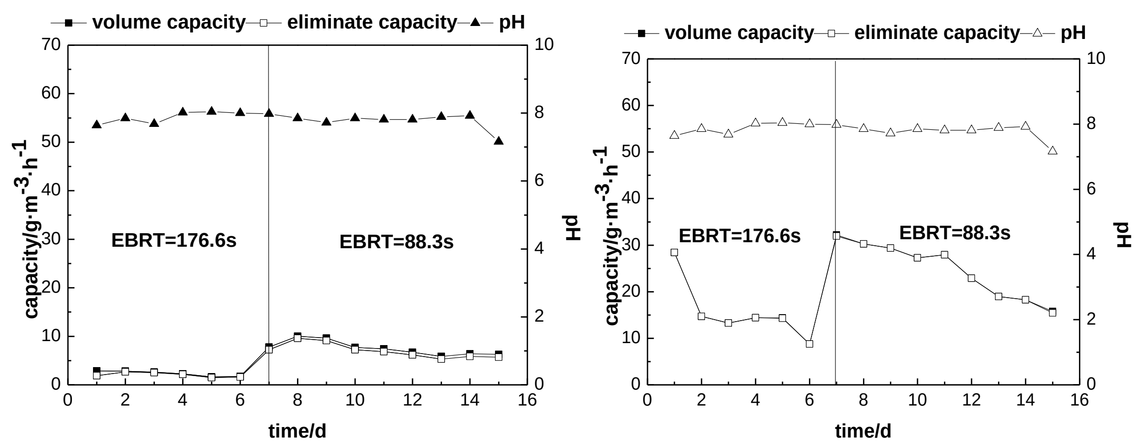


Fig. 7: The curve of cyclohexane and methyl acetate eliminate capacity, volume capacity and solution pH in stable-stage ((a) the curve of cyclohexane eliminate capacity, volume capacity and pH; (b) the curve of methyl acetate eliminate capacity, volume capacity and pH).

It shows in Fig.6 that, after the predominant methyl acetate degrading bacteria was added into BTF, the RE of cyclohexane decreased from 95% to 70%, and began to quickly return to 95% on the second day. And there was no longer sharp changes with the change of inlet concentration of cyclohexane and the change of the EBRT, and maintained above 90% eventually. When the inlet methyl acetate concentration was between 300 and 800 mg·m^{-3}, the degradation efficiency maintained at around 100% and was not fluctuating with the change of EBRT. Fig. 7 shows that cyclohexane and methyl acetate elimination capacity were essentially coincident with the volume capacity, which was not fluctuating with the change of EBRT.

The results show that in the steady state, with the inoculating predominant methyl acetate degrading bacteria, the BTF could efficiently degrade the two kinds of pollutant at the same time and had a good purify performance.

4. Conclusion

The cyclohexane and the methyl acetate degradable domination bacteria *Ochrobactrum intermedium* and *Ochrobactrum anthropi* were selected from activated sludge, and using cyclohexane degradation bacteria to start up the BTF with the ceramsite as packing material. It can be started up in 10 days, and can degrade cyclohexane efficiently compared with the activated sludge. In the stable period, methyl acetate degradable domination bacteria *ochrobactrum* *anthropi* was added into the BTF with the gas of methyl acetate input at the same time. The performance of the BTF to purify the mixture of two different gases was good, and has well anti impact load capacity, which showed obvious stability and adaptability. The results show that, predominant bacteria are the main body of biodegradation of organic waste gas in activated sludge. The microorganisms formed a contaminant capture net by attaching to the filler, and gradually convert VOCs into their own metabolites, thereby realizing the degradation of pollutants.

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