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RESEARCH ON THE TREATMENT OF PIGGERY WASTEWATER FROM BIOGAS DIGESTER BY SBR PROCESS USING SELECTED AMMONIUM - AND NITRITE - CONVERTING BACTERIAL STRAINS

SUMMARY OF ENVIRONMENTAL ENGINEERING DOCTORAL THESIS

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INTRODUCTION

1. The necessary of the study

Pig farming activities have generated a serious threat to the environment, human health and natural ecosystems due to their generation of a huge a mount of wastewater, emissions, and solid waste. Among them, wastewater makes a big environmental concern because it negatively impacts on soil, water and air. According to statistics of the Ministry of Agriculture and Rural Development in 2020, pig farming activities discharged around 75 million cubic meters of wastewater into the environment (approximately 65,7% of the total volume of that of livestock industry). Furthermore, it contains diverse environmental pollutants such as BOD, COD, suspended solids (SS), nitrogen (N) and pathogenic microbes with extremely high concentrations compared to national regulation limitation for them. Nitrogen-containing compounds in wastewater have received the biggest concerns because they have many negative effects on the environment, human and animals health and their removal from wastewater is quite difficult.

The Sequencing Batch Reactor (SBR) technology is the most popuplar technology applied treat pig farming originated wastewate, especially nitrogen-containing compounds. The biological treatment of nitrogen in the SBR is carried out based on a combination of nitrification and denitrification. The conventional nitrification is usually performed by groups of autotrophic bacteria (*Nitrosomonas, Nitrobacter, ...*) but their biotranformation ability to nitrogen-containing pollutants are quite slow becasue they grow are very slow, strongly impacted by many the environmental factors such as pH, oxygen concentration and sun light and receive highly competitions from other bacterial groups. Many recent studies have also shown heterotrophic bacteria have a strong nitifying and denitrifying ability to nitrogen-containing compounds due to their fast growth, high competition with other bactrial groups and high tolerance for the environmental factors, espcially for very high concentration of nitrogen-containing compounds. Furthermore, they, can simultaneously nitrify and denitrify combined with organic matter removal. This indicates that they have a great potential to treat the wastewater contaminated with nitrogen-containing compounds.

Therefore, this study tried to isolate novel heterotrophic nitrifying bacteria groups having the ability to transform nitrogen-containing compounds in wastewater with the research theme ***“Research on the treatment of piggery wastewater from biogas digester by SBR process using selected ammonium - and nitrite - converting bacterial strains”***.

2. Thesis objectives

- To isolate, identify and select some indigenous heterotrophic bacterial strains capable of converting ammonium/nitrite from the slaughterhouse and piggy wastewaters after anaerobic treatment (by biogas digesters); investigate their optimal growth conditions and ability to metabolize ammonium/nitrite; and identify a number of suitable conditions (densities, combination ratios) for the application of the isolated strains into the pig farming wastewater treatment;

- To determine suitable conditions (ratio of anoxic/aerobic time, organic loading rate, nitrogen loading rate) and evaluate the treatment efficiency of SBR technology using the isolated purely bacterial strains in the simultaneous treatment of organic and nitrogen matter in pig farming wastewater after anaerobic treatment.

3. Study contents

Content 1: Isolation, identification and selection of some indigenous heterotrophic bacterial strains capable of biotransforming ammonium and nitrite from the slaughterhouse and piggy wastewater after anaerobic treatment.

Content 2: Determination of the optimal growth and ammonium/nitrite conversion ability of the isolated bacterial strains with different cultivation conditions (temperature, pH, dissolved oxygen (DO), salinity, initial ammonium/nitrite concentration).

Content 3: Determination of the appropriate microbial densities, comparison of ammonium/nitrite conversion ability, investigation of effective combination ratios of isolated strains for the simultaneous treatment of organic matter and nitrogen from pig farming wastewater after anaerobic treatment;

Content 4: Study on the treatment of pig farming wastewater after anaerobic digestion by the SBR technology using the isolated ammonium and nitrite metabolizing bacterial strains at the laboratory scale with the following contents:

- Study on the effect of anoxic/oxic time ratios on the removal efficiencies of COD, ammonium, nitrite, nitrate, and TN.
- Study on the effect of organic and nitrogen loading rates on the COD and TN removal efficiencies.

Chapter 1. LITERATURE OVERVIEW

1.1. Overview of pig farming wastewater in Vietnam

The average volume of wastewater generated by a pig is about 25-30 liters per day for both small and large–scale rearing practices. The concentration values for environmental parameters of the first are 3022 ± 597; 608 ± 87 and 342 ± 92 mg/L for COD, TN and TP, respectively while those of the latter are 860 - 4.590; 130 - 870; 170 - 900 and 250 - 295 mg/L for COD, N-NH_4^+, TN and TP, respectively. The results indicate that the concentration of two important environmental parameters, namely COD and TN are much higher than those of the national standard regulation. Therefore, the direct discharge of untreated piggy wastewater into the environment will cause serious environmental pollution, affecting human health and the life of aquatic organisms.

1.2. Overview of studies on pig farming wastewater treatment

Results of thirteen and fourteen studies in the world and in Vietnam, respectively on pig farming wastewater treatment show that:

(1) Most of the studies focus on the use of biotechnology to treat swine wastewater such as Wetland, UASB, biogas digester, and biofilter technologies which can treat the organic matter with treatment efficiencies of up to 80 - 95% but they show low removal efficiencies for nitrogen(about 30 - 60%).

(2) The sequencing batch reactor (SBR) can achieve high treatment efficiencies for organic matter and nitrogen (about 90-97%). However, the conventional SBR technologies have some limitations as follows: (i) the treatment efficiency of TN is unstable and depends on a number of factors such as ammonium concentration (NH_4^+-N concentration higher than 500 mg/L may inhibit microorganisms performing nitrification process, alkalinity and carbon/nitrogen ratio; (ii) requirement of additional carbon source for the anoxic process. This shows that the SBR technology is quite suitable for the treatment of pig farming wastewater.

1.3. Organic matter and nitrogen biotransformation in an SBR

1.3.1. Biotransformation of organic matter

- Organic matter oxidizing process (providing energy for cells):

$$C_xH_yO_zN + O_2 \xrightarrow[\text{anism}]{\text{Microorg}} CO_2 + NH_3 + H_2O + Q \text{ (energy)} \qquad (1\text{-}1)$$

- Cell synthesis Process (synthetic cell building)

$$C_xH_yO_zN + Q \xrightarrow[\text{anism}]{\text{Microorg}} C_5H_7NO_2 \quad \text{(cytoplasm)} \qquad (1\text{-}2)$$

($C_5H_7NO_2$: average ratio of the main elements in microbial cells).

- Intracellular oxidation process (autooxidation):

$$C_5H_7NO_2 + O_2 \xrightarrow[\text{anism}]{\text{Microorg}} CO_2 + NH_3 + H_2O + Q \qquad (1\text{-}3)$$

1.3.2. Biotransformation of nitrogen

Biotransformation of nitrogen-containing compounds in the SBR is carried out by autotrophic and heterotrophic bacteria for both nitrification and denification processes (Figure 1.1).

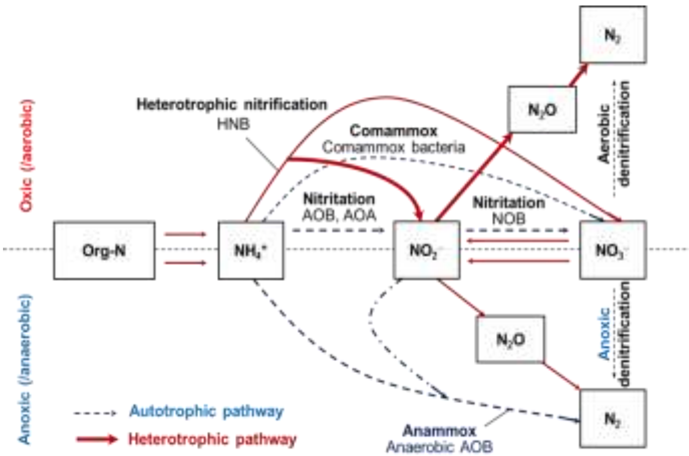

Figure 1.1. Possible microbial nitrogen (N) transformation pathways occurring in biological nitrogen removal (BNR) systems

Anammox: anaerobic ammonia oxidization; **Comammox**: complete ammonia oxidization; **HNB**: heterotrophic nitrifying bacteria; **AOA**: ammonia-oxidizing archaea; **AOB**: ammonia oxidizing bacteria; **NOB**: nitrite-oxidizing bacteria.

The nitrification process is often considered a rate-limiting step and is performed by two main groups of bacteria, autotrophic and heterotrophic. In there, autotrophic groups often grow weakly, are quite sensitive to environmental conditions and are subject to fierce competition from other groups of microorganisms, so the stability of treatment efficiency is not high, while heterotrophic groups are preferred than autotrophs such as: fast growing, can simultaneously nitrify and denitrify combined with removal of organic matter, some species can even tolerate cold, too salty or ammonium-rich environments. These advantages offer great potential for the application of trophic nitrifying bacteria groups for nitrogen treatment in wastewater.

1.4. Overview about Heterotrophic Nitrifying Bacteria (HBN)

- Nitrogen transformation pathways: In most cases, HNB can oxidize ammonia to gaseous nitrogen oxides or nitrogen gas by a full nitrification and denitrification pathway: $NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^-$, $NO_3^- \rightarrow NO$, $N_2O \rightarrow N_2$; Some HNB, can directly oxidize ammonia to nitrogen gas via hydroxylamine as an intermediate product rather than nitrite/nitrate ($NH_4^+ \rightarrow NH_2OH \rightarrow N_2O \rightarrow N_2$)

- Physiological-biochemical features: The carbon/nitrogen (C/N) ratio is suitable for nitrogen metabolism of HBN from 8 - 10; Optimal pH: 5-10; Optimal temperature: 20 - 40 ^oC. In addition, some species can also adapt to other special environmental conditions such as: can withstand cold up to 2 ^oC; salt tolerance at salinity up to 15% or 20%; High ammonium tolerance up to 1.000 mg/L or 2.000 mg/L.

Chapter 2. SUBJECTS AND METHODS

2.1. Subjects, scope and materials research

2.1.1. Research subjects

- *Wastewater:* Wastewater samples for microbiological isolation were collected from biogas digestion systems of a slaughterhouse and four pig farms in Ha Tinh province. Those for testing the treatment capacity of the isolated microbial strains was collected from biogas digestion tanks in several pig farms in Thua Thien Hue province.

- *Isolated bacterial strains:* ammonium/nitrite transformation bacterial strains were isolated and selected from slaughterhouse and piggy wastewater in Ha Tinh province.

- *SBR system:* Includes both anoxic and aerobic cycles

2.1.2. Research scope :

This research was carried out at bench scale at the Microbiology Technology Department of Hue Industrial College and the Hard Bee Hue Science Research and Technology Transfer Joint Stock Company.

2.1.3. Materials research:

All chemicals used for this study were provided by Merck company - Germany and Hanna company - Romania. All reagents have a purity of 99,0 - 99,9% for analytical and laboratory use.

2.2. Research methods

2.2.1. Sample collection and preservation

Wastewater samples for microbiological isolation were collected by specialized sterile plastic bags, and stored at 4 ^oC.

Wastewater samples for SBR operation were daily collected in 20-liter plastic bottles. The bottles were wrapped in black plastic bags to avoid direct sunlight during transportation to the laboratory.

2.2.2. Analytical methods

All environmnetal parameters were measured by standard methods using high-precicion measuringinstruments.

2.2.3. Culture and isolation of ammonium/nitrite transformation bacterial strains

Base mineral media were used to culture and isolate ammonium/nitrite-oxidizingbacterial strains. Bacterial colony formation was observed by bare eyes while the results of ammonium and nitrite transformation were measured by using Nessler and Griess reagents, respectively) with daily checked for a five-day cultivation period. Only isolation tubesthat are positive for the reagent to any degree will be selected for further isolation. The different bacterial colonies were separately and consectively subcultured to new series of cultivation tubes until achieving the pure colonies with uniformity in shape and color. The ammonium/nitrite transformation of the colonies during isolation was checked to eliminate colonies that were not ammonium/nitrite transformation bacteria.

2.2.4. Gram staining method

2.2.5. Bacterial identification

Isolated pure strains were identified by PCR amplification and sequencing of genes encoding 16S rRNA and looked up by BLAST tool.

2.2.6. Investigation of the influence of culture media on the growth and ammonium/nitrite transformation of the isolated bacterial strains

Isolated pure bacterial strains were sharing cultivated with initial microbial density at 106 CFU/mL in a 250 mL conical flask containing 100 mL of 50 mg/L of ammonium/nitrite-containing mineral medium.

- *pH at the values of 5.0; 6.0; 7.0; 8.0; 8.5*
- *Temperature at the values 5; 30; 37; 45; 50^{o}C*
- *DO at the values of 0.1; 4.5; 7.0 mg/L*
- *Salinity: at the values of 1.0; 3.0 and 5.0%*
- *Ammonium/nitrite concentrations: 100; 500 and 750 mg/L.*

2.2.7. Investigation of the treatment ability of pig farming wastewater after biogas digestion by the isolated ammonium/nitrite transformation bacterial strains

2.2.7.1. The effect of microbial density:

1.5 liter of 400 mg/L ± 20 mg/L ammonium/nitrite-containing digested piggy wastewater in a three-liter plastic aeration reactor was spiked with the isolated ammonium/nitrite-oxidizing bacterial strains with a microbial density in the range of 10^3 - 10^6 CFU/mL to investigate their biotransformation ability to ammonia/nitrite. The DO and pH in the reactor were maintained at 4 - 6 mg/L and 7.0 - 7.5, respectively. A negative control was carried out the same but without supplying bacteria. The cultures were investigated for biotransformation of ammonium/nitrite by analyzing three times for the first three-cultivation day period.

2.2.7.2. Comparison of biotransformation ability of ammonium/nitrite:

The experiments were set up the same as those in *subsection 2.2.7.1*. Combinations of isolated ammonium/nitrite transformation bacterial strains in the same ratio with the optimal density determined in the above experiments were used in this study.

2.2.7.3. Investigation of the optimal microbial combination ratios:

A reactor as described in *subsection 2.2.7.1* was used in this study. Wastewater for the experiments was piggery wastewater after biogas digestion with TN of 400 ± 20 mg/L and COD of 1600 ± 80 mg/L. The combination ratios of isolated ammonium oxidizing strains to nitrite transformation strains were 1 : 0, 1 : 1, 2 : 1 and 3: 1.

2.2.7.4. Research on the treatment of piggery wastewater from biogas digester by SBR process using selected ammonium - and nitrite - converting bacterial strains

(1) SBR system:

Table 2.1. The equipment of SBR system

No.	Equipment	Parameter/Function
1	SBR system	- Material: PVC, volume (**V_{SBR}**): 10 liter
2	Air supplier	- Air supply speed: ~ 2 liters/minute - the automatic air suppliier was run and controlled by a timer
3	Piggy wastewater supplying pump	- Metering pump, the automatical pump was controlled by a timer
4	Stirring system	- The suction machine pushes the water to create a vortex to stir, automatic stirrer according to the time set via the timer socket in the experimental modes
5	Electric motor valve	- Diameter: 21 millimeters, automatic discharge of wastewater after treatment according to the time set through the timer socket in the experimental modes
6	Mechanical valve	- Made of PVC, Diameter: 21 millimeters, Discharge excess mud
7	Timer	- Automatically (turn on/off) system devices
8	pH, DO	-Periodic measurements with separate equipment

Figure 2.1. Image of SBR system

(2) Characteristics of piggy wastewater used in SBR system

Table 2.2. Characteristics of piggy wastewater used in the study

No.	Parameters	Range
1	pH	7,0 - 7,5
2	COD_{Cr} (mg/L)	1500 - 2000
3	NH_4^+-N (mg/L)	300 - 400
4	TN (mg/L)	400 - 500

A combination of ammonium/nitrite oxidizing bacteria strains with the optimal density and ratio obtained in the above experiments were added in to the SBR system. DO during the aeration phase was 4 - 6 mg/L, and no pH adjustment was conducted.

(3) Operation Modes/experimental conditions:

- Microbial adaptation period: The bacteria with optimal densisity and ratio and 250 mg/L ± 25 of TN-containing diluted piggy wastewater were applied for this experiment. The air is continuously supplied with a flow rate of 2 liters/min. Input wastewater was supplied with a volume of 4 liters/day (divided into 6 batches of 0.67 liters each, supplying for 10 minutes, settling for 60 minutes. Output wastewater after treatment was discharged with the same volume as the input at the same time. During the time of the study, the experimental conditions remained and the tested wastewater was taken for analysis of the total of nitrogen and the results of TN analysis were compared to the value of this environmental parameter of the level B of national standard regulation on livestock wastewater (QCVN 62-MT:2016/BTNMT). When the tested and standard values were equal, the non-diluted swine wastewater was continuously added to the SBR system for investigation of the biotransformation ability of the isolated pure bacterial strains. Their biotransformation ability was evaluated by analyzing the environmental parameters (COD, N-NH_4^+, N-NO_2^-, N-NO_3^- and TN) of the output wastewater after treatment once per day. Aerobically experimental modes were set up for the SBR system as follows

Table 2.3. Aerobically experimental modes of the SBR system

Fill and draw at the same time (minute)	**Anoxic** (minute)	**Aerobic** (minute)	**Settle** (minute)	**Note**
10	100	100	30	Mode 1
	70	130		Mode 2
	40	160		Mode 3

The treatment efficiency of the different experimental modes above was evaluated by comparing the results of the environmental parameters analysis.

Effect of NLR, OLR on wastewater treatment efficiency:

The effects of OLR and NLR on biotransformation activities of isolated bacterial strains were investigated under the optimal conditions for the best TN removal efficiency.

Table 2.4. The respective NLR and OLR were investigated

NLR (kg/m^3.day)	**0,15**	**0,2**	**0,25**	**0,3**
OLR (kg/m^3.day)	0,6	0,8	1	1,2
Q_{day} (L/day)	**3**	**4**	**5**	**6**
$Q_{\text{mẻ}}$ (L/$_{batch}$)	0,5	0,67	0,83	1
HRT (day)	**3,3**	**2,5**	**2**	**1,7**

COD and TN were analysed for evaluation of treatment efficiency

2.2.8. Calculation and data processing methods

The analytical data are calculated, processed, and presented as mean ± standard deviation on graphs using Microsoft Excel software.

Chapter 3. RESULTS AND DISCUSSION

3.1. Isolation and identification some ammonium/nitrite transformation bacterial strains from wastewater

- Four ammonium transformation bacterial strains were isolated and identified, named: *Bacillus megaterium* HT1; *Bacillus licheniformis* HT1; *Bacillus subtilis* HT1; *Pseudomonas aeruginosa* HT1

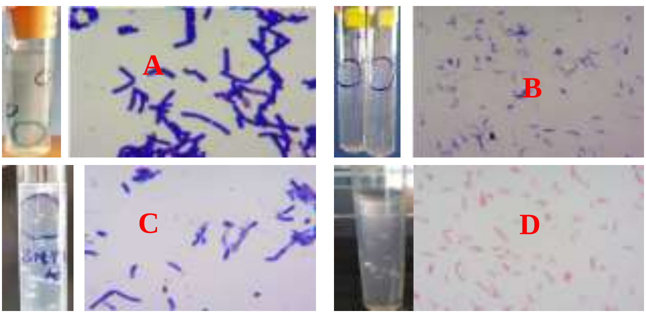

Figure 3.1. Image of colony in test tube and Gram stain of:
B. megaterium HT1 (**A**)*; B. licheniformis* HT1 (**B**);
B. subtilis HT1 (**C**); *P. aeruginosa* HT1 (**D**)

- Two ammonium transformation bacterial strains were isolated and identified: *Lactobacillus fermentum* HT2 *và Pseudomonas stuzeri* HT2.

Figure 3.2. Image of colony in test tube and Gram stain of: *P. stutzeri* HT2 (**A**); *L. fermentum* HT2 (**B**)

3.2. Effect of culture medium on the growth and ammonium/nitrite transformation of isolated bacterial strains

3.2.1. Ammonium transformation bacterial strains

3.2.1.1. Effect of pH

B. megaterium HT1; *B. licheniformis* HT1; *B. subtilis* HT1; *P. aeruginosa* HT1 showed the best growth and ammonium transformation on the medium with pH from 7,0 to 7,5 after two to three days of culture. They completely transformation 50 mg/L of ammonium in three days of culture (Figure 3.3; Figure 3.4; Figure 3.5; Figure 3.6)

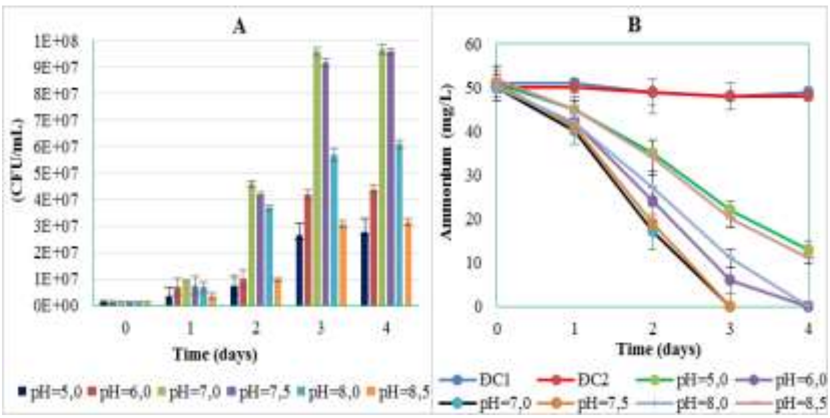

Figure 3.3. Effect of pH on growth (**A**) and ammonium transformation (**B**) of *B. megaterium* HT1

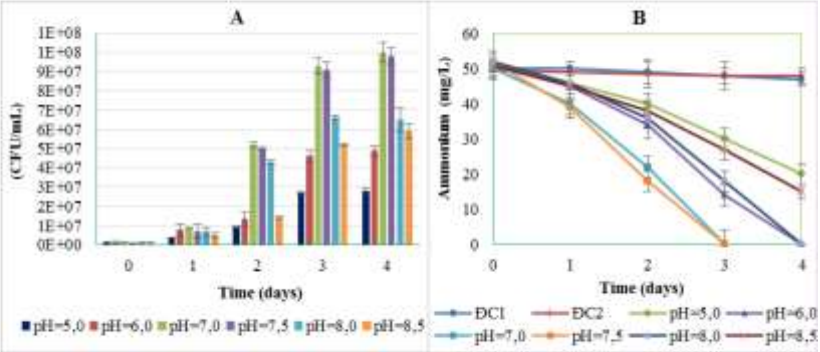

Figure 3.4. Effect of pH on growth (**A**) and ammonium transformation (**B**) of *B. licheniformis* HT1

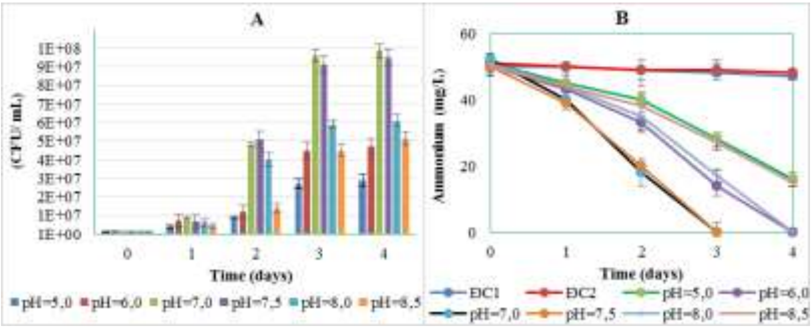

Figure 3.5. Effect of pH on growth (**A**) and ammonium transformation (**B**) of *B. subtilis* HT1

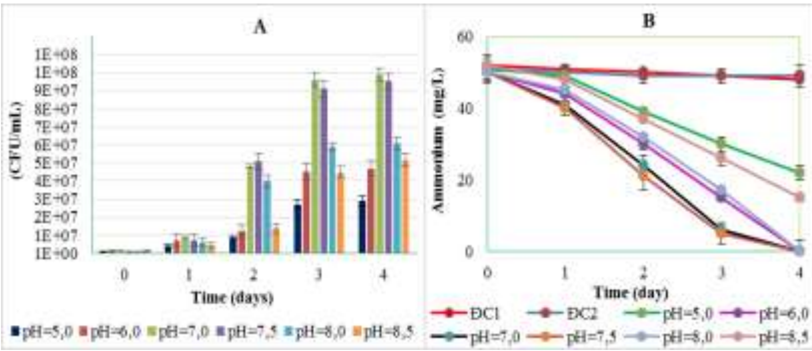

Figure 3.6. Effect of pH on growth (**A**) and ammonium transformation (**B**) of *P. aeruginosa* HT1

3.2.1.2. Effect of DO and temperature

The aerobic environmental cultivation is suitable for growth and ammonium transformation of *B. megaterium* HT1*; B. licheniformis* HT1*; B. subtilis* HT1*; P. aeruginosa* HT1. Low DO (0,1 mg/L) the strains still grow and ammonium transformation, showing that they are adapted good with adverse conditions (low oxygen)

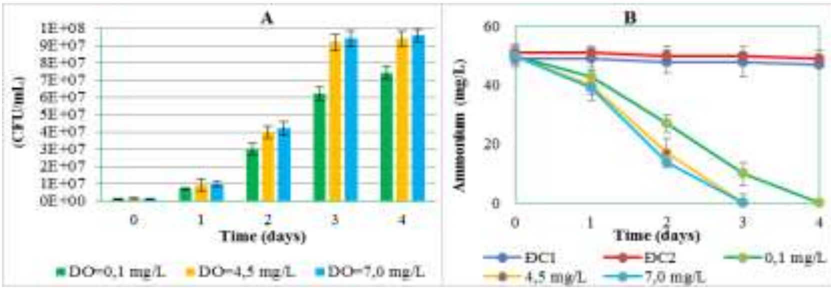

Figure 3.7. Effect of DO on growth (**A**) and ammonium transformation (**B**) of *B. megaterium* HT1

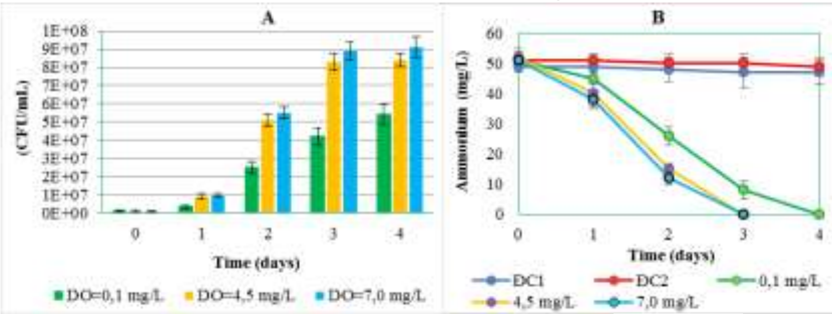

Figure 3.8. Effect of DO on growth (**A**) and ammonium transformation (**B**) of *B. licheniformis* HT1

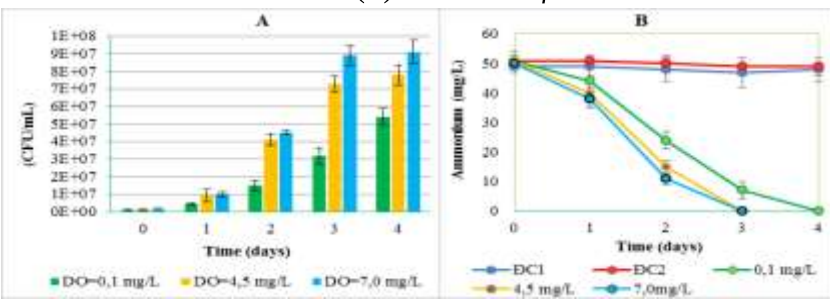

Figure 3.9. Effect of DO on growth (**A**) and ammonium transformation (**B**) of *B. subtilis* HT1

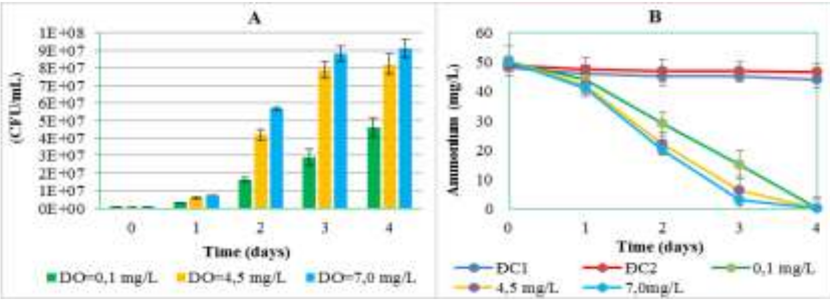

Figure 3.10. Effect of DO on growth (**A**) and ammonium transformation (**B**) of *P. aeruginosa* HT1

Four strains of *B. megaterium* HT1; *B. licheniformis* HT1; *B. subtilis* HT1; *P. aeruginosa* HT1 isolated were all mesophilic bacteria with the preferred growth temperature range of 30 - 37 0C.

3.2.1.3. Effect of salt concentration

Four bacterial strains were isolated: *B. megaterium* HT1; *B. licheniformis* HT1; *B. subtilis* HT1; *P. aeruginosa* HT1 showed their ability to grow and ammonium transformation in the environment with salinity up to 3.0%.

3.2.1.4. Effect of initial ammonium concentration:

Four isolates of bacteria were able to well grow in the media spiked with 100; 500 and 750 mg/L of ammonium, the maximal growth rate was achieved after 2 to 3 days of inoculation. All the isolated cultures also showed that their biotransformation was found in the 740 mg of ammonium-spiked cultures after a five-day-cultivation period while many previous studies indicated that most isolated ammonium-oxidizing bacteria were inhibited with the concentration of ammonium over 150 mg/L.

The isolated pure ammonium-oxidizing strains were also investigated for nitrate formation by cultivating them with three concentrations of ammonium, namely 100; 500; and 750 mg/L. The results showed that both nitrite and nitrate were found in all of the cultures with concentrations of nitrite from 20-80 mg/L and 45-280 mg/L for nitrate. Further experiments with the cultures containing the isolated pure bacterial strains and only 50 mg/L of nitrite but without ammonium. The results showed that nitrite completely disappeared after three days of cultivation while this compound was found in the negative controls.

In this study, a novel bacterial strain of *Pseudomonas aeruginosa* was isolated and showed its biotransformation ability of ammonium and named *P. aeruginosa* HT1. This indicates the biodiversity of ammonium-oxidizing bacteria in nature. However, this strain was not selected for further studies due to the disease-causing bacterium.

3.2.2. Nitrite transformation bacterial strains

3.2.2.1. Effect of pH

The bacterial pure strain of P. stutzeri HT2 could grow and transform nitrite with pH range from 6,0 - 8,5 but the optimal range was from 7,0 - 8.0 (Figure 3.11).

L. fermentum HT2 strain could grow and transform nitrite in pH range from 5,0 - 8,8 but the optimal range was from 6,0 - 7.0 (Figure 3.12)

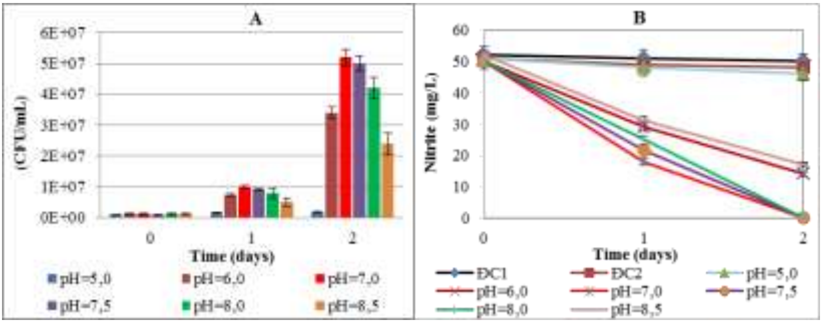

Figure 3.11. Effect of pH on growth (**A**) and nitrite transformation (**B**) of *P. stutzeri* HT2

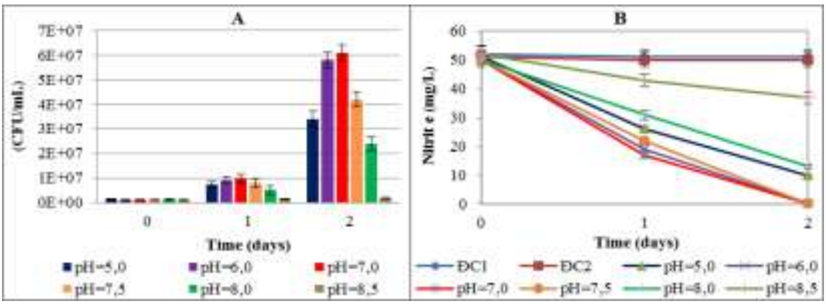

Figure 3.12. Effect of pH on growth (**A**) and nitrite transformation (**B**) of *L. fermentum* HT2

3.2.2.2. Effect of DO and temperature

P. stuzeri HT2 and *L. fermentum* HT2 could grow and transform nitrite at very low DO levels (0,1mg/L)

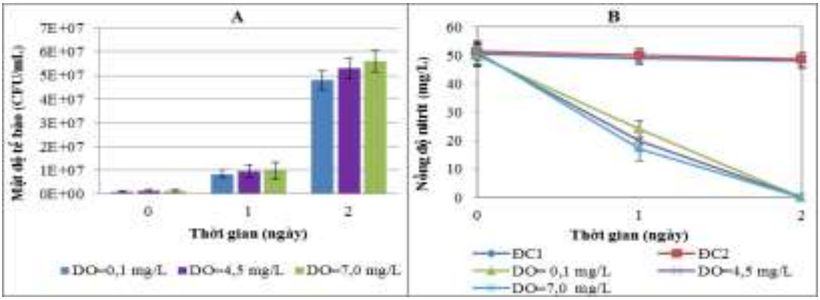

Figure 3.13. Effect of DO on growth (**A**) and nitrite transformation (**B**) of *P. stutzeri* HT2

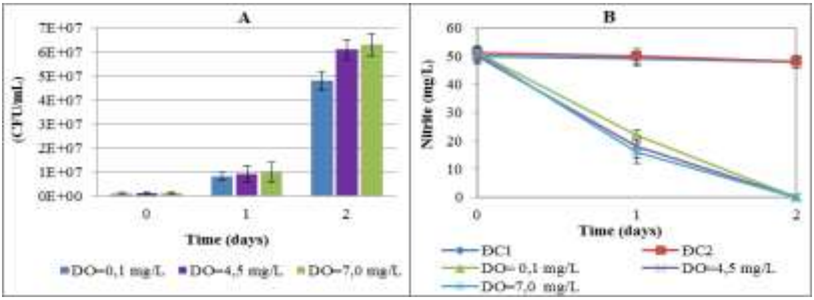

Figure 3.14. Effect of DO on growth (**A**) and nitrite transformation (**B**) of *L. fermentum* HT2

P. stutzeri HT2 và *L. fermentum* HT2 are mesophilic strains, capable of growing and nitrite transformation at the following temperatures: 30; 37, 45, and 50 ^{o}C, of which the best is at 37 ^{o}C.

3.2.2.3. Effect of salt concentration

P. stuzeri HT2 and *L. fermentum* HT2 could grow and convert nitrite in cultureswith salinity up to 3.0%.

3.2.2.4. Effect of initial nitrite concentration:

P. stutzeri HT2 and *L. fermentum* HT2 completely converted 750 mg/L of nitrite after four days of cultivation. The biotranformation activity was not found in the negative controls. This confirmed that the isolated pure cultures were reponsibility for nitrite tranformation.

When two strains of *P. stutzeri* HT2, *L.fermentum* HT2 were inoculated to the medium containing nitrite, the results revealed that nitrate was not found but nitrite was gone after three days of incubation. Further experiments on the two strains in the medium containing 50 mg/L of nitrate without nitrite, the results showed that nitrite was found during incubation but it disappeared after five days of inoculation. When they were tested in the shakes containing both nitrite and nitrate supplied medium, the air bubbles appeared and were evenly distributed in the shakes with the complete disappearance of both nitrite and nitrate. The results indicated that two pure strains of *P. stutzeri* HT2 and *L. fermentum* HT2 could reduce bothe nitrite and nitrate.

Figure 3.15. Air bubbles test-tube of *P. stutzeri* HT2 and *L. fermentum* HT2

The biotransformation ability of *P. stutzeri* HT2 and *L. fermentum* HT2 indicated the biodiversity of ammonium-oxidizing bacteria in nature and their great potential in the nitrogen-containing contaminants in wastewater due to their high-competitive characteristics

3.3. Investigation the ability treated pig farming wastewater after biogas tank by isolated ammonium, nitrite transformation bacterial strains

3.3.1. Effect of initial microbial density on ammonium metabolism

The initial microbial density are 10^5 CFU/mL and 10^6 CFU/mL, the ammonium metabolism of all three strains (*B. megaterium* HT1; *B. licheniformis* HT1; *B. subtilis* HT1) was much higher than other microbial density (10^3 - 10^4 CFU/mL and control experiment). Treatment efficiency after 3 days is 100% at 10^5 CFU/mL and 10^6 CFU/mL microbial density; while at 10^4 CFU/mL and 10^3 CFU/mL microbial densities are 85 - 87 % and 74 - 77 %, the control experiment is 24 - 27 %.

The ammonium treatment efficiency between 10^5 CFU/mL and 10^6 CFU/mL of the three strains was almost the same, so the initial microbial density supplement level of 10^5 CFU/mL wastewater was chosen to apply 3 strains that treated pig farming wastewater after biogas tank.

3.3.2. Comparison of ammonium biotransformation ability of separated and combined strains

There was no difference about removal efficiency of ammonium in pig wastewater after biogas tank of the three strains, but the ammonium removal efficiency of the single strain was 11 - 14% lower than the combination of three strains. Therefore, using a combination of three strains with a 1:1:1 ratio at a density of 10^5 CFU/mL is the most optimal for ammonium treatment in swine wastewater.

3.3.3. Effect of initial microbial density on nitrite transformation

The initial microbial density are 10^5 CFU/mL and 10^6 CFU/mL, the nitrite metabolism of all two strains (*P. stutzeri* HT2, *L. fermentum* HT2) was much higher than other microbial density (10^3 - 10^4 CFU/mL and control experiment). Treatment efficiency after two days is 100% at 10^5 CFU/mL and 10^6 CFU/mL microbial density; while at 10^4 CFU/mL and 10^3 CFU/mL microbial densities are 62 - 69% and 38 - 44%, the control experiment is 15 %.

The nitrite treatment efficiency between 10^5 CFU/mL and 10^6 CFU/mL of the two strains was almost the same, so the initial microbial density supplement level of 10^5 CFU/mL wastewater was chosen to apply two strains that treated pig farming wastewater after biogas tank.

3.3.4. Comparison of nitrite biotransformation ability of separated and combined strains

There was no significant difference in nitrite biotransformation efficiency of *L. fermentum* HT2 and *P. stutzeri* HT2 when they were separately tested. There was yet a significant increase of 12% in nitrite biotransformation efficiency when combining the two strains. Therefore, a mixture of the two strains with a 1:1 ratio at a density of 10^5 CFU/mL was chosen for further experiments of nitrite treatment in swine wastewater.

3.3.5. Investigation of the mixing ratio of isolated ammonium and nitrite transforming bacterial trains to wastewater treatment efficiency

The mixtures made by mixing a mixture of three isolates of ammonium-transforming bacteria (1:1:1) and a mixture of two nitrite-transforming bacterial strains (1:1) with three ratios of 1:0; 1:1; 2:1; 3:1, respectively were cultivated with swine wastewater to investigate their pollutants treatment capacity.

For COD, it was completely removed in all the cultures inoculated with an ammonium or nitrite-transforming strains mixture or a mixture of ammonium and nitrite-transforming strains after 72 hours of incubation while the figure of the negative control was 36%.

The results showed that TN was maximally removed (95%) in the cultures containing the ratio of 2:1 after 60 hours of incubation.

Therefore, the mixture with the ratio of 2:1 was chosen for further studies.

3.3.6. Treatment of swine wastewater after anaerobic treatment by SBR technology applying isolated ammonium and nitrite transforming bacterial strains

3.3.6.1. Effect of discontinuously aeration

- *Effect of aeration mode on COD removal efficiency:* The results indicated that the aeration modes did not have a significant effect on COD treatment efficiency. However, the highest efficiency of COD treatment was found in the third mode with 83-85% of efficiency.

- *Effect of aeration mode on N removal efficiency:*

+ N-NH_4^+ removal efficiency: Similarly, the highest N-NH_4^+ treatment efficiency was also found in the third mode at 75-80% and the efficiency was proportional with aeration time:

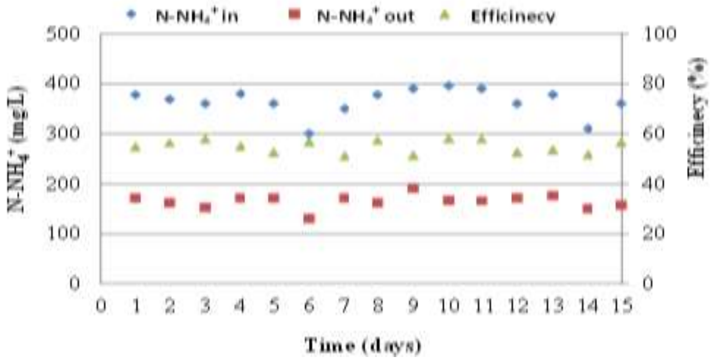

Figure 3.16. N-NH_4^+ treatment efficiency in one mode

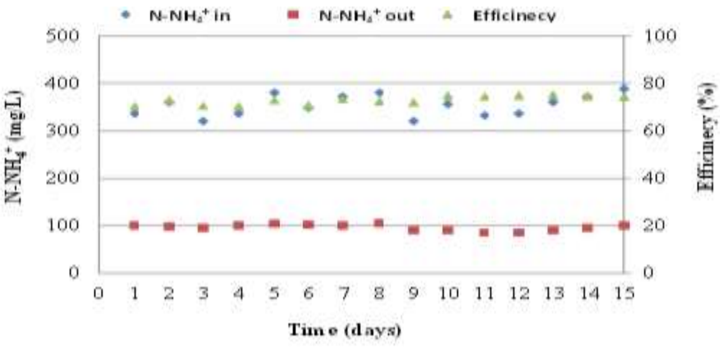

Figure 3.17. N-NH_4^+ treatment efficiency in two mode

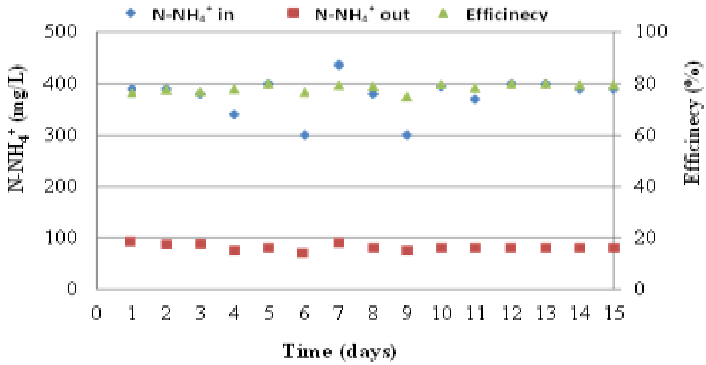

Figure 3.18. N-NH_4^+ treatment efficiency in three mode

+ Nitrate/nitrite reduction efficiency: Changing the aeration/anoxic mode, the efficiency of nitrate/nitrite reduction between aeration modes is different. Mode one: N-NO_2^- and N-NO_3 very low after treatment: 10 -15 mg/L. The second mode, especially the third mode, the amount of NO_3^- was very high compared to the first mode. N-NO_2^- was very low in all 3 modes, it can be seen that NO_2^- has been eliminated effectively.

+ TN treatment efficiency: The highest efficiency of TN removal was found in the second mode with 72%, the first came next with 60% and the least was 54% for the third.

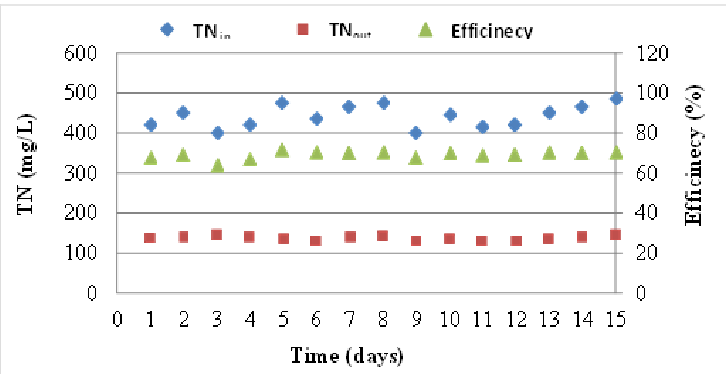

Figure 3.19. TN treatment efficiency of the first mode

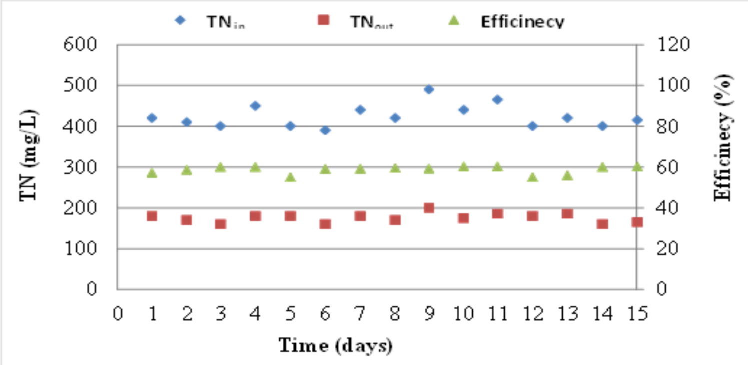

Figure 3.20. TN treatment efficiency in two mode

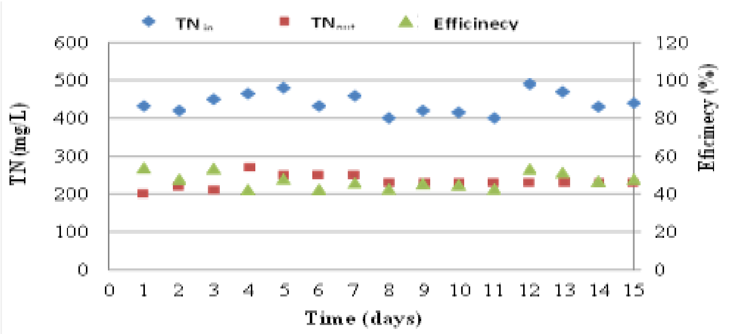

Figure 3.21. TN treatment efficiency of three modes

Comparison of COD, N-NH_4^+ and TN treatment efficiency among the study modes was summarized in Table 3.1.

Bảng 3.1. Comparison of COD, N-NH_4^+ and TN treatment efficiency among the investigated modes

Study mode	**Treatment efficiency (%)**		
	COD	**NH_4^+**	**TN**
Fisrt mode	68 - 75	50 - 58	55 - 60
Second mode	78 - 84	70 - 75	64 - 72
Third mode	83 - 85	75 - 80	42 - 54

The results revealed that the second mode was the best one with anaerobic/aerobic: 70/130 minutes and was chosen for further studies as well as practical application treatment of swine wastewater after anaerobic treatment.

3.3.6.2. Effect of NLR, OLR on wastewater treatment efficiency

- *COD treatment efficiency*: OLR varied in the range of 0.6 - 1.2 kg/m[3]/day with 04 steps (0.6; 0.8; 1.0 and 1.2 kg/m[3]/day), COD treatment efficiency is almost unchanged, ranging from 78 - 85%.

- *TN treatment efficiency:* NLR increased gradually from 0.15; 0.20; 0.25 and 0.3 kg/m[3]/day**,** the TN treatment efficiency gradually decreased, respectively**:** 71 - 75%; 68 - 70%; 65 - 68% and 63 - 65%

The results of the effect of NLR, OLR on wastewater treatment efficiency were shown in Table 3.2.

Table 3.2. Effect of NLR, OLR to wastewater treatment efficiency

OLR (kg COD/m^3.day)	0,6	0,8	1,0	1,2
COD treatment efficiency (%)	78 - 85			
NLR (kg TN/m^3.day)	0.15	0.20	0.25	0,30
TN treatment efficiency (%)	71 - 75	68 - 70	65 - 68	63 - 65

CONCLUSIONS AND RECOMMENDATIONS

1. CONCLUSIONS

1) Four indigenous heterotrophic ammonium–oxidizing bacterial strains from wastewater after biogas of of cattle slaughterhouses and some pig farms in Ha Tinh, named *B. megaterium* HT1, *B. licheniformis* HT1, *B. subtilis* HT1 and *P. aeruginosa* HT1. These strains could grow and oxidized ammonium in the hostile media with poor nutrition, low DO (0,1 mg/L), salinity up to 3% and high ammonium concentration, which show their overwhelming advantages over other autotrophic ammonium-oxidizing bacterial groups (*Nitrosomonas, Nitrobacter, ...*). In addition, they had a great ability to oxidize ammonium up to 750 mg/L after 05 days of incubation to nitrite as an intermediate before they continued to oxidize nitrite to nitrate as the final product.

2) Two indigenous heterotrophic nitrite transforming bacterial strains from wastewater after biogas of cattle slaughterhouses and some pig farms in Ha Tinh, named *L. fermentum* HT2 và *P. stuzeri* HT2. They converted nitrite through a reduction process with a concentration of nitrate up to 750 mg/L after 04 days of incubation. The two strains could grow and transform nitrite in unfavorable media with low DO (0.1 mg/L), salinity up to 3% and high nitrite concentration.

3) A combination of three strains of ammonium-oxidizing bacteria *B. megaterium* HT1, *B. lichniformis* HT1 và *B. subtilis* HT in a ratio of 1: 1 : 1 showed a significant ammonium treatment efficiency higher than that of a single strain. The same was repeated with nitrite-

transforming bacterial strains, a combination of two strains of *L. fermentum* HT2 and *P. stuzeri* HT2 in a ratio of 1: 1 showed nittire treatment efficiency was significantly higher than that of a single strain. The optimal mixing ratio of two groups of ammonium and nitrite transforming bacteria for the nitrogen removal process in swine wastewater was 2 : 1.

4) The results of research on treatment of swine wastewater after anaerobic treatment by SBR combined with the addition two groups isolated of ammonium and nitrite-metabolizing bacteria, the first step shows: (i) The aeration time/ did not significantly impacton COD removal, but had a significant effect on ammonium and TN removal. The optimal ratio is 130 : 70 (minutes); (ii) The OLR did not significantly affect the COD treatment efficiency, while the TN treatment efficiency decreased in the range of 75 - 63% when increasing the NLR in the range of 0.15 - 0.30 kg-N/m^3/day.

2. RECOMMENDATIONS

1) Continue to investigating evaluating the treatment efficiency of the combination of bacterial strains isolated from this study in the swine wastewater treatment systems using SBR technology in situ.

NEW CONTRIBUTIONS OF THE THESIS

1. Completely isolating, selecting and identifying four heterotrophic ammonium metabolizing bacterial strains (*Bacillus megaterium* HT1, *Bacillus licheniformis* HT1, *Bacillus subtilis* HT1 and *Pseudomonas aeruginosa* HT1) and two heterotrophic nitrite metabolizing bacterial strains (*Lactobacillus fermentum* HT2 and *Pseudomonas stutzeri* HT2) from wastewater after biogas of cattle slaughterhouses and some piggy farms in Ha Tinh, these strains of bacteria can completely metabolize high concentrations of ammonium and nitrite (750mg/L) after 4 to 5 cultured days. Simultaneously, they can grow and effectively metabolize in some adverse environmental conditions such as: poor nutrition, low DO (0.1 ≥ mg/L), salinity up to 3%.

2. Determining the appropriate mixing ratio of two ammonium and nitrite metabolizing relected bacteria groups is 2:1 to simultaneously remove of nitrogen and organic matter in pig farming wastewater after biogas, in which, the ratio of *Bacillus megaterium* HT1: *Bacillus licheniformis* HT1: *Bacillus subtilis* HT1 are 1:1:1; and the ratio of *Lactobacillus fermentum* HT2 : *Pseudomonas stuzeri* HT2 are 1:1. Firstly, we are able to evaluate effectiveness COD and TN treatment in

pig farming wastewater after biogas by SBR system in combination with selected strains according to appropriate mixing ratio (2:1) in several operating modes including: Changing the aeration - non aeration time ratio (100/100 minutes, 130/70 minutes and 160/40 minutes) brings the COD treatment efficiency from 68 - 85% and the best TN treatment efficiency from 64 - 72%, at the rate of 130/70 minutes, the results of TN analysis after treatment meets level B of national technical regulation on the effluent of livestock (QCVN 62-MT:2016/BTNMT); Increasing the NLR in the range of 0.15 - 0.30 kg-N/m^3/day the TN treatment efficiency decreased in the range of 75 - 63%.

LIST OF PUBLISHED ARTICLES

1) Nguyen Huu Dong, Nguyen Thi Viet, Dinh Thi Thu Hang, Phan Do Dung, Nguyen Quang Lich, Tran Hoa Duan (2019), *Nitrification of ammonium by pseudomonas aeruginosa strain ht1 isolated from wastewater after biogas treatment of an industrial pig farm in Ha Tinh province*, Hue University Journal of Science: Agriculture and Rural Development, ISSN 2588-1191, Vol. 128, No. 3C, 119-132; DOI: 10.26459/hueuni-jard.v128i3C.5282.

2) Nguyen Huu Dong, Nguyen Thi Viet, Dinh Thi Thu Hang, Phan Do Dung, Nguyen Quang Lich, Tran Hoa Duan (2022), *Research on oxidnitrification of ammonia by three bacterial strains belonging to genus bacillus isolated from wastewater of biogas tanks of industrial pig farms*, Hue University Journal of Science: Earth Science and Environment, ISSN 2588-1183, Vol. 131, No. 4A, 5-20; DOI: 10.26459/hueunijese.v131i4A.6759.

3. Nguyen Huu Dong, Nguyen Thi Viet, Dinh Thi Thu Hang, Phan Do Hung, Tran Hoa Duan (2022), *Ammonia oxidation capacity of bacillus bacteria in swine wastewater after biogas treatment*, Hue University Journal of Science: Natural Science: pISSN 1859 -1388, eISSN 2615 - 9678, Indexing: ACI (Asean Citation Index), Vol. 131, No. 1D, 77-87; DOI: 10.26459/hueunijns.v131i1D.7006;

4. Dinh Thi Thu Hang, Nguyen Thi Viet, Phan Do Hung, Tran Hoa Duan, Nguyen Dang Giang Chau, **Nguyen Huu Dong** (2022), *Nitrite metabolism of several bacterial strains isolated from abattoir and swine wastewater after biogas treatment*, Hue University Journal of Science: Natural Science: pISSN 1859 -1388, eISSN 2615 - 9678, Indexing: ACI (Asean Citation Index), Vol. 131, No. 1D, 105-114; DOI: 10.26459/hueunijns.v131i1D.7006.