Original Research

Induction Conditions and Kinetic Properties of Quinoline Biodegradation by a Newly Isolated Bacterial Strain

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Abstract

In order to strengthen the removal efficiency of heterocyclic nitrogenous compounds in industrial wastewater, a new bacterial strain identified as *Bacillus* sp. LH-1 was isolated, which could use quinoline as its sole carbon, nitrogen and energy source. The inducing conditions and kinetic properties of quinoline biodegradation by the strain were explored. The results showed that the strain *Bacillus* sp. LH-1 exhibited maximum tolerance of 700 mgL^{-1}quinoline concentration. After the strain was activated under the conditions of 200 mgL^{-1} quinoline for 120 h, the quinoline degradation was triggered, and the quinoline degradation rate increased approximately 10 times, from 2.030mg (L•h)$^{-1}$ to 21.508 mg (L•h)$^{-1}$. When the initial concentration of quinoline was 300 mgL^{-1}, more than 97% quinoline degradation occured within 12 h at pH 7 and at a temperature of 37ºC. The degradation kinetics of quinoline by strain *Bacillus* sp. LH-1 followed Haldane's model and indicated that the strain isolated in this study could be used for degradation of heterocyclic nitrogenous compounds in industrial wastewater.

Keywords: *Bacillus* sp. LH-1, biodegradation kinetics, induction condition, quinoline

Introduction

Heterocyclic nitrogenous compounds are of significant concern from an environmental perspective because of their toxic and carcinogenic properties, lethal effect on the natural biogenic environment, and severe odor. Given these threats, it is critical to prevent the discharge of these substances into the environment [1-3].

Quinoline and its derivatives are typical heterocyclic nitrogenous compounds, widely present in coal tar, oil shale and petroleum.

Quinoline is a popular industrial raw material and acts as a solvent for dyes, paints, fungicides and wood-treatment chemicals [4], and is also used as a substrate in the production of medicines such as fluoroquinolone antibiotics. A significant anthropogenic source of environmental pollution by quinoline and their derivatives is coal processing. The global manufacture of coal tar in cooking plants reaches a value 15×10^6 tons per year, whereas the content of quinoline is 0.2-0.3%.

Moreover, quinoline can be found in creosote, which is obtained during coal processing. Concentrations of quinolone in wastewater generated during shale oil production reach 2-50 mgL^{-1}. Concentrations of quinoline are about 10 mg/L in coking wastewater and 40-80 mg/L in semi-coking wastewater [5-6].

With a lone pair of electrons on the N-atoms in the ring system, quinolines and other N-heterocyclic compounds are more polar than homocyclic analogues. This makes these industrial wastes significantly more water soluble than other substances. Quinolines have been documented as toxic pollutants due to their carcinogenic and mutagenic properties [7].

Many treatment methods are used to degrade quinoline. Physical or chemical treatments are generally supplemental treatments because of their high operating cost and generation of secondary pollutants [8]. The main biological treatments include conventional activated sludge, or A^2/O and SBR processes [9-11]. However, the refraction and toxicity of quinoline and its derivatives in wastewater adversely affect the performance of biological treatment systems and result in failure to meet current effluent discharge standards.

In recent years, research has focused on bioaugmentation technology using special strains to enhance organic pollutant removal from wastewater [12-13]. Bacteria play an important role in the energy cycle and material cycle of the natural ecosystem. The practical application also shows that it is feasible to deal with recalcitrant substances by using microbial metabolism through biological enhancement.

Over the past decade, studies have found several bacteria with the excellent capability to degrade quinoline. These include *Arthrobacter* sp. [14], *Microbacterium* sp. [15]*, Burkholderia* sp. [16], *Rhodococcus* sp*., Desulfobacterium* sp. [17]*, Comamonas* sp. [18]*, Achromobacter* sp. [19] and *Pseudomonas* sp, which is the dominant genus among quinoline-degrading bacteria. Previous studies have comprehensively focused on its biodegradation pathway, relevant enzymes, and degradation genes [20-23]. Many bacteria can degrade quinoline, but their efficiencies are either low or have poor tolerance. Use of technical means to activate the strains improves its degradation ability for specific pollutants and provides a richer source of superior strains and more channels for the bioaugmentation of wastewater treatment systems.

The goal of this study was to identify new quinoline-degrading bacterial strains, explore their degradation properties, and assess the impact of acclimation conditions on the quinoline biodegradation rate. Degradation kinetics were also studied to describe the quinoline degradation process. The study identifies valuable sources of bacteria for the microbial degradation of quinoline and provides useful information about heterocyclic nitrogenous compounds and their relationship to degrading bacteria. These results will provide insights to support bioaugmentation in industrial wastewater treatment or the implementation of in situ bioremediation at contaminated sites.

Experimental

Reagents and the Culture Medium Used in this Study

Quinoline at purity levels greater than 99% was purchased from Aladdin. Methanol at purity levels greater than 99.9% was purchased from Fisher for high-performance liquid chromatography (HCLP) analysis. All other chemicals used were of analytical grade, purchased from local suppliers. Activated sludge samples were collected from the Xi'an Beishiqiao Wastewater Treatment Facility in Xi'an, China.

A Luria-Bertani (LB) medium was used to enrich the bacteria. A mineral salt medium (MSM) was used to isolate a quinoline-degrading strain containing the following ingredients (per liter): Na$_2$HPO$_4$ 4.26 g, KH$_2$PO$_4$ 2.65 g, MgSO$_4$ · 7H$_2$O 0.2 g, CaCl$_2$ 0.02 g, and 1 ml of a trace element solution. The trace element solution contained (per liter): MnSO$_4$ · 4H$_2$O 0.2 g, ZnCl$_2$ 0.01 g, H$_3$BO$_3$ 0.07 g, CuCl$_2$ · 2H$_2$O 0.005 g, Na$_2$MoO$_4$·2H$_2$O 0.04 g, FeSO$_4$ · 7H$_2$O 1.5 g, and CoCl$_2$ · 6H$_2$O 0.004 g [16].

The solid medium included 1.8% agar. Different concentrations of quinoline were filtered through a 0.22 um pore size filter and then added to the sterile MSM as the sole carbon, nitrogen, and energy source.

Domestication and Isolation of Superior Strains for Quinoline Degradation

Activated sludge samples were inoculated and cultivated for approximately 24 h in a rotary shaker at 37ºC with 180 rpm rotating speed. All microbes in the supernatant were enriched in the LB medium at 37ºC in a rotary shaker for 24 h. Afterwards, the culture was inoculated in the MSM to acclimate the quinoline-degrading microbes. The cultures were acclimated to increasing concentrations of quinoline through successive transfers. The initial concentration was 50mgL^{-1}, which was gradually increased by incremental amounts of 50 mgL^{-1} to the highest concentration of 500 mgL^{-1}. Next, quinoline-degrading bacteria colonies were purified using plate-streaking technology on an MSM plate containing 500 mgL^{-1} of quinoline.

Identifying Quinoline-Degrading Bacteria

The genomic DNA of LH-1 was extracted using the method described by Liu et al. [24]. 16s rDNA fragments of the screened dominant strains for quinoline degradation were amplified with the primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CGGYTACCTTGTTACGACTT-3'). PCR reaction,

BLAST program and the phylogenetic tree construction were performed according to Liu et al. [25].

Inducing Conditions of the Dominant Strain for Quinoline Biodegradation

Bacteria suspensions (OD$_{600}$ = 1.5±0.2) were added using the same dose as the MSM containing quinoline, at 100 mgL^{-1}, 200 mgL^{-1}, and 300 mgL^{-1}. Then, bacteria were collected at regular intervals to create new bacterial suspensions to degrade quinoline at a concentration of 300 mgL^{-1}. The effects of acclimation time and initial acclimation concentration on the microbial degradation rate were investigated.

The same process was repeated using the MSM with quinoline at 200 mgL^{-1}; in this test, glucose, acetic acid, methanol, pyridine, phenol, phthalic acid, citric acid, sucrose and lactose were added respectively at 100 mgL^{-1} concentrations. The effects of these additional carbon sources on microbial acclimation time and degradation rate were also investigated.

Tolerance and Influencing Factors of Quinoline Biodegradation

(1) Quinoline tolerance test. The quinoline-degrading bacteria LH-l were inoculated in a sterile LB medium containing 300 mgL^{-1} quinoline and cultivated overnight at 37ºC. After that, the cells were centrifuged and washed three times using an inorganic salt solution (pH = 7) and then suspended in the inorganic salt medium. Cell density was brought to OD$_{600}$ = 1.5±0.2. The cells were added based on the same dosing quantities as with the MSM culture medium, at quinoline concentrations of approximately 100 mgL^{-1}, 200 mgL^{-1}, 300 mgL^{-1}, 400 mgL^{-1}, 500 mgL^{-1}, 600 mgL^{-1}, 700 mgL^{-1}, and 800 mgL^{-1}. Making the initial OD$_{600}$ = 0.15±0.02, interval sampling was used to analyze the residual quinoline concentration and bacterial growth. The initial pH value of the medium was adjusted to 7.0 using HCl and NaOH before autoclaving. The growth media and all solutions were autoclaved for 25 min at 121ºC and 105 Pa. All cultivation was conducted at 37ºC in a rotary shaker at a speed of 180 rpm.

(2) Influencing factors of quinoline biodegradation. The microbial strain was inoculated in 50 mL of the LB medium and then grown at 37ºC in a rotary shaker at 180 rpm. After 24 h of incubation, the cell cultures were washed and transferred to fresh sterilization MSM medium containing a concentration of 200 mgL^{-1} quinoline for the acclimation stage. Cells were harvested during the late exponential growth phase, at the 120±15 h point. The cells were then washed and prepared into the bacteria suspension (OD$_{600}$ = 1.5±0.2) as inoculums.

Using quinoline as the sole carbon and energy source, quinoline biodegradation was studied at pH levels of 4, 5, 6, 7, 8, and 9, which are the pH of an inorganic salt solution without quinoline. The initial inoculant doses were 0.04, 0.067, 0.086, 0.117, and 0.165 gL^{-1}. The tested temperature levels were 25ºC, 30ºC, 37ºC, and 42ºC. During these experiments, samples were run at each level for each variable with the other variables fixed, and samples were periodically removed from the medium and analyzed for cell density and residual substrate concentrations.

The degradation rate of quinoline is expressed as:

$$K = \frac{S_0 - S_t}{T_t - T_0} \tag{1}$$

...where S_t and S_0 (mgL^{-1}) are the substrate concentrations of T_t time and the initial substrate concentrations of T_0 time, respectively.

Kinetics Analysis of Quinoline Gegradation

The LH-1 strain was cultured in LB liquid medium and acclimated to the late exponential growth phase with 200 mg L^{-1} quinoline. Using the method above, bacterial cells were harvested and inoculated into 100 ml MSM, containing approximately 100-700 mgL^{-1} quinoline. The beginning value of the OD$_{600}$ was approximately 0.15±0.02. Incubations were grown at 37ºC with shaking at 180 rpm. Cell density and residual quinoline concentrations were determined over a 45 h period.

The substrate inhibited cell growth. As such, Haldane's equation was selected for the kinetics elements of the study [26-27].

Analysis Methods of Quinoline and Cell Density

Samples were collected from the culture at different time points to measure the optical density of cells and the quinoline concentrations, which were determined using HPLC (Thermo, U3000) equipped with a C18 column (Thermo, 100 × 2.1 mm inner diameter, 1.7 m particle size). Samples were centrifuged at 8000 rpm for 10 min. The supernatant for each sample was collected and filtered through a 0.22 μm pore size filter for analysis. The mobilephase, consisting of a methanol and water mixture (65:35,v/v), was introduced to the column at a flow rate of 0.2 mLmin^{-1}. Quinoline was detected at a 275 nm wavelength; its retention time was 3.65 min. The pH was measured using a pH meter (PHS-3C, Lei Ci).

The cell density of the microbial culture was estimated using a UV spectrophotometer (TU-1901, Persee) by measuring its absorbance (OD) at a wavelength of 600 nm. All tests were done in triplicate.

GC/MS Analysis of Metabolites

Possible metabolites were analyzed with GC/MS (Agilent 7890 GC/7000A, DB-5MS narrow bore column,

30 m × 0.25 mm × 0.25 μm). Reaction products were extracted with ethyl acetate and dried over anhydrous Na₂SO₄. Helium was used as the carrier gas with a flow rate of 1 ml.min⁻¹, injector volume of 1 μL and injector temp of 280ºC. The oven temperature was programmed at 40ºC for 2 min, followed by a linear increase of 5ºC/min to 200ºC, holding at 200ºC for 5 min, followed by a linear increase of 7ºC/min to 280ºC and holding at 280ºC for 5 min. MS analysis was performed at electron energy of 70 eV and ion source temp of 230ºC. The structures of metabolites were confirmed from the fragmentation patterns of the mass spectra, through comparison with those predicted for known compounds.

Results and Discussion

Screening and Identification of the Dominant Strain for Quinoline Degradation

Three bacterial colonies capable of degrading quinoline were isolated from the activated sludge of a municipal wastewater treatment plant using the streak plate method. The 16S rRNA gene fragments of strains with the ability to degrade quinoline were named LH-1, LH-B and LH-X and were deposited in the GenBank data library with the accession numbers KX442614, MF062572 and MF062570, respectively. Among them, strain LH-1 had the best degradation effect and stability when quinoline was provided as the sole source of carbon, nitrogen, and energy, so the follow-up study is aimed at LH-1.

Strain LH-1 formed smooth, convex, opaque, and wet colonies. The 2-3 mm diameter circular colonies were milky white and slightly yellow and could be easily scraped off nutrient agar plates after being incubated at 37ºC for 1-2 days. Each individual bacterium grew aerobically and was gram-positive, short, and rod-shaped. 16srRNA gene fragments of strain LH-1 were obtained by PCR amplification, and then submitted for sequencing. The BLAST search of the sequences indicated that strain LH-1 was closely related to the species in genus of *Bacillus*, and also exhibited the highest similarity (99.93%) to *Bacillus* sp. and thus we tentatively classified strain LH-1 as *Bacillus* sp. LH-1 (KX442614; Fig. 1). Some researchers have found that *Bacillus* sp. can degrade phenol, benzo[a]pyrene [28], pyridine [29], Polyethylene [30] and waxy crude oil [31]. This suggests that *Bacillus* sp. could have a great potential application in the biodegradation of organic contaminants and remediation of water and soil.

Tolerance Test of the Quinoline Concentrations for the Dominant Strain

The effect of the strain *Bacillus* sp. LH-1 in degrading quinoline was studied over an initial 100-800mgL⁻¹ quinoline concentration (Fig. 2a). Results showed that the strains could grow at quinoline concentrations ranging from 100-700 mgL⁻¹. The quinoline removal rate reached 93.43%, 95.05%, 98.59%, 96.72%, 98.19%, 98.33% and 98.19% at concentrations of 100 mgL⁻¹, 200 mgL⁻¹, 300 mgL⁻¹, 400 mgL⁻¹, 500 mgL⁻¹, 600 mgL⁻¹, and 700 mgL⁻¹, respectively. However, even after 420 h, the strain *Bacillus* sp. LH-1 could not degrade quinoline at a concentration of 800mgL⁻¹, manifesting a strong substrate inhibition.

However, there was a long lag time before the cells began to accommodate the high quinoline concentration and start to degrade it. The lag time increased and the strain *Bacillus* sp. LH-1 cell biomass became antiblastic as the initial quinoline concentration increased. At a 100 mgL⁻¹ quinoline concentration, the lag time was approximately 90 h; at a 700 mgL⁻¹ quinoline concentration, the lag time increased to 350 h. After the inhibitory stage, degradation began at 20-35 h. Both cell growth and quinoline degradation occurred in this time frame. In the first 50 h, cell concentrations significantly
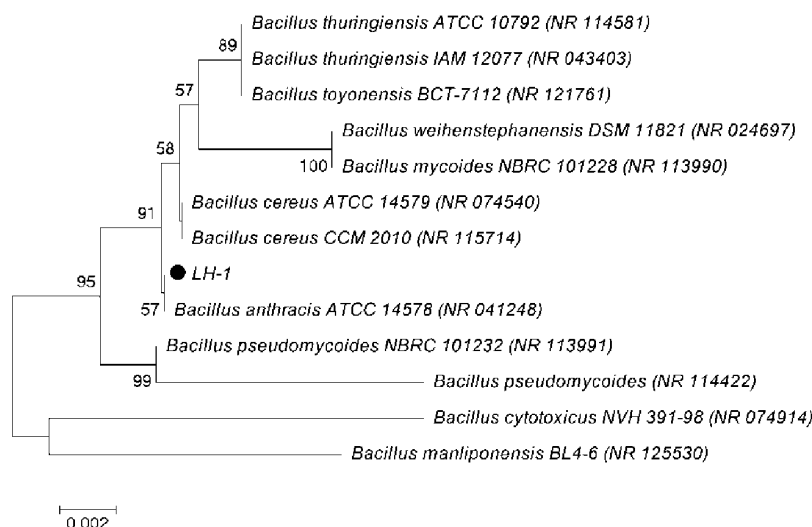

Fig. 1. Phylogenetic tree based on 16S rDNA sequences of strains named LH-1 and its related species.
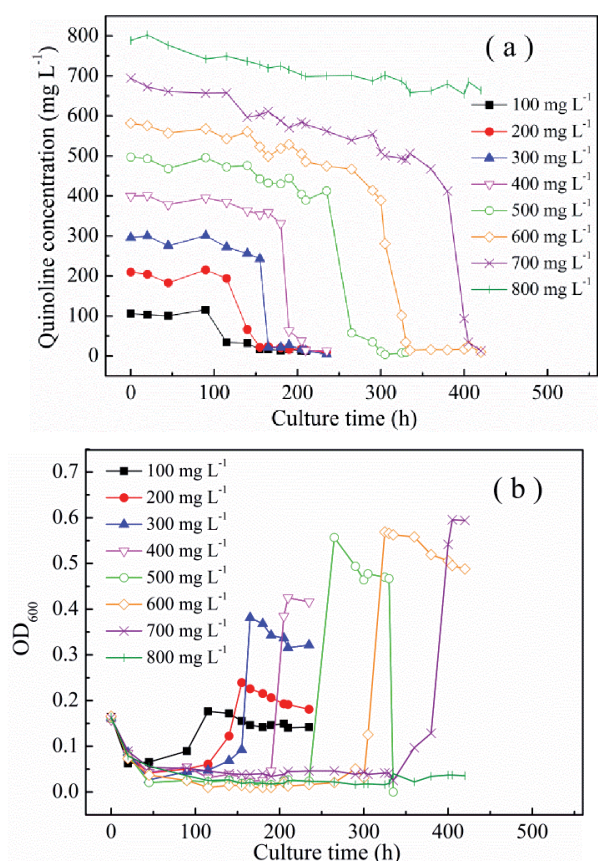
Fig. 2. Influence of initial quinoline concentration on a) quinoline degradation and b) biomass variation of the strain *Bacillus* sp. LH-1 at pH 7 and 37ºC.
A: quinolone B: uinoline+glucose C: quinoline+acetic acid
D: quinoline+methanol E: quinoline+pyridine
F: quinoline+phenol G: quinoline+phthalic acid
H: quinoline+citric acid I: quinoline+sucrose
J: quinoline+lactose

Fig. 3. Inducing conditions of quinoline degradation for strain *Bacillus* sp. LH-1 at pH 7 and 37ºC: a) activation of the acclimation time and the quinoline concentration on strain *Bacillus* sp. LH-1 for the quinoline biodegradation and b) activation of different carbon sources on strain *Bacillus* sp. LH-1 for quinoline biodegradation; error bars represent standard deviation of triplicate tests.

declined due to the toxic effects of quinoline (Fig. 2b). However, after the inhibition period, the bacteria appear to have adapted to the quinoline toxicity, and then started to use it as a nutrient.

In the 150-300 mgL^{-1} range, quinoline can be used by most quinoline-degrading bacterial strains. Only selected bacterial strains, even with immobilized cells, can degrade more than 300 mgL^{-1} of quinoline [22]. For example, in one study, *Pseudomonas* sp. strain BW003 degraded 192-911 mgL^{-1} of quinoline within 3-8 h [21]. In this study, *Bacillus* sp. strain LH-1 grew at concentrations of 100-700 mgL^{-1} quinoline, with a degradation rate exceeding 95%. However, LB enrichment caused long-term inhibition of the strains. As such, this study examined how acclimation conditions quantitatively affect quinoline degradation.

Inducing Conditions of Quinoline Degradation for Strain *Bacillus* sp. LH-1

To improve quinoline degradation efficiency, lower concentrations were used to acclimate strains.
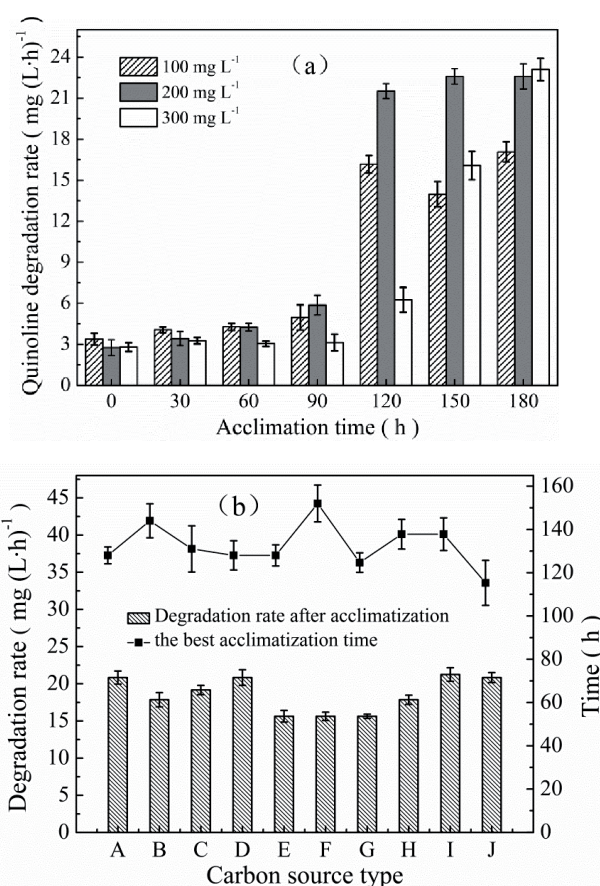
Samples collected at different points in the acclimation process were analyzed to quantitatively determine how acclimation conditions impact the ability of strain *Bacillus* sp.LH-1 to degrade quinoline.

The initial quinoline acclimation concentration and acclimation time significantly affected the microbial degradation rate (Fig. 3a). Strains were acclimated for 120 h at initial quinoline concentrations of 100 mgL^{-1} and 200 mgL^{-1}, the largest degradation rates were 16.167 mg(L·h)$^{-1}$ and 21.508 mg(L·h)$^{-1}$. It took 180 h to achieve a maximum degradation rate of 23.095 mg(L·h)$^{-1}$ at an acclimation concentration of 300 mg L^{-1}.

An acclimation concentration of 200 mgL^{-1} positively affected quinoline degradation. The bacteria synthesized an inducible enzyme in the process of degrading quinoline. Acclimation may allow bacteria to adapt to the environment in advance, and may promote the generation of this inducible enzyme. Hence, acclimation in small concentrations of quinoline may improve the bacteria's ability to degrade high concentrations of quinoline. The quinoline degradation rate of the strain *Bacillus* sp. LH-1 increased as acclimation time

increased. The average degradation rate of the strain *Bacillus* sp.LH-1 strain was only 2.030 mg(L·h)$^{-1}$ without an acclimation period. The rate increased significantly, reaching 21.508 mg(L·h)$^{-1}$ after a 120 h acclimation period (200 mgL^{-1}). This rate was 10.6 times more than before acclimation in a system containing 300 mgL^{-1} quinoline.

This experiment also investigated how introducing other organic sources affected the quinoline degradation rate when acclimated at a quinoline concentration of 200 mgL^{-1} (Fig. 3b). Adding a carbon source to support quinoline acclimation played no significant role, as the best acclimation time remained more than 120 h.

Previous studies have shown that bacteria require long-term domestication to degrade organic matter. This is particularly true when degrading refractory organic matter [5, 32]. The same applies to degrading quinoline; without domestication, microbial metabolism efficiency is low and unstable for specific pollutants. Many scholars have cultivated bacteria in an inorganic salt solution to the logarithmic phase containing target pollutants, which was also the initial bacterial acclimation process [19, 27]. However, few researchers have described the acclimation characteristics in detail, resulting in only a vague understanding of the quantitative characteristics of acclimation conditions during quinoline biodegradation.

The best acclimation period for the strain *Bacillus* sp.LH-1 was 120±15 h. This outcome occurs during the logarithmic phase of the MSM system containing 200 mgL^{-1} quinoline, regardless of whether there is a supplementary carbon source. The appropriate acclimation concentration and timeframe benefitted acclimation efficiency; the result was a two-fold effect with only half the effort.

The follow-up dynamic experiment will assess the acclimation of 200 mgL^{-1} quinoline in an inorganic salt medium; bacteria are cleaned and prepared in a suspension that is part of the corresponding reaction system. Research was also done to assess the influence of temperature and pH on quinoline degradation, and quinoline degradation kinetics after acclimation.

Influencing Factors of Quinoline Biodegradation

Fig. 4a) shows how initial inoculant dosage affects quinoline degradation by the strain *Bacillus* sp. LH-1 at 37ºC with a quinoline concentration of 300 mgL^{-1} at pH 7. The degradation process sped up slightly at an increased inoculant dosage, but results were generally similar when the initial inoculant dosages were 0.086 gL^{-1}, 0.117 gL^{-1}, and 0.165 gL^{-1}. At the same time, Fig. 4b) shows that bacteria biomass had an inhibitive effect due to quinoline toxicity in the early phase. Biomass experienced some stagnation and slow growth, but there does not appear to have been significant biomass reduction, which shows that after acclimation, bacteria adapts better to the quinoline environment

and the inhibition period is greatly reduced. When the added bacteria levels were greater than 0.086 gL^{-1}, the bacterial competition for the substrate slightly changed the overall degradation efficiency, but the final biomass was maintained at a higher level. Considering both bacteria growth and quinoline degradation, 0.086 g L^{-1} was the ideal amount of bacteria when 10% (v/v) of the cell suspension was inoculated to the media and the initial OD$_{600}$ was approximately 0.15.

Experiments to assess optimal pH levels for quinoline degradation were performed at 37ºC with a quinoline concentration of 300 mgL^{-1} (Fig. 4c). The best initial pH range was 5-7, indicating that *Bacillus* sp. LH-1 strain cell growth and quinoline degradation were higher in slightly acid media. Quinoline degradation by *Bacillus* sp. LH-1 and biomass growth were inhibited in an alkaline environment at pH values of 8 and 9; the lag period was approximately 30 hours (Fig. 4d), which may be because an environment that is too alkaline can damage the cells' surface potential and enzyme activity, suppress quinoline degradation, and even crack cells and destroy cellular structures. A strongly acidic environment at a pH of 4 is also unfavorable to bacterial growth.

pH of solution may also affect the presence of the substrate quinoline, which exists as a positively charged protonated form under acidic conditions and in the form of neutral molecules under alkaline conditions. Normally, neutral molecules are more susceptible to bacterial attack and degradation, but because the addition of quinoline will increase the pH of the MSM, at the same time quinoline degradation will release ammonia nitrogen in the process, so the pH increases during the whole degradation process.

Therefore, a meta-acid environment rapidly becomes more neutral, resulting in a neutral culture as the reaction progresses. The final result shows that MSM has the highest efficiency of quinoline degradation under conditions of neutral partial acidity. This encourages bacteria growth and increased enzyme activities. There can be enzyme disintegration or substrate dissociation at the active center of the group if the system contains too much acid and alkali, which affects enzyme and substrate combinations, enzyme denatured inactivation and reduced substrate use.

Fig. 4e) shows the effect of temperature on quinoline biodegradation after the strain *Bacillus* sp. LH-1 acclimation. At a temperature range of 20-42ºC, quinoline degraded well in aerobic conditions. Quinoline degradation rate was highest at 37ºC, decreasing in descending order at 30ºC, 42ºC, and 25ºC. Biomass growth adapted to quinoline degradation (Fig. 4f). The results indicate that strain *Bacillus* sp.LH-1 is a mesophilic type of bacteria, with an optimal temperature of 30-37ºC. This result is consistent with other research, which concluded that mesophilic bacteria had advantages in degrading quinolone [4, 7].

In this study, the best pH and temperature for cell growth and quinoline degradation were close to
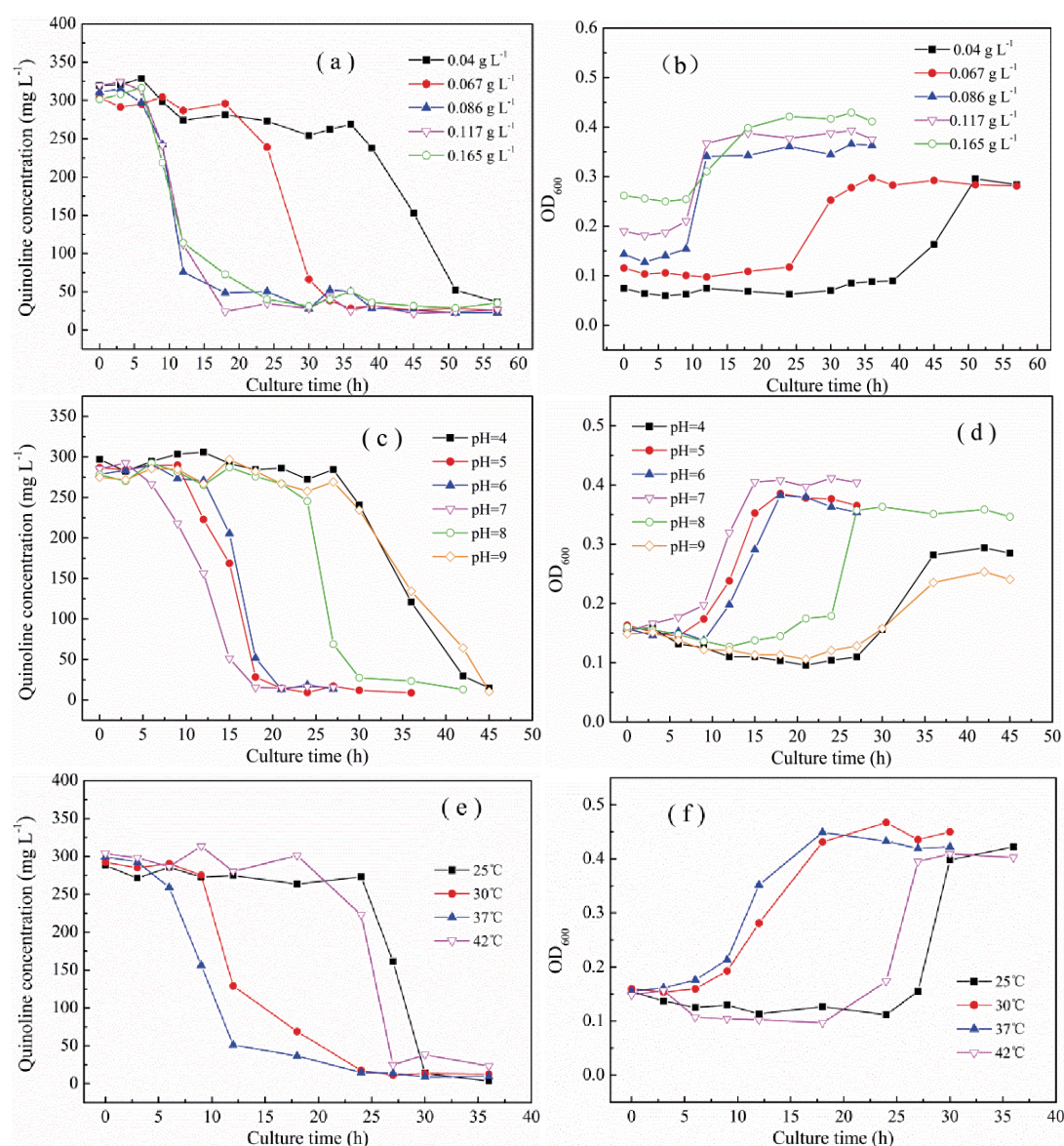
Fig. 4. Influence of different factors on quinoline degradation and biomass variation of the strain *Bacillus* sp. LH-1: a) influence of the inoculation dosage on quinoline degradation; b) influence of the inoculation dosage on the biomass of *Bacillus* sp. LH-1; c) influence of pH on quinoline degradation; d) influence of pH on biomass of *Bacillus* sp. LH-1; e) influence of temperature on quinoline degradation; and f) influence of temperature on biomass of *Bacillus* sp. LH-1.
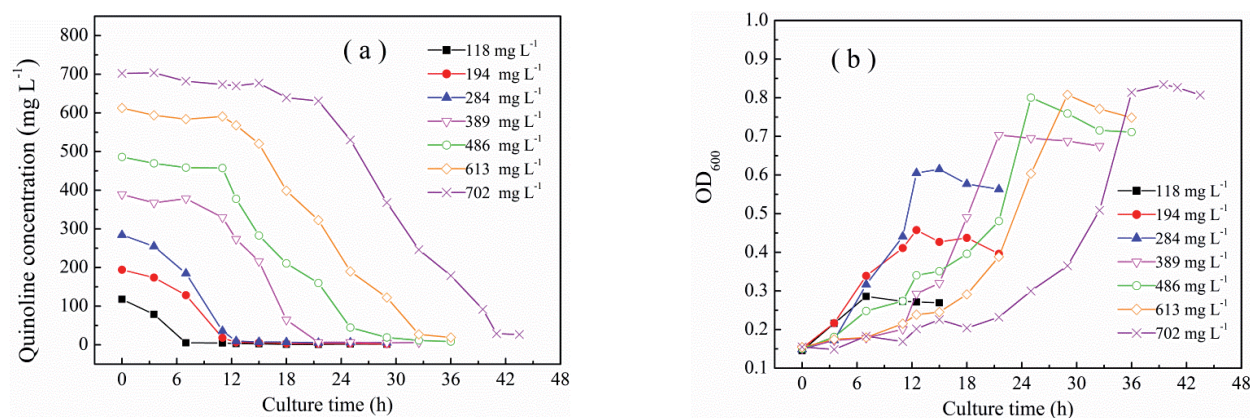

Fig. 5. Quinoline degradation rate a) and biomass variation of the strain *Bacillus* sp. LH-1 b) under the conditions of different initial quinoline concentrations after the strain was activated.
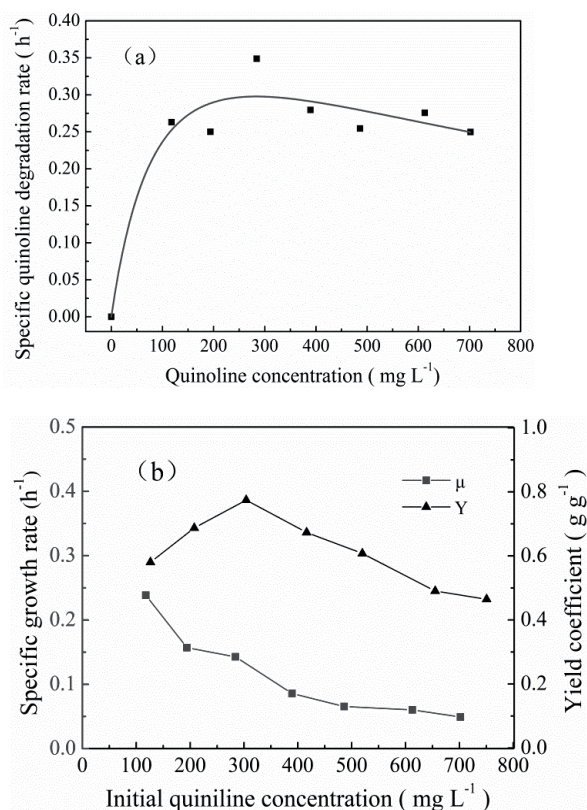
Fig. 6. Specific growth rate, yield coefficient and specific growth kinetics of the strain *Bacillus* sp. LH-1 under the conditions of different initial quinoline concentrations: a) experimental and predicted specific growth kinetics of the strain and b) specific growth rate and yield coefficient of the strain.

a solution with a pH of 7 and at 37ºC. In these conditions, more than 96.55% of quinoline at an initial inoculant pure strain dose of 0.087 gL^{-1} was degraded within 12 h.

Kinetic Characteristics of Quinoline Biodegradation by *Bacillus* sp. LH-1

Quinoline degradation dynamics were further investigated after LH-1 acclimation. More than 95% of quinoline was removed at concentrations of 100-300 mg/L within 12 h of inoculation without a lag phase (Fig. 5). In contrast, there was a lag phase when the quinoline concentration increased from 400 to 700 mgL^{-1}. A high initial quinoline concentration resulted in the longest degradation time; however, the quinoline removal rate was not related to the initial concentration, and remained between 95.62-98.29%.

Haldane's mathematical model was used to generate kinetic parameters of q_{max} = 0.529 h^{-1}, K_S = 10.058 mgL^{-1}, and K_i = 727.733 mgL^{-1} (correlation coefficient R^2 = 0.9015). Fig. 6a) shows the contrast between the theoretical and experimental values. The regression curve fit the experimental data very well. Based on the differential equation, the *Bacillus* sp. LH-1 strain degrades the quinoline best at a concentration of 283.007 mgL^{-1}. At lower quinoline concentrations, the quinoline degradation rate is lower due to the lack of good carbon sources of energy and limited cell growth. When the quinoline concentration exceeds 283.007 mgL^{-1}, the high quinoline concentration inhibits assimilation and substrate degradation.

As the initial substrate concentration increased, the specific growth rate decreased; this may be the result of intense substrate inhibition (Fig. 6b). The maximum specific growth rate was 0.2384 h^{-1}, which occurred at a very low substrate concentration. The higher substrate

real-world conditions. This suggests that quinoline pollution can be eliminated by applying degrading bacteria as part of bioaugmentation treatments. The strain *Bacillus* sp. LH-1 growth was optimized in
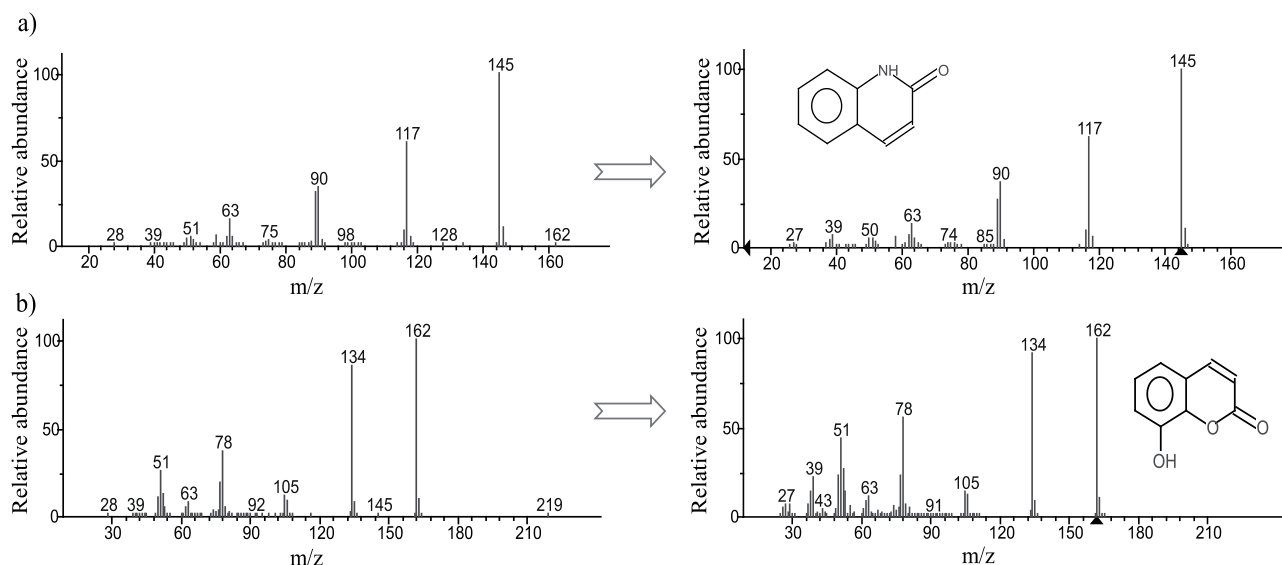

Fig. 7. Mass spectra of quinolone metabolites by *Bacillus* sp. LH-1. a) and b): comparisons of mass spectrum data of 2-hydroxyquinoline and 8-hydrocumarinwith authentic standards.

concentration led to a stronger inhibitory response. Bacteria yields in the rapid biodegradation phase first increased and then decreased as the initial quinoline concentration increased. The maximum yield was 0.774 when the quinoline concentration was 300 mgL⁻¹. This result was consistent with Haldane equation's estimate of the best quinoline concentration. At a larger Y value, more biomass is obtained from the degradation unit mass matrix, with highly efficient bacteria.

In this study, quinoline degradation by *Bacillus* sp. created a pink compound. This is consistent with other studies that have tested quinoline biodegradation using different microbes, and indicates that quinoline biodegradation by different bacteria may produce colorful intermediates [4]. 2-hydroxyquinoline and 8-hydrocumarinwere were detected by GC/MS during the quinoline biodegradation (Fig. 7). Preliminary speculation is that the *Bacillus* sp. biodegradable quinoline via the 8-hydroxycoumarin pathway. Quinoline biodegradation intermediates and metabolic pathways using the strain *Bacillus* sp. LH-1 will be further analyzed both qualitatively and quantitatively in the future. These results broaden the ways in which *Bacillus* sp. may be used in the field of environmental protection research, especially with respect to biodegrading heterocyclic nitrogenous compounds.

Conclusions

A new bacterial strain identified as *Bacillus* sp. LH-1 was isolated and which can use quinoline as its sole carbon, nitrogen, and energy source. The strain grew well in quinoline concentrations of 100-700 mgL⁻¹. When the strain was triggered under the conditions of 200 mgL⁻¹ quinoline concentration and kept for 120 h, the degradation rate of quinoline significantly increased from 2.030 mg (L·h)⁻¹ to 21.508 mg (L·h)⁻¹, and the other organic carbon sources did not appear to promote or inhibit quinoline degradation efficiency. After the strain was activated, more than 96.55% of the quinoline (initial quinoline concentration was 300 mgL⁻¹) were degraded within 12 h, and the biodegradation kinetics were consistent with Haldane's model (the kinetic parameters were: q_{max} = 0.529 h⁻¹, K_S = 110.058 mgL⁻¹, K_i = 727.733 mgL⁻¹ and R^2 = 0.9015). *Bacillus* sp. can be used in the degradation process of refractory organic compounds, and the development of the bacillus bacterial agent could provide an effective source and technical support for biological enhancement of wastewater treatment.

Acknowledgements

Conflict of Interest

The authors declare no conflict of interest.

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