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MICROBIOLOGY

Isolation of 2 simazine-degrading bacteria and development of a microbial agent for bioremediation of simazine pollution

JIANGWEI ZHU, YAN ZHAO, XIAOLOU LI, LEI WU, LI FU, NING YANG, JUN YIN & RONG HUANG

Abstract: Simazine was one of the most commonly used herbicides and was widely used to control broadleaf weeds in agriculture and forestry. Its widespread use had caused wide public concern for its high ecological toxicity. In order to remove simazine residues, 2 strains capable of effectively degrading simazine were isolated from the soil and named SIMA-N5 and SIMA-N9. SIMA-N5 was identified as *Bacillus licheniformis* by 16SrRNA sequence analysis, and SIMA-N9 was *Bacillus altitudinis*. According to the degradation ratio of simazine in a certain period of time, the degradation ability of different strains was evaluated. The degradation efficiency of simazine (5 mg/L) by SIMA-N9 could reach about 98% in 5d, and the strain SIMA-N5 could reach 94% under the same conditions. In addition, the addition of Pennisetum rhizosphere soil during the process of degrading simazine by strain SIMA-N9 could effectively improve the degradation efficiency. The strain SIMA-N9 has been developed as a microbial agent for the bioremediation of simazine contamination in soil. The new microbial agent developed by using SIMA-N9 has achieved satisfactory application effects. Based on the research results already obtained in this study, it was considered that strain SIMA-N9 and its live bacterial agent could play an important role in bioremediation of simazine pollution. This study could not only provide a set of solutions to the simazine pollution, but also provide a reference for the treatment of other pesticide pollution.

Key words: *Bacillus altitudinis*, *Bacillus licheniformis*, biodegradation, s-triazine herbicides, live bacteria preparation.

INTRODUCTION

The worldwide human population boost and expansion of industry and agriculture as well as climate change have resulted in a consequential increase in the chemical wastes upon natural ecosystems (Dauda et al. 2019, Islam et al. 2019). Especially in agricultural production, the extensive use of pesticides such as herbicides, insecticides and fungicides has caused serious adverse effects on the environment. Simazine (CAS:122-34-9) was one of the most commonly used s-triazine herbicides and was widely used to control broadleaf weeds in agriculture and forestry, especially in sugarcane, corn and orchard fields (Li et al. 2018, Silva & Iyer 2014). Its widespread use has caused wide public concern for its moderate persistent in the soil and aquatic environments and high ecological toxicity (Morgante et al. 2010, 2012, Garmouma et al. 2001). Under different environmental conditions, the half-life of simazine was usually from 1 month to 5 months, with an average of about 2 months (Spurlock et al. 2000). In many European countries, simazine was specifically prohibited due to its potential endocrine and

carcinogenic activity (Sai et al. 2015, Zhang et al. 2011). But it was still one of the most widely used herbicides in the United States, Australia and many other countries (Morgante et al. 2012, Flores et al. 2009, José et al. 2010). Up to now, simazine has been used in China for more than 30 years. According to statistics, in 2006, the United States used about 5-7 million pounds of simazine in agricultural and non-agricultural fields (Marriage et al. 2010). Therefore, the rapid removal of simazine from the contaminated site was considered essential for the safe ecological environment and human health. The United States EPA's Office of Water had established a Maximum Contaminant Level (MCL) for simazine in drinking water of 4.0 parts per billion (ppb). In order to remove simazine from the polluted environment, some techniques have been practiced in the past, such as ozonation, Fenton's oxidation, bioreactor, photodegradation and microbial degradation (Addorisio et al. 2011, Chen et al. 2019, Kang et al. 2020, Sathishkumar et al. 2014, Zhu et al. 2020b). In addition, membrane treatment technology may play an important role in the removal of simazine from water (Meng et al. 2020, Zhang & Jiang 2019, Zhang et al. 2021). Among the various technologies available for simazine mineralization, the microbial degradation showed better prospects than other methods (Mondragón-Parada et al. 2008, Wan et al. 2014). Microbial degradation usually played a major role in attenuating s-triazine herbicides from contaminated environment.

For bioremediation of simazine-contaminated soils, it was of practical importance to select highly efficient degrading strains or consortia that have strong viability in soil. There have been some reports about simazine biodegradation by strains of bacteria (Guo et al. 2014, Iwasaki et al. 2007), fungi (Blaszak et al. 2011), or microbial community (Grigg et al. 1997, Castro-González et al. 2011). The use of microorganisms for bioremediation of simazine-contaminated sites was receiving increasing attention as a popular alternative to chemical remediation because it was cost effective and environmentally friendly (Satsuma 2010, Zhu et al. 2019a). However, the degradation efficiencies of the simazine-degrading strains were not satisfactory in most cases, and no simazine-degrading strain has been used for commercial purposes. Therefore, in order to solve the environmental problems caused by simazine pollution, it was necessary to isolate high-efficiency simazine-degrading strains and study their degradation characteristics (Caracciolo et al. 2007).

Extensive research has addressed the behavior of simazine in the soil, the identification and isolation of the degrading strains, and the biodegradation pathway of simazine and related s-triazines. In the biodegradation process of simazine, intermediate compounds such as ammeline, ammelide and cyanuric acid were usually produced (Caracciolo et al. 2005, Raquel et al. 2005). Some intermediates may have higher stability or other biological activities and may have a negative impact on the surrounding environment and related organisms (Morgante et al. 2012, Caracciolo et al. 2005). Therefore, the attention should also be paid to these intermediate metabolites (Navarro et al. 2000, Kearney et al. 1965). In addition, many factors usually affect the biodegradation of simazine, such as pH, temperature and plant roots (Morán et al. 2010, Behki & Khan 1994, Zhu et al. 2019b). Some plants (eg. *Pennisetum alopecuroides*) could also affect the degradation of simazine in soil (Singh et al. 2004, Liao & Xie 2008, Lu et al. 2020). Therefore, in addition to screening high-efficiency simazine-degrading strains, various factors affecting the biodegradation of simazine should also be highlighted.

In this study, two strains capable of effectively degrading simazine (SIMA-N5 and SIMA-N9) were isolated. The degradation efficiency and characteristics of two simazine-degrading strains were studied and compared. The effects of some plants' rhizosphere soil on the biodegradation of simazine and the important intermediate metabolites of simazine were also analyzed. In addition, based on the higher simazine degradation efficiency possessed by strain SIMA-N9, it had been formulated into a microbial agent that could degrade simazine residues. The microbial agent containing SIMA-N9 had achieved initial success and would be commercialized. And it was expected to be used to remove simazine and other s-triazine herbicide residues in soil or water. The isolated simazine-degrading bacteria may also be used for the development of bioreactors or the production of degrading enzymes. The flow chart of the experiment was shown in Fig. 1.

MATERIALS AND METHODS

Sampling of soils

0~10 cm soil layer was collected from farmland in Changzhou, China, in which simazine was used for more than six years. The depth of the sampled layer was 0 cm, 5 cm and 10 cm, respectively. A total of 30 soil samples were collected. The collected soil samples were used to isolate simazine-degrading bacteria and for subsequent research.

Chemicals and Media

Chemicals: simazine (98.6%), ammelide (99.0%) and cyanuric acid (99.5%) were purchased from Sigma-Aldrich Company and Dr. Ehrenstorfer GmbH. Other chemicals used were of analytical grade.

Media: (I). Mineral salt medium (Lanyi Biotechnology Co., Ltd., China): Ammonium sulfate 0.1 g, Dipotassium hydrogenphosphate 0.1g, Calcium sulfate 0.05 g, Ferrous sulfate heptahydrate 0.01 g, Magnesium sulfate heptahydrate 0.2 g, H_2O 1.0 L, pH 7.0. (II). Isolation medium: mineral salt medium containing 5.0 mg/L simazine. (III). LB (Luria-Bertani) medium (Lanyi Biotechnology Co., Ltd.): yeast extract 5

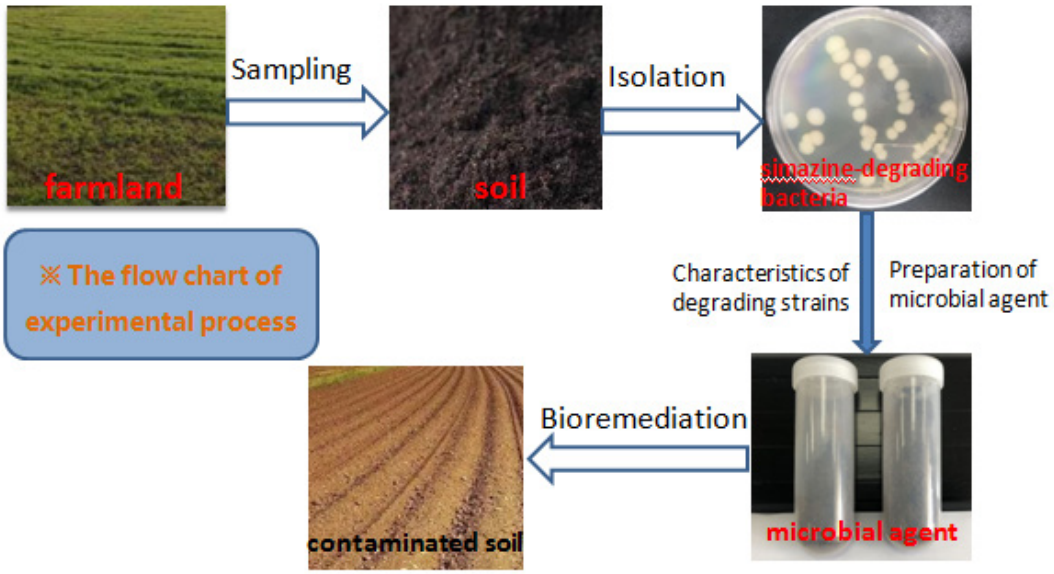

Figure 1. The flow chart of the experiment.

g, peptone 10 g, NaCl 10 g, H_2O 1.0 L, pH7.0. (IV). Enrichment medium (Lanyi Biotechnology Co., Ltd.): beef extract 5 g, yeast extract 5 g, peptone 7 g, NaCl 5 g, H_2O 1.0 L, pH7.0.

Isolation of simazine degrading bacteria

Five grams of soil samples were mixed with 100 ml isolation medium, and incubated at 30 °C. The content of simazine in medium was determined regularly. Transfer 5 ml of culture solution with 3-day degradation efficiency >80% to an enrichment medium containing 5.0 mg/L simazine, and continuously subculture for more than 5 times. After verifying the degradation ability again, the above-mentioned enrichment culture solution was inoculated into an inorganic salt culture plate containing simazine for incubation at 30 °C. The eugonic microbial colonies were selected, and obtained a pure culture by repeated scribing. The new isolates were identified by 16S rRNA sequence (Wang & Xie 2012, Wang et al. 2012, Kou et al. 2020). A phylogenetic tree was made by using ClustalX1.83 and MEGA 5.0.

Preparation of bacterial inoculant

Simazine-degrading strains, *Bacillus licheniformis* SIMA-N5 and *Bacillus altitudinis* SIMA-N9 have been isolated. Two strains were incubated individually in LB medium amended with 5 mg/L simazine at 30°C. After overnight incubation, the cells were collected by centrifugation and rinsed with sterile water. Then, the cells were resuspended, and approximately 8×10^6 cells/ml. This cell suspension will be used as inoculant for subsequent research (Wang & Xie 2012, Ding et al. 2019, Fan et al. 2020).

Degradation of simazine by strain SIMA-N5 and SIMA-N9

6% strain SIMA-N5 inoculant was inoculated in mineral salt medium containing 5 mg/L simazine, and incubated at 30°C (100rpm). The concentrations of simazine, ammelide and cyanuric acid were determined every 24h. The strain SIMA-N9 was tested according to the same procedure.

3% strain SIMA-N5 inoculant and 3% SIMA-N9 inoculant were simultaneously inoculated in mineral salt medium containing simazine (5 mg/L), and incubated under the same conditions as above. The concentrations of simazine, ammelide and cyanuric acid were determined regularly.

Tolerance of strain SIMA-N5 and SIMA-N9 on simazine

The concentration of simazine in LB medium was adjusted to 25, 50, 100, 200 and 500 mg/L by applying simazine wettable powder, respectively. Then the strain SIMA-N5 (or SIMA-N9) was inoculated and incubated at 30 °C with shaking. OD_{600} was regularly recorded to evaluate the resistance of strains to simazine.

Table I. The parameters of MS/MS analysis.

Compound	Ionization mode	Precursor ion	Product ion	Fragmenter	CE
Simazine	positive	202	96/104	130	13/21
Ammelide	negative	127	84/42	120	15/15
Cyanuric acid	negative	128	85/42	120	14/20

Degradation of simazine by combined application of strain SIMA-N9 and fresh soil

The simazine wettable powder was dissolved in water and added into LB medium, the concentration of simazine in LB medium was 30 mg/kg. LB medium containing simazine (30mg/L) was divided into 5 groups, each one was 250ml. ①Add 7.5ml of the strain SIMA-N9 inoculum and 5g fresh soil to the first group (fresh soil was taken from idle farmland without crops, the depth of the sampled soil layer was about 12 cm. n=5). ②Add 7.5ml of the strain SIMA-N9 inoculum and 5 g Pennisetum (*Pennisetum.alopecuroides* (Linn.) Spreng.) rhizosphere soil, the depth of the sampled soil layer was about 12 cm, n=5. ③Add 7.5ml of the strain SIMA-N9 inoculum and 5 g soybean (*Glycine max* (Linn.) Merr.) rhizosphere soil, the depth of the sampled soil layer was about 12 cm, n=5. ④Add only 7.5ml of the strain SIMA-N9 inoculum, n=5. ⑤Control group. Finally, all 5 groups were incubated at 30°C, the residual simazine was determined regularly.

Degradation of simazine by combined application of strain SIMA-N9 and soil solution

First, the soil of idle farmland, the rhizosphere soil of Pennisetum and the rhizosphere soil of soybean were collected separately, and the depth of the sampled soil layer was about 12 cm. Then, 50 g of soil was mixed with 100 ml of sterile saline and stirred evenly. The mixture was first filtered by three layers of gauze and then filtered by filter paper. Finally, the soil filtrate was filtered and sterilized by sterile syringe filter with 0.2μm Supor® membrane(Pall Corporation). The sterilized soil solution was stored in a freezer for subsequent study.

LB media containing simazine (30mg/L) were divided into 5 groups, each one was 250ml. ①Add 7.5ml of the strain SIMA-N9 inoculum and 5 ml sterilized soil solution(from idle farmland) to the first group. ②Add 7.5ml of the strain SIMA-N9 inoculum and 5 ml sterilized Pennisetum rhizosphere soil solution. ③Add 7.5ml of the strain SIMA-N9 inoculum and 5 ml sterilized soybean rhizosphere soil solution. ④Add 7.5ml of the strain SIMA-N9 inoculum and 5 ml of sterile saline. ⑤Control group. Finally, all 5 groups were incubated at 30°C, the residual simazine and OD_{600} in medium were determined regularly.

Construction of a microbial agent for effective simazine degradation

Preparation steps for microbial agent containing strain SIMA-N9

Option A: The strain SIMA-N9 inoculant was mixed with a sterile enrichment medium (1:9, v/v), the mixture was incubated for 1 day at 30°C. The mixture of rice hull powder, dried peat soil and fresh Pennisetum rhizosphere soil (6:3:1, w/w) was used as carrier for the preparation of microbial agent, and each 100 g carrier was mixed with 25 ml pre-prepared mixture containing SIMA-N9. The final mixture was incubated at 30°C for 15h, then put them in the sterile vial and stored at 0°C.

Option B: The mixture of rice hull powder and dried peat soil (6:4, w/w) was used as carrier. Other steps were the same as in Option A.

Degradation of simazine in soil by the microbial agent

An aqueous solution of simazine wettable powder was added to the fresh soil to make the concentration of simazine in soil 30 mg/kg. The soil containing simazine was then dispensed into a 30 cm x 30 cm plastic box with a soil depth of 12 cm and the soil weight in each box was the same.

1.0 g of the microbial agent was dissolved in 45 ml of sterile water and then sprinkled all over a box of soil. 45 ml sterile water was sprinkled in control group. Finally, the residual simazine in soil was determined regularly after incubation.

Determination of simazine and its metabolites

Soil samples were treated in the following steps: 10.0 g soil sample was weighed into 250 ml beaker. 10.0 g anhydrous Na_2SO_4 and 100 ml ethyl acetate were added to each sample. Samples were homogenized for 0.5 min (12,000 rpm), and allowed to settle for 0.5 hours. Filter the extract by quantitative filter paper, 10 ml of filtrate was blown to dryness by nitrogen at 55 °C. The dry extract was redissolved in 2 ml of CH_3OH, then filter into a vial through a 0.22 micron filter (Merck Millipore). All extracts were stored at 0 °C until analysis (Navarro et al. 2000, Chung et al. 2011).

Liquid samples were centrifuged to obtain cell-free supernatant, which were then analyzed by LC-MS/MS on 1260HPLC-6430 Triple quadrupole mass spectrometer (Agilent, USA). Simazine, ammelide and cyanuric acid were separated on a C18 column (Agilent, 3.0×100 mm, 1.8-Micron) with a flow rate of 0.3 ml/min. 0.1% HCOOH aqueous solution and CH_3CN were used for HPLC gradient elution. MS/MS analysis was performed in ESI mode and transitions were measured in MRM (Zhu et al. 2020a).

Statistical analysis

The statistical analysis of the experimental data was carried out with Excel2013 (Microsoft Software). Data were plotted using Origin (version 8.1).

RESULTS AND DISCUSSION

Characterization of simazine degrading strains and degradation of simazine by the strain SIMA-N5 and SIMA-N9

2 strains capable of effectively degrading simazine were isolated from soil and named as SIMA-N5 and SIMA-N9. SIMA-N5 was bacillus, 0.5-0.8×2.5-3.5 μm, Gram positive, aerobic bacteria, motile, and formed pale-yellow and opaque colonies on LB medium. It was positive in tests for oxidase, gelatinase and catalase, but negative for D-glucose fermentation, amylase and urease (Zhao et al. 2019, Song et al. 2020, Xu et al. 2020). SIMA-N5 was identified as *Bacillus licheniformis* according to 16S sequence (GenBank accession No. MN067735).

SIMA-N9 was curvulate bacillus, Gram positive, facultative aerobe, mobile, 0.7-0.8×2.0-3.0 μm, and it formed grayish and opaque colonies on LB medium. It was positive for urease, amylase, catalase and indole test, but negative for oxidase, gelatinase and methyl red test. SIMA-N9 was identified as *Bacillus altitudinis* (GenBank accession No. MN067807, phylogenetic tree was shown in Fig. 2). The phylogenetic tree (neighbor-joining tree) was constructed by using ClustalX1.83 and MEGA 5.0.

Degradation of simazine by strain SIMA-N5 and SIMA-N9

The results in Fig. 3 and Fig. 4 showed that simazine could be rapidly degraded by SIMA-N5 and SIMA-N9. The degradation efficiency of simazine (5 mg/L) by SIMA-N5 could reach about 98% in 6d, while the strain SIMA-N9 could reach 100%. It could also be seen from Fig. 5 that SIMA-N9 degraded simazine slightly faster than SIMA-N5. But this did not mean that simazine has been completely degraded. Ammeline, ammelide and cyanuric acid were important metabolites in the degradation of simazine (Caracciolo et al. 2005,

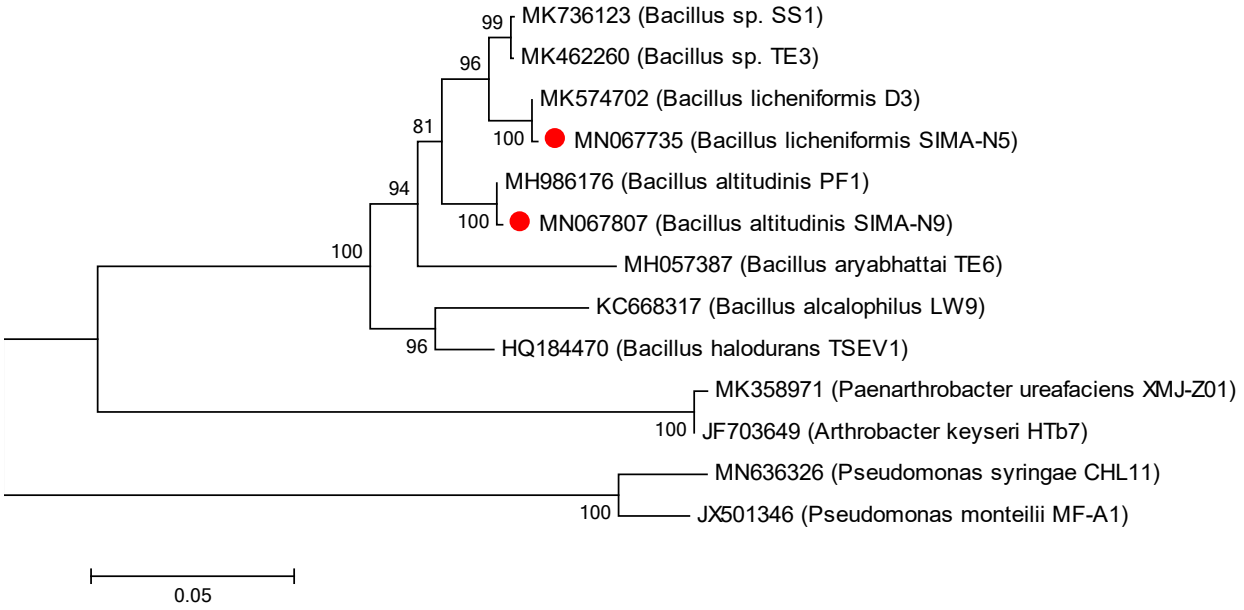

Figure 2. Phylogenetic tree of strain SIMA-N5 and SIMA-N9.

Xia et al. 2015). Ammeline was easy to convert to ammelide or cyanuric acid, resulting in a very low concentration of ammeline in the medium, so ammeline was not analyzed separately in this study. The concentrations of ammelide and cyanuric acid rose first and then fell in Fig. 3 and Fig. 4, which indicated that ammelide and cyanuric acid could be further degraded although there was a staged accumulation of intermediate metabolites during the degradation process. In addition, it was found that the accumulation of ammelide and cyanuric acid during the degradation of simazine by strain SIMA-N5 was significantly higher than that of the strain SIMA-N9 by comparing Fig. 3 with Fig. 4. It could be speculated that strain SIMA-N9 could degrade ammelide and cyanuric acid more rapidly. The rapid release of intermediate metabolite accumulation would also facilitate degradation of the parent compound, and it may be an important reason why strain SIMA-N9 could degrade simazine more rapidly than strain SIMA-N5. In this study, two strains were also combined for the degradation of simazine, and the results in Fig. 5 show that the combined application of strain SIMA-N5 and strain SIMA-N9 did not achieve better results. This might be due to the competition or antagonism between the two strains, which would affect the degradation efficiency.

Tolerance of strain SIMA-N5 and SIMA-N9 on simazine

If the concentration of simazine was increased from 25mg/L to 200mg/L (Fig. 6), OD_{600} of strain SIMA-N5 did not change significantly ($P>0.05$). However, 200 mg/L of simazine significantly inhibited the growth of strain SIMA-N9 (Fig. 7). 500 mg/L of simazine would lead to a greater reduction in OD_{600} of strain SIMA-N9, while also could significantly inhibit the growth of strain SIMA-N5. Based on the available data, it was preliminarily concluded that the strain SIMA-N5 was better tolerant to simazine than the strain SIMA-N9. In addition, although 500 mg/L of simazine could significantly inhibit the growth of strain SIMA-N5 and SIMA-N9, it did not cause them to stop growing, which also indicated that the two strains were well tolerated to simazine.

Degradation of simazine by strain SIMA-N9 and soil (or the sterilized soil solution)

Based on the higher simazine degradation efficiency of strain SIMA-N9, strain SIMA-N9 would be selected for subsequent application studies (but did not deny the application potential of strain SIMA-N9). The results shown in Fig. 8 indicated that the combination of strain SIMA-N9 and fresh soil for the degradation of simazine could achieve higher degradation efficiency. The highest degradation efficiency was obtained when strain SIMA-N9 was combined with the rhizosphere soil of Pennisetum, and the 6-day degradation rate of simazine (30 mg/kg) was 100%. If soybean rhizosphere soil or idle farmland soil was combined with strain SIMA-N9 for degradation of simazine, its degradation efficiency was significantly higher than that of strain SIMA-N9 alone, which might be due to the fact that some microorganisms and enzymes in soil were involved in the degradation of simazine or play a supporting role. These microorganisms and soil enzymes in soil may be involved in the deamination, dealkylation or dechlorination of simazine, but cannot completely mineralize simazine. They may only participate in the degradation of the intermediate metabolites of simazine, but it will facilitate the mineralization of simazine (Douglass et al. 2017, Silvia et al. 2011, Jin et al. 2020). In this study, the application of Pennisetum rhizosphere soil significantly improved the degradation efficiency of simazine, but it was not confirmed which factor in the rhizosphere soil was working, possibly soil microbes, soil enzymes, or other biological factors(Douglass et al. 2017, Min et al. 2019).

The soil solution used here was filtered to remove microorganism by 0.2μm Supor® membrane to eliminate the effects of soil microorganisms on the experiment. The results in Fig. 9 indicated that the combination of the rhizosphere soil solution of Pennisetum and strain SIMA-N9 for the degradation of simazine could achieve higher degradation efficiency than other soil solutions. The degradation efficiency of simazine in the combination of soybean rhizosphere soil solution (or idle farmland soil solution) and strain SIMA-N9 was similar to that of strain SIMA-N9 alone ($P>0.05$). In addition, according to the results shown in Fig. 10, although the combination of Pennisetum rhizosphere soil solution and strain SIMA-N9 could obtain higher simazine degradation efficiency, OD_{600} of strain SIMA-N9 in the experimental group of Pennisetum rhizosphere soil solution was not significantly different from that of soybean rhizosphere soil solution group (or idle farmland soil solution). This indicated that the highest simazine degradation efficiency obtained by the combination of the rhizosphere soil solution of Pennisetum and strain SIMA-N9 in Fig. 9 was not due to the accelerated growth of strain SIMA-N9. It was concluded that the rhizosphere soil solution of Pennisetum may contain some enzymes or other compounds that could promote the degradation of simazine, and such enzymes or other related compounds may be valuable for the treatment of simazine pollution. The application of the rhizosphere soil of Pennisetum in Fig. 8 led to an increase in the degradation efficiency of simazine, which could not rule out the role of microbes in Pennisetum rhizosphere soil. In Fig. 8, the degradation efficiency of simazine in the rhizosphere soil of soybean (or idle farmland soil) combined with strain SIMA-N9 was higher than that of strain SIMA-N9 alone, but this phenomenon was not observed after the soil solution was sterilized by filtration in Fig. 9, so it was inferred that this phenomenon in Fig. 8 was mainly due to the action of microorganisms in the soil.

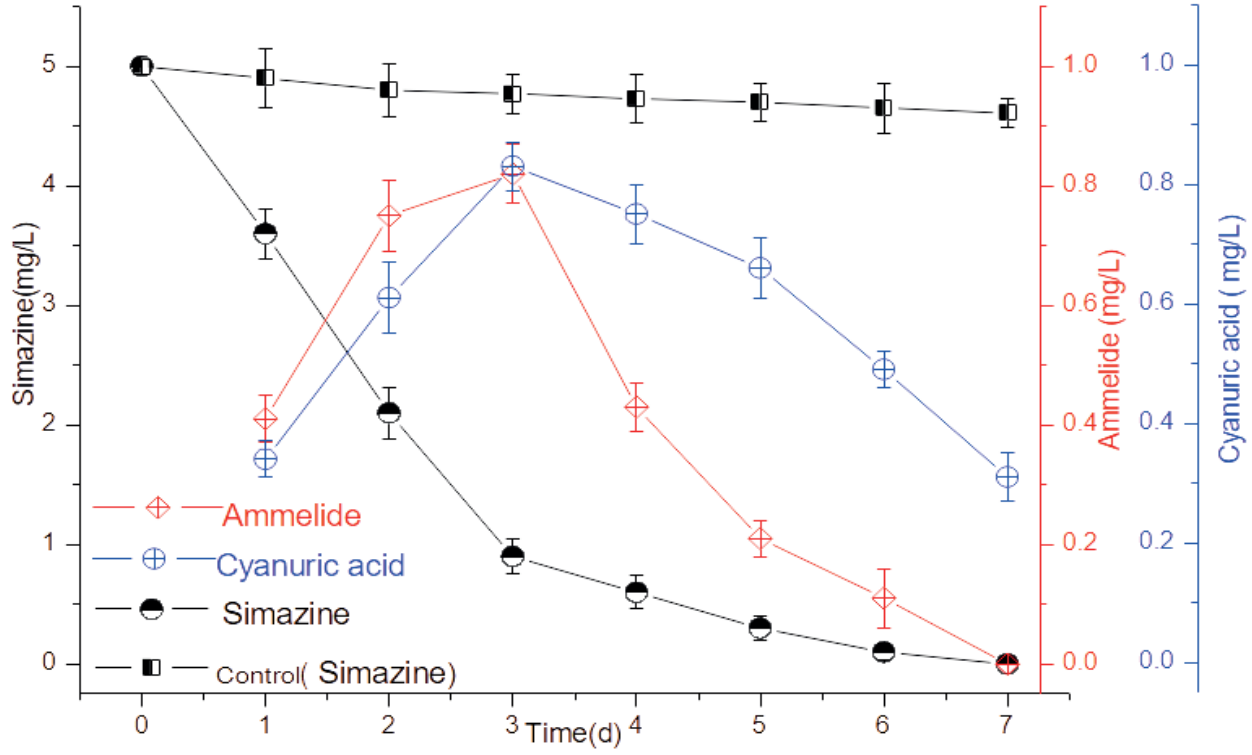

Figure 3. The curve on degradation of simazine by the strain SIMA-N5 (n=5).

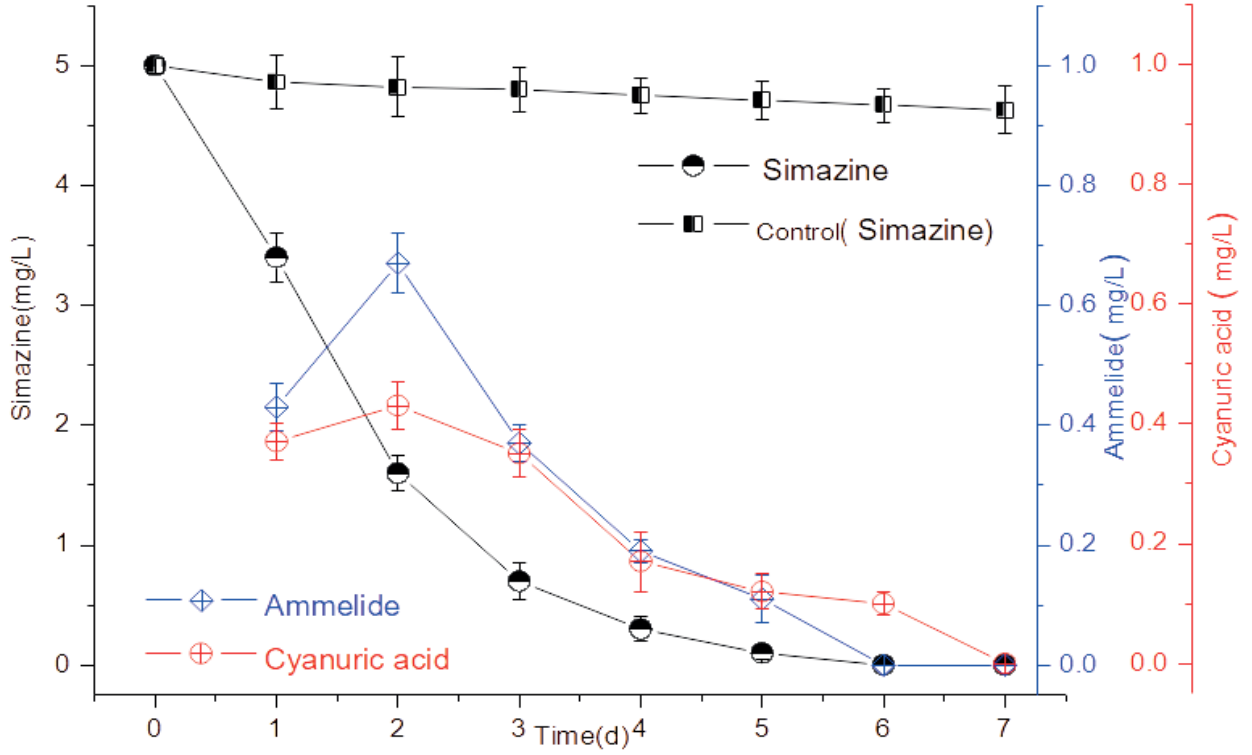

Figure 4. The curve on degradation of simazine by the strain SIMA-N9 (n=5).

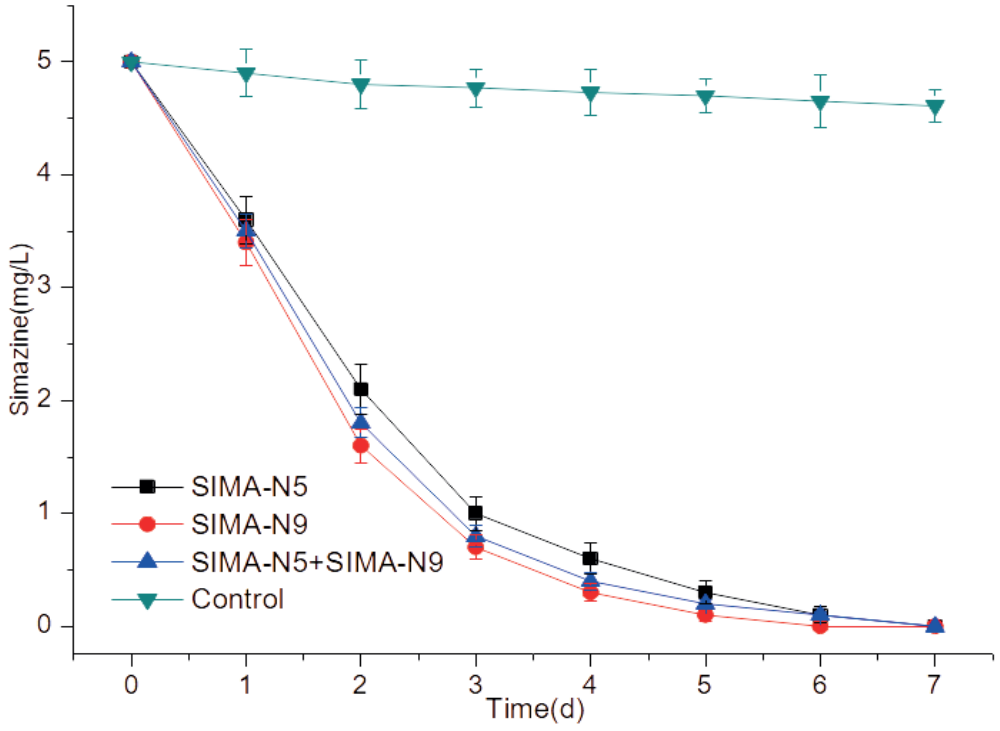

Figure 5. The curve on degradation of simazine by different microorganisms (n=5).

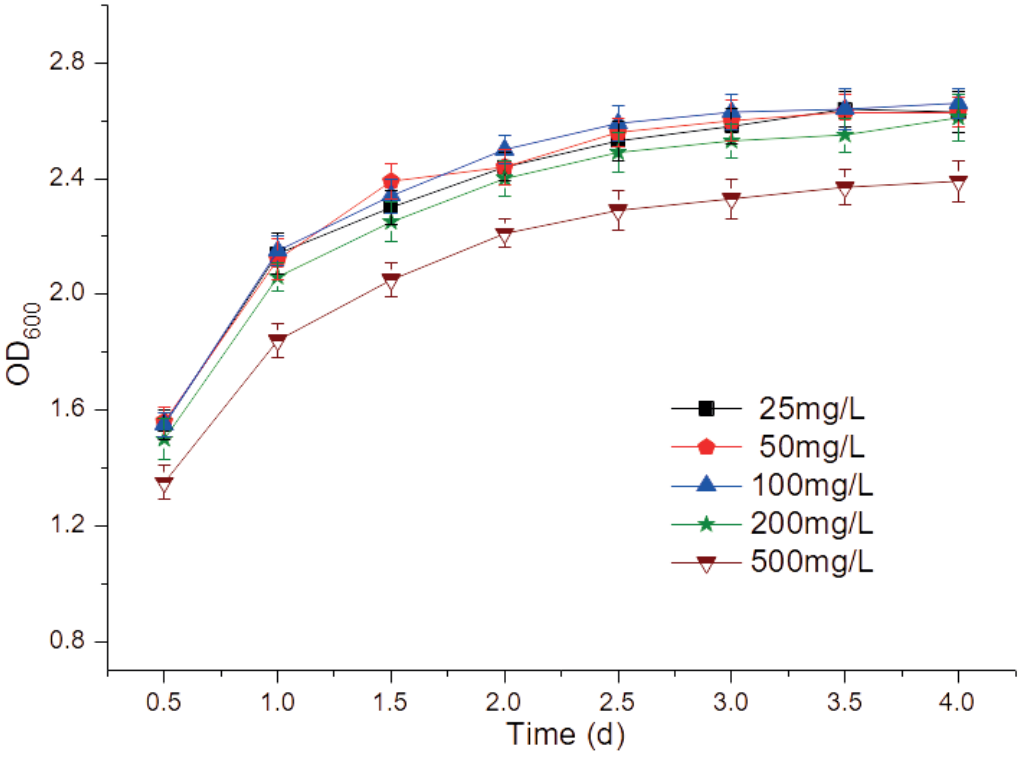

Figure 6. Effect of concentration of simazine on the growth of SIMA-N5 (n=5).

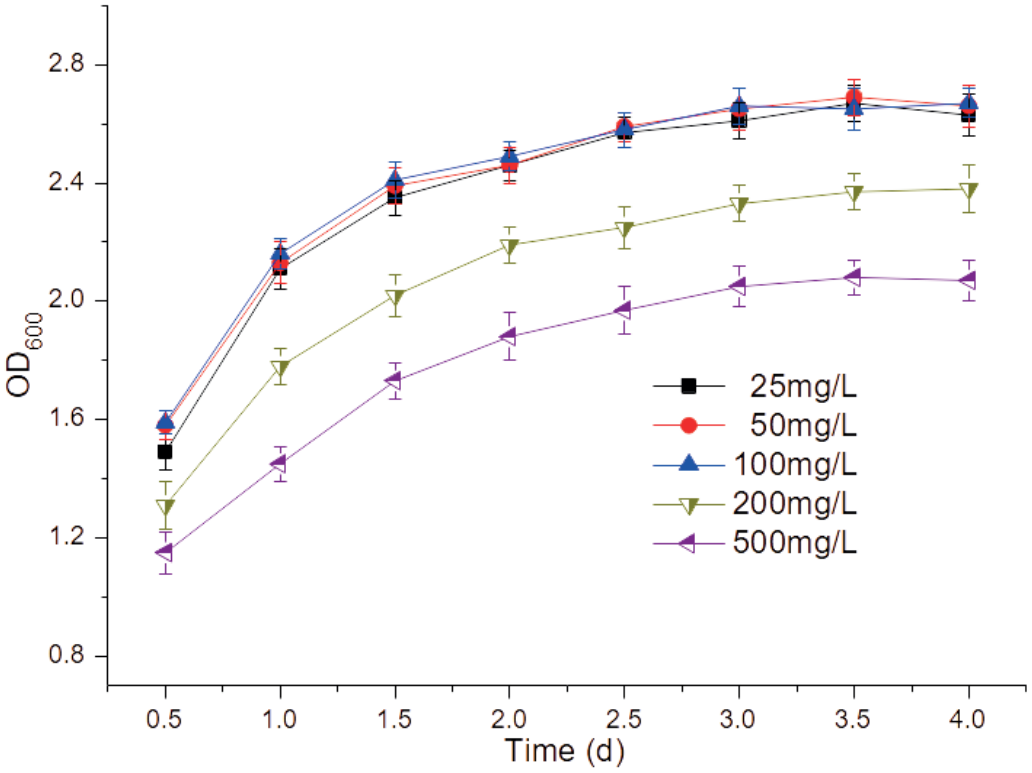

Figure 7. Effect of concentration of simazine on the growth of SIMA-N9 (n=5).

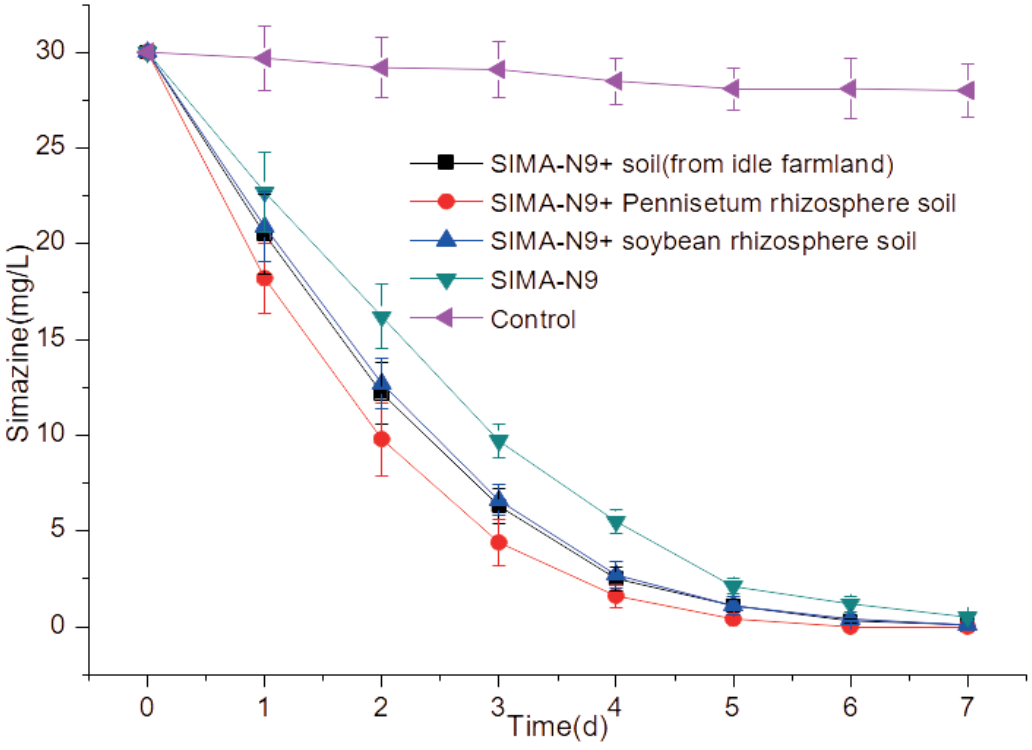

Figure 8. Degradation of simazine by strain SIMA-N9 and soil (n=5).

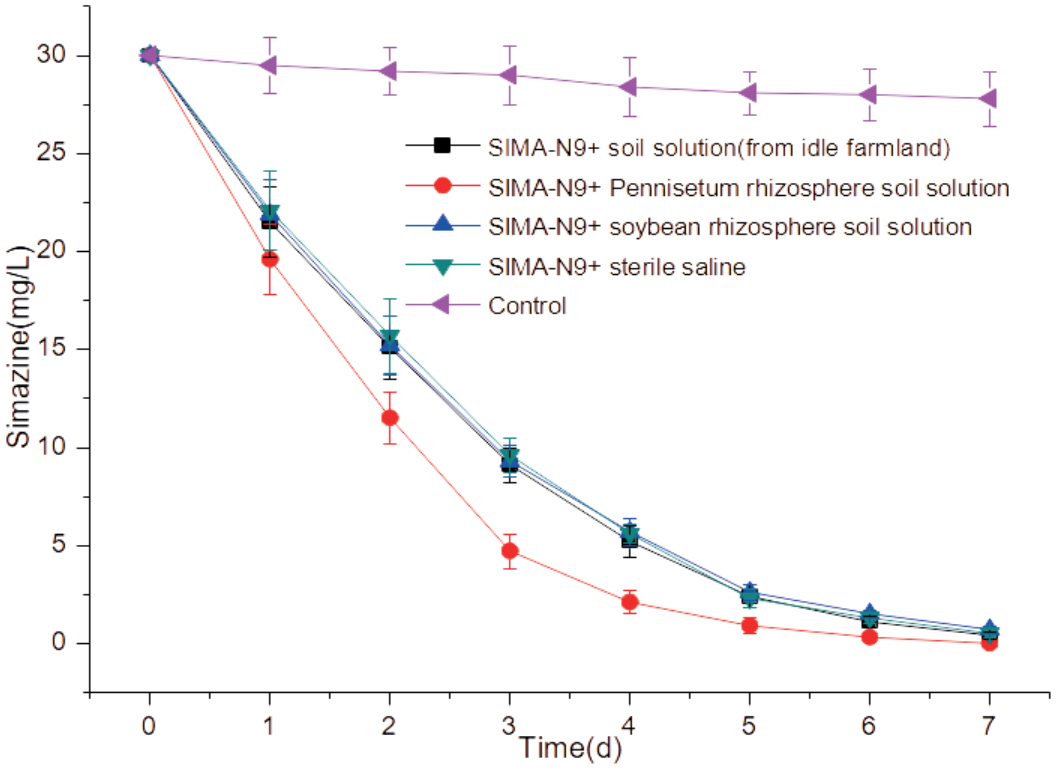

Figure 9. Degradation of simazine by strain SIMA-N9 and the sterilized soil solution (n=5).

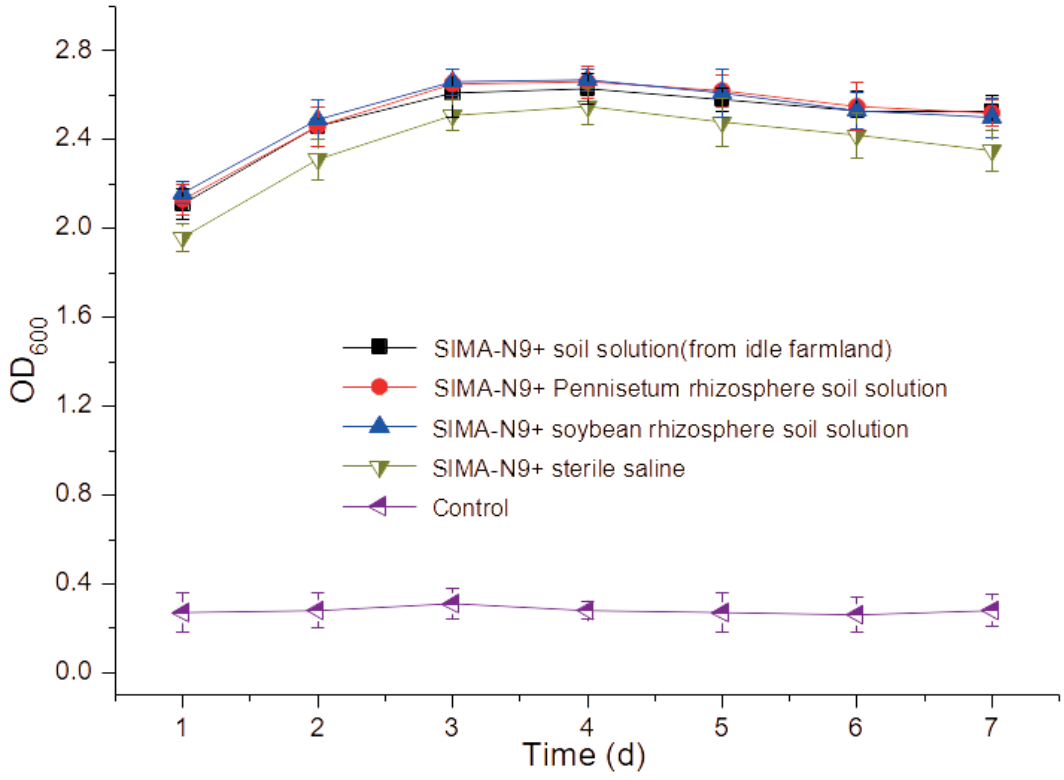

Figure 10. OD_{600} curve during the degradation of simazine by strain SIMA-N9 and soil solution (n=5).

Degradation of simazine by the microbial agent

Bioaugmentation with pure cultures or microbial consortia was an attractive option for removing simazine or other s-triazine herbicides from the contaminated sites (Hernandez et al. 2010, Zhu et al. 2021, Herrera et al. 2019). In general, in order to ensure the effect of bioaugmentation, it was necessary to prepare an efficient microbial agent which was easy to store and use. According to the above experimental data, it was predicted that the proper addition of the rhizosphere soil of Pennisetum during the preparation of the microbial agent would be beneficial to improve the degradation efficiency of simazine. The experimental results of degrading simazine in soil by using the above microbial agents were shown in Fig. 11. The microbial agent containing the rhizosphere soil of Pennisetum could achieve a 7-day degradation efficiency of 100% for simazine (30 mg/kg). If the rhizosphere soil of Pennisetum was not added during the preparation of the live bacterial agent, the final simazine degradation efficiency was slightly reduced (Fig. 11). Therefore, in the preparation of the live microbial agent, it was also necessary to pay attention to some factors such as related adjuvants. In the process of microbial degradation under artificial intervention, the survival of bioaugmentation strains was an issue that must be paid attention to (Zhu et al. 2018, Cho et al. 2008, Xu et al. 2019). It was speculated that SIMA-N9 could survive well in soil or microbial agent because the microbial agent containing SIMA-N9 could effectively degrade simazine in soil. The degradation efficiency of simazine was 32% after 7 days in the control group (no microbial agent), which should be attributed to the role of soil enzymes, indigenous microorganisms and other soil biochemical factors.

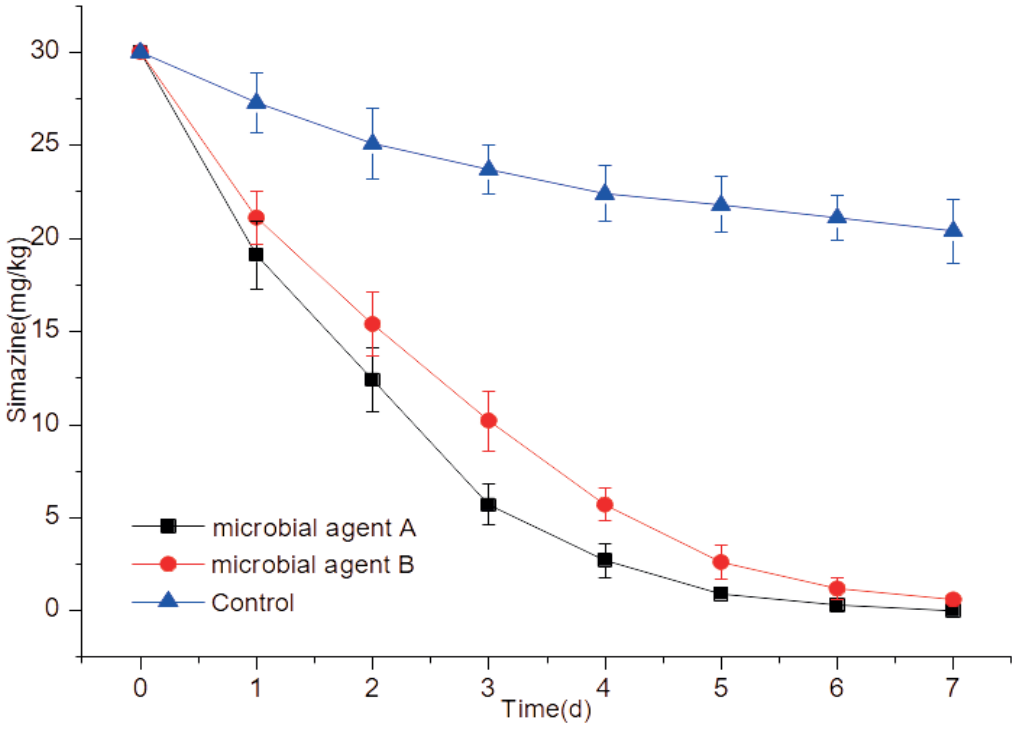

Figure 11.
The curve on degradation of simazine in soil by microbial agent (Microbial agent B did not contain the rhizosphere soil of Pennisetum) (n=5).

CONCLUSIONS

2 simazine degrading strains were isolated from soil, and they were *Bacillus licheniformis* SIMA-N5 and *Bacillus altitudinis* SIMA-N9, respectively. The strain SIMA-N9 degraded simazine slightly faster than SIMA-N5. The combined application of strain SIMA-N5 and strain SIMA-N9 could not achieve higher degradation efficiency, which should be attributed to the competition or antagonism between the two strains. In addition, Pennisetum rhizosphere soil could effectively improve the biodegradation efficiency of simazine by strain SIMA-N9. It was concluded that the pennisetum rhizosphere soil solution sterilized by filtration may contain some enzymes or other compounds that could promote the degradation of simazine. The promotion effect of Pennisetum rhizosphere soil on the biodegradation of s-triazine herbicides was confirmed again, but the related mechanism need to be further studied. The strain SIMA-N9 had been successfully prepared as a microbial agent for degradation of simazine residues in soil. The microbial agent containing SIMA-N9 and Pennisetum rhizosphere soil had achieved satisfactory experimental results, and the degradation efficiency of simazine (30 mg/kg) by this microbial agent could reach 97% after 5d. These results suggested that strain SIMA-N9 and its microbial agent could be used to treat simazine pollution.

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