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Isolation and Identification of Ammonia Nitrogen Degradation Strains from Industrial Wastewater

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ABSTRACT

Nine strains of ammonia nitrogen degradation strains from C_1 to C_9 were isolated from industrial wastewater to study their degradation and conversion of ammonia nitrogen. The results showed that C_2 strain with a high degradation activity of ammonia nitrogen, and the ammonia nitrogen degradation rate of the activated C_2 strain was 93% within 24 h when the initial concentration of ammonia nitrogen was 200 mg/L under the conditions of inoculation 10%, temperature 35˚C, pH 7.0, rotation 200 r/min. And C_2 was identified as *Bacillus amyloliquefaciens*.

Keywords: Industrial Wastewater; Ammonia Nitrogen; Degradation Strain; Degradation Characteristics

1. Introduction

With the rapid development and growth of Chinese economy, fertilizer, petrochemical and other industries, wastewater from chemical products production process which includes many pollutants such as phenolic compound, quinoline, ammonia nitrogen will cause certain damage to the environment and it will do more harm to human health. As we know, ammonia nitrogen discharged into water bodies will result in water quality decline or water eutrophication and will pollute the environment health, thus poses a threat to the ecological balance [1]. Biological nitrogen removal method is acknowledged to be a more economical, efficient method. When applied in treat wastewater, it is the most promising wastewater treatment method [2]. Ammonia nitrogen pollution in coke wastewater is becoming a major problem [3]. It is caused by cooled coal waste gas and ammonia produced by high temperature after dry distillation, or is caused in the process of wastewater treatment during which microbes react with inorganic nitrogen or organic nitrogen by means of biochemical reaction and chemical reaction. In this paper, we isolated and identified an efficient degradation of ammonia nitrogen capacity strains from industrial wastewater and conducted preliminary physiological and biochemical identification and provide alternative bacteria for the development of many types of highly efficient microbial agents.

2. Materials and Methods

2.1. Materials

1) The source of sample

Sediments of Miyun Longtan sewage treatment plant (saved in the refrigerator at 4˚C).

2) The main culture medium

a) The enriched medium: deionized water 500 mL, $C_6H_{12}O_6$ 2.5 g, $(NH_4)_2SO_4$ 1.0 g, K_2HPO_4-$3H_2O$ 0.5 g, NaCl 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, $FeSO_4 \cdot 7H_2O$ 0.2 g, pH 7.2 - 7.4, 121˚C stream sterilization 20 mins.

b) The isolation medium: add 20% agar to the enriched medium, 121˚C stream sterilization 20 mins.

c) The screening culture medium: $(NH_4)_2SO_4$ was added gradually, other components with the same as the isolation medium.

d) The activation medium: deionized water 500 mL, beef extract 1.5 g, peptone 5 g, NaCl 2.5 g, pH 7.2 - 7.4, 121˚C stream sterilization 20 mins.

2.2. Methods

1) Culture conditions

The sampling water was added at a ratio of 10% to enrichment culture medium containing 100 m/L phenol and cultured at 30˚C on a rotary shaker at 150 rpm·1 mL 5% $(NH_4)_2SO_4$ solution was added in the enrichment culture medium to acclimation training same time each day, and continuing about 7 days. The enriched microorganisms were inoculated into new culture medium with a 5% inoculation quantity, and then repeat the above operation, continuing 7 days. The bacteria were cultured for two weeks with NH_4^+ as the only nitrogen resource. To obtain pure cultures, the strains were separated with a dilution-plate method.

2) Identification

a) Morphology [4,5]

Some morphological characteristics of colonies was identified by the naked eye, such as shape, size, surface, edge, uplifted shape, transparency, wetness, roughness, colonies and medium color change, *et al*.

b) Physio-biochemical characteristics [6]

The physiological and biochemical reactions of sampled bacteria were tested, including Starch hydrolysis, (M.R) M.R test, Glucose fermentation, Sucrose fermentation, Indol test, (V.P) V.P test, Gelatin test, Citrate test, *et al*.

c) Molecular identification

The strain of the genome sequence is analyzed by 16SrDNA identification methods. Gene sequences were compared with those in the GenBank database using batch BLAST.

3) Ammonia nitrogen degradation experiment [7]

The startup of the experiments was obtained by inoculating 50 mL mineral cultures with 5 mL bacterial suspension which OD is about 1.50, this cell suspension is available by inoculating bacteria for 1 day in liquid medium and then washed by distilled water. The influence factors of ammonia nitrogen concentration, inoculum volume, pH value, temperature, shaking revolution and adding carbon on phenol degradation were investigated. The ammonia nitrogen concentration increased from 50 to 600 mg/L, the inoculum volumes were 3%, 5%, 10%, 20%, the initial pH values were 5, 6, 7, 8, 9 and 10, the temperature were 15˚C, 20˚C, 25˚C, 30˚C, 40˚C and 45˚C, the shaking revolution are 50, 100, 150, 200, 250 r/min, the adding carbon are 0, 0.5, 1, 1.5, 2.0 and 2.5 g/L glucose. During the period of batch culture, all samples were periodically taken for the concentration of ammonia nitrogen. All experiments were carried out in duplicate.

4) Analytical methods

In this article, the method of Nessler's reagent photometry was used to monitor the detection of ammonia nitrogen determination, and turbidimetry was used to determine the growth of the bacteria.

3. Results and Analysis

3.1. Identification of Fungi

1) Colony observation (**Table 1**)

2) Identification with PCR amplification

The strain was sampled from the wastewater treatment system of the Sediments of Miyun Longtan in Beijing and isolated under the laboratory condition. It was identified based on Gram staining, biochemical tests, and 16S rRNA sequence determination. The results showed that the strain C_2 was identified as *Bacillus amyloliquefaciens.* **Figure 1** showed the phylogenetic tree of the degrading bacteria C_2.

Table 1. Physio-biochemical characteristics of the degrading bacteria physio-biochemical characteristics C_2.

The characteristic of physiology and biochemistry	C_2
Starch hydrolysis	–
(M.R) M.R test	–
Glucose fermentation	+
Sucrose fermentation	+
Indol test	–
(V.P) V.P test	–
Gelatin test	+
Citrate test	–

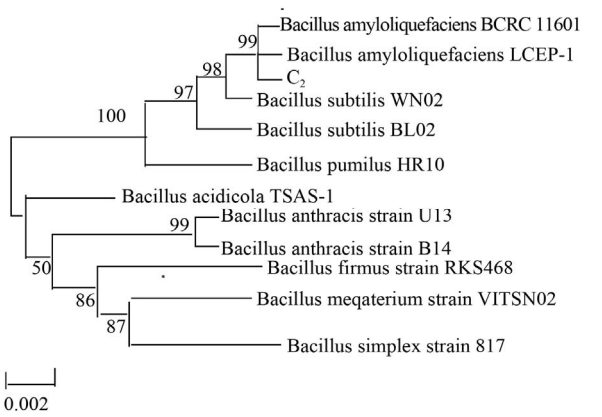

Figure 1. Phylogenetic tree of the degrading bacteria.

3.2. Ammonia Nitrogen Degradation

1) The effect of temperature

The temperature is an important factor of microbial survival, it influences the growth of microorganism and the absorption and utilization of growth substances [7] mainly through changing the activity of the enzyme. **Figure 2** showed the effect of temperature on the ammonia nitrogen degradation using C_2. The temperature ranges from 15˚C to 45˚C. It can be seen that the optimal values of temperature was observed as 35˚C, and decreased gradually thereafter.

2) The effect of pH values

The initial pH values of the culture media affect microbial development. Having pH values that are either too high or too low are not conducive to cell growth [8,9]. **Figure 3** showed the ammonia nitrogen degradation at the different pH values, ranging from 5 to 10. When the pH value ranged from 5 to 7, the degradation rate kept increasing. The degradation rate reached a maximum of 90% at pH 7. After that, the degradation rate then decreased. This indicated that partial alkaline environment was conducive to the degradation of ammonia nitrogen by C_2.

3) The effect of ammonia nitrogen concentration **Figure 4** showed ammonia nitrogen degradation at the ammonia nitrogen of 50 - 600 mg/L with 10% starting inoculums. 50, 100, and 200 mg/L ammonia nitrogen could

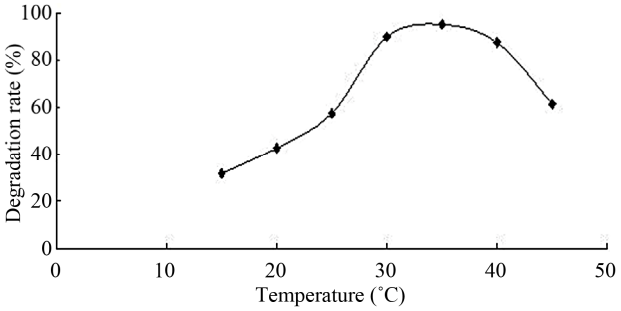

Figure 2. The effects of temperature on ammonia nitrogen degradation by C_2.

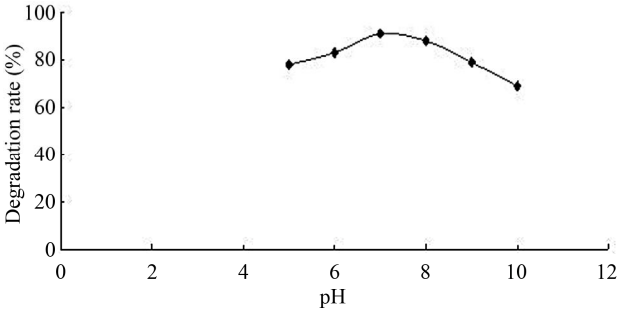

Figure 3. The effects of pH on ammonia nitrogen degradation by C_2.

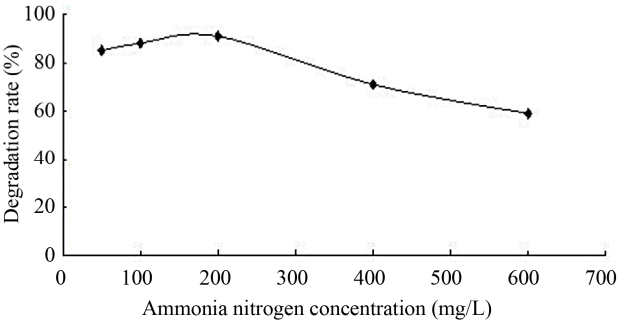

Figure 4. The ammonia nitrogen concentration degradation by C_2 in the mineral medium containing initial ammonia nitrogen concentration from 50 to 600 mg/L with 10% starting inoculums.

be degraded above 80% by C_2 within 48 h respectively. While the ammonia nitrogen degradation rates of the sample of 400, and 600 mg/L, were decreased. It exhibited a remarkable augment of substrate inhibition, which could be also demonstrated by the longer lag phase of cell growth. The production and accumulation of various intermediates may be responsible for the decreased cell mass yield [10].

4) The effect of inoculums volume

Figure 5 indicated the effect of inoculums volume on ammonia nitrogen degradation after 24 h. The cells inoculated with 10% starting inoculums manifested the high ammonia nitrogen-degrading velocity. The reasons were accounted as following: the increase of the quantity shortens the lag phase of the bacteria, which can quicken degradation of ammonia nitrogen.

5) The effect of carbon addition

The microbe with carbon source acts as growth nutrients, so different carbon sources have a different but great effect on the efficiency for the degradation of strains [11-13]. **Figure 6** showed the effect of adding carbon concentration on the ammonia nitrogen degradation. The C_2 degradation rate decreased when the sucrose concentration increased, because the overloaded glucose inhibited the ammonia nitrogen degradation. This indicated that ammonia nitrogen consumed in medium was utilized to synthesize new cells.

6) The effect of shaking revolution

Shaking revolution is a reflection of the bacterial ability to get oxygen in the process of the growth, bacteria will get more oxygen with a high speed, and get lower oxygen with a low speed [14,15]. **Figure 7** indicated the effect of shaking revolution on ammonia nitrogen degradation after 48 h. It can be seen that the optimal values of speed were observed as 200 r/min, and decreased gradually thereafter.

4. Conclusions

4.1. An Efficient Ammonia Nitrogen Degradation Strain Was Isolated

Through the wastewater sludge concentration in training,

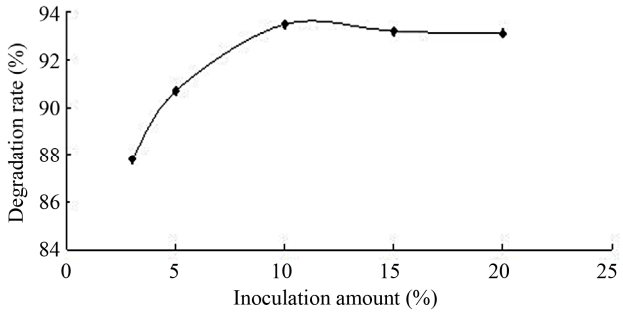

Figure 5. The effect of inoculums volume on ammonia nitrogen degradation.

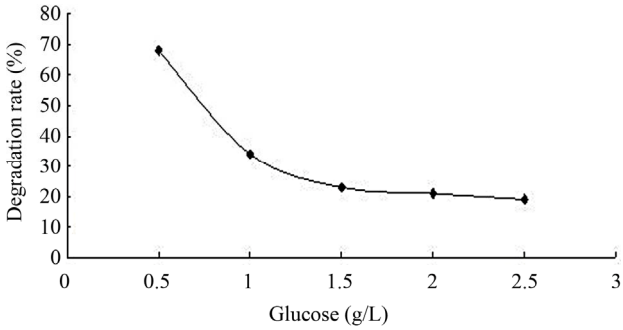

Figure 6. The effects of sucrose addition on ammonia nitrogen degradation by C_2.

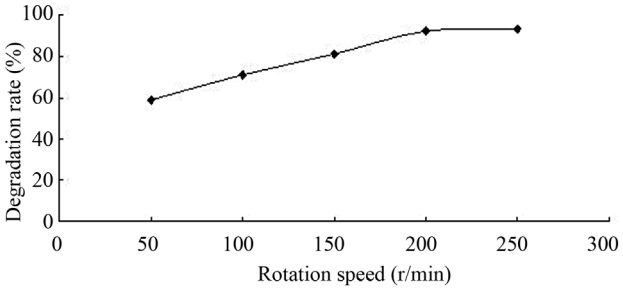

Figure 7. The effect of shaking revolution on ammonia nitrogen degradation by C_2.

an efficient ammonia nitrogen degradation strain C_2 was isolated, and was identified as *Bacillus amyloliquefaciens.*

4.2. Efficient Degradation Characteristics of Strains on Degrading the Ammonia Nitrogen Wastewater

Through the simulation of ammonia nitrogen wastewater, we set up different environment conditions, include the time, inoculums volume, temperature, pH value, ammonia nitrogen concentration, carbon addition and shaking revolution. The results showed that the C_2 strains have an excellent degradation ability in the ammonia initial concentration of 200 mg/L, pH 7.5, temperature 35˚C, 10% inoculum, shaking revolution 200 r/min, and the degradation rate was 93% after cultured for 48 h.

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