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Biodegradation of 2-methylisoborneol by *Bacillus idriensis* isolated from biological activated carbon

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ABSTRACT

2-Methylisoborneol (2-MIB) is one of typical odorants in potable water sources, which is hardly removed by conventional water treatment process. In this study, three bacterial strains capable of removing 2-MIB singly from drinking water were isolated from activated carbon of sand filter. They were identified to be *Shinellazoogloeoides*, *Bacillus idriensis* and *Chitinophagaceae bacterium*, respectively, based on 16S rRNA gene sequence analysis. In mineral salts medium containing 2-MIB as the sole carbon sourse, removal efficiencies of 20 µg/L 2-MIB in three days were 23.3%, 32.9% and 17.0% for *Shinellazoogloeoides*, *Bacillus idriensis* and *Chitinophagaceae bacterium*, respectively. Interestingly, the *Bacillus idriensis* isolate was not sensitive to the growth temperature, pH and initial concentration of 2-MIB, and could tolerate a rather high concentration of 2-MIB. Moreover, we found that the biodegradation of 2-MIB was significantly improved with the presence of cometabolism carbon source such as glycerol and glucose. After 20 d incubation, the concentration of 2-MIB was reduced from 2 mg/L to 368.2 µg/L and 315.4 µg/L by *Bacillus idriensis* in the absence or presence of glycerol, respectively.

Keywords: 2-MIB; Biodegradation; *Shinellazoogloeoides; Bacillus idriensis*; *Chitinophagaceae bacterium;* Cometabolism

1. Introduction

Taste and odour (T&O) causing compounds, in particular, 2-methylisoborneol (2-MIB) and geosmin, are a problem for water authorities as they are recalcitrant to conventional water treatment. Removing T&O compounds from drinking water is a significant challenge for water authorities internationally. 2-MIB is a secondary metabolite produced by members of *cyanobacteria* and *actinomycetes* [1–3]. It impart an earthy/musty odour that can be detected by humans at low nanogram per litre levels.

2-MIB cannot be readily removed by conventional water treatments, therefore advanced treatment processes such as activated carbon adsorption, ozonation and advanced oxidation technologies are required [4–9]. Previous studies indicated that biological treatment could be an alternative method for 2-MIB removal [10–14]. The biodegradability of 2-MIB in water indicates that there is potential for using biological filtration processes as a viable treatment option for removing these T&O compounds. Biological filtration systems for the removal of contaminants are becoming more attractive to water suppliers as they are generally of low technology, requiring little maintenance and infrastructure. Furthermore, such systems are able to remove these contaminants without the addition of other chemicals that may have the potential to produce by-products that are undesirable in drinking water.

Several studies have demonstrated bacterial degradation of 2-MIB, although intermediates and products have in most cases not been identified or well documented. In 1988, it was reported for the first time that microorganisms could be used for biodegrading 2-MIB. Yagi et al. seeded GAC (granular active carbon) filter columns with a bought bac-

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terium of *Bacillus subtilis* and they found that 56% of 2-MIB was biodegraded compared with control without bacterium [15]. In the same year, Izaguirre et al. reported 2-MIB can be used as the sole carbon source to be biodegraded by microorganisms [16]. 2-MIB of mg/L concentration can be removed after about 90 d by bacteria enriched from a lake; *Bacillus fusiformis* and *B. sphaericus* isolated from lake water were capable of decreasing 2-MIB at mg/L and ng/L levels to the concentration below odor threshold concentration (OTC) [17]. Similarly, another group reported *B. subtilis* isolated from biological activated carbon filter was capable of removing 2-MIB at mg/L level [18]. In addition, *Candida spp.* isolated from a slows and filter [19] and *Enterobacter spp.* isolated from the backwash water of a pilot biological filter [9] were both identified as 2-MIB degraders. Other bacterial strains such as *Pseudomonas spp.* and *Flavobacteriumspp.* from the surface of biological activated carbon were also reported [20,21].

As yet, the pathways for the biodegradation of 2-MIB is still unknown, although Tanaka et al. [9] were able to identify two possible dehydration products, 2-methylcamphene and 2-methylenebornane. Oikawaet al. [22] confirmed this by excising the entire camoperon from a camphor degrading *Pseudomonas putida*, where its subsequent transformation into *Escherichia coli* demonstrated the acquired ability of 2-MIB degradation. To further elucidate the kinetics and products of 2-MIB biodegradation, it is necessary to isolate single bacterium with the ability to degrade 2-MIB. This study focused on the isolation and identification of 2-MIB-degraders, examination of their 2-MIB degradation performances and optimization of potential factors affecting their degradation efficiency.

2. Materials and methods

2.1. Materials

Solid 2-MIB (98%) used in this study was obtained from Wako Pure Chemicals, Ltd. (Osaka, Japan). Sterile 2-MIB solution (20 mg/L) was prepared by dissolving 2-MIB in Ultra-pure water treated by a Milli-Q system (Millipore Pty Ltd., USA). The solution was then filtered through a sterile 0.22 μm-pore-size micro porous membrane (MPM) and stored in fridge at 4°C before use.

The glassware was washed three times with Ultra-pure water, and all of the glass wares and Ultra-pure water were sterilized at 121°C for 30 min before use.

2.2. Enrichment procedures

Enrichment of 2-MIB degraders was conducted with a mineral salts medium (MSM, NH_4NO_3 0.1%, K_2HPO_4 0.1%, $MgSO_47H_2O$ 0.05%, KCl 0.02% (W/V), pH 7) as reported by Tanaka et al. [9]. Portions of 100 mLMSM were placed in 250 mL Erlenmeyer flask sealed with absorbent gauze and then sterilized at 121°C for 30 min. Activated carbon samples were obtained from a carbon filter of a water plant in Beijing, and stored in the bottle containing the influent of the filter at 4°C. About 5.0 g carbon was added into 10 mL sterile PBS buffer (0.1 mol/L).The buffer was shaken vigorously for 15 min.

The culture of PBS buffer for inoculation was poured into the Erlenmeyer flask filled with 100 mL MSM. The 2-MIB solution was spiked through the sterile 0.22 μm pore-size MPM. The initial concentration of 2-MIB in MSM was controlled at 100 ng/L. The flasks were then covered with sealing film (12 × 12 cm, Solarbio, Beijing, China) and rotated in an incubator shaker at 30°C and 120 rpm for 5 d. 10% of the MSM culture was spiked into another 100 mL MSM to select 2-MIB degraders, and incubated repeatedly with the increase of 2-MIB concentration (200 ng/L, 500 ng/L, 1 μg/L, 5 μg/L, 10 μg/L and 20 μg/L). Experimental operation was performed in a sterile operating station.

2.3. Isolation of 2-MIB degraders

For isolation of 2-MIB degraders, LB agar medium (peptone 1%, yeast extract powder 0.5%, NaCl 0.5%, agar 1.5% (W/V), pH 7) was supplemented and autoclaved [21]. Followed by cooling to about 40°C the medium was poured into 90 mm vitreous plates. After application of 1 mL cultured MSM on LB agar medium in triplicate, these plates were covered with the lids, and sealed tightly. They were placed upside down and incubated at 30°C for 2 d. Various cultures were streaked onto the LB agar medium at least twice for purification. Three pure strains were isolated. The pure cultures were dissolved in 1mL sterile PBS buffer (0.02 $mol{\cdot}L^{-1}$, pH 7), and centrifuged at 12000 rpm for 3 min. The cells in each vial were washed twice with 1 mL sterile PBS buffer and stored at 4°C.

2.4. Identification of bacteria

The purified isolates from various cultures were identified by a commercial laboratory, TaKaRa Biological Company (China). The 16S ribosomal RNA (rRNA) gene was PCR-amplified from genomic DNA isolated from the enriched culture. The purified culture of 10 μL was spiked into the sterile water, and then denatured at 100°C followed by centrifugation. The supernatant was taken as the template and mixed with 1 μL of master ready reagent (Takara 16SrRNA Bacterial Identification PCR Kit, Code No.D310), 25 μL of PCR premix, 0.5 μL of forward primer (20 pmol/μL), 0.5 μL of reverse primer 2, 23 μL of 16S-free H_2O. PCR cycling parameters used were as follows: 94°C for 20 s, 55°C for 20 s, 72°C for 1.5 min and circulated for 30 times. Five micro liters of amplified sample was used to conduct an Agarose Gel Electrophoresis (AGE), followed by Gel Extraction Purification using Takara Agarose Gel DNA Purification Kit Ver.2.0 (Code No.DV805A). Sequence analysis was performed with Seq Forward, Seq Reverse and Seq Internal as the primers. Sequence similarity searches were conducted using the National Center for Biotechnology Information BLASTnet work service (Blastn). Similar sequences, from previously cultured bacteria, were obtained from GenBank and aligned against the DNA sequences of strain 1 to strain 3.

2.5. Degradation performance of bacteria

The pretreatment of bacteria was continuous culture in the MSM with 20 μg/L 2-MIB, and enrichment culture in LB medium. Cell suspensions was taken under the conditions

of 25°C and 5000 rpm for 5 min. Centrifugal sedimentation which full of bacteria was washed by ultra-pure water under the same conditions for 3 times. Bacteria liquid was diluted to OD_{600} of 1. All of the glasswares, ultra-pure water and the centrifuge tube were sterilized at 121°C for 30 min before use.

The prepared bacterial suspension were added into MSM containing 2-MIB at an initial dose of 20 μg·L^{-1} and kinds of extra carbon sources at 0.2 g·L^{-1}. These flasks were rotated in an incubator shaker at 30°C and 120 rpm for 2 weeks. Samples of 20 mL were transferred into a cap vials from the Erlenmeyer flask at 2 and 3 d. The concentration of 2-MIB was determined within 7 d using GC-MS. Research on optimization of the degradation efficiency factors was carried out using the bacterium with the highest degradation efficiency. The prepared bacterial suspension were added into MSM containing 2-MIB at an initial dose of 20 μg/L. These flasks were rotated in an incubator shaker at 5, 10, 15, 20, 25, 30, 35°C and 120 rpm for 2 weeks. OD_{600} of the samples were measured at day 0, day 3 and day 5. Similar experiments with optimization of environmental pH, initial concentration of 2-MIB , dosage of bacteria, kinds of cometabolism carbon and concentrations of cometabolism carbon, were also performed.

2-MIB removal experiments with 2-MIB at an initial concentration of 2 mg/L were carried out in 2000 mL Erlenmeyer flask with 1000 mL MSM. The flasks were tightly capped and rotated in an incubator shaker at 30°C and 120 rpm for 20 d. Samples of 40 mL were transferred into a cap vials from the Erlenmeyer flask at 2, 3, 4, 5, 7, 10, 15, 20 d, respectively. The samples were filtered through sterile 0.22 μm pore-size MPMs and then transferred 20 mL into crimp cap vials. The concentration of 2-MIB and biomass of living bacteria (plate counting) were measured.

2.6. Analytical method

The concentration of 2-MIB was determined by headspace solid-phase micro extraction (HS-SPME) coupled with GC-MS [21,23]. The Injection mode was taken by manual injection. Analyses were carried out in a gas chromatograph (Agilent 6890N) coupled to a mass selective detector (Agilent 5975C). The GC was equipped with a capillary column (HP-5 ms, 30 m by 0.25 mm by 0.25 μm film thickness, Hewlett-Packard). The column temperature was held at 40°C for 2 min, then programmed to increase at 8°C/min to 240°C, which was held for 5 min. Helium of purity 99.999% was used as the carrier gas with a column flow rate of 1.0 mL/min in constant flow mode. Individual liquid sample (20 mL) was supplemented with 0.3 g/mL NaCl and incubated in crimp cap vials, then incubated in heating magnetic stirrer at 60°C with rotational speed of 1000 r/min. After 50 min of incubation, SPME fiber was immersed into the head space of the liquid sample vial for 5 min,which was agitated so that the analyte was absorbed on to the SPME fiber.

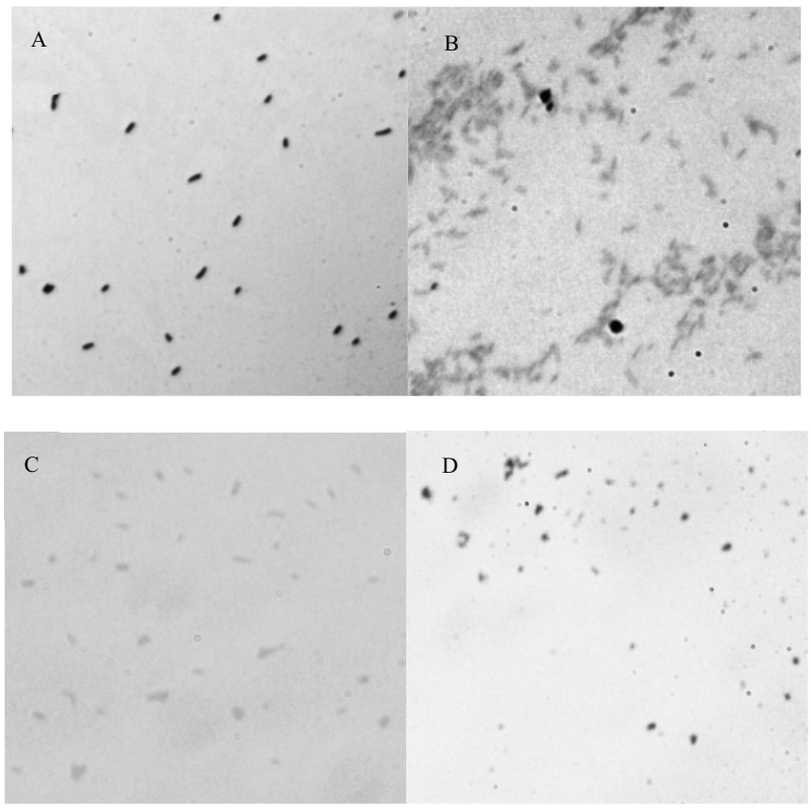

Fig. 1. Micrograph of degrading bacteria by 100 times oil immersion lens A:S1,B:S2,C: the logarithmic phase of S3, D: stable phase of S3.

Table 1
Morphological analysis of degrading bacteria

Bacterial colony	S-1	S-2	S-3
Colony size	2 mm	2–3 mm	1–3mm
Colony color	White	Milky white	Yellow
Morphological characteristics	Semitransparent, minimal elevation, round with a glassy surface	Semitransparent, round with smooth edge	Smooth, Wet and convex
Single cell shape	Bacilli	Bacilli	Bacilli (Cocci)
Colony formation time	2 d	2 d	3–4 d
Gram staining	G+	G+	G+

Biomass of samples was detected by OD_{600} or plate counting method (GB/T 5750.12-2006, China). OD_{600} was analyzed by the spectrophotometer DR 5000 HACH. The process of plate counting was same as isolation of 2-MIB degraders. The plate with 30–300 colony forming units (CFU) was utilized.

3. Results and discussions

3.1. Isolation of 2-MIB degraders

Three kinds of bacteria, (designated as S1, S2, and S3, respectively), were observed from the agar containing 2-MIB as the sole carbon source after 72 h inoculation. All of them were gram-positive. The colony-forming time of S1 and S2 was about 24 h, which was faster than S3. The initial formation of colonies of S3 is about 1 mm growing for 3 d. At 6–7 d, the colony morphology changed to flat and with diameter of 3 mm. Micromorphology of degrading bacteria was observed by 100 time soil immersion lens. The logarithmic phase of S3 was bacilli, and stable phase of S3 changed to cocci. Morphological characteristics and physio-biochemistry of homogenous colonies are summarized in Table 1.

The suspension of each kind of bacteria were applied to the surface of mineral salts solid medium coated with 2-MIB (20 μg/L) in vitreous plates. Colonies of S2 and S3 could be observed from the medium after 7 d of inoculation and they were all 1 mm.

3.2. Identification and phylogenetic analysis of the bacteria

The agarose gel electrophoresis (Fig. 2) was supplied to help identifying the genera of 2-MIB degraders. The 16S rRNA phylogeny results (Fig. 3) identified the species of these isolates. According to the DNA sequences which were compared with the NCBI Gene Database, the isolated strain of S1 shares 100% 16SrRNA gene sequence identity with its nearest relative of *Shinellazoogloeoides*, while S2 shares 99% with *Bacillus idriensis* and S3 shares 98% with *Chitinophagaceae bacterium*. These bacteria may play a vital role in the biodegradation of 2-MIB within the Erlenmeyer flask. Satoshi Hanada had reported a member of the family *Chitinophagaceae* isolated from a hot spring [24], and the logarithmic phase of this bacterium was bacilli while stable phase changed to be cocci. That was quite similar with *Chitinophagaceae bacterium* (S3). This is the first report of these bacteria capable of degrading 2-MIB.

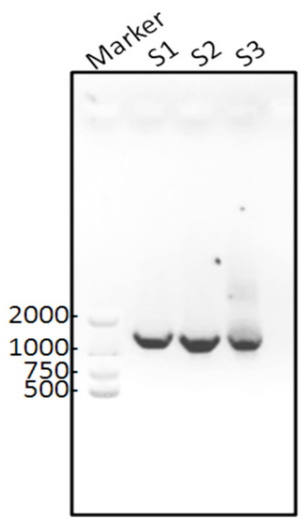

Fig. 2. Agarose gel electrophoresis of amplified 16S rRNA fragment of three strains.

3.3. Degradation efficiency for three bacteria

The comparison of the degradation efficiency for three kinds of the bacteria is to examine the biodegradation rate of the same concentration of single bacterial isolate on 2-MIB over a period of time. Since culture temperature and rotation speed are quite high, the natural volatilization of 2-MIB was determined as about 3% per 24 h. The comparison of the degradation efficiency of the three kinds of bacterial was as shown in Fig. 4. *B. idriensis* had the highest degradation efficiency, the biodegradable removal rate for 2-MIB (20 μg/L) after 2 d was 26.6%, and 32.9% for 3 d, while 23.3% for *Shinellazoogloeoides* and 17% for *Chitinophagaceae bacterium* after 3 d.

3.4. Optimization of degradation efficiency affecting factors

3.4.1. Temperature

The optimum temperature for most bacteria growth is between 28–35°C. The growth rate of *B. idriensis* and its degradation efficiency of 2-MIB under different temperatures were investigated in this study (Fig. 5). The same amount

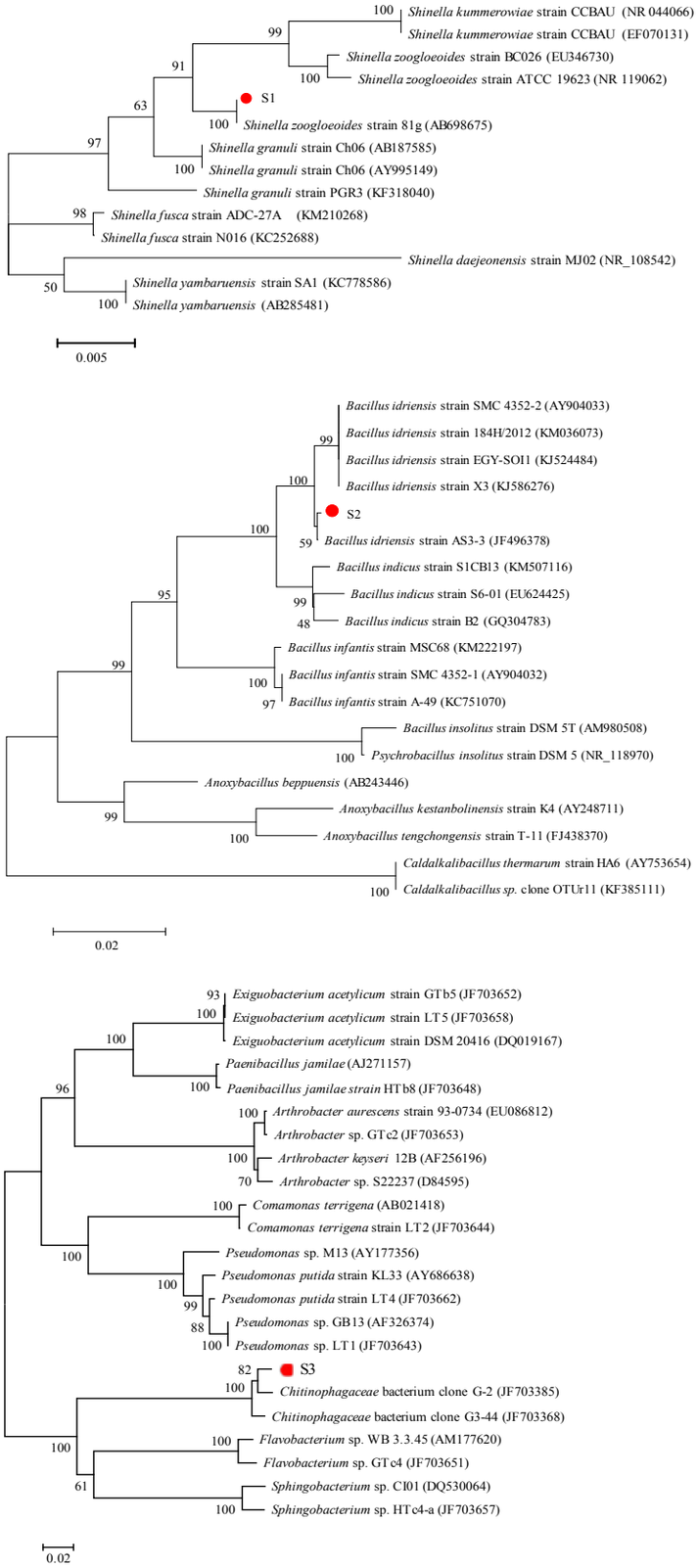

Fig. 3. Neighbor-joining trees showing the phylogenetic relationships of the predominant strains in the isolated culture with the closest-matching species.

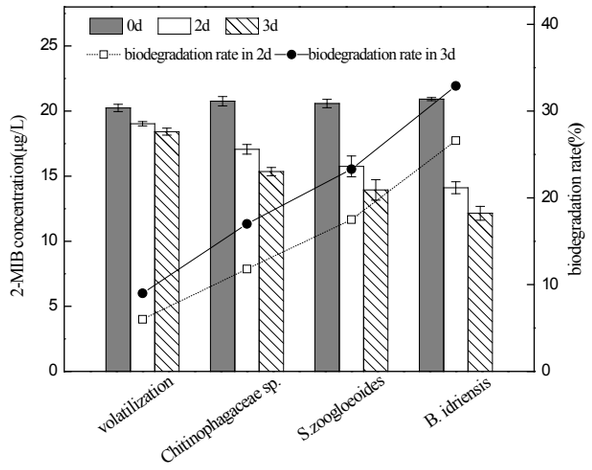

Fig. 4. Degradation efficiency of 2-MIB by three strains.

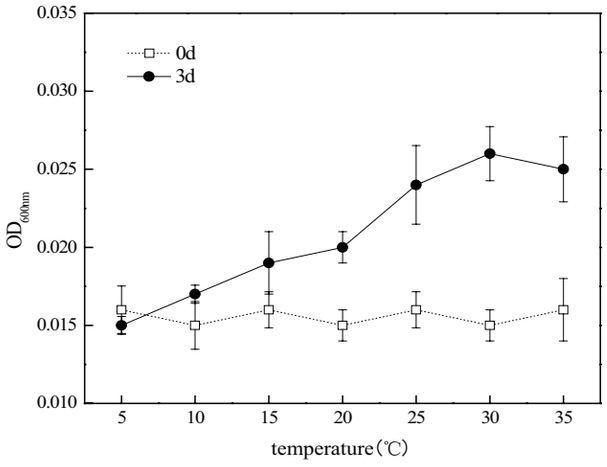

Fig. 5. Effect of temperature on the growth of *B.idriensis.*

of *B. idriensis* were added to MSM containing 20 μg/L2-MIB and placed in various temperatures between 5–35°C, then the OD_{600} value of the medium were measured at day 3. When the temperature was below 10°C, there was very slow bacterial growth and was accelerated along with the increasing temperature, indicating that the efficiency of 2-MIB was gradually increased. The bacteria reached the maximum growth rate when the temperature was between 25–35°C, which agree with the general conditions of most microorganisms. Thus, the culture temperature was selected to be 30°C.

3.4.2. pH

The optimum pH for bacteria growth is generally between 6 and 9, and most microorganisms grow in neutral or alkaline environment. In this experiment, HCl or NaOH was added to MSM containing 20 μg/L2-MIB to adjust pH. The bacteria hardly grew at pH 5 and began to grow slowly at pH 6, indicating that acidic environment was not suitable for *B. Idriensis* growth. The bacteria grew fastest in neutral

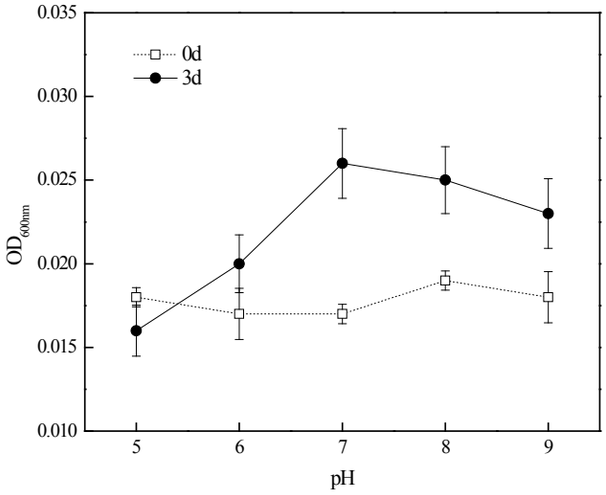

Fig. 6. Effect of pH on the growth of *B.idriensis.*

or weak alkaline environment. The results were as shown in Fig. 6. Thus, in this experiment, pH 7 was chosen as the most suitable growing condition.

In addition, the pH of growth medium were measured at day 3. It was also found that the pH of culture medium was slightly increased after bacteria growth. A final pH of 7.24 was detected compared with the initial neutral condition (pH 7), probably due to constant lysis of the thallus cells during their growing process. The hydrolysis of the cytoplasmic components and enzymes would raise the pH value. The change was most significant in the medium with pH 7, indicating that *B. idriensis* grew most quickly and had the highest degradation efficiency of 2-MIB in a neutral environment.

3.4.3. 2-MIB initial concentration

As the carbon source, 2-MIB offers the energy necessary for the growth and reproduction of *B. idriensis*. It is necessary to consider the utilizability of low concentrations of 2-MIB by the strain and the tolerance of high concentrations of 2-MIB. This study examined the utilization of different concentrations of 2-MIB by monitor changes in absorbance before and after growth of bacteria (Fig. 7). With the increase of initial 2-MIB concentration, the degradation and utilization of thallus to 2-MIB was also increased accordingly, especially in the concentrations of 20 μg/L and 100 μg/L. When the concentration of 2-MIB was up to 500 μg/L, the growth rate of strain was a bit higher compared to that at 100 μg/L, indicating that the strain had good tolerance to 2-MIB at high concentrations and could use high concentrations of 2-MIB as the energy substance for its growth and reproduction. Meanwhile, it can also be observed that after the concentration reached 100 μg/L, the bacteria growth did not change much along with the increasing concentration, indicating that in the bacteria tolerance range, 2-MIB of 100 μ/L fully meets the growth needs of the amount of bacteria (OD_{600}0.16).

Those study demonstrated that *B. idriensis* was able to degrade 2-MIB at ng/L level, and its utilization of 2-MIB at μg/L level was higher. Moreover, it has good tolerance

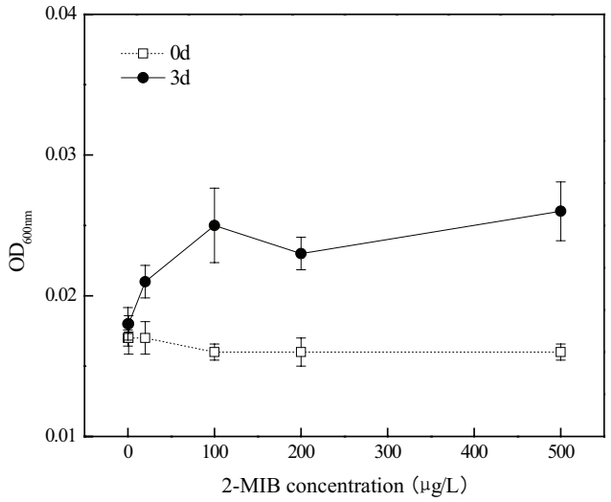

Fig. 7. Effect of initial concentration of 2-MIB.

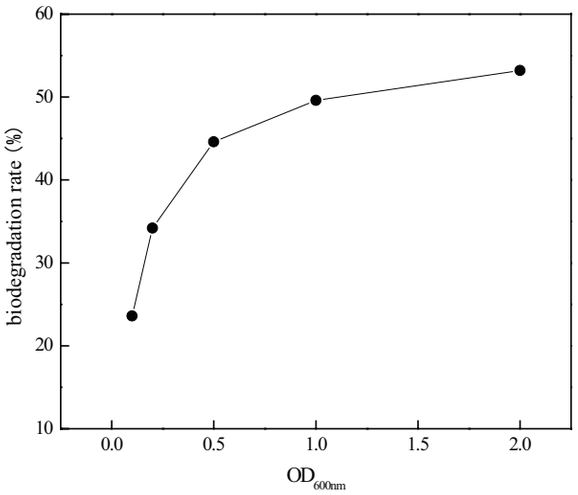

Fig. 8. Effect of Initial dosage of bacteria.

to the high concentration of 2-MIB at 500 μg/L, therefore, *B idriensis* is able to degrade 2-MIB at different levels of concentration.

3.4.4. Initial dosage of bacteria

Larger amount of microorganisms often have higher metabolic capacity. However, the concentration range most suitable for strain growth should be determined. In Fig. 8, When the initial dosage was OD_{600} = 2, the biodegradable removal rate is 53.2%. However, compared to the dosage of OD_{600} = 0.5, the extent of degradation efficiency increase is much less than that of the increase of the dosage of bacteria. These experiments indicated that once the initial dosage of bacteria is beyond a certain range, it might be no longer appropriate for the bacteria to grow. Taking into account the economic applicability and the adaptability of bacteria growth and reproduction, the initial dosage of OD600 = 0.2 in the inorganic medium with 20 μg/L of 2-MIB was selected as the best dosage for strain.

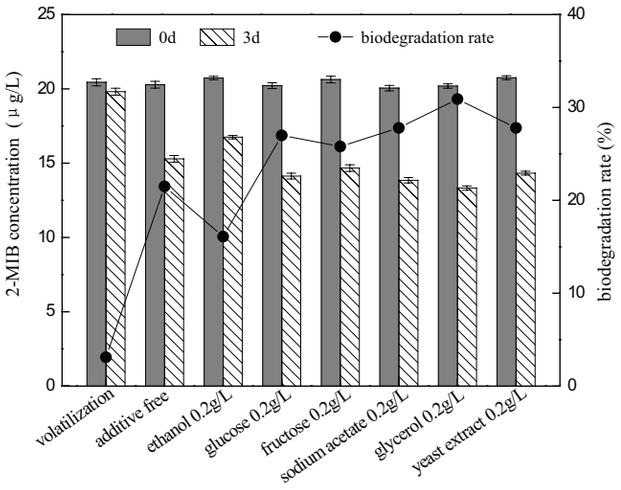

Fig. 9. Effect of cometabolism carbon.

3.5. Co-metabolic carbon source

3.5.1. Influence of different carbon sources on degradation efficiency of B. idriensis

When *B. idriensis* preserved by the glycerol was activated, it was found that *B. idriensis* grew very fast in MSM containing both glycerol and 2-MIB, suggesting that the strain might use glycerol as the carbon source. There have been a lot of research reports on organic small molecules being degraded as co-metabolic carbon source [25,26]. It has been previously reported that the removal rate of degrading bacteria to geosmin had been improved significantly after adding the methanol or ethanol.

In order to investigate the degrading ability of 2-MIB when there's co-existence of other competing carbon sources, some carbon sources most commonly reported were selected: ethanol, glucose, fructose, sodium acetate, glycerol and yeast extract, their impact on *B. idriensis* degrading 2-MIB was shown in Fig. 9. Except ethanol, when there is presence of other carbon sources, the degradation ability of strain to 2-MIB could be improved significantly. Glucose, fructose, sodium acetate, glycerol are the direct carbon sources of small molecules, and are often used to offer energy for bacterial culture. In this study, *B. idriensis* had higher utilization of the external carbon sources. When there were glucose, fructose, glycerol and other external carbon sources, the bacterial growth rate was significantly increased, indicating that these carbon sources can participate in the metabolic activity of growth and reproduction of *B. idriensis* strain. Although the impact of the different external carbon sources on 2-MIB degradation efficiency is measured only at day 3, but the contrast of the degradation efficiency was sufficient to explain that the glucose, glycerol and other co-metabolic carbon sources can directly affect the biodegradation of *B. idriensis* to 2-MIB. Glucose, glycerol and other small molecules of carbon sources can

be used directly as a primary energy source for microbial growth, thereby improving the thallus growth environment, enhancing the growth rate of the thallus, and it also accelerates the bacteria to produce key enzyme to biodegradate 2-MIB to a certain extent [25], thereby increasing the degrading efficiency of degrading bacteria.

3.5.2. Influence of different concentrations of co-metabolic carbon sources

This study also examined the impact of different concentrations of glycerol, and glucose co-existing with 2-MIB on thallus growth and biodegradation rate. The study respectively investigated the changes of thallus growth and 2-MIB biodegradable removal rate when 2-MIB (20 μg/L) coexisting with glycerol or glucose with the final concentrations of 0.05 g/L, 0.1 g/L, 0.2 g/L, 0.5 g/L, 1 g/L, 2g/L, and the results were as shown in Fig. 10.

When the external carbon source of glucose coexisted with 2-MIB, the growth rate of *B. Idriensis* had been significantly improved. With the increasing glucose concentration, the thallus growth rate was also gradually increased. It indicated that glucose content was positively correlated to slow growth of bacteria. Meanwhile, when there was glucose, the biodegradable removal of 2-MIB was also improved to some extent. When glucose content was 0.5 g/L, the biodegradation rate of 2-MIB reached a peak. When there was higher concentrations of glucose, its impact on biodegradation rate was little or tended to decrease. It can be concluded that when different glucose concentrations coexist with 2-MIB at 0.05–2 g/L, the thallus growth rate and 2-MIB degradation rate were increased than when there's only 2-MIB, and there's the highest biodegradation rate of 2-MIB in the concentration range of 0.2–1 g/L.

When glycerol coexists with 2-MIB, thallus growth rate was also significantly improved, indicating that different concentrations of glycerol had the promoting effect on thallus growth. However, when there was the glycerol with the concentration of up to 1–2 g/L, the promotion to biodegradation of 2-MIB is not obvious, on the contrary, it was the suppressed compared to a concentration of 0.2 g/L, and the thallus growth rate is slightly lower when glycerol concentration is higher than 1 g/L, indicating that the existence of the high concentrations of glycerol is inappropriate for Bacillus growth and the biodegradation of 2-MIB. The highest removal rate was reached when the concentration of glycerol is in the range of 0.2–0.5 g/L. The existence of co-metabolic carbon source offers a wealth of nutrients for thallus growth, which to some extent can promote *B. idriensis* to produce enzymes used to degrade 2-MIB, thereby increasing the degradation efficiency to 2-MIB. However,too much external carbon source not only affects the growth rate of the bacteria, but also reduces the enzymatic reaction, which is not suitable for the biodegradation process to 2-MIB.

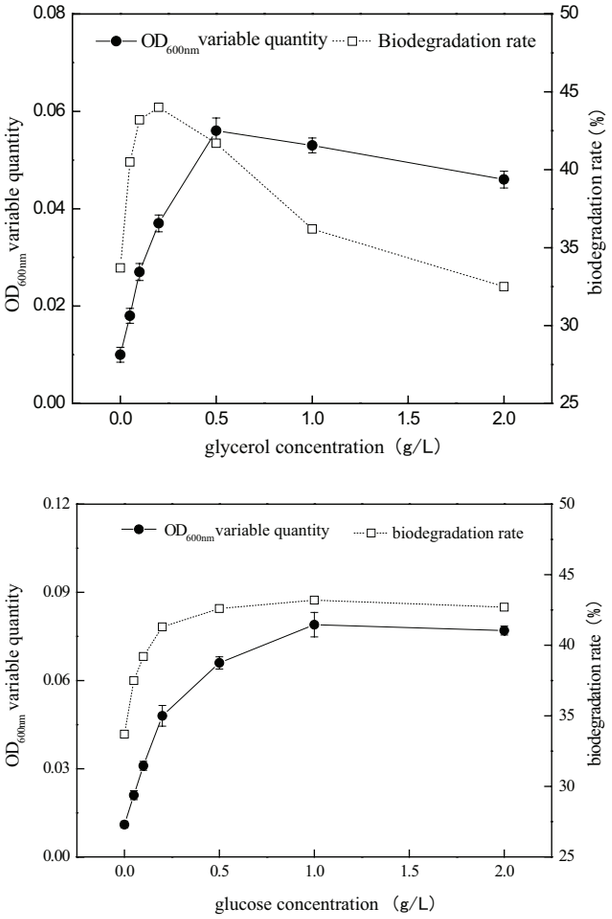

Fig. 10. Effect of different concentrations of cometabolism carbon.

3.6. Growth curve of B. idriensis

After bacteria *B. idriensis* was added to MSM with high concentration of 2-MIB, the bacterial growth was slow in the first 1–2 d. Then the number of viable bacteria increased sharply in the logarithmic growth phase from day 3 to day 5. The stationary phase of the bacteria was from day 6 to day 15, and the stable period was longer, which was helpful for bacteria degradation of 2-MIB. The decline phase was from day 15 to day 20. However, the cardinal number of viable cells was still larger, indicating that *B. idriensis* had stronger viability and metabolism in MSM containing 2 mg/L of 2-MIB.

When glycerol (0.5 g/L)and 2-MIB exist simultaneously, the growth curve of *B. Idriensis* has some changes, and the adaptation period was one day in advance. The logarithmic growth phase was from day 2 to day 5, the bacteria growth rate was significantly accelerated than that without the glycerol, and the number of viable cells also increased obviously .The stable period of the bacteria was from day 5 to day 8. Day 8 to day 20 was for decline phase and there was no significant reduction of the number of viable cells in this phase, which was possible because of the presence of glycerol accelerating the growth of bacteria. With the glycerol consumption and the impact of dead cells on the environment, as a result the number of viable cells had a sharp decline on day 8 to day 10.

In the degradation period of up to 20 d, *B. Idriensis* can make the degradation of 2 g/L 2-MIB to 368 μg/L, show-

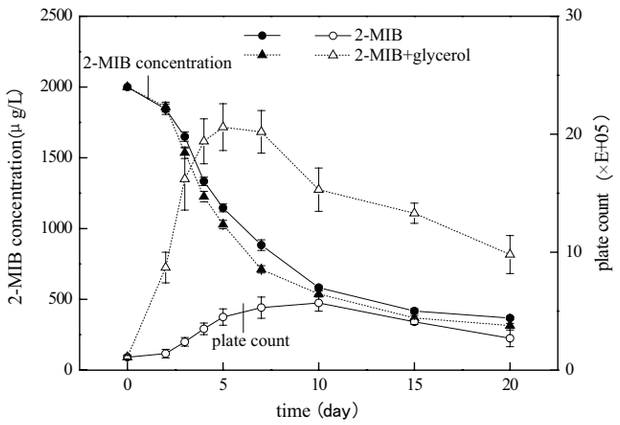

Fig. 11. Concentrations of 2-MIB and growth curve in MSM.

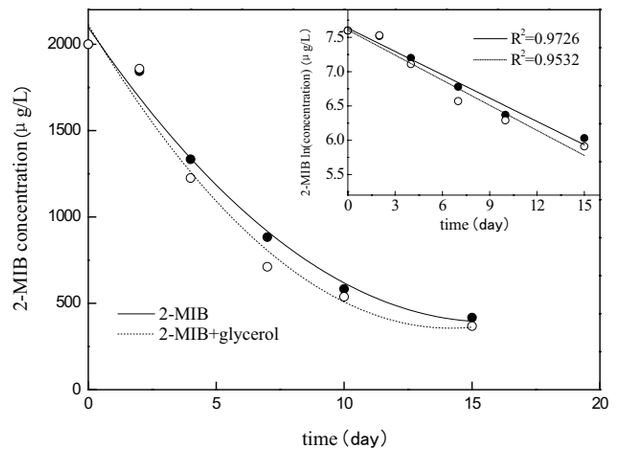

Fig. 12. Kinetics of 2 mg/L 2-MIB biodegradation for *B.idriensis.*

ing thalli have stronger tolerance and degradation towards odor substance with super-high concentration. The biodegradation efficiency of *B. Idriensis* is higher than that of other reports on *Bacillus* [15,16], and similar to that of other strains as *Pseudomonas spp.* and *Flavobacterium spp.* [20,21]. The degradation rate of 2-MIB is accelerated significantly when the thalli entering into the logarithmic growth phase (day 2–day 5) with the presence of glycerol. When the number of viable cells is significantly reduced, there's no significant change in the degradation rate of 2-MIB, thus it can be inferred that the dramatic decrease of the number of the viable cells on 8–10 d is due to glycerol is the direct carbon source for growth of thallus at the beginning, when the glycerol consumption in the medium is reduced to a certain level, the thalli begin to take 2-MIB as the carbon source, which may also explain the number of viable cells maintains at a higher level rather than have any significant decrease on day 10 to day 20. However, due to the presence of glycerol, *B. Idriensis* thalli grow vigorously, the continuous microbial secretion of metabolites and disruption of cell death leads to the changes of pH and dissolved oxygen, seriously affecting the later biodegradation of *B. idriensis* towards 2-MIB. This also explains the degradation efficiency under the presence of glycerol within 7 dis significantly higher than the case of containing only 2-MIB, but there's ultimately little difference in the degradation rate of above two.

The linear relationship between the natural logarithm values of the fraction of remaining 2-MIB versus time was consistent with that of a pseudo first order mechanism of degradation. The degradation of 2 mg/L 2-MIB in 15 d is shown in Fig. 12. The rate constants was 0.113 d^{-1} with R^2 0.9726 for *B. idriensis*. When there is presence of extra carbon sources with 2-MIB, the linear relationship was still consistent with that of a pseudo first order mechanism of degradation.

4. Conclusions

Through the initial discussion on the physiological and biochemical characteristics of three types of bacteria, where the *Shinellazoogloeoides* and *Bacillus idriensis* were bacillus, *Chitinophagaceae bacterium* was bacillus in the logarithmic growth phase, but it was coccus after the stable period. *Bacillus idriensis* and *Chitinophagaceae bacterium* could form colonies on inorganic salt medium coated with 20 μg/L2-MIB. Through evaluation and comparison of degradation efficiency of the three 2-MIB degrading bacteria, where the highest degradation efficiency of all was *Bacillus idriensis*, removal efficiency of 20 μg/L2-MIB in three days was 32.9%.

The factors affecting growth and reproduction of *B. idriensis* and the degradation efficiency of 2-MIB were optimized: the suitable temperature for growth of bacteria was between 25–35°C, and the optimum pH was neutral, the initial dosage of strains should be best around OD = 0.2, 2-MIB concentration had little impact on *Bacillus idriensis* to degrade 2-MIB, the degradation efficiency was higher under μg/L concentration.

The effects on degradation efficiency of 2-MIB under the presence of various co-metabolic carbon source were investigated, when there's co-existence of glycerol, yeast extract, glucose, fructose with 2-MIB, the degradation efficiency was improved significantly. The concentration change of co-metabolic glycerol and glucose had a greater impact on the degradation efficiency of the degrading bacteria, indicating that the co-metabolic carbon source can also promote *B. idriensis* thalli to generate enzyme degrading 2-MIB to some extent in addition to offer rich nutrients for thalli growth. Further research about the bacteria was carried out with the degradation mechanism on enzymatic levels, and the improved bacteria can make the efficient degradation of 2-MIB in potable water.

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