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EFFECTS OF ENVIRONMENTAL CONDITIONS ON PROPANIL DEGRADING ACTIVITY OF *Acinetobacter baumannii* DT

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ABSTRACT

Effects of various environmental conditions on propanil degrading activity of *Acinetobacter baumannii* DT were investigated. Results showed that both propanil degradation and bacterial growth rate were reduced when bacteria were cultured in extreme conditions, such as high acidic or alkaline levels or high salinity. Moreover, the propanil degradation activity of *A. baumannii* DT decreased in contaminated water. The propanil dissipation rate was higher in herbicides-contaminated soil (treated soil) than in herbicide-free soil. In soil inoculated with *A. baumannii* DT, propanil removal was enhanced. Even though the propanil degrading activity of *A. baumannii* DT were reduced under extremely stressful conditions, this bacterium retained a good potential to degrade propanil in real environmental conditions.

Keywords: *Acinetobacter baumannii* DT, environmental factors, extreme conditions, propanil.

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INTRODUCTION

Pesticides have been intensively applied in agriculture, which is an issue of global concern. Propanil has been extensively used to control grasses and sedges worldwide, including Vietnam. This post-emergence contact herbicide, which inhibits the photosynthesis process of broadleaf weeds (Tomlin, 2009), is usually applied to flooded paddy fields, corn fields and other fruit gardens. Therefore, propanil and its metabolites have been detected in surface water, groundwater and soil (Dabrowski et al., 2002; Silva et al., 2006; Primel et al., 2007). For example, propanil was detected to be up to 3.6 mg/L in irrigation water in Brazil (Primel et al., 2007).

Propanil has acute toxicity to a number of aquatic species (Pothuluri et al., 1991; Mitsou et al., 2006; Darren et al., 2009), leading to death from acute poisoning, especially methemoglobinemia (Darren et al., 2009). 3,4-dichloroaniline is a common major product of propanil transformation. This metabolite also causes adverse health and ecotoxicity effects (Salazar et al., 2008). Propanil and 3,4-dichloroaniline may enter natural aquatic systems, and reach water supply resources or accumulate in the soil, where the substrates are difficult to be biodegraded because of the environmental conditions unsuitable for microorganisms to degrade them.

Propanil may be degraded by physical, chemical and biological pathways. This compound can undergo chemical hydrolysis within a wide range of pH levels, and photo-degraded under direct sunlight with a half-life of 12 hours (Dahchour et al., 1986). However, biodegradation is considered to be an effective and environmentally friendly method to remediate pesticides and other organic compounds. Several bacteria and fungi can transform propanil to 3,4-dichloroaniline; *e.g.*, *Fusarium oxysporum* (Reichel et al., 1991), *Paracoccus* sp. FLN-7 (Zhang et al., 2012)**,** *Ochrobactrum* sp. PP-2 (Zhang et al., 2019a) and *Spirosoma sordidisoli* TY50^T (Zhang et al., 2019b). However, these isolates require the addition of co-substrates for the biotransformation of propanil. They also can not degrade 3,4-dichloroaniline. Moreover, propanil may accumulate in extreme environments such as soils with high alkaline and acidic values, high salinity, and low oxygen content. However, most studies have been carried out in artificial media. Thus, evaluation of propanil biodegradation under extreme natural conditions should be conducted to investigate the possibility of biodegrading propanil and its delivatives at contaminated sites. In this study, the propanil degrading activity of *A. baumannii* DT isolated from soil (Oanh et al., 2020) was investigated under extreme conditions, in natural water and in soil.

MATERIALS AND METHODS

Cultivation media and bacteria

The mineral medium (MM medium) used was prepared according to Nguyen & Ha (2019) consisting of 1,419.6 mg/L Na_2HPO_4, 1,360.9 mg/L KH_2PO_4, 98.5 mg/L $MgCl_2$, 5.88 mg/L $CaCl_2 \cdot 2H_2O$, 8.4 mg/L $NaHCO_3$, 1.16 mg/L H_3BO_4, 1.15 mg/L $ZnSO_4 \cdot 7H_2O$, 0.38 mg/L $CuSO_4 \cdot 5H_2O$, and 0.24 mg/L $CoCl_2 \cdot 6H_2O$, 1.0 g/L $(NH_4)_2SO_4$ and 1.0 g/L succinate. pH was adjusted to 7.0. The medium was sterilized at 121 ^oC for 15 min before use. Propanil (99.6%) and other chemicals were purchased from Sigma-Aldrich (Singapore) and Merck (Germany).

The *Acinetobacter baumannii* DT isolate used in this study has been proven to degrade propanil and 3,4-dichloroaniline effectively under laboratory conditions (Oanh et al., 2020). The 16S rRNA sequence of this isolate has been deposited in GenBank and available in NCBI under the accession number MN658561.1.

Evaluation of propanil degradation under various conditions

Bacteria cultured in Luria-Bertani (LB) medium for 12 hours were used for inoculation. Propanil degradation was carried out in 150 mL-bottles containing 40 mL of the MM medium. 0.1 mL of the inoculum was

transferred to the MM medium to provide an initial bacteria concentration of 10^6 CFU/mL, except for studying the effects of cell numbers on the degradation, in which the initial inoculum ranged from 10^5 to 10^8 CFU/mL.

For the effects of pH values on degradation, NaOH (10 M) and HCl (5 M) were used to adjust pH levels. In another experiment, NaCl was added at the dose range of 1.0−5.0% to determine the effects of salinity on propanil degradation and bacterial growth.

For the effects of oxygen levels on bacterial activities, experiments with oxygen restriction in anoxic media were carried out using bottles sealed with rubber septa and aluminum crimps during the incubation processes. The anaerobic condition was created by bubbling nitrogen gas into the bottles for 20 min. Rezasurin (0.4 mM) was used as an indicator to confirm the anaerobic condition. Liquid samples were collected from bottles to determine the degradation during incubation process using syringes and needles. Bacteria activities under normal conditions were determined in bottles capped with cotton plugs. The cap was opened when collecting liquid samples.

To evaluate the propanil degradation in relatively clean natural water, water samples were collected from the Tien River (a branch of the Mekong River) at Cao Lanh City. Additionally, contaminated water was collected from a trench in a fruit garden in Lap Vo District, Dong Thap Province, where farmers have been using herbicides including propanil to kill grasses. The chemical components of those water samples were analyzed according to the APHA method (APHA, 1998).

All experiments were performed at least three replicates. Incubation was conducted at 30 ^{o}C with a rotation speed of 150 rpm. Propanil was added at 0.1 mM. Degradation in abiotic controls was also measured to compare with degradation by *A. baumannii* DT.

Propanil degradation in soil

One soil sample was collected from a depth of 10−50 cm in a corn field where farmers have been using herbicides occasionally (contaminated soil). Another soil sample was collected from mountain named Nui Cam, An Giang Province, where no herbicide has been used (uncontaminated soil). The soil samples were broken up, crumbled, then sieved through a mesh with 2 mm in diameter. Soil components were determined according to the soil texture triangle (Soil Science Division Staff, 2017). Other physicochemical properties were analyzed using the APHA method (1998).

The bacteria inoculation and propanil degradation in soil were conducted as described by Duc (2017). The propanil degradation was also determined in soil without inoculation. Propanil in soil was extracted with acetone as described by Milan et al. (2012). The extraction efficiencies of propanil from contaminated and uncontaminated soil were 92.0% and 94.6%, respectively.

Analytical methods

Bacteria numbers in liquid media were counted based on colony-forming units (CFU) on LB agar plates. Propanil concentrations were measured using reversed-phase high-performance liquid chromatography (HPLC) as described in a previous study (Oanh et al., 2020). The HPLC profiles were compared with standards to obtain the results.

RESULTS AND DISCUSSION

Effects of pH levels on propanil degradation

Propanil degradation by *A. baumannii* DT and its growth on the substrate at various pH levels revealed that the optimum pH was 7.0 (Fig. 1). The degradation rates decreased in acidic and alkaline conditions. The overall biodegradation rate was proportional to the bacteria growth in both acidic and acidic conditions, suggesting that biodegradation capacity of individual bacterium is maintained well at wide ranges of pH, with the range of acidic pH being wider.

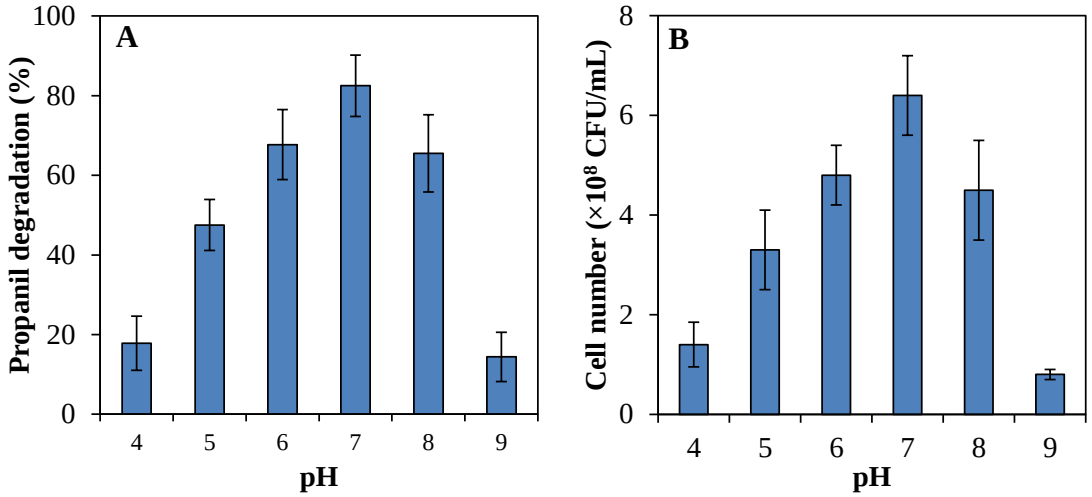

Figure 1. Effects of pH on propanil degradation (A) and bacterial growth (B) of *A. baumannii* DT for 10 hours at 0.1 mM propanil

Effects of salinity on propanil degradation and bacterial growth

When applied to soil, propanil may contaminate water. The contaminated water will flow to brackish zones and eventually into marine water. Thus, determining the acceptable range of NaCl concentrations for *A. baumannii* DT to degrade propanil is critical for field application of this bacterium. Results showed that no significant differences in degradation and growth rates were observed within the range of 0−2% NaCl. However, degradation and growth decreased with the dose at NaCl concentrations > 2% (Fig. 2).

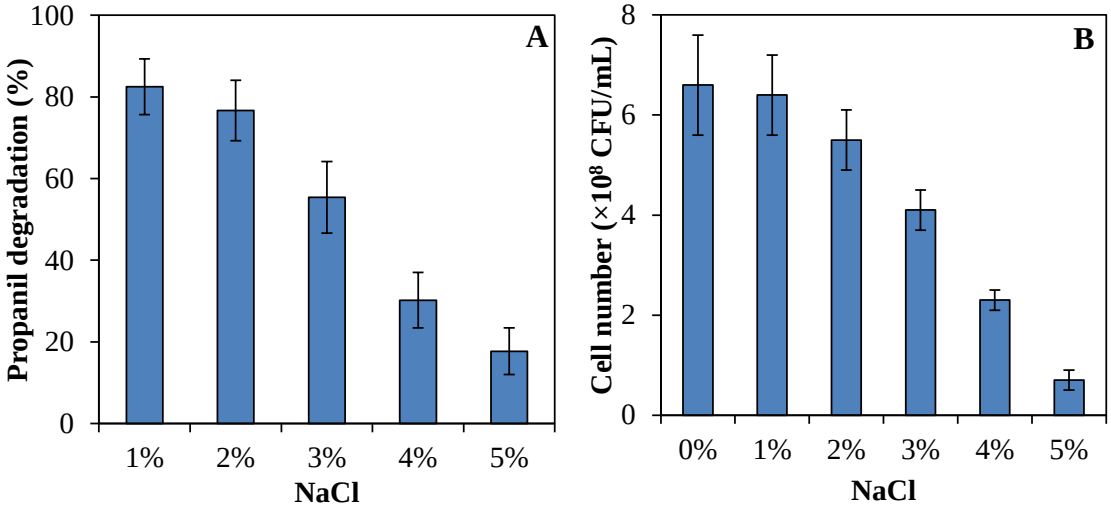

Figure 2. Effects of salinity on propanil degradation (A) and bacterial growth rates (B) of *A. baumannii* DT at 0.1 mM propanil for 10 hours

Effects of *A. baumannii* DT inoculum concentrations on the efficacy of propanil degradation

Increasing bacterial cell concentrations enhanced the propanil degradation rate. An initial inoculation dose of 10^8 CFU/mL

resulted in complete degradation of 0.1 mM propanil within 10 hours. At lower initial inoculum concentrations, the degradation rates were slower (Fig. 3). However, propanil degradation was mostly complete by 15 hours regardless of initial incubation doses.

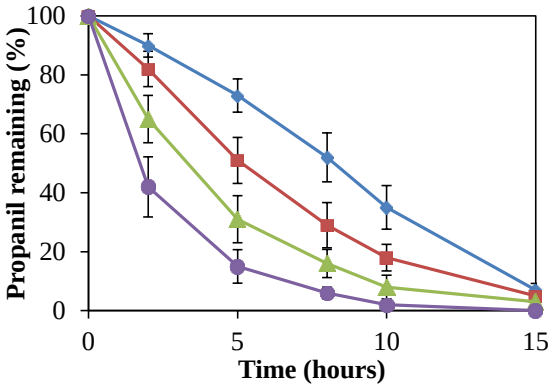

Figure 3. Effects of initial inoculation dose of *A. baumannii* DT on propanil degradation at 0.1 mM propanil. The initial bacteria concentration (CFU/mL) were 10^8 (circle symbol), 10^7 (trangle symbol), 10^6 (square symbol) and 10^5 (diamond symbol)

Effects of oxygen concentrations on propanil degradation and bacterial growth

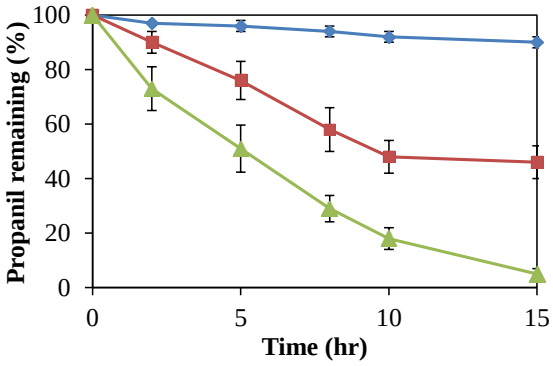

Figure 4. Effects of oxygen on propanil degradation at 0.1 mM. The degradation was performed at normal (triangle symbol), restricted oxygen (square symbol) and anoxic (diamond symbol) conditions

Propanil may contaminate soil and sediment where the oxygen is restricted. The evaluation of propanil degradation by *A. baumannii* DT in anoxic and anaerobic condition will provide useful information about the application of bacteria for propanil remediation in soil and sediment. Propanil degradation was initially normal but markedly decreased with time under anaerobic conditions (Fig. 4). The degradation was slow under limited oxygen condition with no progress after the first 10 hours, probably due to oxygen in the medium being exhausted. These results suggest that *A. baumannii* DT can degradade propanil effectively only under an aerobic condition.

Propanil degradation by *A. baumannii* DT in environmental water collected from the river and the contaminated trench

As shown in table 1, water collected from the Tien River was quite clean, while trench water was of poor quality regarding total suspended solids, total nitrogen, chemical oxygen demand (COD) and bacteria numbers. However, propanil was not detected in both water sources.

Then, propanil degradation by *A. baumannii* DT was performed in two extremely different water samples, river water and trench water. Figure 5 showed that the degradation by indigenous microorganisms in two types of water samples was not significant. Even though adding ammonium sulfate and succinate as supplemental nutrients increased the degradation process, the degradation rates of *A. baumannii* DT in natural water were significantly lower compared to the rates in MM medium shown in Fig. 3.

Propanil degradation by *A. baumannii* DT in sterile and non-sterile river water was mostly comparable. Propanil degradation by *A. baumannii* DT in non-sterile trench water was significantly lower than that in the sterile one. Moreover, regardless of the experimental conditions, propanil degradation in trench water was always slower than in the river water. These results suggest that high numbers of native microorganisms and higher pH levels of the trench water might confer inhibitory effects on propanil degradation of *A. baumannii* DT.

Table 1. Chemical components of water samples collected from the Tien River and a trench

Parameters	Unit	The Tien River water	Trench water
pH		7.3	7.7
Total suspended solids	mg/L	70.2	217.7
Total nitrogen	mg/L	0.44	1.02
Total phosphorus	mg/L	0.06	0.09
Dissolved oxygen	mg/L	7.12	6.25
Chemical oxygen demand	mg/L	2.01	3.51
Propanil	mg/L	0.0	0.0
Sulfate	mg/L	5.6	7.1
Chloride	mg/L	6.8	7.3
Calcium	mg/L	7.1	6.1
Magnesium	mg/L	3.3	3.7
Sodium	mg/L	3.5	4.7
Potassium	mg/L	0.8	0.6
Bacteria	CFU/mL	3×10^5	7×10^6

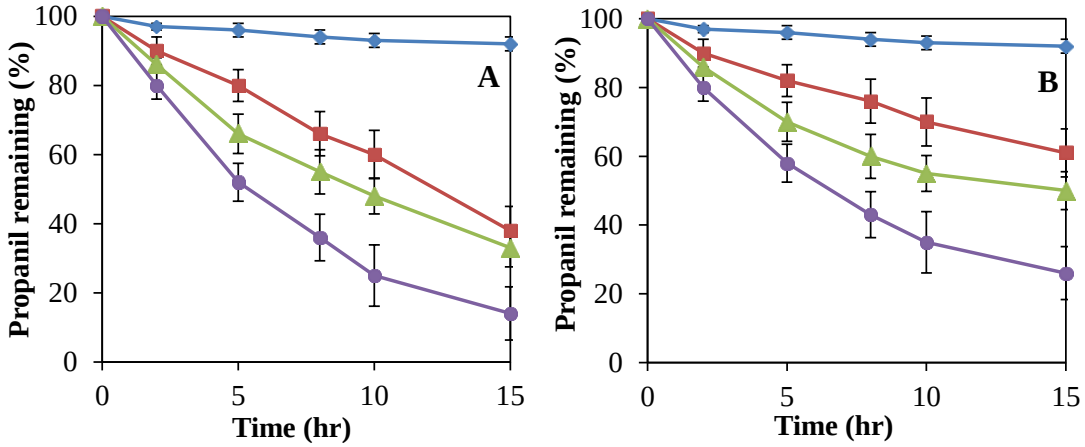

Figure 5. Degradation of propanil in water collected from the Tien River (A) and a trench in a fruit garden (B). The degradation was performed in non-sterile water without *A. baumannii* DT (diamond symbol), non-sterile water with *A. baumannii* DT (square symbol), sterile water with *A. baumannii* DT (triangle symbol), and sterile water with *A. baumannii* DT supplemented with $(NH_4)_2SO_4$ and succinate (circle symbol)

Effects of soil components on the propanil degradation in soil

Soil samples collected from two sites had different components (Table 2). Corn field soil (treated soil) contained higher concentrations of silt, clay, total organic carbon, nitrogen and phosphorus, while soil from the mountain (untreated soil) contained a higher amount of sand and other minerals. Propanil was not detected in both soil samples (Table 2).

Figure 6 showed that the propanil concentrations decreased in all treatments. Propanil dissipation was lowest in mountain soil. The highest degradation was found in corn field soil, in which most propanil disappeared after 10 days. Inoculating *A. baumannii* DT into soil enhanced the degradation rates. Previous studies also reported quick transformations of propanil in soil (Burge, 1972; Milan et al., 2012). Degradation in untreated soil was slower compared to in

treated soil, probably due to the activities of native microorganisms. Microorganisms in treated soil were adapted to propanil and might also degrade the substrate.

Table 2. Chemical and physical properties of dried soil samples

Parameter	Soil collected from corn field	Soil collected from Nui Cam
Sand (%)	45.2 ± 4.54	60.8 ± 6.06
Silt (%)	25.4 ± 4.40	26.6 ± 4.52
Clay (%)	29.4 ± 4.44	12.7 ± 3.36
pH	6.8 ± 0.54	6.6 ± 0.36
Total Organic Carbon (%)	1.12 ± 0.11	0.76 ± 0.06
Nitrogen (%)	0.096 ± 0.01	0.055 ± 0.01
Phosphorus (%)	0.066 ± 0.01	0.097 ± 0.01
Potassium (%)	0.018 ± 0.00	0.028 ± 0.00
Zink (mg/kg)	0.55 ± 0.03	0.96 ± 0.06
Copper (mg/kg)	0.56 ± 0.07	0.90 ± 0.08
Iron (mg/kg)	9.4 ± 0.10	22.4 ± 0.17
Propanil (mg/kg)	0	0

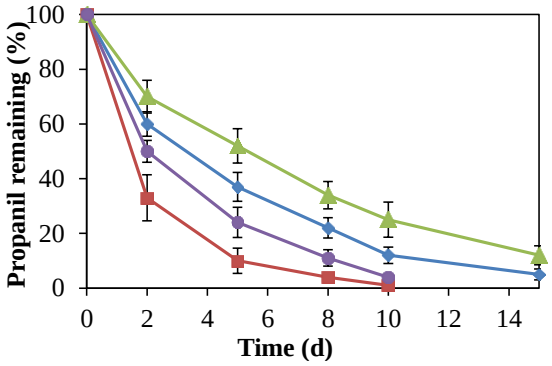

Figure 6. Propanil degradation in soil at 0.1 mM/kg soil. The treatments consisted of untreated soil without bacteria inoculation (triangle symbol), treated soil with inoculation with *A. baumannii* DT (circle symbol), treated soil without bacteria inoculation (diamond symbol), and treated soil inoculated with *A. baumannii* DT (square symbol)

CONCLUSION

The effects of various environmental factors on the propanil degradation by *A. baumannii* DT were evaluated. Higher levels of acidity, alkalinity and salinity reduced bacterial activities. Degradation was also slower in contaminated water than in MM medium or clean river water. Inoculating this isolate into untreated and treated soils increased degradation. Although the degradation and growth of *A. baumannii* DT was affected by extreme environmental conditions, this bacterial strain showed a good potential for propanil degradation in real environmental conditions.

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