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ORIGINAL PAPER

Biodegradation of ethanethiol in aqueous medium by a new *Lysinibacillus sphaericus* strain RG-1 isolated from activated sludge

Shungang Wan · Guiying Li · Taicheng An · Bin Guo · Lei Sun · Lei Zu · Ailing Ren

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Abstract In the present study, a new bacterial strain isolated from activated sludge has been identified as *Lysinibacillus sphaericus* based on its morphology, physiochemical properties, and the results of 16S ribosomal RNA (rRNA) gene sequence analysis. This new bacterial strain uses ethanethiol as both carbon source and energy source. The key factors for controlling the degradation efficiency of ethanethiol by this strain were found to be initial ethanethiol concentration, temperature, and pH value of solutions. Under the optimized conditions, as well as 4 mg l^{-1} ethanethiol, 30°C, and pH = 7.0, almost 100% ethanethiol can be degraded within 96 h and sulfate as a final product was detected in aqueous medium. The degradation reaction of ethanethiol over this newly isolated strain can be described by pseudo first-order equation in which the maximum degradation rate constant K and the minimum half-life were respectively calculated to be 0.0308 h^{-1} and 22.5 h under the optimal conditions.

Keywords *Lysinibacillus sphaericus* · Identification · Wastewater treatment · Biodegradation · Ethanethiol

S. Wan · G. Li (✉) · T. An · L. Sun · L. Zu
The State Key Laboratory of Organic Geochemistry and Guangdong Key Laboratory of Environmental Protection and Resources Utilization, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China
e-mail: ligy1999@gig.ac.cn

B. Guo (✉) · A. Ren
Colleges of Environmental Science and Engineering, Hebei University of Science and Technology, Shijiazhuang 050018, China
e-mail: gbin69@163.com

S. Wan · L. Sun · L. Zu
Graduate School of Chinese Academy of Sciences, Beijing 100049, China

Introduction

Odors emission is a notorious environmental problem (Luo and Lindsey 2006) and can cause serious annoyance to the neighborhood (Nimmermark 2004; Sheridan et al. 2003). Among of these odorous compounds, volatile organic sulfur compounds (VOSCs) released into the environment from various special industries occupy enormous quantity, and can cause serious environmental problem threatening the public health (Burgess et al. 2001). These VOSCs mainly include dimethyl sulfide, dimethyl disulfide, methanethiol, carbon disulfide, carbonyl sulfide, and ethanethiol (Cheng et al. 2005; Hort et al. 2009; Muezzinoglu 2003). The presence of VOSCs in waste treatment process has received intensive attentions and drawn significant attention because of their very low odor threshold value, high toxicity, and potential corrosive effect. Compared with natural emissions,

the local pollution is mainly caused by the anthropogenic emission, such as the anaerobic or aerobic degradation of organic matters (Burgess et al. 2001; Gostelow et al. 2001; Tsang et al. 2008), heat of organic matters (Rappert and Muler 2005; Shareefdeen et al. 2005; Sironi et al. 2007); industrial applications of VOSCs in semi-conductor industries (Hwang et al. 2007), paper making industry (Yoon et al. 2001), and so on. Thus, a variety of feasible and inexpensive technologies have been consecutively attempted to removal these VOSCs from the water and atmosphere systems (Smet et al. 1998). For example, various physicochemical processes, such as scrubbing, adsorption, condensation, and oxidation (Burgess et al. 2001; Smet et al. 1998), have been developed. However, these conventional physicochemical methods are impractical for treating low molecular weight VOSCs, and particularly are still high-costly (Mathur and Majumder 2008; McNevin and Barford 2000). In contrast, biological treatment of odors has been validated as a cost-effective option during the past few decades (Cohen 2001; Easter et al. 2005), especially for the VOSCs treatment (De Bo et al. 2003; Ho et al. 2008a, b; Sercu et al. 2005). However, the practical application of this method is still limited because no microbial species that can achieve the efficient removal of VOSCs have been isolated from the natural environment. Although some of aerobic microorganisms, such as *Hyphomicrobium* MS3 (Smet et al. 1999), *Hyphomicrobium* S, *Thiobacillus thioparus* E6 and DW44, were isolated to degrade VOSCs, the use of these microorganisms for the biological treatment of wastewater containing ethanethiol or ethanethiol off gas is seldom reported (Park et al. 1993; Smet et al. 1998). Among the VOSCs, ethanethiol is significantly toxic to human being. It is classified as an odorous, strong, and colorless liquid VOSC with a low odor threshold concentration of 0.0007 mg m^{-3}. Its vapor is a severe irritant for skin and olfaction exposure to cause depression, gastrointestinal upset, unconsciousness, headache, nausea and anorexia. It is desired to develop an effective biodegradation route to remove the ethanethiol from environment. However, none of an individual bacterial strain capable of decomposing ethanethiol in aqueous medium has been reported.

In the present study, a new bacterial strain for achieving the efficient decomposition of VOSCs in aqueous medium was reported. Ethanethiol was selected as a representative of VOSCs. The isolated and identified new aerobic bacterium can utilize ethanethiol as sole carbon and energy source. The operation parameters of this bacterial strain for efficiently removing ethanethiol have been optimized. In addition, the reaction kinetics for the biodegradation ethanethiol by the isolated bacterial strain was also investigated.

Materials and methods

Chemicals and growth medium

Ethanethiol (Acros, 99+ %, Geel, Belgium) used for bacterial growth and biodegradation was purchased from J&K Chemical Ltd. All other chemicals (analytical grade reagents with more than 99% purity) used for the preparation of aqueous medium and biochemical experiments were obtained from Guangzhou Chemical Reagent Co., Inc., China. The enrichment culture medium A contains (g l^{-1}) 2.0 beef extract, 10 peptone, 5.0 NaCl, and 1.5 ml ethanethiol. The enrichment culture medium B contains (g l^{-1}) 2.0 beef, 10 peptone, 5.0 NaCl, 10 sodium hyposulfite and 10 agar. Phosphate buffer solution was chosen to prepare mineral salts medium (MSM). The MSM contains (g l^{-1}) 1.2 $K_2HPO_4{\cdot}3H_2O$, 1.2 KH_2PO_4, 0.2 $MgSO_4{\cdot}7H_2O$, 0.4 NH_4Cl, 0.01 $FeSO_4{\cdot}7H_2O$, and 1 ml of trace element stock solution. The trace element stock solution contains (g l^{-1}) 0.2 $CaCl_2{\cdot}2H_2O$, 0.2 $MnSO_4{\cdot}4H_2O$, 0.1 $CuSO_4{\cdot}2H_2O$, 0.2 $ZnSO_4{\cdot}7H_2O$, 0.09 $CoCl_2{\cdot}6H_2O$, 0.12 $Na_2MoO_4{\cdot}2H_2O$ and 0.006 H_3BO_3.

Isolation of bacteria

Initially, activated sludge obtained from a domestic wastewater treatment plant was used as microbial source. Bacterial strains capable of degrading ethanethiol were isolated from the activated sludge following the modified enrichment culture technique according to the previous paper (Smith and Kelly 1988). In brief, one milliliter of the sludge homogenate was taken with sterile measuring cylinder from the domestic wastewater treatment plant. The samples were mixed with 10, 20, 30, 40, and 50 ml of the enrichment culture medium A, and then were incubated at 30 ± 1°C with constant shaking at 120 rpm

for 7 days. Subsequently, an aliquot of 0.5 ml of the culture was plated on the enrichment culture medium B, and the plates were incubated at 30 ± 1°C for further 3 days. The colonies obtained on the plates were carefully observed for their uniformity and difference. The resulting colonies on the plates were repeatedly reinoculated into the enrichment culture medium A and plated on the enrichment culture medium B for further several times, respectively. After these procedures, one pure bacterial strain was isolated from the activated sludge to grow profusely, and was selected for the ethanethiol biodegradation on the basis of its growth.

Identification of bacterial strain

Optical microscope (Leica DMBX, Wetzlar, Germany) was used for the morphological observation. Physiological, colony morphology, cultural and biochemical characteristics of the strain were identified by China Center of Industrial Culture Collection. The bacterial strain was further confirmed by 16S rRNA gene sequence analysis. Genomic DNA was isolated using standard procedures described in reference (Goldberg and Ohman 1984). A 100 ng extracted genomic DNA was used as a template for PCR amplification. Forward primer (5′-GAGCGGATA ACAATTTCACACAGG-3′) and reverse primer (5′-CGCCAGGGTTTTCCCAGTCACGAC-3′), purchased from TaKaRa Biotechnology Co., Ltd., Dalian, China, and were designed to amplify the 16S rRNA. PCR amplification condition was as follows: each PCR mixture (50 μl) was composed of 5 μl genomic DNA templates, 4 μl of dNTP at 2.5 mM, 5 μl of 10× buffer, 0.3 μl of 5 Unit DNA polymerase, 0.5 μl of each primer at 10 μM, and 34.7 μl sterile water. The PCR was performed in a PTC-100 Peltier Thermal Cycle with a hot starting performed at 94°C for 5 min, followed by 30 cycles at 94°C for 60 s, 55°C for 60 s, and 72°C for 90 s. A final 5 min extension at 72°C was included after the cycles of the PCR amplification. PCR products were subsequently separated and visualized in 1.0% agarose gels. The objective DNA fragment was excised and purified with using TaKaRa agarose gel DNA purification kit Ver 2.0 (Code No. DV805A, TaKaRa, Dalian Co. Ltd.) following the manufacturer's instructions. The purified sample was chosen for sequencing by TaKaRa Biotechnology (Dalian) Co., Ltd. Nucleotide sequence similarities were determined using BLAST software in GenBank for biotechnology information databases.

Biodegradation kinetics

In order to understand the biodegradation pattern of ethanethiol with the newly isolated strain, various operating parameters, such as the initial concentration of ethanethiol, the pH value and the temperature, were investigated in culture MSM in detail. For each batch experiment, one of the parameters was varied while keeping the others constant. In addition, the negative index of the least-squares regression method was applied to analyze the experiment data. Therefore, a 250 ml of serum bottle was employed as a biodegradation reactor to carry out the experiment. The reactors were sealed with Teflon-coated silicone septum to avoid the volatilization of ethanethiol during the experiment. In the biodegradation experiment, only 50 ml working volume in the serum bottles was used. Different volume of ethanethiol saturation gas from the headspace of a glass container containing ethanethiol solution was injected into the cultures of reactors through Teflon-coated silicone septum by an Agilent syringe. Because mass transfer is not a limiting step at low concentrations (Reardon et al. 2000), ethanethiol diffuses from gas to liquid phase and balances in vapor–liquid two phases. Attaining equilibrium in the reactor is of paramount importance in the experiments. A reactor containing pure culture MSM was employed to measure equilibrium time. The concentration of ethanethiol in the liquid was selected as the initial concentration after equilibrium. The interval samplings were done at the top of the reactor to collect headspace gaseous samples. When the optimum conditions were determined, the biodegradation of samples in other six parallel serum bottles were carried out under the optimal conditions. And 20 ml liquid samples were sampled from their respective system at different time to determine the concentration of generated sulfate and the population of bacteria. Before the experiment, strain RG-1 was cultured in the medium A for 36 h with continuous shaking. The culture was collected in the late logarithmic growth phase, and then washed with MSM by centrifugation at 3,000×g for 20 min for three times. The obtained cell pellet was re-suspense in the MSM by adjusting the optical density to 1.0 U. Finally, a 10 ml of the cell

suspension was inoculated into 40 ml MSM with ethanethiol. MSM without adding inoculums was served as a blank test for the loss of substrates by oxidation as well as for bacterial contamination.

Analytical methods

The concentrations of microorganisms were measured at its maximum absorption wavelength of 600 nm by using an UV–Visible spectrophotometer (Thermo Spectronic Helios α series, USA). The results showed 1.0 U of optical density was equivalent to a concentration of 26.9 mg l^{-1} according to the calibration curve equation. The concentrations of ethanethiol in the aqueous solutions were determined by using a headspace gas chromatograph (GC) method (Kolb and Ettre 1997; Takashi et al. 2007). The concentration of ethanethiol was measured by a GC (HP 5890, Series II, equipped with a split/splitless injector and a flame ionization detector) equipped with a HP-5 MS capillary column (0.32 mm × 0.25 μm × 30 m, Agilent Technologies). The column temperature was initially kept at 40°C for 2 min, then programmed to 100°C at 5°C min^{-1}, and finally maintained at 100°C for 1 min. 300 μl gaseous samples taken at given intervals from the headspace of serum bottle by a 500 μl gas-tight locking syringe (Agilent, Australia) were injected into the GC for ethanethiol concentration determination. And each sample was assayed and averaged in triplicate. The concentrations of the sulfate were determined by barium chromate spectrophotometer according to the standard method of Environmental Protection Industry Standard of the People's Republic of China (HJ/T 342-2007).

Results and discussion

Characteristics of ethanethiol degrading bacterium

The morphological, physiological, and biochemical characteristics of the newly isolated strain RG-1 were listed in Table 1. The strain is a Gram-negative, non-flagellated and short rod (diameter: 0.9–1.1 μm; length: 1.2–2.2 μm). The colonies shows salmon pink, smooth on the surface and trim at the edge. The tests were positive for gelatin, phenylalanine deaminase and catalase, while negative for indole and mannitol. Sugar utilization test found that this strain cannot use either xylose or glucose as carbon source. On the basis of the phenotypic and physiochemical characteristics, the newly isolated strain RG-1 can be tentatively identified as genus *Bacillus*.

Table 1 Biological characteristics of isolated *L. sphaericus* strain RG-1

Biochemical and culture conditions	Results
Gram staining	Gram-negative
Glucose	−
Hydrolysis of starch	−
Arabinose	−
Mannitol	−
Catalase	+
Indole	−
Xylose	−
V-P test	−
Gelatin liquefaction	+
Production of glucose	−
Nitrate reduction	−
Phenylalanine deaminase	+
Growth at temperature (10–50°C)	+
Growth on NaCl (2.0–7.0%)	+
Morphology under microscope	
Cell type (shape)	Spherical rods
Color	Yellowish
Size	(0.9–1.1) × (1.2–2.2) μm
Surface	Smooth
Arrangement	Coherent cluster
Density	Opaque
Elevation	Convex
Motility	Positive

+ Positive reaction, − negative reaction

16S rRNA gene sequencing and phylogenetic analysis

To identify the phylogeny of the RG-1 strain, total genomic DNA was isolated from pure cultures by standard protocol described in "Identification of bacterial strain" section. Figure 1 shows the gel purified PCR product of isolated strain RG-1. The corresponding gene fragment was 1471 bp and was submitted for sequencing subsequently. The detailed nucleotide sequences of the16S rRNA gene of

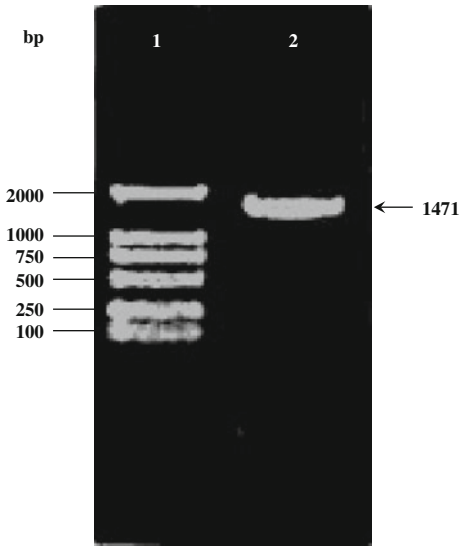

Fig. 1 Agarose gel electrophoresis of polymerase chain reaction (PCR) product after purification from *L. sinibacillus sphaericus* strain RG-1 using primers that amplify DNA. *Line 1* DNA Marker DL 2 000; *line 2* product from *L. sphaericus*

isolated strain can be found in GenBank (FJ544252). The newly isolated strain was deposited in China General Microbiological Culture Collection Center (CGMCC No. 2808) and named RG-1. After the amplified fragment was sequenced, and the BLAST procedure was used to search sequence homology in the NCBI GenBank database for identification. This sequence was compared with those of the type strains of 19 *Bacillus* species based on the 16S rRNA gene sequence alignment and phylogenetic tree analyses (as seen in Fig. 2). In order to validate the reproducibility of the branching pattern, a bootstrap analysis was performed using the program MEGA4. A bootstrap value of 100 indicates that a branching pattern is confirmed in all the resampling, whereas a bootstrap value of 60 indicates that the branching pattern is reproduced only in 60% of the resamplings. In this study, the high bootstrap value was obtained as 100 for the strain RG-1 as compared with *Lysinibacillus sphaericus* and *Lysinibacillus fusiformis*, and also drawn into the phylogenetic trees. However, the results derived from the 16S rRNA gene sequence analysis indicated that the strain RG-1 is much closer to *L. sphaericus* PRE16 (EU880531) with 99% sequence similarity than to *L. fusiformis* NRS350 (AF169537) with 97% sequence similarity. The high bootstrap value was also obtained as 95 for the strain RG-1 as compared with *L. sinibacillus sphaericus* PRE16 (EU880531). On the basis of phenotypic and genotypic characteristics, the strain RG-1 can be identified as a typical member of the genus *Lysinibacillus*.

Biodegradation kinetics study

The developed pure bacterial strain RG-1 was further examined for its ethanethiol biodegradation properties under different conditions. The results of the equilibration test showed that ethanethiol could approach its balance in vapor–liquid two phases within 60 min. The effect of the initial concentration of ethanethiol on the biodegradation performance was determined by varying its initial concentration at fixed pH value of 7.0 and temperature of 30°C. Figure 3a shows the degradation trends of ethanethiol with an initial concentration ranging from 2 to 32 mg l^{-1} in MSM. The total removal efficiency (RE) of ethanethiol increases with increasing the time at fixed initial concentration. The REs at 96 h are 98.5, 96.1, 91.5, 81.2, 79.8 and 78.1% for fixed initial ethanethiol concentration of 2, 4, 8, 16, 24 and 32 mg l^{-1}, respectively. Almost 100% of ethanethiol with an initial concentration of 2–4 mg l^{-1} can be removed. With increasing the initial concentration from 8 to 16 mg l^{-1}, the ethanethiol REs decreases rapidly from 91.5 to 81.2%. With further increasing the concentration, the RE begins to level off, showing a distinct difference. The above REs are measured based on a cumulative result of 96 h biodegradation. It must be mentioned that the RE of ethanethiol is also very high at lower initial concentration within shorter time intervals. For example, when, nearly half of ethanethiol can be degraded within first 12 h for 2 mg l^{-1} initial concentration of ethanethiol. In contrast, within the same reaction time, only 17.0% of ethanethiol can be removed at 32 mg l^{-1} initial concentration. Afterwards, all the degradation curves smoothly drop with increasing the reaction time. In addition, the data shown in Fig. 3 were also controlled by the blank sample. It was found that <5.0% loss ethanethiol was observed in the sterile control.

The reasons why low REs were obtained at high initial ethanethiol concentration may be substrate inhibition and overloading for the bacteria. At low initial ethanethiol concentrations, the bacteria were not saturated by substrate, and slight increase of the concentration normally would result in longer

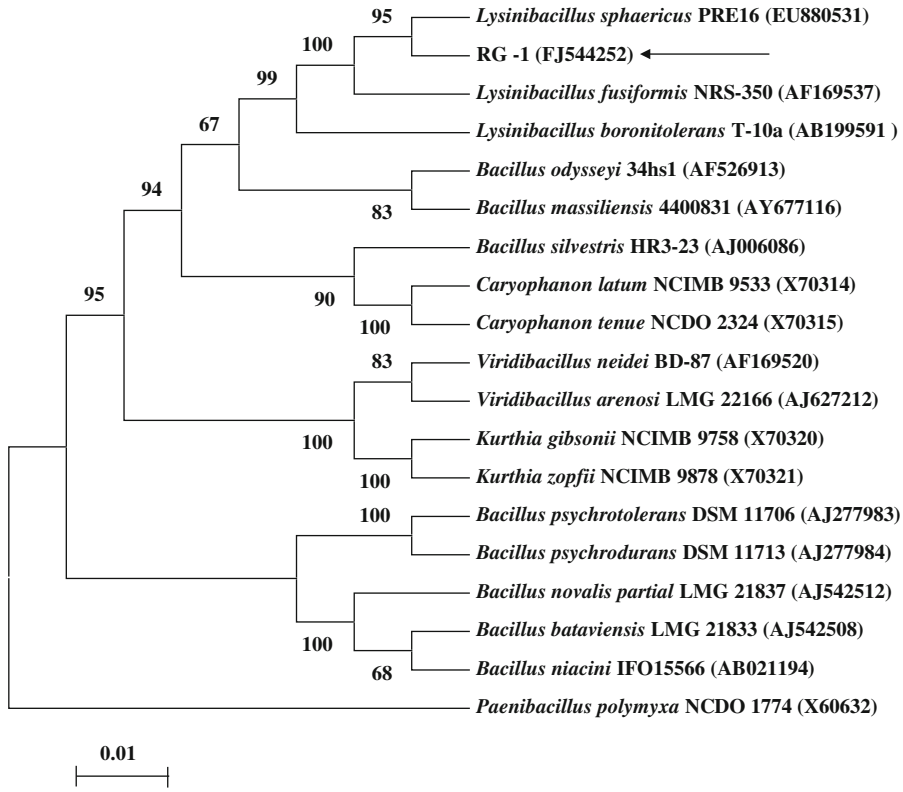

Fig. 2 Phylogenetic tree of the strain RG-1 based on 16S rRNA gene sequence with other reference gene sequences. The phylogenetic tree was constructed by using the neighbor-joining method on the program Mega 4.0. The evolutive distance was based on the Kimura 2p model. The *numbers at the forks* indicated the bootstrap values in percentage. *Bar* indicates the nucleotide difference per sequence position. GenBank accession numbers are given in *parentheses*. *Paenibacillus polynyxa* NCDO 1774 (X60632) was used as the outgroup

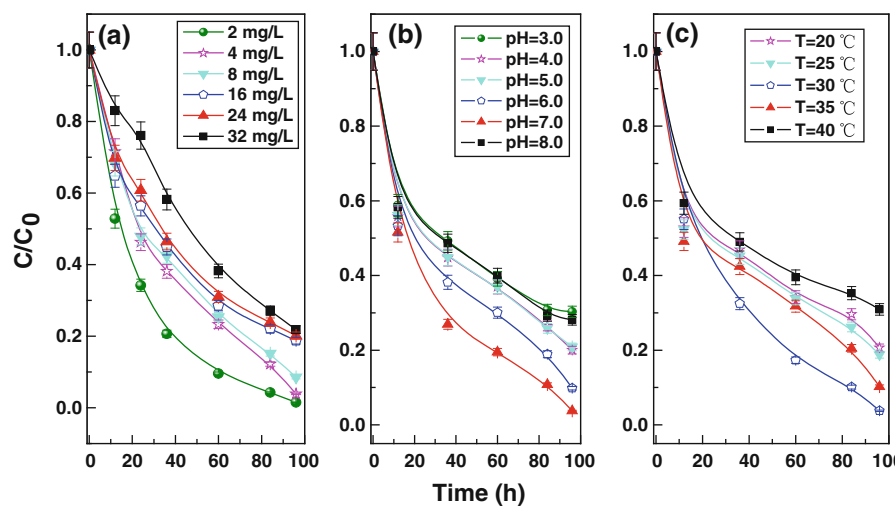

Fig. 3 **a** Effects of initial concentration of ethanethiol on the removal efficiencies in batch culture, at 30°C and at pH value 7.0 for 96 h; **b** effects of pH value on the removal efficiencies in batch culture for 4 mg l^{-1} ethanethiol at 30°C for 96 h; **c** effects of temperature on the removal efficiencies in batch culture for 4 mg l^{-1} ethanethiol at pH value 7.0 for 96 h

degradation reaction time rather than the maximum REs. Thus, ethanethiol can be almost completely degraded by strain RG-1 within 96 h as the initial ethanethiol concentrations ranging from 2 to 4 mg l^{-1}. At high initial concentrations, it produced higher concentration gradients which improved mass

transfer of ethanethiol between the gas phase and the liquid phase, and resulted in a reaction limitation. As the initial ethanethiol concentration increasing, the lag phase of bacteria may be extended at higher concentrations of substrate as described in previous reference (Arutchelvan et al. 2005). Thus, more time needed to achieve the same RE for the higher concentrations in the batch studies. In addition, the concentration of bacteria keep constantly after 96 h of growth and removal capacity is limited. Thus, overloading of substrate for the bacteria may result in the decrease of the RE with the increasing initial ethanethiol concentration.

The effect of pH value on the REs by the strain RG-1 was also investigated. Figure 3b shows the relationship between the REs and the pH value for 4 mg l^{-1} ethanethiol at 30°C. As seen in figure, the RE increases gradually from 69.7 to 96.3% with increasing the pH value from 3.0 to 7.0, and then decreases to 71.8% at pH = 8.0. The highest RE of 96.3% was achieved at pH = 7.0, which is due to that the biodegradation reaction of ethanethiol is strongly affected by super-acidity and super-alkalinity because of the inhibitory activity of intracellular enzyme of bacteria. It is also noteworthy that, the degradation experiments were carried out in buffer solutions and no significant change of pH values was observed comparing with the initial pH values in the batch experiments by the end of 96 h. Thus, the optimum pH value in the aqueous medium for the growth of this newly isolated strain RG-1 is 7.0 which is also used to degrade the ethanethiol.

Temperature is another key parameter for the biodegradation of ethanethiol. Hence, the relationship between the REs and the reaction temperature of aqueous medium was also investigated at fixed initial concentration of 4 mg l^{-1} and pH value 7.0, and the REs as a function of temperature at the different intervals were also plotted in Fig. 3c. The results revealed that the newly isolated strain RG-1 can grow at the range of 20–40°C. As the medium temperature increased from 20 to 25 and to 30°C, the total RE of ethanethiol increased slowly from 79.4 to 81.2 and then quickly to 96.2% after 96 h of degradation. It is worth mentioning that 30°C marks a turning point, the RE reached to the maximum point at this temperature. That is to say that the RE decreased slowly then steeply with the temperature increase further. It must be noted that the REs did not changed so widely around the optimum temperature. The possible reason is that the intracellular enzyme of bacteria consists of protein and a non-protein. The intra- and intermolecular bonds that hold protein in their secondary and tertiary structures can be disrupted by abrupt changes in temperature. Therefore the catalytic activity of an enzyme is temperature sensitive.

Data of the ethanethiol concentration, the bacteria concentration and the sulfate product at optimal conditions were shown in Fig. 4. It can be found that the decrease of ethanethiol concentration was accompanied by an increase in the concentration of strain RG-1 and the sulfate during the biodegradation process. Therefore, this result revealed that strain RG-1 was able to resist ethanethiol toxicity and could utilize ethanethiol as a sole carbon and energy source to grow. And the S atom in the ethanethiol could be ultimately converted to SO_4^{2-} ions by the strain RG-1.

Biodegradation kinetic modeling

To further confirm the effect of variables on the RE, the negative index of least-squares regression by the return was employed to provide a systematic analysis for biodegradation process, and the statistical method was used to confirm the assumption that the data can be represented by pseudo first order kinetics. The fitting results were shown in Fig. 5. From the figure, it was found that even at the higher initial concentrations, the plots of C_t versus the degradation time obey an exponential decay, and the negative index

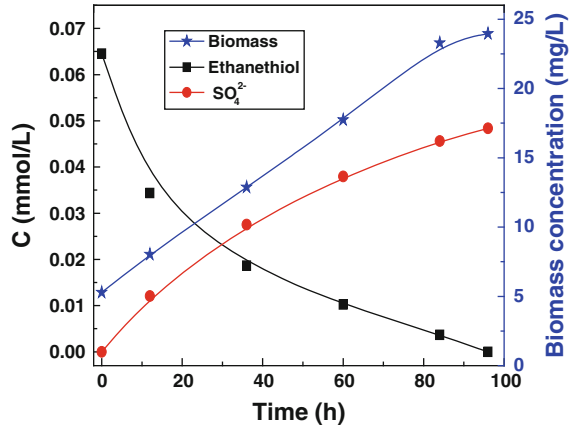

Fig. 4 The relationship between the consumption of ethanethiol, the production sulfate concentration and the concentration of strain RG-1 at the optimal conditions

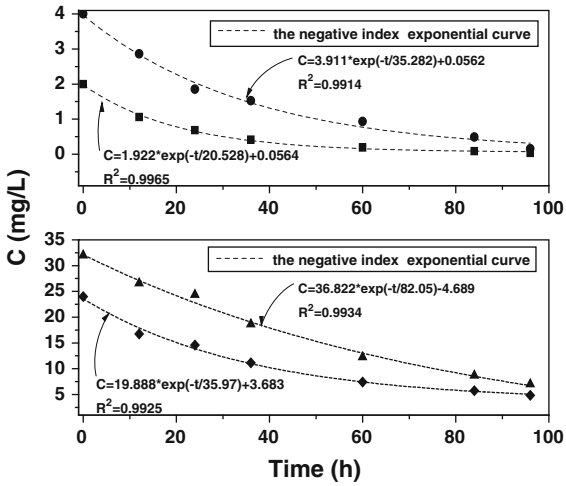

Fig. 5 Negative index of least-squares regression results of batch biodegradation experimental data at different initial concentration

exponential curve matched with the biodegradation curve very well. This indicates that the change of the ethanethiol concentration follows pseudo first-order kinetic model in the biodegradation process. The biological degradation rate constant and the half life were determined by the algorithm $\ln(C_0/C_t) = Kt$, where C_0 and C_t is the concentration of ethanethiol at $t = 0$ and t, respectively; K and t is the biodegradation rate constant (h^{-1}) and the degradation time (h), respectively.

The equation parameters listed in Table 2 were obtained using linear regression with the algorithm $\ln(C_0/C_t) = Kt$ at different initial concentration, pH values, and temperatures at different batch experiments. With increasing the initial concentration of ethanethiol from 2 to 32 mg l^{-1}, the degradation rate constant K gradually decreases from 0.0411 to 0.0156 h^{-1}, and the half-life increases from 16.9 to 44.4 h. Thus, higher initial ethanethiol concentration will inhibit the biodegradation of ethanethiol. Besides the initial concentration of ethanethiol, the pH value also affects the rate constant K and the half life. As seen in Table 2, with increasing the pH value from 3.0 to 8.0, the degradation rate constant K firstly increases from 0.0142 h^{-1} at pH = 3.0 to 0.0308 h^{-1} at pH = 7.0, and then rapidly decreased to 0.0147 h^{-1} at pH = 8.0. In contrast, the half-life exhibits the reverse changing tendency. The half-life decreases from 48.8 to 22.5 h with increasing the pH value from 3.0 to 7.0. By further increasing the pH value to 8.0, the half-life increases to 47.2 h. Therefore, the optimum pH value for degradation of ethanethiol over this newly isolated strain RG-1 is 7.0. In addition, the effect of temperature on the degradation rate reveals that the degradation rate constant K gradually increases from 0.0166 to 0.0309 h^{-1} as the temperature increases from 20 to 30°C, and then decreases remarkably to 0.0135 h^{-1} at 40°C. The half-life decreases from 41.8 to 22.4 h with increasing the temperature from 20 to 30°C, and then increases to 51.3 h with further increasing the temperature to 40°C. Thus, the temperature is an important parameter for the newly isolated strain RG-1, and the optimum growth temperature is 30°C.

Table 2 Degradation dynamic equation of different concentration of ethanethiol, pH value and temperature

	Kinetic equation	K	Half-life (h)	R^2
C_0 (mg l^{-1})				
2	$\ln C_t = -0.0411t + 0.6931$	0.0411	16.9	0.9929
4	$\ln C_t = -0.0289t + 1.3863$	0.0289	23.9	0.9722
8	$\ln C_t = -0.0242t + 2.0794$	0.0242	28.6	0.9916
16	$\ln C_t = -0.0188t + 2.7726$	0.0188	36.9	0.9886
24	$\ln C_t = -0.0177t + 3.1781$	0.0177	39.2	0.9919
32	$\ln C_t = -0.0156t + 3.4657$	0.0156	44.4	0.9978
pH value				
3	$\ln C_t = -0.0142t + 1.3863$	0.0142	48.8	0.9505
4	$\ln C_t = -0.0169t + 1.3863$	0.0169	41.0	0.9675
5	$\ln C_t = -0.0169t + 1.3863$	0.0169	41.0	0.9692
6	$\ln C_t = -0.0224t + 1.3863$	0.0224	30.9	0.9693
7	$\ln C_t = -0.0308t + 1.3863$	0.0308	22.5	0.9712
8	$\ln C_t = -0.0147t + 1.3863$	0.0147	47.2	0.9577
T (°C)				
20	$\ln C_t = -0.0166t + 1.3863$	0.0166	41.8	0.9516
25	$\ln C_t = -0.0176t + 1.3863$	0.0176	39.4	0.9615
30	$\ln C_t = -0.0309t + 1.3863$	0.0309	22.4	0.9828
35	$\ln C_t = -0.0216t + 1.3863$	0.0216	32.1	0.9552
40	$\ln C_t = -0.0135t + 1.3863$	0.0135	51.3	0.9500

C_t concentration of ethanethiol at different time; t biodegradation time; K_i: biodegradation rate constant

Conclusion

A new bacterium was isolated from the activated sludge in a domestic wastewater treatment plant. This

new bacterial strain RG-1 is Gram-negative, non-flagellated and short rod with diameter of 0.9–1.1 μm and length of 1.2–2.2 μm. It belongs to genus *L. sphaericus* according to its 16S rRNA gene sequence analysis and physiological characteristics. Ethanethiol can be efficiently degraded by strain RG-1 in wide ranges of initial concentration of ethanethiol, pH value, and the temperature. The S atom in the ethanethiol was ultimately converted to SO_4^{2-} ions. The biodegradation reaction kinetics of ethanethiol over the new bacterial strain RG-1 was found to follow the pseudo first-order kinetic model in which rate constant K and half time are two parameters determined by the reaction conditions. Under optimized conditions (4 mg l^{-1} ethanethiol, pH = 7.0 and 30°C), enthanethiol can be totally biodegraded within 96 h. The present work may lay the foundation for further treatment of ethanethiol bearing wastewater and ethanethiol laden gas.

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