

**EFFECTS OF BIOGAS SLURRY ON *CAPSICUM* SPP. GROWTH AND  
CONTROL OF SOIL-PATHOGENS**

by

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## PREFACE

The research contained in this thesis was completed by the candidate while based in the School of Life Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The research was financially supported by the National Research Foundation (NRF).

The contents of this work have not been submitted in any form to another university and, except where the work of others are acknowledged in the text, the results reported are due to investigations by the candidate.



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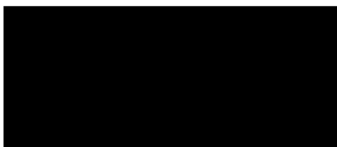
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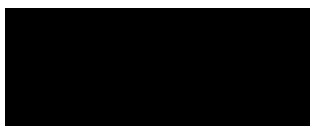
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- (i) the research reported in this thesis, except where otherwise indicated, is my original research;
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## DECLARATION 2: PUBLICATIONS

My role in each paper and presentation is indicated. The \* indicates the corresponding author.

### Chapter 3

1. Wang Z, Zhang L, Sun G, Zhou W, Sheng J, Ye X, Olaniran AO and Kana EBG\* 2022. Adsorption characteristics of three types of soils on biogas slurry ammonium nitrogen. *Frontiers in Environmental Science* 10:942263. <https://doi.org/10.3389/fenvs.2022.942263>.

This original research paper was published on 21 June 2022. The experiment was conceptualized, designed and carried out in the laboratory by Wang Zichen, Wang Zichen also collected, analyzed the data and wrote the paper under the guidance of the other authors.

### Chapter 4

2. Wang Z, Sun G, Zhang L, Zhou W, Sheng J, Ye X\*, Olaniran AO\*, Kana EBG, Shao H 2022. Aging characteristics and fate analysis of liquid digestate ammonium nitrogen disposal in farmland soil. *Water* 14(16):2487. <https://doi.org/10.3390/w14162487>.

This original research paper was published on 12 August 2022. The experiment was designed and carried out in the laboratory by Wang Zichen, Wang Zichen also collected, analyzed the data and wrote the paper under the guidance of the other authors.



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Date: November 2022

## ABSTRACT

The biogas slurry discharged from intensive large-scale livestock and poultry farms, and biogas projects was more than 1.3 billion tons each year in the world. It has exceeded the maximum fertilizer carrying capacity of plants growth on limited nearby farmland when used as fertilizer, and its application is greatly affected by agricultural seasonality. It is therefore necessary to explore alternative treatments for more efficient utilization of biogas slurry. This raises the possibility of using biogas slurry in soil pretreatment for continuous cropping in protected land to achieve a dual purpose of efficient digestion of biogas slurry per unit area of farmland, and prevention and control of soil-borne diseases in protected land. Additionally, there is a lack of knowledge on the maximum amount ( $Q_m$ ) of biogas slurry ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) that could efficiently be absorbed by the soil. There is also a scarcity of reports on the fate of liquid digestate ammonium nitrogen disposal in farmland soil. It is imperative to know the fate of liquid digestate ammonium nitrogen as well as the prevention and control of continuous cropping obstacles such as soil-borne diseases for subsequent crops cultivation. In this study, indoor simulation and field experiments were carried out to investigate the effects of biogas slurry on *Capsicum* spp. growth and control of soil-borne diseases under controlled environment. Firstly, the adsorption of biogas slurry  $\text{NH}_4^+\text{-N}$  of three types of soils (silty loam, loam, and sandy loam) was investigated in a composite kinetic process that comprised two stages of rapid and slow reactions. Rapid adsorption predominantly occurred within 0–1 h, and the adsorption capacity accounted for 35.24–43.55% of the total adsorption. The ExpAssoc equation produced a good fit for the adsorption kinetic behaviour in the three soil types. The equilibrium adsorption were described by the Langmuir equation, the Freundlich equation, the PIPlatt model, and the Langevin model isotherm, among which the Langevin model had the best fit. The theoretical saturated  $Q_m$  fitting results of  $\text{NH}_4^+\text{-N}$  were 1038.41–1372.44 mg/kg in silty loam, 840.85–1157.60 mg/kg in loam, and 412.33–481.85 mg/kg in sandy loam. The optimal values were 1108.55 mg/kg, 874.86 mg/kg, and 448.35 mg/kg for silty loam, loam, and sandy loam, respectively.

The  $Q_m$  value was positively correlated with soil organic matter, total nitrogen, available phosphorus, available potassium, cation exchange capacity, and particle content of 0.02–0.002 mm, but significantly negatively correlated with soil pH.

Moreover, the simulation experiment of indoor static soil column was used to determine the time-effect of absorbing  $\text{NH}_4^+\text{-N}$  from liquid digestate in saturated water content soil as well as analyze the migration and transformation characteristics and fate ratio of  $\text{NH}_4^+\text{-N}$  from liquid digestate. After 3 days of application, the overlying water  $\text{NH}_4^+\text{-N}$  concentration decreased by 63.5–80.7%, and the reduction rate of total  $\text{NH}_4^+\text{-N}$  was 65.8–82.3%. At Day 4, the  $\text{NH}_4^+\text{-N}$  concentration of pore water in the 0–10 cm soil layer reached the peak value (24.41–28.91 mg/L). Similarly, after 7 days, the  $\text{NH}_4^+\text{-N}$  concentration adsorbed by the 0–10 cm soil layer reached the peak value (66.42–86.89 mg/kg with water-soluble  $\text{NH}_4^+\text{-N}$ , 98.42–121.15 mg/kg with ion-exchanged  $\text{NH}_4^+\text{-N}$ ). Finally, after 15 days, the overlying water  $\text{NH}_4^+\text{-N}$  concentration decreased by 97.0–98.7%, with a reduction rate of 97.9–99.2%, while the proportion of  $\text{NH}_4^+\text{-N}$  absorbed in the 0–10 cm soil layer accounted for 63.5–76.3%. The disposal is mainly based on soil sorption and pore water migration. A rapid disposal was observed between 0 and 3 days, and a complete safe digestion was observed after 15 days.

Furthermore, a pilot field application was carried out to assess the applicability of the biogas slurry. During soil pretreatment, soil ammonium nitrogen, soil pH, and soil nitrate nitrogen were closely related to the growth of soil bacteria and fungi. The biogas slurry pretreatment with a concentration of 495  $\text{m}^3/\text{hm}^2$  was beneficial to the growth of *Capsicum* spp. This could significantly improve the survival rate of *Capsicum* spp. seedlings, the flowering plants rate, the fruit-bearing plants rate and the plant height, with potential to significantly reduce the rate of rigid seedlings. Interestingly, the use of biogas slurry to pretreat protected soil can increase soil total nitrogen, total phosphorus, available phosphorus, available potassium, organic carbon content and soil pH value. In addition, this could inhibit the growth of soil bacteria, fungi, actinomycetes, and fusarium, as well as influence the soil microbial population, diversity and uniformity of species distribution in the community.

This study has demonstrated the potential of using biogas slurry as an effective measure

to overcome the bottleneck of sewage treatment in livestock and poultry farms towards the sustainability of a green environment. The time required for the discharge to avoid surface water pollution so as to provide theoretical basis and technical guidance for the efficient elimination of liquid digestate in farmland under the safety of water environment was also elucidated. Finally, the application of biogas slurry to pretreat protected soil with the dual goals of preventing and controlling soil-borne pathogens and efficiently absorbing biogas slurry in a unit area of farmland was demonstrated.

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## **CHAPTER 1: INTRODUCTION**

In recent years, the animal husbandry industry of China has gradually developed into an important pillar industry of China's agriculture and rural economy. According to the China Rural Statistical Yearbook (2019), the output value of animal husbandry in 2018 was 2869.7 billion yuan, accounting for 25.3% of the total output value of agriculture, forestry, animal husbandry and fishery. The total output of meat in the country was 86.2 million tons, the output of poultry eggs was 31.3 million tons, and the total output of milk was 31.8 million tons. This industry has made important contributions to meeting the national consumption of meat, eggs, milk and other animal products and improving the living standards of residents. However, with the development of livestock and poultry breeding and the improvement of the degree of intensification and specialization, the excreta of livestock and poultry breeding has also increased significantly. The subsequent waste disposal is facing huge challenges and has become an important source of agricultural source pollution. It is estimated that in 2017, the total output of livestock and poultry excreta in China was about 3.8 billion tons, and in 2020, it exceeded 4.2 billion tons (Zou et al., 2020). Dealing with livestock and poultry excreta in an effective way has become a serious challenge. In 2017, the Communist Party of China (CPC) Central Committee issued a call to vigorously promote the high-efficiency ecological circulation planting and breeding mode, speed up the centralized treatment of livestock and poultry manure, promote the healthy development of large-scale biogas, and promote the pilot of agricultural waste resource utilization with the county as the unit. The general office of the State Council of China stated that biogas and biological natural gas were the recommended treatment channels for livestock and poultry manure, and agricultural organic fertilizer and rural energy were the main utilization channels. As of the end of 2019, the central government has supported 585 large animal husbandry counties to promote the utilization of livestock and poultry manure resources. The Ministry of Agriculture and Rural Affairs has increased the number of pilot counties to replace chemical fertilizers with fruit,

vegetable and tea organic fertilizers to 175 per year. The utilization area of solid manure returned to farmland in China has exceeded 8.5 million hectares per year. However, solid manure has been effectively used, but the progress of liquid manure treatment is still slow, and it is urgent to expand consumption and treatment channels and innovate efficient resource utilization methods.

Biogas production by anaerobic fermentation of livestock and poultry excreta is an effective way of treating and utilizing the excreta. By the end of 2020, China has built 43.0 million rural household biogas construction, 128,976 small and medium-sized biogas projects, and 10,122 large-scale biogas projects (Wang et al., 2022). The process of producing methane from organic matter through microbial metabolism is called anaerobic fermentation (Jain et al., 2015). While producing biogas, a large amount of residues such as biogas slurry are also produced. The liquid and solid residues after biogas fermentation are called biogas slurry and biogas residue, respectively. The biogas slurry accounts for more than 90% of the total fermentation residue (Dong et al., 2021). China's annual production of biogas slurry has exceeded 1.1 billion tons (Ma et al., 2018). Due to its composition, low commercial values, high storage and transportation cost, there are serious risk of secondary pollution (Liang et al., 2013). The treatment and utilization of biogas slurry has been a continuous focus of research (Jiang et al., 2011; Wang et al., 2014; Nkoa, 2014; Hagos et al., 2017).

The treatment methods of biogas slurry in developed countries are more inclined to be stored and used as fertilizer to realize field consumption and utilization (Song et al., 2021). However, due to the large scale of intensive aquaculture and the huge output of biogas slurry in China, a large amount of land will be occupied for long-term storage, and the farmland consumption capacity around most farms is limited. The direct discharge of untreated biogas slurry cannot meet the requirements of the discharge standards for livestock and poultry breeding, which is easy to cause farmland pollution (Cheng et al., 2018). At present, for the treatment of biogas slurry, traditional sewage treatment processes can realize its purification, such as activated sludge method, membrane concentration method, chemical flocculation method, etc., but the



improvement of disposal efficiency needs further research and refinement, and it also faces waste of resources and high cost input. Biogas slurry recycling fundamentally reduces the effluent discharge of biogas slurry, saves water for dilution, and improves the buffering capacity and gas production of the system (Cao et al., 2018). However, in the actual operation process, biogas slurry recycling also has a series of challenges such as ammonia nitrogen inhibition, volatile organic acid accumulation, and accumulation of refractory substances such as cellulose (Deng et al., 2016). Biogas slurry from livestock and poultry farms has complex components. As a high-concentration organic wastewater, its colloidal substance content is high. There are pollutants such as inorganic matter, organic matter and sediment in the liquid. It has the characteristics of high ammonia nitrogen concentration, low carbon nitrogen ratio and poor biodegradability (Yan et al., 2013). In addition, biogas slurry is also rich in macro elements such as nitrogen, phosphorus and potassium, trace elements such as copper, iron and zinc, and microbial metabolites such as amino acids, hydrolase and vitamins (Kirchmann and Witter, 1992; Walsh et al., 2012; Abubaker et al., 2012) and some biologically active substances such as plant hormones and some antibiotics (Han et al., 2014; Qin et al., 2001). Most of these substances are soluble and can promote plant growth. Compared with traditional nitrogen, phosphorus and potassium fertilizers, biogas slurry as fertilizer can improve the overall maximum yield of crops and the nitrogen mineralization capacity, and increase the soil nitrogen supply capacity during the planting season. Therefore, biogas slurry can be used as a quick acting fertilizer for plants (Qin et al., 2001). Timely and appropriate application of biogas slurry is critical to ensuring the sustainable production capacity of farmland (Tao and Dong, 2017). A large number of research findings have shown that the return of biogas slurry to the field can improve the quality of agricultural products and improve soil conditions (Shen et al., 2010), and partially or fully replace inorganic fertilizers (Shi et al., 2019), an important bridge to realize the combination of planting and breeding with circular agriculture. In addition, biogas slurry has a certain inhibitory effect on a variety of crop pathogenic fungi (Tao et al., 2011; Li et al., 2013). The biogas slurry of 21 large-scale

biogas projects in stable operation in Jiangsu Province have been shown to have different degrees of inhibitory effect on the growth of strawberry Fusarium wilt, and the biogas slurry at different storage stages has a significant effect on its antibacterial effect (Ma et al., 2011).

The large amount of acid or physiological acid fertilizers, pesticides and high-intensity single planting mode applied in protected vegetable cultivation can easily cause soil quality degradation, and promote soil-borne diseases (Cao et al., 2004; Perez-Brandan et al., 2014), soil acidification, secondary salinization, nutrient imbalance and other continuous cropping obstacles (Ju et al., 2006; Shi et al., 2009), resulting in a significant drop in vegetable output, seriously affecting farmers' economic income and threatening the sustainable development of vegetable planting, worldwide (Zhu et al., 2013). It is reported that the worldwide vegetable industry loses as much as one billion US dollars every year due to pathogen damage (Lamour et al., 2012). Research on the methods for preventing and controlling soil degradation in facilities has important scientific value and practical significance for promoting sustainable agricultural development. Soil-borne diseases are diseases caused by plant pathogenic fungi, bacteria, nematodes and viruses remaining in the soil, over-reproducing when the conditions are suitable, and infecting from the roots and stems of crops (Cai and Huang, 2016). Among them, the root rot of facility vegetables caused by pathogenic fungi is more serious, and a large number of dead trees will appear after entering the colonization period. The initial symptoms are wilting and death of a single plant, and even the death of the whole plant in the later stage. In severe cases, the yield can be reduced by as much as more than 60%. Soil acidification provides a suitable living environment for the growth of pathogenic fungi. The accumulation of phenolic acid allelochemicals in soil affects the permeability of root cell membranes, which is the main reason for the auto-toxic effect of continuous cropping of vegetables, and can provide pathogenic fungi with carbon source and energy (Xie et al., 2014). Reductive Soil Disinfestation (RSD) has been proved to be a very strong method to "cure" the infection of soil borne pathogens (Cai et al., 2015; Momma et al., 2013; Blok et al.,

2000; Katase et al., 2009; Runia et al., 2010; Butler et al., 2012; Shrestha et al., 2014). The traditional approach of RSD is to prevent air from diffusing into the soil by applying a large amount of decomposable organic materials, irrigation and film covering, so as to create a strong soil reduction condition in a short time and achieve the goal of killing soil-borne pathogens. The biogas slurry is weakly alkaline, which helps to alleviate soil acidification. The biogas slurry is rich in organic carbon and ammonium nitrogen nutrients, which can provide abundant carbon sources and energy for the growth of microorganisms, thereby affecting the number and community structure of soil microorganisms (Tao et al., 2014) and regulating the activity of microorganisms in soil (Li et al., 2007).

Hence, this study focuses on the application of biogas slurry to treat degraded soil for vegetable farming based on the RSD method, and intends to achieve the dual goals of high-efficiency consumption of biogas slurry per unit area of farmland and prevention and control of soil-borne diseases in facilities. The study intends to provide theoretical basis and practical knowledge for expanding the use of biogas slurry, preventing and controlling soil-borne diseases of facilities vegetable fields, and improving the utilization level of waste resources combined with crop cultivation.

The objectives of this study were to:

- Provide insights on the maximum adsorption capacity of the plough layer soil for ammonium nitrogen in biogas slurry, and provide knowledge on the amount of biogas slurry ammonium nitrogen that can be efficiently absorbed by the soil of biogas slurry pretreatment facilities.
- Elucidate the safe cycle of biogas slurry consumption in saturated water content soil and the final fate of ammonium nitrogen in biogas slurry, so as to provide theoretical support for the safe consumption of biogas slurry pretreatment soil.
- Determine the impact of biogas slurry pretreatment on the growth of subsequent crops (such as *Capsicum* spp.), analyze the response changes of soil microorganisms and soil properties, and elucidate the effect of reducing

the continuous cropping obstacle of subsequent crops.

The thesis has been structured into six chapters. Chapter one is the introduction and provides an overview of the background, purpose and significance of the study. Chapter two is a literature review providing an overview of advance in farmland consumption of biogas slurry. Chapter three is a study of adsorption characteristics of three types of soils on biogas slurry ammonium nitrogen predicting the maximum amount of biogas slurry application. Chapter four is a study of aging characteristics and fate analysis of liquid digestate ammonium nitrogen disposal in farmland soil elucidating the safe cycle of biogas slurry disposal. Chapter five focus on the application effect of assessing biogas slurry pretreatment on soil properties, soil microflora and growth of *Capsicum* spp. Each of these chapters (Chapters' three to five) is self-contained and is presented in the format of an independent scientific paper. Chapter six provides a general discussion based on the result findings presented in chapters' three to five, as well as provides a general overview of the major findings and highlights their contribution and relevance; challenges and discuss the future perspectives on the research topic.

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## **CHAPTER 2: LITERATURE REVIEW**

### **(ADVANCEMENT IN FARMLAND CONSUMPTION OF BIOGAS SLURRY – A REVIEW)**

#### **2.1 Introduction**

Biogas slurry is one of the products of anaerobic fermentation (Jain S et al., 2015; Lou M et al., 2022), which refers to material produced by biodegradable organic wastes such as livestock and poultry manure, agricultural and forestry wastes, human excrement, urine, and kitchen waste (Lu G et al., 2021). These wastes undergo anaerobic fermentation in a closed container to produce methane, carbon dioxide and other gas residues (Deng L et al., 2017). This process is made up of the solid matter called biogas residue, and the liquid matter (biogas slurry) (Han M et al., 2014). Biogas slurry has complex components (Hu Y et al., 2017; Zou M et al., 2020), which are rich in nitrogen, phosphorus, potassium, copper, iron, zinc, manganese, amino acids, organic acids, hydrolases, vitamins and other components that are beneficial to plant growth and development (Rodriguez-Navas C et al., 2013; Shen Q et al., 2014; Wang X et al., 2021; Lou M et al., 2022). It also contains substances such as 8-hydroxy-3,4-dihydroquinoline-2-ketone and 3,4-dihydroquinoline-2-ketone that have inhibitory effects on pests and diseases (Qin W et al., 2001; Huo C et al., 2011; Yin F et al., 2021) as well as heavy metal components such as mercury, cadmium, chromium, arsenic and lead that are harmful to human beings (Song C et al., 2011; Huang H et al., 2018). According to estimates, China's annual production of biogas slurry has exceeded 1.12 billion tons (Ma Y et al., 2018; Zou M et al., 2020; Ke L et al., 2022). The safe consumption and treatment of biogas slurry has become a problem that must be faced and solved in the prevention and control of agricultural source waste pollution in China, and the urgency of the situation has attracted the attention of both government and scientists.

Traditional sewage treatment processes can realize the purification of biogas slurry,

such as the oxidation pond method (Zu B et al., 2009; Jin H et al., 2011; Wan J et al., 2017), artificial wetland method (Zhu G et al., 2012; Lang L, 2017), activated sludge method (Yetilmezsoy K et al., 2009; Wang Z et al., 2013), membrane concentration method (Bai X et al., 2015; Zhan Y et al., 2018; Wu L et al., 2019; He Q et al., 2021), chemical flocculation method (Luján-Facundo MJ et al., 2019; Zhang M and Gao D, 2020), but the improvement of treatment efficiency needs further research and refinement. This approach also faces the challenge of waste of resources and high cost input (Song Y et al. 2021). For the direct treatment of biogas slurry, the resource utilization of biogas slurry is more in line with the requirements of sustainable development and a green environment (Zhang C et al., 2014; Nicholson F et al., 2017). Soil fertilization is a major way of waste resource (biogas slurry) utilization (Haraldsen TK et al., 2011; Robles Á et al., 2020), but biogas slurry discharged from intensive large-scale livestock and poultry farms and biogas projects have exceeded the carrying capacity of adjacent farmland. Similarly, biogas slurry discharged is greatly affected by agricultural seasonality. So it is particularly necessary to expand diversified treatment and utilization (Wang Z et al., 2016; Liu C et al., 2021). In order to provide a reference for the development of new ways of eco-friendly and efficient consumption in farmland, there is a need to understudy the approaches, challenges, application methods, and advantages of using biogas slurry.

## 2.2 Composition of biogas slurry

The physicochemical properties of biogas slurry are closely related to the raw materials and fermentation concentration of anaerobic fermentation (Li H et al., 2016). Through the search and analysis of over 750 documents on biogas slurry release to the field in China in the past 20 years from January 2000 to December 2019 (Dong Y et al., 2021a), the nutrient composition of biogas slurry with different fermentation raw materials are shown in **Table 2.1**. Other research data show that the water content of biogas slurry is as high as 95% or more (Sheets JP et al., 2015), with a weak alkalinity. The amino acids, vitamins and plant hormones content in biogas slurry are shown in **Table 2.2**. The heavy metals, antibiotics and other residual substances content are

shown in **Table 2.3**. Since the composition of biogas slurry varies greatly, the composition of biogas slurry should be determined before its resource utilization to achieve scientific and reasonable safe digestion.

Table 2.1 The nutrient composition of different types of biogas slurry (Dong Y et al., 2021a; Wang X et al., 2021)

Element in biogas slurry	PM		DM		CM		MM	
	Range	Average	Range	Average	Range	Average	Range	Average
pH	4.23–9.20	7.52	6.10–9.20	7.75	6.77–8.50	7.80	6.15–8.20	7.37
TN (mg/L)	0.80–7280.00	1166.71	32.00–6500.00	1488.59	400.00–5700.00	3226.13	0.04–5900.00	1369.31
TP (mg/L)	0.54–2220.50	291.60	10.00–3700.00	561.67	49.00–4650.00	959.71	0.03–3900.00	665.90
TK (mg/L)	0.33–8880.00	1144.26	11.00–9650.00	1679.10	390.00–4400.00	2858.31	0.12–3200.00	1240.21
NH <sub>4</sub> <sup>+</sup> -N (mg/L)	66.53–1800.00	597.53	80.35–1098.00	493.47	ND	ND	250.50–787.80	519.15
NO <sub>3</sub> <sup>-</sup> -N (mg/L)	0.19–472.16	67.84	0.70–223.70	71.53	ND	ND	ND	ND
DP (mg/L)	0.16–1730.00	261.40	80.00–1860.00	416.88	ND	ND	0.16–201.10	76.68
DK (mg/L)	0.86–5010.00	986.47	263.20–2500.00	1418.33	ND	ND	0.84–2316.70	764.73

ND: no data; PM: biogas slurry using pig manure as fermentation raw material; DM: biogas slurry using cow dung as fermentation raw material; CM: biogas slurry using chicken manure as fermentation raw material; MM: biogas slurry using two or more of straw, human excrement, pig manure, cow dung, chicken manure and other household waste mixture as fermentation raw material; TN: total nitrogen; TP: total phosphorus; TK: total potassium. NH<sub>4</sub><sup>+</sup>-N: ammonium nitrogen; NO<sub>3</sub><sup>-</sup>-N: nitrate nitrogen; DP: available phosphorus; DK: available potassium.

Table 2.2 Concentration of amino acids, plant hormones and B vitamins in a biogas slurry (Huo C et al., 2011; Shen Q et al., 2014)

Amino acids	Contents (mg/L)	Amino acids	Contents (mg/L)	Plant hormones	Contents (μg/L)	B vitamins	Contents (mg/L)
Cysteine	2.92	Arginine	0.63	Indole acetic acid (IAA)	332	B1	0.089
Serine	2.07	Proline	0.58	Gibberellin (GA4)	0.857	B2	0.022
Threonine	1.41	Valine	0.56	Gibberellin (GA19)	1.47	B6	0.530
Lysine	1.05	Leucine	0.45	Gibberellin (GA53)	0.271	B11	0.078
Glycine	1.01	Methionine	0.36	Cytokinin (iPR)	0.00194	B12	0.009
Tyrosine	0.88	Alanine	0.36	8-hydroxy-3,4-dihydroquinoline-2-ketone	737.5		
Aspartic acid	0.76	Phenylalanine	0.33	3,4-dihydroquinoline-2-ketone	177.5		
Isoleucine	0.75	Glutamate	0.31				
Histidine	0.63						

Table 2.3 The heavy metals, antibiotics and other residual substances content in biogas slurry (Shen Q et al., 2014; Jing D et al., 2016; Huang H et al., 2018; Wang X et al., 2021)

Element in biogas slurry	PM		DM		CM	
	Range	Average	Range	Average	Range	Average
Hg (mg/L)	0-0.167	0.028	0-0.119	0.024	0-0.054	0.014
Cd (mg/L)	0-7.51	0.126	0-0.190	0.039	0-4.3	0.367
As (mg/L)	0-13	0.868	0.001-4.576	0.235	0.01-5.21	0.548
Pb (mg/L)	0-36.07	0.710	0.008-1.056	0.199	0-2.430	0.345
Cr (mg/L)	0-24.18	0.657	0-3.146	0.301	0.001-10.18	1.085
Ni (mg/L)	0-5.85	0.317	0.027-0.063	0.045	0.088-0.55	0.281
Cu (mg/L)	0-99	4.50	0.02-30.03	2.63	0-2.12	0.78
Zn (mg/L)	0-205.43	9.11	0.1-68.15	8.31	0-13.94	4.06
Cl (mg/L)	150-3647.5	917.1	850.5-963	906.8	540-1087	813.5
Na (mg/L)	88.5-559	287.1	994.45	994.45	172.29	172.29
Se (mg/L)	0-0.232	0.049	0.002-0.022	0.012	0.011	0.011
Mn (mg/L)	0-50.8	6.815	0.231-124.6	61.092	0-50.8	7.534
Fe (mg/L)	0.0014-6.05	2.505	0.0084-48.3	18.56	0.0054-13.3	4.962
Mg (mg/L)	0.0057-253.34	109.6	0.352-553	225.06	0.0109-89.46	32.82
Ca (mg/L)	0.0042-264	81.65	0.785-769	280.8	0.0426-96.1	61.14
Oxytetracycline (mg/L)	0.0001-0.994	0.1456	0.5748	0.5748	0.0759-0.4007	0.2383
Tetracycline (mg/L)	0-0.9821	0.0296	0.0208-0.5608	0.2908	0.0289-12.862	4.3106
Chloromycin (mg/L)	0.0002-0.642	0.0415	ND	ND	ND	ND
Norfloxacin (mg/L)	0-0.204	0.0191	0.0054-0.1189	0.0641	0.056-0.2048	0.1065
Ciprofloxacin (mg/L)	0.0002-0.0513	0.0052	0.016-0.0227	0.0183	0.005-0.0071	0.0058
Enrofloxacin (mg/L)	0-0.1513	0.0108	0.0058-0.089	0.0520	0.0073-0.0676	0.0519

ND: no data; PM: biogas slurry using pig manure as fermentation raw material; DM: biogas slurry using cow dung as fermentation raw material; CM: biogas slurry using chicken manure as fermentation raw material.

## 2.3 Advance in farmland consumption of biogas slurry

### 2.3.1 Approaches of using biogas slurry in farmland

#### 2.3.1.1 Seed soaking

The abundant nitrogen, phosphorus, potassium, various trace elements, growth hormones and other substances in biogas slurry can be absorbed and utilized by seeds through seed soaking and infiltration. It can accelerate the formation and metabolism of seeds during the dormant period, thereby promoting seed germination. Reports have shown that the proper concentration of biogas slurry and soaking time could improve the germination rate of seeds and promote the growth of seedlings. Soaking seeds with 50% biogas slurry for 5 hours had the best comprehensive effect on

the germination of marigold seeds and seedling growth (Yuan D et al., 2011). For instance, the germination rate and seedling rate of watermelon seeds treated with 40% biogas slurry for 24 hours was observed to give the best performance (Cheng W et al., 2018). Similarly, soaking seeds with 25% biogas slurry for 5 h had significant effect on seed germination and seedling growth of *Astragalus membranaceus var. mongholicus* (Bunge)P.K.Hsiao (Lu G et al., 2019). In addition, soaking seeds with biogas slurry can increase crop yield. The seed soaking treatment of wheat seeds with biogas slurry with better maturity and longer fermentation time can increase the germination rate by about 13% compared with the water treatment. Resulting in the seeds emerging 3 days earlier, the leaf length, leaf width, dry weight of seedlings increase by 1.70 cm, 0.10 cm, 0.70 g respectively. And the maturity period shortens by 2 days with the yield per hectare increases by 379.50 kg (Shi L et al., 2019). Hence, biogas slurry could be a potential fertilizer for improving agricultural productivity and a sustainable green environment

### **2.3.1.2 Foliar fertilizer using biogas slurry**

Biogas slurry is often used as foliar fertilizer for it contains a variety of available nutrients and amino acids which can promote plant growth, increase yield and improve quality (Yan L et al., 2019; Yang W et al., 2020). Biogas slurry has been directly used as foliar fertilizer to spray on fruit trees and vegetables, which can significantly increase chlorophyll content and yield (Zhao X et al., 2011; Hao S et al., 2012). For instance, proper application of biogas slurry sprayed on the leaves can improve the growth, yield and quality of tomato (HL2109 produced by Beijing Yinyue Seed Industry) plants (Jia L et al., 2017). Similarly, investigating the effect of biogas slurry application on the high-efficiency production of walnuts, Bi T et al. (2020) observed that the biogas slurry has a good promotion effect on the improvement of walnut quality and the prevention and control of pests and diseases. The authors pointed out that when biogas slurry is used as leaf fertilizer and pest control, it should be diluted and sprayed on the back of leaves. The application of biogas slurry has become a hotspot in the research field in recent years. Likewise, foliar topdressing of biogas slurry concentrate can increase the yield of cucumber by 6% and tomato by 8% (Xue S et al., 2013). Adding humic acid

and other nutrients to biogas slurry concentrated to 10% of the original volume, and compounding into organic fertilizer with large, medium and trace elements, as a foliar fertilizer, it can significantly improve the yield and quality of Chinese cabbage. The yield of Chinese cabbage increased by 23.3%, and the content of vitamin C and soluble sugar increased by 68.5% and 43.1%, respectively. At the same time, soil fertility and enzyme activity were improved, and soil nutrient content increased significantly (Fan B et al., 2015). In addition, topdressing biogas slurry concentrate can increase *Capsicum* spp. yield (Cui W et al., 2021), and increase the content of vitamin C, soluble sugar, and protein in *Capsicum* spp., among which the vitamin content increases by 18.32% compared with the market foliar fertilizer (Fu Y, 2018).

### **2.3.1.3 Base fertilizer using biogas slurry**

Biogas slurry as basic fertilizer is the most traditional approach of farmland application of biogas slurry. Compared with chemical fertilizer group, under the treatment condition of biogas slurry as base fertilizer (52.5 t/hm<sup>2</sup>) and root irrigation twice (0.25kg/root · time), the length, diameter, leaf area and chlorophyll content of sweet melon (Xitian No. 1, provided by Xi'an Hejia Seedlings Co., Ltd) vine can be increased as well as the weight of melon and the melon plant can be increased (Wu Z et al., 2015). A study by Li and Jiang (2016), shows that when the concentration of biogas slurry is between 10% and 20%, it is favourable for the growth of container seedlings of *Dendrobium candidum* on the substrate of water moss, and between 10% and 30%, was found to be favourable for the growth of disk seedlings of *Dendrobium candidum* on the substrate of sawdust pine bark. Under the condition of the total application amount of biogas slurry with 600 t/hm<sup>2</sup> (base fertilizer: top dressing=1:1), the total panicle number of rice and the yield could be increased, and the content of heavy metals in grains did not increase (Shao W et al., 2017). Using livestock manure to ferment biogas slurry as base fertilizer in autumn and topdressing in the next spring can improve the yield and quality of spring tea and ensure the content of heavy metals in soil and tea within the safe range. However, when the biogas slurry is applied alone, potassium depletion will occur. Therefore, it is necessary to pay attention to the supplement of potassium

in practical application (Hu Z et al., 2020). Practically, the treatment of biogas slurry should be carried out according to the ratio of base fertilizer to fruit expanding fertilizer of 1:1. There was no significant difference in plant height and stem diameter when the biogas slurry was applied at 70–110 t/hm<sup>2</sup> compared with applying compound fertilizer 600 kg/hm<sup>2</sup> treatment. When the total application amount of biogas slurry was 180 t/hm<sup>2</sup> (base fertilizer 90 t/hm<sup>2</sup>, fruit expansion fertilizer 90 t/hm<sup>2</sup>), it could promote the growth of melon plants and dry matter accumulation, improve fruit quality, and can be popularized in melon production (Wang L et al., 2021a).

#### **2.3.1.4 Top dressing fertilizer using biogas slurry**

The application of biogas slurry instead of chemical fertilizer for crop top dressing can increase the content of nitrogen and phosphorus in the soil, and it increases with the increase of the application amount of biogas slurry. The yield of rice treated with pure nitrogen 396 kg/hm<sup>2</sup> of biogas slurry is greater than that treated with chemical fertilizer, and the utilization rate of nitrogen and phosphorus is the highest (Wang W et al., 2014). Compared with no biogas slurry topdressing treatment, 10 times of biogas slurry topdressing treatment can increase the yield of *angelica sinensis* by 112.89kg, an increase of 58.01%. This significantly reduces the disease index of angelica hemp (*Angelica sinensis* (Oliv) Diels. Mingui No. 1) mouth disease and the preventive effect can reach 82.3%. The special grade yield of *angelica sinensis* increased by 7.4%, and the first-class yield of *angelica sinensis* increased by 2.4% (Zhou J, 2015). By using nutrient balance method, it was found that there was no significant difference in muskmelon dry matter quality, nutrient content and carrying capacity of roots, stems, leaves and fruits between biogas slurry integrated water and fertilizer topdressing group and fertilizer group, which proved that biogas slurry integrated water and fertilizer could completely replace fertilizer topdressing (Gao X, 2019).

#### **2.3.1.5 Hydroponics**

Using biogas slurry to replace the inorganic nutrient solution of hydroponic cash crops for vegetable cultivation is one approach of resource utilization of biogas slurry. The celery was hydroponic in biogas slurry of different concentration by the technology of



bio-floating bed. After 80 days of planting, the celery which was hydroponically cultured in 30–40 times diluted biogas slurry achieved high environmental and economic benefits (Zhang L et al., 2011). In the concentration range of 3%–5%, the stepwise addition and one-time addition of chicken manure biogas slurry can increase the chlorophyll content, biomass and vitamin C content of water spinach in the solar greenhouse, and reduce the nitrite content (Wang H et al., 2013). Compared with ordinary soil cultivation treatment, biogas slurry soilless cultivation treatment can significantly increase the number of lateral roots and total yield of water spinach by 45.4% and 12.8%, respectively as well as reduce nitrate nitrogen content in water spinach by 31.5% and increase soluble sugar content by 68.5% (Yu C et al., 2015). In another related study using biogas slurry as a nutrient substitute for the second growth stage of lettuce (*Lactuca sativa*, Jinshulu Seed), the replacement of nutrient solution with biogas slurry had a better effect on lettuce yield, photosynthetic characteristics and quality. The replacement ratio of 40% biogas slurry has the best effect, and the yield is 66.97% higher than that of the control (lettuce hydroponics with a nutrient solution prepared by the original Yamasaki formula) (Yang X et al., 2017). After the biogas slurry deamination was pretreated and diluted 5–10 times, lettuce was hydroponically cultured for 35 days. Then compared with hydroponics in nutrient solution, the relative growth of lettuce increased by 60%, the leaf width became wider by 4–5cm, the number of leaves increased by 2 pieces on the average, the carotenoids content increased by 20.4%, and the content of nitrate nitrogen from 2.11%–4.02% compared to that of chemical nutrient solution group (Liang F et al., 2018).

Biogas slurry hydroponic microalgae is a new type of resource treatment process with potential and stable operation. Compared with traditional biochemical methods, it can improve the nitrogen removal efficiency of biogas slurry by about 20% (Odlare M et al., 2011b), and obtain higher efficiency functional microalgae products. Five species of microalgae were cultured with pig manure fermentation biogas slurry, and the nitrogen removal ability and sugar accumulation degree were investigated. *Chlorella vulgaris* ESP-6 showed the best sugar production capacity, with the maximum sugar content and average daily sugar production capacity of 61.50 % and 395.73 g/L

respectively. The ammonia nitrogen removal rate and daily average removal concentration were 96.30% and 91.7 mg/L, respectively, which underscored the good purification ability of microalgae on biogas slurry. Accumulating more carbohydrates in microalgae cells can be regarded as a new strategy for sugar production, which fully proves the value of biogas slurry hydroponic microalgae utilization and the regeneration potential of biogas slurry waste resources (Tan F et al., 2016).

#### **2.3.1.6 Animal feed**

The use of biogas slurry as animal feed and feed additive is another environmentally friendly approach to solve the comprehensive utilization of biogas slurry to realize both ecological and economic benefits. The literature reports are mostly found in empirical research and attempts. For instance, feeding pigs with biogas slurry can achieve the effects of promoting growth, shortening the fattening period and improving the ratio of meat to feed. It is estimated that using biogas slurry as a feed additive for pigs can save about 50 kg of feed per pig, and shorten the fattening period by 20–40 days (Wu X, 2006), which can increase the weight of pigs by 0.2 kg per day and reduce the swine morbidity (Zhang H et al., 2009). The anatomical analysis and meat quality analysis after slaughtering live pigs with biogas slurry showed no abnormality in the main organs and meat quality and no infectious diseases and parasitic diseases detected (Wang H and Zhang Z, 2006). The number of heterotrophic bacteria in the sediments of fish ponds with biogas slurry or biogas slurry combined with feed was higher than that of cattle dung or biogas slurry combined with inorganic fertilizers. Similarly, the sediment-water interaction in fish ponds with biogas slurry was better than the conventionally fertilized fish ponds (Das M et al., 2013). Fish farming with biogas slurry can increase the yield and economic benefits of feeding and filter-feeding fish, however, attention should be paid to the amount, frequency and timing of biogas slurry dosing (Jing D et al., 2016).

## **2.3.2 Challenges of using biogas slurry in farmland**

### **2.3.2.1 Water environment**

#### **2.3.2.1.1 Surface runoff**

The nitrogen and phosphorus nutrients in biogas slurry are mostly and readily available nutrients. When the nitrogen and phosphorus nutrients provided in the biogas slurry exceed the needs of crop growth, they will continue to accumulate in the soil, in the face of heavy rainfall, improper irrigation and poor drainage system, it is easy to cause nutrient loss, leading to eutrophication of rivers and lakes (Song D et al., 2020). A case study is the paddy field engineering approach to biogas slurry valorization. The use of paddy field engineering for digesting biogas slurry is different from the utilization of paddy field fertilizer, which is a new way to deal with biogas slurry (Wang Z et al., 2016). The first 3 days after irrigation is a critical period for the digestion of biogas slurry in paddy fields (Wang Z et al., 2015a; Wang Z et al., 2015b; Yang R et al., 2017), and it is also a critical period for controlling nitrogen loss in paddy field runoff (Jiang L et al., 2011; Li S 2011; Wang Z et al., 2016). The risk of nutrient loss in the application of biogas slurry in paddy fields increased with the increase of application years, which was not only reflected in the soil fertility index and nutrient accumulation rate, but also reflected in the low proportion of soil C:P and N:P (Dong Y et al., 2021b). There may be a risk of nutrient loss in paddy soil with continuous application of biogas slurry for 4 years. To reduce the risk, the construction of farmland infrastructure such as farmland ecological interception ditch system should be strengthened, and a series of agronomic, biological and other supporting measures such as fertilizer and water management should be taken.

#### **2.3.2.1.2 Downward leaching**

While the application of biogas slurry increases the nutrients such as nitrogen and phosphorus in the soil (Wang J et al., 2019), these nutrients may also be leached downward with the biogas slurry and rainwater. It may pose a potential threat to farmland health and even cause secondary environmental pollution (Tan F et al., 2016). For example, when biogas slurry is applied in vegetable fields, soil nitrogen and

phosphorus nutrients are surplus, phosphorus accumulations while, nitrogen leaching loss occur in surface soil (Kang L et al., 2011). The pollution risk of digesting biogas slurry by paddy field engineering measures to the infiltration water is mainly concentrated in base tiller stage. Ammonium nitrogen pollution is the main risk, nitrate nitrogen pollution risk is small, and the degree of pollution varies with the depth of water seepage. The amount of biogas slurry digested in the paddy field at the base tiller stage should be controlled within 211.76 t/hm<sup>2</sup>. The capacity of the paddy field in panicle fertilizer stage to digest biogas slurry is relatively strong and the risk of pollution is small. The single digested biogas slurry amount (< 423.53 t/hm<sup>2</sup>) can be regarded as a safe amount in a rice growth cycle (Wang Z et al., 2016). A 3-year field trial of the mixed application of biogas slurry and irrigation water during the wheat-maize rotation was carried out in the North China Plain. It was found that the use of medium-concentration biogas slurry instead of chemical fertilizers is a reasonable method to ensure high crop yield, high nitrogen usage efficiency and reduction of nitrate leaching losses (Du H et al., 2019). The leaching amount of ammonium nitrogen produced by the application of biogas slurry in autumn fallow period is related to the growing season of crops, the amount of biogas slurry and the application method of biogas slurry. The increase of biogas slurry application rate increases the risk of ammonium nitrogen leaching. Meanwhile, biogas slurry injection treatment increases the leaching potential of ammonium nitrogen compared to the spray treatment. Field experiments for three consecutive years showed that the content of ammonium nitrogen in soils of each biogas slurry nitrogen treated soil was lower than that of no nitrogen application, indicating that no leaching of ammonium nitrogen occurred.

### **2.3.2.2 Soil environment**

#### **2.3.2.2.1 Heavy metals and antibiotic residues**

Due to the use of different chemical compounds in animal feeds with various chemical additives and antibiotics being abused, the utilization rate of heavy metal elements and antibiotics by livestock and poultry is low. Hence, a considerable part of heavy metal and antibiotic pollutants are left in the faeces (Ma H, 2014; Qian M et al.,

2016). During the anaerobic fermentation process, the heavy metals and antibiotic pollutants enriched in the manure will also remain in the biogas slurry (Wei D et al., 2014; Huang H et al., 2018; Shen A et al., 2019). Although the content is very low, if biogas slurry is applied blindly for a long time, it will cause the risk of excessive heavy metals and residual antibiotic pollutants in farmland, which will destroy the farmland ecosystem causing food security problems (Lai X et al., 2018; Zhou Y et al., 2020). Detection and analysis of soil and crops after application of biogas slurry showed that Cd, As, Pb, Ni, Cr, Cu and Zn accumulated in different degrees in soil, and Cd, As, Pb, Ni, Cr and Zn were enriched in different degrees in crops (Bian B et al., 2015; Zhou L and Lv L, 2017; Wang Y, 2018). Long term or high concentration application of biogas slurry fermented with pig manure, chicken manure, cow manure and other raw materials will lead to the accumulation of heavy metals in the soil. However, due to the different types of livestock and the different types and amounts of feed added, the content of heavy metals in the biogas slurry will be different. Similarly, after a long-term biogas slurry application, the accumulation of heavy metals in the soil will be different (Liu X et al., 2018). When the application amount of biogas slurry is low, it can help to reduce the effectiveness of three heavy metals such as lead, copper, and zinc in the soil. On the other hand, when the dosage is high, although the availability increases, but heavy metal soil environmental pollution increases. Therefore, reasonable control of the application amount of biogas slurry will reduce the pollution of heavy metals in the soil (Han J et al., 2021). A standard control of feed additives to block the input of heavy metals and antibiotics is the key link for the subsequent safe utilization of livestock and poultry manure biogas slurry. In this regard, the Chinese government has formulated safe use specifications for additives in feed, and issued announcement policy on the complete prohibition of antibiotics in Chinese feed from 2020.

#### **2.3.2.2.2 Secondary salinization**

As a renewable water resource, biogas slurry can provide a large amount of nitrogen and phosphorus nutrients while solving the water shortage in arid and semi-

arid areas. Therefore, biogas slurry irrigation is one of the important ways of recycling and using wastes at present (Wu G et al., 2014; Cui B et al., 2019). However, biogas slurry also contains excess sodium ions, potassium ions and bicarbonate ions. Improper irrigation may cause excessive accumulation of soil salt, leading to soil salinization and potential pollution risks to farmland soil. For instance, in the vegetable planting base of Yining, Xinjiang, China, five consecutive years of biogas slurry irrigation show that with increase of years of biogas slurry irrigation, salt accumulates in farmland soil, resulting in secondary salinization of the soil in facility vegetable field (Yang L et al., 2012). Also, the nutrient and salinity accumulation in the soil of protected vegetable field applied with pig manure biogas slurry for 0, 1, 3, 5 and 7 years was investigated. The results showed that the available nitrogen, organic matter, total copper, total zinc and electrical conductivity in the soil showed an increasing trend year by year. After 7 years, each index was 3.4, 1.5, 3.3, 1.3, 3.9, 1.88 and 4.74 times of that in the soil without the application of pig manure biogas slurry, respectively. This led to the rapid accumulation of salt while simultaneously increasing soil nutrients that put the soil at a risk of soil pollution (Guo Q et al., 2020). In another study, the biogas slurry microbial fertilizer prepared by anaerobic digestion of kitchen waste was applied to single season rice and winter wheat. It was observed that the water-soluble total salt and chloride ion in winter wheat and rice soil showed weak accumulation, although no salt forcing phenomenon occurred (Sun T et al., 2020). The field experiment used  $\text{Na}^+$  concentration of about 35 mmol/L biogas slurry to irrigate oil sunflower for a long time. Under the low irrigation amount ( $150 \text{ m}^3/\text{hm}^2$ ), the agronomic profile of oil sunflower did not change much, and the difference of  $\text{K}^+/\text{Na}^+$  was not significant. But under high irrigation ( $600 \text{ m}^3/\text{hm}^2$ ), various agronomic indicators of oil sunflower growth were inhibited, and  $\text{K}^+/\text{Na}^+$  of each tissue decreased by 57%–88%. The high pH damage caused by alkaline salt is higher than that of salt ion osmotic stress. The  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  in the slurry should be controlled, and the pH of wastewater should be adjusted to reduce damage to crops. Alkaline salt could damage the ion homeostasis of oil sunflower to a greater extent, affecting the germination and growth of oil sunflower

(Fan J et al., 2022). Using biogas slurry on farmland as regenerated water resources for the irrigation of farmland. Therefore, when applying biogas slurry in agriculture, consideration should be given to controlling the amount of salt accumulation in the soil from the source and reducing the risk of soil salinization.

### 2.3.2.3 Atmospheric environment

More than 70% of the nitrogen in the biogas slurry in the form of  $\text{NH}_4^+\text{-N}$  (Jin H et al., 2012), can be directly decomposed into gaseous ammonia and volatilized after being applied to the soil (Jin H et al., 2011). Therefore, the application of biogas slurry will increase the amount of soil ammonia volatilization (Deng O et al., 2011; Jin H et al., 2013; Li H et al., 2019a), and become the most important contributor to the loss of  $\text{NH}_3\text{-N}$  after returning to the field (Martines AM et al., 2010; Cheng J et al., 2018). The amount of biogas slurry application, application time, temperature, and application method will all affect the amount of ammonia volatilization. The larger the amount of biogas slurry applied in the field, the larger the amount of ammonia volatilization (Wu H et al., 2012). For instance, in a pot experiment with medium soil fertility, it was found that the total amount of ammonia volatilization from farmland under conventional chemical fertilizer treatment was  $77.0 \text{ kg/hm}^2$ , while the amount of ammonia volatilization from 100% biogas slurry treatment and 75% biogas slurry plus 25% pig manure organic fertilizer treatment was higher, which were  $120.7 \text{ kg/hm}^2$  and  $88.0 \text{ kg/hm}^2$ , respectively (Zhou W et al., 2019). Moreover, when biogas slurry was applied at low temperature in autumn fallow period, the peak value of ammonia volatilization in spraying and injection treatment was  $0.22 \text{ kg}/(\text{hm}^2 \cdot \text{d})$  and  $0.65 \text{ kg}/(\text{hm}^2 \cdot \text{d})$  respectively (Liu C et al., 2021; Wu H et al., 2012; Yuan Y et al., 2019). Furthermore, a study by Jin H et al. (2013), pointed out that more than 58% of ammonia volatilization loss is related to environmental temperature after biogas slurry has been applied to soil. When the temperature during biogas slurry application is higher, the amount of ammonia volatilization increases significantly. Studies also show that after the application of biogas slurry, the volatilization of ammonia was higher than in the previous week, after which the volatilization of ammonia gradually decreased and

became stable (Yang R et al., 2017; Gao B et al., 2022). In addition to the influence of environmental temperature on ammonia volatilization, there is a positive correlation between the ammonia volatilization flux and the  $\text{NH}_4^+\text{-N}$  concentration of biogas slurry in the field surface water (Tian Y et al., 2007; Yang S et al., 2012; Li X et al., 2015).

The reason for the increase in ammonia volatilization rate is not only due to the increase in  $\text{NH}_4^+\text{-N}$  content, environmental temperature, and the presence of surface water in soil (Jin H et al., 2013). But also due to the large amount of soluble organic carbon in biogas slurry, which can stimulate the mineralization of soil organic nitrogen (Sommer SG et al., 2003; Martines AM et al., 2010). Usually the amount of ammonia volatilization loss correlate with the proportion of biogas slurry application, and the proportion of nitrogen loss caused by ammonia volatilization could reach 20% (Gao B et al., 2022). Ammonia volatilization in paddy field application of biogas slurry can account for 42.2%–72.0% of total nitrogen loss (Li H et al., 2019a). However, in conventional storage of biogas slurry, 25%–35% of N will be lost in the form of  $\text{NH}_3\text{-N}$  (Wang Y et al., 2016; Wang Y et al., 2017), much higher than the loss of ammonia volatilization from farmland reuse. Therefore, in the valorization of biogas slurry, it is necessary to clarify the whereabouts of the ammonium nitrogen, not only to reduce the loss of ammonia volatilization, but also to evaluate the risk of ammonia volatilization loss.

#### **2.3.2.4 Crop safety environment**

Appropriate biogas slurry concentration and dosage can promote plant growth and development, as well as improve yield and quality. However, when the concentration and dosage are improperly applied, the growth and development of crops will be inhibited. Ammonia, phenols, hydrogen sulfide and high chemical oxygen demand (COD) in biogas slurry may cause anoxic death of plant roots and slow growth development of plants (Gao M et al., 2019). Under the condition of equal amount of nitrogen, phosphorus and potassium, COD content is the key limiting factor affecting the use of biogas slurry in farmland. Low amount of biogas slurry COD ( $1566 \text{ kg/hm}^2$ ) promotes seedling growth, accelerates the peak supply of soil available phosphorus,



while high amount of biogas slurry COD (3132 kg/hm<sup>2</sup>) inhibits seedling growth, and delays the peak supply of soil available phosphorus. The optimal application safety threshold of COD is 1102–1442 kg/hm<sup>2</sup> and the maximum application safety threshold is 2208–2884 kg/hm<sup>2</sup>. This factor needs to be taken into consideration for farmland biogas slurry safe usage and efficiency (Wang Z et al., 2019). Moreover, when the concentration of ammonium nitrogen and lactic acid in biogas slurry is higher than 336 and 61 mg/L, respectively, it could produce phytotoxicity to seed germination (Zhang Y et al., 2021). Excessive application of biogas slurry increases NH<sub>4</sub><sup>+</sup>-N concentration and electric conductivity (EC) value in soil solution, resulting in inhibited seedling growth, decreased plant height, and increased root yellowing rate. The maximum safe absorption threshold of NH<sub>4</sub><sup>+</sup>-N in biogas slurry-water mixture by seedlings was 314.0 mg/L. It is also found that the EC value of biogas slurry increases with the increase of biogas slurry concentration, a possible synergistic effect between EC and NH<sub>4</sub><sup>+</sup>-N concentration still needs further studied (Zhang L et al., 2021).

In addition, the application of biogas slurry can increase the content of heavy metals in plants. For instance, Shao W et al. (2017), observed a varying concentration of Hg, As, Cr and Pb in rice straw. Also, the application of high-concentration pig farm fermentation biogas slurry (1.8×10<sup>5</sup> kg/hm<sup>2</sup>) significantly increase the copper content in lettuce and in Chinese Cabbage, but lower than the limit range stipulated by the national food hygiene standards (Ye J et al., 2014; Yang J et al., 2015). When the biogas slurry contains 4 times the nitrogen equivalent, it will increase the excessive enrichment of Cu and Zn elements, which will have a negative effect and reduce the yield and quality of plants such as corn (Li J et al., 2021). 5-year irrigation experiment using pig manure biogas slurry in rice-wheat rotation field in Dongtai, Jiangsu, China, the detected Zn in the grains of wheat and rice increased by 24% and 16%, respectively, compared with the control (Tang Y et al., 2019a).

### **2.3.2.5 Pathogen transmission**

Livestock and poultry manure contains a variety of pathogens (including bacteria, fungi, parasites, viruses, etc.), these pathogens can survive in the anaerobic

fermentation process, and may remain in biogas residue and biogas slurry (Nag R et al., 2020). The top 10 pathogens associated with the farming of livestock and poultry manure biogas slurry in Ireland with potential risks were assessed: *Cryptosporidium parvum*, *Salmonella*, *Norovirus*, *Streptococcus pyogenes*, *Escherichia coli enteropathy*, *Mycobacterium*, *Salmonella typhi* (followed by *Salmonella paratyphi*), *Clostridium*, *Listeria monocytogenes*, and *Campylobacter coli* (Nag R et al., 2020). It shows that untreated livestock manure biogas slurry agricultural use can introduce pathogens into farmland and spread disease through the food chain (mainly ready-to-eat crops) (Nag R et al., 2019). Hence, before the biogas slurry is used for agriculture, it is necessary to ensure the pathogenic presence standardization. This can be achieved by high-temperature, ultraviolet radiation and other treatments for sterilization and disinfection.

### **2.3.3 Application methods of using biogas slurry in farmland**

#### **2.3.3.1 Drip irrigation**

The biogas slurry is applied in the planting of protected vegetables and melons and fruits, and drip irrigation measures are mostly adopted, which have the advantages of uniform application, cost saving, production increase and nutrient improvement. Strict filtration and blockage prevention systems need to be put in place in drip irrigation. Compared with spraying biogas slurry, drip irrigation with biogas slurry can make the available nutrients in the substrate higher, as well as improve the growth of crops (Li S et al., 2014). Using the method of biogas slurry aeration drip irrigation to conduct a plot test on leeks in a greenhouse, it was found that when the concentration of biogas slurry was 80%, with aeration coefficient of 1.0, the yield of leeks was the highest up to 230.50 kg/667m<sup>2</sup>, an increase of 28.53% compared with the control. At the same time, the content of vitamin C in chives increased by 77.78%, while the content of soluble sugar increased by 91.20%, and the content of soluble protein increased by 70.59% (Chu C et al., 2013). Similarly, the drip irrigation biogas slurry treatment in watermelon, cucumber, strawberry, grape and tomato fruit cultivation achieved 13.9% increase in watermelon fruit weight (Zhou G et al., 2014), 15.7% increase in strawberry fruit weight (Yuan C et al., 2013), and 18.1% increase in tomato fruit weight (Sui H et al.,

2016). The strawberry yield increased by 18.1% (Yuan C et al., 2013), grape yield increased by 18.3% (Wang Z and Ma D, 2018), cucumber yield increased by 47% (Wang Z, 2018) and tomato yield increased by 20.7%–59.4% (Wang X et al., 2013; Ma D et al., 2017). Increase in the fruit soluble total sugar, Vitamin C, titratable acid, and improved fruit firmness were observed (Sui H et al., 2016).

Furthermore, after the biogas slurry was diluted with water, it was dripped into the saline-alkali soil. The results showed that the soil fertility was significantly improved, the soil pH was reduced, and the desalination effect was significant in the 0–20 cm soil layer, but salt accumulation occurred below the 20 cm soil layer (Wang X et al., 2018). From the economic point of view, integrated drip irrigation of biogas slurry, water and fertilizer greatly reduces the manpower and material costs of the previous use of biogas slurry distribution vehicles to transport to the fields. It also realizes the resource utilization of biogas slurry and reduces the application of chemical fertilizers (Gao X et al., 2019). Compared with the biogas slurry flood irrigation treatment, the biogas slurry water and fertilizer integrated drip irrigation treatment significantly improved the yield and quality of pear fruit, and the soil nitrogen nutrient accumulation was significantly reduced. Likewise, comparing the use of biogas slurry with conventional fertilization, 43% of chemical fertilizer usage can be avoided and can reduce soil nitrogen accumulation (Wang L et al., 2021b). The integrated drip irrigation of biogas slurry, water and fertilizer for pear trees also significantly improves the yield and quality of pear fruit, providing an economical and effective fertilization mode for precise fertilization of pear trees.

### **2.3.3.2 Ditch irrigation and flood irrigation**

At present, the methods used to absorb biogas slurry in farmland are still mainly furrow irrigation, flood irrigation and surface application, which not only requires a large amount of labor and high labor intensity, but also has large loss of ammonia volatilization. Long-term application may cause secondary soil salinization, heavy metal accumulation, and increased groundwater contamination risk (Wang Y, 2010; Li J et al., 2021). It was found that the irrigation of biogas slurry could increase

the root system and the yield of *Codonopsis pilosula*, towards the commercialization level of *Codonopsis pilosula* (An J and Song Z, 2011). Similarly, the furrow irrigation biogas slurry application significantly increased the yield of tomato. The yield increase rate was 10%, and the vitamin C content increased was 1.54 times better compared with the control (Zhang F et al., 2013). In ditch irrigation, when the amount of biogas slurry applied was the same, the content of ammonium nitrogen and nitrate nitrogen in the soil treated by furrow application was higher than that of surface application and deep ploughing (Yuan Y et al., 2019). Moreover, biogas slurry flood irrigation significantly increases the content of soil organic matter, total nitrogen, alkali-hydrolyzed nitrogen, available potassium. Long-term application will increase the risk of secondary soil salinization.

### **2.3.3.3 Spraying application**

The spraying of biogas slurry is more common with foliar spraying and soil surface spraying. It is necessary to pay attention to the spraying concentration and spraying amount. Studies have shown that spraying 60% concentration of biogas slurry or root application of biogas slurry, the yield of tomato, radish, celery, and anal bean can significantly be increased. Similarly, in celery (*Apium graveolens* L.) production, spraying 40% concentration of biogas slurry has the best yield increase effect (Jiang H et al., 2007). Spraying nectarine leaves with different concentrations of biogas slurry can increase the nutrition of nectarine leaves, and at the same time, the single fruit weight, soluble sugar content and sugar-acid ratio of nectarine fruit are also improved (Wang C, 2010). Foliar spraying of biogas slurry can effectively increase the single melon quality of cantaloupe and improve its quality. Furthermore, spraying 75% concentration biogas slurry has the best effect (Song B et al., 2016; He M et al., 2018). And foliar spraying of biogas slurry can increase the yield of apple trees and increase the content of vitamin C and soluble sugar in the fruit (Chen J et al., 2017). The use of biogas slurry spray irrigation can improve the soil content of alkali-hydrolyzed nitrogen, available phosphorus and available potassium in the deep soil (Wang K et al., 2019).

#### 2.3.3.4 Compatible application

The application of biogas slurry is no longer limited to drip irrigation, furrow irrigation, flood irrigation, spraying and other conventional means. In order to maximize the benefits of biogas slurry resources and promote its high value usage, the application of biogas slurry in combination with chemical fertilizer (Xu K et al., 2020), solid organic fertilizer (Zhou W et al., 2019), biochar (Yuan J et al., 2022), duckweed (Song D et al., 2020), as well as pesticide (Cheng H et al., 2018; Ran Y et al., 2020; Qi B et al., 2021), has become desirable.

A study found that 150 mL/m<sup>2</sup> biogas slurry plus 27g/m<sup>2</sup> urea combined application was beneficial to increase the yield of dandelion, increase the content of vitamin C, nitrate and soluble protein (Kang X et al., 2019). In related studies, the combined application of pig manure biogas slurry and earthworm fertilizer significantly improve the yield and quality of flat peach fruit (Li H et al., 2019b), while, the combination of biogas slurry and biochar increase the mass fraction of soil water stable aggregates (Li C et al., 2014). Moreover, the combined application of biogas slurry and biochar for 3 years effectively increase the mass fraction of soil water-stable aggregates with a particle size of >0.25 mm, which is 13.0%–36.3% higher than that of the control (Yuan J et al., 2022). When biochar application is constant (12 t/hm<sup>2</sup>), soil water-stable aggregate organic carbon shows a trend of increasing gradually with the increase of biogas slurry concentration, as well as increase in the range (4.8% to 37.1%) of soil organic matter (Zheng J et al., 2020b). When the ratio of biogas slurry is constant, with the increase of biochar dosage, the soil quality gradually decreases (Zheng J et al., 2020a). The application of 6% biochar from biogas residues can significantly reduce the leaching amount of biogas slurry nitrogen in lime-soil, and the leaching amounts of total nitrogen, ammonium nitrogen, nitrate nitrogen and nitrite nitrogen were reduced by 12.06, 11.82, 1.14, and 0.103 kg/hm<sup>2</sup>, the declines were 35.89%, 52.99%, 25.53%, and 23.25%, respectively (Jiang T et al., 2021).

Furthermore, combined application of biogas slurry concentrate and chemical

fertilizer increased the rapeseed yield by 9.7% (Luo L, 2010). Also, combined application of biogas slurry concentrates with chemical fertilizer and chicken manure significantly improve the quality of tomato with the contents of vitamin C and sugar increased by 9.36% and 49.52%, respectively, while the nitrite content was decreased by 27.05% (Liu J, 2013). Similarly, the application of biogas slurry concentrated with amino acid formula fertilizer increased banana yield by 4.09%, banana fruit protein by 10.67%, and vitamin C by 3.32%. It also increased the pH value of acidic soil and soil organic matter content by 2.98% and 3.93%, respectively (Gao L et al., 2017). While, foliar spraying of amino acid biogas slurry increased the pulp hardness and soluble solid content of cantaloupe by 13.9% and 7.7%, respectively (Chen N et al., 2021). Similarly, the addition of nutrients to biogas slurry concentrate, combined with berberine showed a strong inhibitory effect on tomato botrytis cinerea (Liu J et al., 2018). A related study using chicken manure biogas slurry concentrate diluted 300–500 times and mixed with pyridaben reduced by 10% can reduce the amount of pesticide application by 10%–20%. It not only achieves the purpose of pest control, but also delays the enhancement of pest resistance, and reduced medication costs (Liu M et al., 2019). Also, concentrated chicken manure biogas slurry and flonicamid were used in a combination to control apple yellow aphid, when the pesticides were reduced by 10% to 20%, the control effect was better than or equal to the conventional dosage of flonicamid (Wang H et al., 2019).

### **2.3.4 Advantages of using biogas slurry in farmland**

#### **2.3.4.1 Soil fertilization**

Farmland application of biogas slurry can improve the physical and chemical properties of farmland soil (Möller K, 2015; Niyungeko C et al., 2020; Tang Y et al., 2021) while effectively valorizing the biogas slurry (Chen S et al., 2015). This has a direct positive effect on increasing soil organic matter, maintaining soil structure, and fertility (Xu C et al., 2013; Liu X et al., 2018; Yu D et al., 2018).

The decrease in soil organic matter content is one of the reasons for the deterioration of soil structure and the reduction of soil productivity (Yılmaz E et al.,

2019). The application of biogas slurry rich in organic matter to farmland can increase the content of organic matter, especially dissolved organic matter in the soil, thereby improving soil structure (Song Z et al., 2011; Yan L et al., 2019; Huang H et al., 2021). For instance, the pig manure biogas slurry can increase the organic matter content of the topsoil to 3.0 kg/hm<sup>2</sup> (Chen Y et al., 2011). After 5 years of biogas slurry irrigation, the soil organic carbon content increased significantly by 90.3% compared with the soil with chemical fertilizers (Yang L et al., 2012). However, some studies applying biogas slurry from chicken manure, pig manure and cow manure on the soil in comparison to the control had no significant effect on soil organic matter content (Li Y et al., 2021b). The input of nitrogen from biogas slurry promotes the consumption of organic carbon by non-autotrophic microorganisms (Wang W et al., 2010), thereby offsetting the accumulation of organic matter carried by biogas slurry in the soil (Huang J et al., 2016b). The effect of biogas slurry application on soil organic matter content is related to the application mode and the concentration of biogas slurry. Under the same nitrogen substitution conditions in biogas slurry, the increase of organic matter content in the injection treatment is higher than that in the spray treatment (Liu C et al., 2021). The increase of soil organic matter content was proportional to the amount of biogas slurry application (Huang J et al., 2013). The soil organic matter content of all the fertilizers applied gradually decreased with the growth of corn, while the biogas slurry treatment was the opposite. At the mature stage, the organic matter content of all the treatments with biogas slurry was found to increased (Cui Y et al., 2020).

Consumption of biogas slurry on farmland can enhance soil permeability, water retention and fertilizer retention capabilities, and avoid damage to soil environment, an advantage that chemical fertilizers do not have (Li J et al., 2021). The 3-year application of biogas slurry (165.1 and 182.1 t/hm<sup>2</sup>) improved the nutrient content of yellow soil under rice-rape rotation and promoted the formation of aggregate soil structure (Xu M et al., 2019). With the increase of the amount of biogas slurry in the mixed solution, the stability indicators of dry-fed red soil aggregates: soil >0.25 mm water-stable aggregate content (WR0.25), aggregate mean mass diameter (MWD), geometric mean diameter

(GMD), the aggregate stability rate (AR) showed an upward trend, and the fractal dimension (D) showed a downward trend. After long-term biogas slurry irrigation, soil porosity, soil aggregate structure and microorganisms in soil increased (Ruan R et al., 2021).

Similarly, the application of biogas slurry can effectively adjust the proportion of various nutrients in the soil, enhance the balanced nutrient absorption capacity of crops, increase crop resistance to diseases (Nzila A, 2018), improve soil organic quality, and structure (Shahbaz M et al., 2014). For example, soil ammonium nitrogen and soil nitrate increased by 47.8% and 19.0% compared with the control, respectively (Chen S et al., 2015). Also, after applying biogas slurry formulated fertilizer in the orchard, the soil organic matter content in each soil layer increased from 2.98% to 3.93%, the available phosphorus increased from 5.59% to 18.64%, and the available potassium increased from 25.20% to 39.20% (Gao L et al., 2017). Compared with the control treatment (no fertilization), the application of chicken manure biogas slurry, pig manure biogas slurry and cow manure biogas slurry under isonitrogen conditions could improve soil inorganic nitrogen, total nitrogen, available phosphorus, available potassium, pH and electrical conductivity. It is worth noting that the increase in soil nitrate nitrogen in pig manure biogas slurry treatment is the largest, followed by chicken manure biogas slurry treatment (Li Y et al., 2021b). The maximum growth rate of available phosphorus, total phosphorus, available nitrogen and total nitrogen were 81.12%, 12.66%, 84.88%, and 127.70% in winter wheat soil samples after biogas slurry fertilizer was applied in paddy and wheat rotation fields. Continuous application of biogas slurry could increase the contents of total nitrogen, total potassium and alkaline hydrolyzed nitrogen in farmland soil (Lai X et al., 2018; Qiao F et al., 2018). The content of soil organic matter, cation exchange capacity, electrical conductivity, total soil nitrogen, total phosphorus, total potassium, alkali-hydrolyzed nitrogen, available phosphorus, available potassium and NH<sub>4</sub>-N content of paddy field with continuous application of biogas slurry four (4) years were significantly higher than those without biogas slurry application (Dong Y et al., 2021b). Interestingly, a tea garden with extremely low soil



fertility level reached high fertility level after continuous application of biogas slurry for 2 years. The soil indexes of the treatment with biogas slurry for 4 years were significantly improved compared with the treatment without biogas slurry (Dong Y et al., 2022).

#### **2.3.4.2 Improvement in crop production**

Achieving the increase in crop yield is the primary object of concern for biogas slurry fertilizer utilization (see **Table 2.4**). Meta-analysis method was used to quantitatively analyze the effect of biogas slurry application on crop yield under different conditions (Zheng J et al., 2019). The results showed that: the effect of biogas slurry application on crop yield of yield of wheat, corn, tomato and rice were all improved. Moreover, the impact of biogas slurry on farmland could be soil type or climate specific. For example, biogas slurry application in northwest and north China increased crop yield significantly compared with other regions, while that in southwest and east China was slightly lower.

Identifying the physicochemical properties, application period and application amount of biogas slurry plays an important role in formulating technical measures for safe and efficient use of biogas slurry (Odlare M et al., 2011a; Liu X et al., 2018; Xu M et al., 2019). Applying 50% biogas slurry instead of chemical fertilizer can obtain the same corn yield as chemical fertilizer treatment (Wu H et al., 2012). Similarly, biogas slurry fermented by pig urine and feces significantly increase the yield of corn. When the concentration was controlled within the range of 60–90 t/hm<sup>2</sup>, maximum corn yield was obtained (Chen B, 2010). Also, many studies have shown that the application of biogas slurry was beneficial in rice cultivation to increase rice yield (Tang W et al., 2010; Huang H et al., 2013; Huang J et al., 2016a; Wang G et al., 2018). It was also reported that the complete replacement of fertilizer with full biogas slurry will significantly reduce rice yield (Wang W et al., 2014; Wang Z et al., 2016). Hence, the application of appropriate biogas slurry is critical in improving rice than conventional fertilization (Hou F et al., 2019).

Table 2.4 Yield-increasing effect of applying biogas slurry to farmland

Slurry type	crops	Production increase range		References
		Comparison with conventional chemical fertilizer	Comparison with no fertilization	
PM	Rice	0.2%–20.4%	1.0%–102.5%	(Huang H et al., 2013; Huang J et al., 2016a; Shao W et al., 2017; Song S, 2017; Wang G et al., 2018; Hou F et al., 2019; Xu K et al., 2020; Sun G et al., 2021)
	Wheat	2.9%–22.4%	97.1%–217.5%	(Huang H et al., 2013; Song S, 2017)
	Com	0.6%	5.6%–13.2%	(Chen B, 2010; Wu J et al., 2014)
	Barley	1.1%–2.0%	31.9%–111.9%	(Yang Z et al., 2019)
	Watermelon	0.2%–24.9%	ND	(Zhou G et al., 2014; Cao Y et al., 2015; Cheng W et al., 2018)
	Pear	12.0%	3.1%–7.4%	(Hao S et al., 2012; Wang L et al., 2021b)
	Grape	ND	10.7%–18.3%	(Wang Z and Ma D, 2018)
	Peach	ND	9.7%–43.7%	(Li H et al., 2019b)
	Cabbage	ND	75.4%–133.9%	(Lin S et al., 2019)
	Tea	ND	9.3%–93.4%	(Hu Z et al., 2020)
DM	Corn	ND	59.2%–81.7%	(Qiao F et al., 2018)
	Melon	8.8%–32.2%	8.6%–33.0%	(Song B et al., 2016; He M et al., 2018; Chen N et al., 2021)
CM	Grape	30.0%–170.0%	ND	(Hao Y, 2019)
	Corn	9.0%–26.2%	12.9%–107.7%	(Cui Y et al., 2020; Li J et al., 2021)
	Apple	2.0%–3.5%	42.8%–67.0%	(Gao W et al., 2020)
MM	Leafy vegetables	9.1%–45.1%	45.1%	(Zhao F et al., 2010; Wang H et al., 2013; Li S et al., 2014)
	Rice	4.6%–7.7%	4.1%	(Huang L et al., 2014; Zhang Y, 2018)
	Wheat	ND	28.2%–71.1%	(Wang J et al., 2019)
	Tomato	0.49%–21.59%	15.6%–39.8%	(Sui H et al., 2016; Jia L et al., 2017)
	Cabbage	8.5%–41.2%	ND	(Ma X and Tao Z, 2019)
	Tangerine	11.8%–24.8%	32.4%–56.4%	(Xi H et al., 2021; Liu Y et al., 2022)

ND: no data; PM: biogas slurry using pig manure as fermentation raw material; DM: biogas slurry using cow dung as fermentation raw material; CM: biogas slurry using chicken manure as fermentation raw material; MM: biogas slurry using two or more of straw, human excrement, pig manure, cow dung, chicken manure and other household waste mixture as fermentation raw material; TN: total nitrogen; TP: total phosphorus; TK: total potassium.  $\text{NH}_4^+\text{-N}$ : ammonium nitrogen;  $\text{NO}_3^-\text{-N}$ : nitrate nitrogen; DP: available phosphorus; DK: available potassium.

### 2.3.4.3 Quality improvement

The digestion of biogas slurry in farmland can improve the nutritional quality of cultivated crops (increase the content of protein, soluble solids, reducing sugar and minerals in crops) (Tang W et al., 2010; Wu J et al., 2014). This will turn enhance the commodity economic value. For instance, the increasing use of biogas slurry in

irrigation of rapeseed cultivation improve, Fe, Mn, Cu and Zn mineral content, while the content of oleic acid, Ca and Mg in rapeseed increased first and then decreased. The optimal quality of rape was achieved when biogas slurry was applied in the range of 78.8–101.3 t/hm<sup>2</sup> (Wu J et al., 2013). The application of biogas slurry can increase the protein content of rice and improve the nutritional quality of rice (Tang W et al., 2010; Wang G et al., 2018). But some studies also shown that the application of biogas slurry has little effect on the nutritional quality of rice. (Moreno-García B et al., 2017; Yang X et al., 2021). Based on the 8–9 years data analysis of long-term biogas slurry application, an improvement in the rice yield, taste value of rice, and gel consistency of rice when compared with those obtained from chemical fertilizer treated soil (Sun G et al., 2021). The application of biogas slurry promoted the accumulation of vital components such as polysaccharides, carotenoids, flavonoids and betaine in lycium barbarum fruit, thereby improving the nutritional quality of lycium barbarum and its efficacy (Zhou Q, 2012). Similarly, the application of nitrogen fertilizer and fermented pig manure in the cultivation of Chinese cabbage showed lower content of amino acids and soluble sugar when compared with the application of pig manure biogas slurry alone (Xu P et al., 2014). Likewise, in a related study, the effects of biogas slurry treatment with concentration of 25%, 50%, 75% and original liquid on the quality of *Capsicum* spp. were studied. The results showed that with the increase of biogas slurry concentration, the chlorophyll content, vitamin C content, soluble sugar content and organic acid content of *Capsicum* spp. were improved (Cui Y et al., 2019). It was also found that applying biogas slurry to replace chemical fertilizer could significantly increase the soluble sugar, soluble solids and sugar acid ratio of muskmelon (Gao X et al., 2019).

#### **2.3.4.4 Bacteriostatic**

Biogas slurry undergoes long-term anaerobic fermentation to produce a variety of biologically active substances, such as organic acids, vitamin B12, gibberellin, that inhibit the proliferation of soil bacteria, fungal and viruses (Huo C et al., 2011). In addition, the high concentration of NH<sub>4</sub><sup>+</sup>-N in the biogas slurry has the potential of

killing pests and pathogenic bacteria (Cao Y et al., 2013a; Cao Y et al., 2013b). For instance, fresh biogas slurry from cattle farm has strong inhibition effect on *Botrytis cinerea* (Hyphomycetes), *Phytophthora capsici*, *Alternaria solani*, *Colletotrichum gloeosporioides* Penz., and *Botrytis capsici*. However, when biogas slurry storage time was increased, the inhibition rate of biogas slurry against *phytophthora capsici* and *fusarium solani* decreased significantly (Shang B et al., 2011). Similarly, concentrated biogas slurry remarkably inhibit the growth of cotton verticillium wilt mycelium (the inhibitory effect of 0.50% concentrated solution biogas slurry on cotton verticillium wilt disease was 64.89%). The biogas slurry also prevent spore production, conidial germination and microsclerotia germination. (Feng Z et al., 2018). Likewise, the application of biogas slurry had effective repellent effect on adult brown rice plant hopper (Huang L et al., 2014). The spraying of biogas slurry with 66.6% concentration had the best repellent effect (Ma X and Tao Z, 2019).

Furthermore, the application of biogas slurry was effective in the prevention and control of soil borne diseases of crops (Min YY et al., 2011; Cao Y et al., 2016b; Li Y et al., 2020). Although nitrogen input is considered to be the key factor to stimulate soil microbial biomass carbon (Möller K, 2015), however, a large amount of ammonium nitrogen in biogas slurry may play a role in inhibiting microbial growth in the short term. The bacteria population in soil decreased, after biogas slurry was applied in pot culture system (Cao Y et al., 2013b). For example, irrigation with high concentration of biogas slurry in broccoli field reduces soil fungi by 55.03% (Yang Z et al., 2017), thus significantly reducing the plant disease index. Similarly, when biogas slurry was applied to watermelon, a significant inhibitory effect on Fusarium wilt was observed, and the disease index was lowered by 36.4% compared to the control treatment (Cao Y et al., 2016b). Further analysis showed that the inhibition of basidiomycota and mortierella growth was the reason for the decrease disease index (Wang L et al., 2021a). Remarkably, root irrigation with biogas slurry effectively prevent and cure astragalus root rot, the same inhibitory effect was obtained when this was repeated many times (Sang D and Yong S, 2011). Likewise the inhibitory effect of 1.25% biogas slurry

concentrate on cotton verticillium wilt by root irrigation reached 78.01% (Feng Z et al., 2018).

#### **2.3.4.5 Prevention and control of soil acidification**

Soil acidification is one of the main factors affecting agricultural productivity as well as negatively impacting the environment. Soil acidification will destroy the structure of biological cell membranes, reduce microbial activity of soil microorganisms, consequently, crop growth and productivity. Prevention and control of soil acidification is of great significance to maintaining sustainable agricultural development (Wang A et al., 2019). The consumption of biogas slurry in farmland can effectively regulate the proportion of various nutrients in the soil, enhance the ability of soil to buffer acid and alkali changes. It can reduce the pH in alkaline soil (Lin S et al., 2019) and increase the pH in acidic soil, thereby improving soil quality (Zheng X et al., 2016b; Wang K et al., 2019). Studies have shown that irrigation with biogas slurry in coastal poplar forests and coastal saline-alkali rice-wheat rotation fields cause pH reduction (Tang Y et al., 2019b; Zhu R, 2019). For instance, soil pH of alkaline paddy field treated with biogas slurry for 4 years was significantly lower than that of soil without biogas slurry application (Dong Y et al., 2021b). Compared with conventional fertilization treatment, biogas slurry application can effectively inhibit soil acidification caused by long-term application of chemical fertilizers (Sun G et al., 2021). Similarly, a 3-year field experiment was carried out on the yellow soil under rice-rape rotation as the experimental site, and it was found that the application of biogas slurry (165.1 and 182.1 t/hm<sup>2</sup>) could increase the soil pH value (Xu M et al., 2019). Lower concentration of biogas slurry do not prevent soil acidification, while higher biogas slurry concentration inhibited the growth of acidobacteria, thereby reducing soil acidification (Wang L et al., 2021a). Likewise, long term application of biogas slurry, resulted in increasing trend of soil pH of cultivated Hongmeiren citrus. The soil pH after 4 years biogas slurry application was significantly higher than that of the conventional fertilization (Liu Y et al., 2022). Also, the application of biogas slurry in economic fruit plantation such as tea garden (Li W et al., 2021), grapefruit (Huang H et al., 2021),

apple (Gao W et al., 2020) and citrus (Xi H et al., 2021) showed similar results of increasing soil pH value.

#### **2.3.4.6 Improved microbial structure and soil enzyme activity**

The role of soil organisms in underground ecological processes are vital to maintaining a healthy farmland (Cao Z, 2013). An important group of soil organisms are the microorganisms; as decomposer in the food web (Li Y et al., 2018), they occupy more than 80% of food web biomass (Li Y et al., 2021a). These microbes participate in the decomposition and synthesis of soil organic matter, the fixation and release of nutrients, and the degradation of pollutants. The impact of farmland application of biogas slurry on underground ecological processes will inevitably lead to changes in microbial community structure, metabolic characteristics and functional diversity, which in turn can be used as important indicators for the evaluation of the health of farmland.

Soil microbial biomass C/N ratio reflects the composition of soil microbial flora. The lower the microbial biomass C/N ratio, the more bacteria in the soil. Application of biogas slurry can increase the culturable quantity of soil bacteria (Feng D et al., 2014), fungi (Tang Y et al., 2021) and actinomycetes (Yu F et al., 2010) to a certain extent. In a related study, after biogas slurry application, the ratio of soil microbial biomass C/N decreased by 25.2%–48.0% (Liu Y et al., 2022). The application of biogas slurry promoted the proliferation of soil bacteria, and the activity of soil bacteria increased significantly with long-term application of biogas slurry on farmland (Chai Y et al., 2019). The ratio of bacteria and fungi (B/F) in the soil is usually used to evaluate the soil microbial flora (Sun Y et al., 2021). A high B/F value indicates that the soil is a "bacterial type" with high fertility and less damage to the soil, while a low B/F value indicates that the soil is a "fungal type" with low fertility and high damage to the soil. For instance, the treatment of biogas slurry mixed with chemical fertilizer reduced the B/F value. Increase in the concentration of biogas slurry application resulted in B/F value that initially decreased and then increased, while, the application of pure biogas slurry increased the B/F value considerably (Zheng X et al., 2015). Hence, the B/F value

of soil can be kept stable or even increased by using appropriate biogas slurry, and consequently, improving the soil fertility.

Nitrogen, phosphorus, potassium, organic matter, growth hormone, humic acid, cellulose and other substances in biogas slurry can further promote the growth and enrichment of soil dominant bacteria (Lv W et al., 2011) as well as promote microbial alpha diversity by improving soil structure and increasing organic matter (Abubaker J et al., 2013; Cao Y et al., 2013b; Xu C et al., 2013; Cao Y et al., 2016a; Cao Y et al., 2016b; Wentzel S and Joergensen RG, 2016). For example, paddy soil with biogas slurry applied continuously for 6 years, campylobacter, proteus and acidobacter were the dominant bacteria, which shows that biogas slurry can improve soil microbial structure and consequently, soil quality and soil fertility (Chen Z et al., 2020). Moreover, with increase in biogas slurry concentration resulted in actinomycetes population, however, excessive biogas slurry application inhibits the growth of actinomycetes (Xu M et al., 2019; Wang L et al., 2021a). The Chao1 index and Shannon index of soil bacteria treated with 180 t/hm<sup>2</sup> biogas slurry were higher than those of control treatments. However, the Chao1 index of fungi was lower than that of chemical fertilizer (100 t/hm<sup>2</sup> and 220 t/hm<sup>2</sup> treatments). The application amount of biogas slurry at 180 t/hm<sup>2</sup> can improve the bacteria richness and diversity, while reducing the diversity of fungi (Wang L et al., 2021a).

In addition, the application of biogas slurry in farmland has a certain impact on the activities of soil animals. When the concentration of biogas slurry increased from 0 to 300m<sup>3</sup>/hm<sup>2</sup>, the density of soil animals increased by 94%, the number of groups increased by about 2, and the dominance index increased by 9.4% ( $P<0.05$ ). When 66% of biogas slurry was used to replace chemical fertilizer, soil animal density, number of groups and dominance index were the highest. The principal component analysis showed the application of the biogas slurry alone or mixed with the chemical fertilizer, collembola, prestoma, and ortychia were the most sensitive, and they could be used as indicators of the response of small arthropods in the soil to digesting biogas slurry.

Proper application of biogas slurry in farmland can increase soil enzyme activity

(see **Table 2.5**) thereby improving soil carbon and nitrogen transformation. Studies have shown that applying biogas slurry increased the activities of soil phosphatase, protease, dehydrogenase, sucrase, catalase and urease. After 4 years of biogas slurry application, the activities of the six (6) enzymes were significantly higher than those of the control (Liu Y et al., 2022).

Table 2.5 Improvement of soil enzyme activity by digested biogas slurry in farmland

Soil type	Crops	Slurry type	Total nitrogen consumption of slurry (kg/hm <sup>2</sup> )	Years	Improvement range of soil enzyme activity		References
					Comparison with no fertilization	Comparison with conventional chemical fertilizer	
Yellow loamy paddy soil	Rice-rape	PM	157.5–694.9	3	Urease 31.8%–74.6%, catalase 4.4%–85.1%, sucrase 30.4%–228.6%	Urease 21.7–61.1%, catalase 19%–111%, sucrase 45.4%–266.4%	(Feng D et al., 2014)
Retention paddy soil	Rice-wheat	PM	210.3–540.9	3	Urease 30.5%–79.5%, protease 19.1%–41.4%, phosphatase 11.3%–29.7%, catalase 6.4%–40.1%, sucrase 0.2%–39.3%, amylase 53.1%–161.4%, cellulase 15.8%–104.8%, lactase 30.1%–65.2%	Urease 26.7%–74.8%, protease 9.1%–43.3%, phosphatase 7.3%–8.8%, catalase 14.9%–30.2%, sucrase 3%–49.4%, amylase 28%–145.7%, cellulase 38.5%–48.3%, lactase 13.3%–37.1%	(Song S, 2017)
Medium loam Red soil	Wheat	PM	60–180	1	Urease 57.5%–72.5%, protease 31.7%–62.6%	Urease 10.5%–21.1%, protease 6%–30.1%	(Guan T et al., 2010)
Aeolian sandy soil	Peanut	PM	36–240	2	Urease 16.2%–62.3%, dehydrogenase 48.6%–133.1%	Urease 11.3%–24.1%, dehydrogenase 33.8%–120.5%	(Zheng X et al., 2015)
ND	Grape	DM	190–1160	2	ND	Urease 41%–113.8%, phosphatase 32.4%–106.4%, sucrase 62.7%–98%	(Hao Y, 2019)
ND	Cucumber	(P+C)M	37.5–150	1	Polyphenol oxidase 13.49%–14.75%, cellulase 68.7%–71.9%, chitinase 41.0%–57.5%	ND	(Zhang H and Wang G, 2011)
ND	Cabbage	(P+S)M	504–675	3	ND	Urease 2.4%, protease 95.4%–139.7%, phosphatase 50.5%–137.6%, invertase 55.7%–64.0%, Urease 53.8%–100.0%, protease 23.1%–100.0%, phosphatase 20.2%–42.3%, catalase 107.0%–127.5%, dehydrogenase 36.6%–96.0%, sucrase 47.4%–111.8%	(Hao X et al., 2011)
Paddy soil	Citrus	(P+D)M	450	4	ND	ND	(Liu Y et al., 2022)

ND: no data; PM: biogas slurry using pig manure as fermentation raw material; DM: biogas slurry using cow dung as fermentation raw material; (P+C)M: biogas slurry using pig manure and chicken manure as fermentation raw material; (P+S)M: biogas slurry using pig manure and straw as fermentation raw material; (P+D)M: biogas slurry using pig manure and cow dung as fermentation raw material.

## 2.4 Prospects

It is important to reduce the pollution risk of biogas slurry disposal through its valorization. There are numerous advantages of biogas slurry application as listed above. However, farmland application of biogas slurry needs to be further studied to:



1) establish fitting models for different components of biogas slurry adsorbed by soil, maximum adsorption capacity of different types of soil and environmental factors affecting soil adsorption; 2) explore the transformation and characteristics of biogas slurry components in its farmland application, the time cycle of safe application, as well as assessment of associated potential risks; 3) develop combined technologies to increase biogas slurry utilization and valorization; and 4) analyze the mechanism and microecological mechanism of biogas slurry digested in farmland to improve soil fertility, and yield to establish the theoretical and practicable application of biogas slurry on farmland.

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# CHAPTER 3: ADSORPTION CHARACTERISTICS OF THREE TYPES OF SOILS ON BIOGAS SLURRY AMMONIUM NITROGEN

This chapter has been published as detailed below:

Wang Z, Zhang L, Sun G, Zhou W, Sheng J, Ye X, Olaniran AO and Kana EBG 2022. Adsorption characteristics of three types of soils on biogas slurry ammonium nitrogen. *Frontiers in Environmental Science* 10:942263. <https://doi.org/10.3389/fenvs.2022.942263>.

## 3.1 Abstract

Using farmland to digest biogas slurry is an effective measure to overcome the bottleneck of sewage treatment in livestock and poultry farms. However, there is limited research on the soil adsorption characteristics of biogas slurry ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ). In addition, the maximum adsorption capacity ( $Q_m$ ) of farm soil is unclear. In this study, three typical farmland tillage layer soils (silty loam, loam, and sandy loam) were used to analyze adsorption characteristics through adsorption kinetics experiments (adsorption for 0.25, 0.5, 1, 2, 4, 6, 12, 18, or 24 h with  $\text{NH}_4^+\text{-N}$  concentrations of 42.90 mg/L) and thermodynamic experiments (adsorption for 3 d with  $\text{NH}_4^+\text{-N}$  concentrations of 54.25, 88.66, 105.85, 133.71, 178.80, 273.54, and 542.87 mg/L). The  $Q_m$  value was fitted by models, and its relationship with soil properties was discussed. The results showed the following: (1) the adsorption of biogas slurry  $\text{NH}_4^+\text{-N}$  by the three types of soils was a composite kinetic process that comprised two stages of rapid and slow reactions. Rapid adsorption predominantly occurred within 0–1 h, and the adsorption capacity accounted for 35.24–43.55% of the total adsorption. The ExpAssoc equation produced a good fit for the adsorption kinetic behaviour in the three soil types. (2) The equilibrium adsorption could be described by the Langmuir equation, the Freundlich equation, the PIPlatt model, and the Langevin model isotherm, among which the Langevin model had the best fit, with a coefficient of determination  $R^2$  close to 1. The theoretical saturated  $Q_m$  fitting results of  $\text{NH}_4^+\text{-N}$  were 1038.41–1372.44 mg/kg in silty loam, 840.85–1157.60 mg/kg in loam, and 412.33–481.85 mg/kg in sandy loam. The

optimal values were 1108.55 mg/kg, 874.86 mg/kg, and 448.35 mg/kg for silty loam, loam, and sandy loam, respectively. (3) The  $Q_m$  value was significantly positively correlated with soil organic matter, total nitrogen, available phosphorus, available potassium, cation exchange capacity, and particle content of 0.02–0.002 mm ( $P < 0.01$ ), but significantly negatively correlated with soil pH ( $P < 0.05$ ). This study can provide a reference for the safe application of biogas slurry on farmland.

**Keywords:** *biogas slurry, wastewater, ammonium nitrogen, soil absorption, kinetics, thermodynamics*

### **3.2 Introduction**

Biogas slurry is the residual liquid substance produced by anaerobic fermentation in biogas engineering using biodegradable organic wastes, such as human and livestock manure or various agricultural and forestry wastes. It is produced together with methane, carbon dioxide, and other gases under certain conditions of water content, temperature, and the action of methane bacteria in closed containers (Han et al., 2014). Biogas production has become an important energy-saving and emission-reduction technology for the harmless treatment and energy utilization of livestock and poultry manure in China. According to recent estimates, the biogas slurry produced by the anaerobic fermentation of livestock and poultry manure is more than 1.3 billion tons each year (Lu et al., 2010; Zhu and Huang, 2010). Returning biogas slurry to farmland soil as fertilizer is an effective method of economical use (Bradford et al., 2008; Ning et al., 2019; Liu et al., 2020). However, unreasonable application will not only reduce the use efficiency of biogas slurry, but it will also cause the environmental pollution of farmland water and the reduction of crop yields (Gao et al., 2011; Chen et al., 2013; Wang et al., 2016). The safe utilization of biogas slurry resources is expected to become an increasingly important topic in agriculture, energy use, and environmental protection and thus urgently needs to be investigated.

Biogas slurry is rich in a variety of nutrients required for plant growth and development, such as nitrogen, phosphorus, potassium, copper, zinc, organic acids, hydrolases, and amino acids. The total nitrogen (TN) content of biogas slurry is 0.53–

3.24 g/kg (Jin et al., 2011; Ni and Zhang, 2017). Many researchers have studied the use of biogas slurry nitrogen as a measurement parameter for farmland reuse and discussed the safe dosage for fertility enhancement, yield, quality improvement, and environmental emission reduction. For example, Wang et al. (2010) found that applying biogas slurry equivalent to 2–3 times the amount of chemical fertilizer could obtain a *Capsicum* spp. yield similar to that of conventional chemical fertilizer treatment, as well as improve *Capsicum* spp. quality, increase the contents of soil organic matter (SOM), TN, available nitrogen, phosphorus, and potassium, and save on fertilizer costs. Yang et al. (2017) showed that compared with chemical fertilizer, the application of an equal amount of biogas slurry nitrogen led to no significant differences in the rice yield, nitrogen utilization rate, or soil residual inorganic nitrogen, while the ammonia volatilization per unit rice yield was significantly reduced by 22.6%. Most of these studies were based on the nitrogen fertilizer required for crop growth and replaced chemical nitrogen fertilizer with different proportions of biogas slurry nitrogen, but they did not consider whether the applied biogas slurry nitrogen exceeded the maximum nitrogen adsorption capacity of the topsoil.

Ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) is the main component of biogas slurry nitrogen, accounting for 46.42%–92.86% of TN (Ham and DeSutter, 1999; Jin et al., 2012; Ni and Zhang, 2017). In addition to being consumed and retained by ammonia volatilization, biological absorption (Li et al., 2021), and soil adsorption, a considerable portion of  $\text{NH}_4^+\text{-N}$  over-applied to soil can leach into deep soil through nitrification or into the water table along with surface runoff, thus causing nitrogen pollution in groundwater and surface water (Kithome et al., 1998; Wang et al., 2016; He et al., 2021). A large number of studies reported that the nitrate content in the areas adjacent to concentrated animal feeding operations exceeded the safety standards, thereby creating a bottleneck for livestock and poultry feeding development (Ciravolo et al., 1979; Feinerman et al., 2004). Considering the environmental behavior of  $\text{NH}_4^+\text{-N}$ , adsorption is an important process that can block and delay the further migration and transformation of nitrogen in order to inhibit nitrogen loss (Li et al., 2021). Studies of

the soil adsorption of  $\text{NH}_4^+\text{-N}$  have focused on the use of a single chemical ammonium salt solution to explore the adsorption mode, deduce the adsorption mechanism, and estimate the adsorption capacity, generally using  $\text{NH}_4\text{Cl}$  solution (Xue et al., 1996; Li et al., 2009; Tian, 2011; Cong et al., 2017). Solutions of  $\text{NH}_4\text{NO}_3$ ,  $(\text{NH}_4)_2\text{SO}_4$ , and  $\text{NH}_4\text{H}_2\text{PO}_4$  have also been used (Dalal, 1975). Furthermore, the soils used in previous research were mostly vermiculitic-type clay loam, kaolinitic sandy soils, and some tropical soils (Dalal, 1975; Lumbanraja and Evangelou, 1994; Wang and Alva, 2000; Kumar and Kothiyal, 2011). There are few studies on the adsorption characteristics of ammonium nitrogen biogas slurry solutions co-existing with complex components (Kumar and Kothiyal, 2012; Zhao et al., 2013), and there is a lack of research on the adsorption characteristics of ammonium nitrogen from biogas slurry in common agricultural production land topsoil.

Studying the adsorption characteristics of biogas slurry ammonium nitrogen in typical farmland soils and predicting the maximum adsorption capacity of the cultivated layer soil are significant for guiding the safe digestion of biogas slurry in farmland. In this study, three types of common farmland soils were collected: silty loam, loam, and sandy loam. Through analyses of adsorption kinetics and adsorption thermodynamics, the adsorption characteristics of these soils for biogas slurry ammonium nitrogen were analyzed, kinetic models were fitted, and the relationships between soil physical and chemical properties and adsorption capacity were explored to provide a scientific basis for the safe application of biogas slurry in farmland and the prevention and control of water pollution.

### **3.3 Materials and methods**

#### **3.3.1 Materials**

Biogas slurry was collected from a large pig farm (Jiangsu Yangyu Ecological Agriculture Co., Ltd.) in Xinjie Town of Taixing City, China (**Fig. 3.1**). The pig farm has 138 standardized brick-and-concrete pens with a structure area of more than 100,000  $\text{m}^2$ . The farm produces around 120,000 commercial pigs annually. It has

sewage collection tanks of 1,200 m<sup>3</sup>, fermentation tanks of 4,000 m<sup>3</sup>, oxidation ponds of 35,000 m<sup>3</sup>, one sewage treatment system, and a high-efficiency organic fertilizer farm with an annual output of more than 10,000 tons. The biogas slurry sample was generated from pig feces through primary anaerobic fermentation in a continuous stirred tank reactor (CSTR) and a secondary anaerobic fermentation using a black-film-sealed storage process. Once collected, the samples were stored in sealed plastic barrels and were mixed well and filtered through a 0.25-mm mesh screen before the analysis. Samples were then diluted corresponding to the NH<sub>4</sub><sup>+</sup>-N concentration with deionized water for backup use. The basic quality indices of the biogas slurry are shown in **Table 3.1**.



Figure 3.1 Site image of manure and sewage treatment facilities in the pig farm.

Table 3.1 Chemical composition of biogas slurry.

	pH	TN (mg/L)	NH <sub>4</sub> <sup>+</sup> -N (mg/L)	NO <sub>3</sub> <sup>-</sup> -N (mg/L)	TP (mg/L)	TK (mg/L)	COD (mg/L)	EC (mS/cm)	TS (g/L)
Biogas slurry (unfiltered)	7.95	630.29	558.72	42.40	23.97	380	636.67	5.15	2.25
Biogas slurry (filtered through a 0.25-mm mesh screen)	7.97	624.53	550.41	42.36	23.70	375	626.67	5.12	2.24

TN: total nitrogen; NH<sub>4</sub><sup>+</sup>-N: ammonium nitrogen; NO<sub>3</sub><sup>-</sup>-N: nitrate nitrogen; TP: total phosphorus; TK: total potassium; COD: chemical oxygen demand; EC: electrical conductivity; TS: total solid.

The experimental soils were collected from the 0–20 cm plough layer of basic

farmland in the plain water network area of the Yangtze River Basin in China. The soil textures were silty loam, loam, and sandy loam. The soils were dried naturally, while stones, plant roots, and other DEBRIS found in the soils were removed. The soils were then crushed with a round wooden stick, sieved through a 2-mm mesh screen, and finally fully mixed and placed into a clean plastic storage box for future use. The particle composition and basic physical and chemical properties of the soils are listed in **Tables 3.2** and **3.3**.

Table 3.2 Soil particle composition of three soils.

Soil number	2–0.2 mm (%)	0.2–0.05 mm (%)	0.05–0.02 mm (%)	0.02–0.002 mm (%)	<0.002 mm (%)	Soil texture	Soil classification system of China	Sampling point
Xinbei	0.64	29.90	22.54	30.39	16.54	Silty loam	Wushan soil	Dongnancun, Xixiashu, Xinbei
Jintan	0.53	34.96	19.70	30.64	14.17	Loam	Permeable paddy soil	Luocun, Xuebu, Jintan
Taixing	0.27	61.56	22.58	10.14	5.45	Sandy loam	High sandy soil	Lidangcun, Xinjie, Taixing

Table 3.3 Physical and chemical characteristics of three soils.

Soil number	pH	SOM (g/kg)	TN (g/kg)	NH <sub>4</sub> <sup>+</sup> -N (mg/kg)	NO <sub>3</sub> <sup>-</sup> -N (mg/kg)	TP (g/kg)	AP (mg/kg)	AK (mg/kg)	CEC (cmol/kg)	EC (μS/cm)
Xinbei	6.45	29.09	1.16	8.93	56.97	0.57	13.10	122.91	16.27	492.67
Jintan	5.43	19.70	0.79	25.58	26.26	0.31	7.98	126.45	9.57	244.67
Taixing	8.19	5.27	0.16	3.17	10.50	0.54	6.29	65.70	5.83	174.97

SOM: soil organic matter; TN: total nitrogen; TP: total phosphorus; AP: available phosphorus; AK: available potassium; CEC: cation exchange capacity; EC: electrical conductivity.

### 3.3.2 Adsorption kinetics experiments

Soil samples (10 g) were weighed and placed in 250-mL conical bottles. Two hundred millilitres of biogas slurry (NH<sub>4</sub><sup>+</sup>-N concentration 42.90±0.66 mg/L) was added to each conical bottle at a soil:water ratio of 1:20, and three drops of toluene were added to inhibit microbial activity. Biogas slurry without soil was used as a blank control. The conical bottles were sealed with a silica gel plug, placed in a thermostatic oscillator (set temperature 25±1°C), and oscillated at 140 r/min for 0.25, 0.5, 1, 2, 4, 6, 12, 18, or 24 h. The conical bottles were taken out for biogas slurry sample collection, and a 20-mL biogas slurry sample was taken each time. The biogas slurry sample was centrifuged at a speed of 3800 r/min for 10 min. The concentration of NH<sub>4</sub><sup>+</sup>-N in the supernatant was determined by an automatic flow analyzer (SKALAR SAN<sup>++</sup>). The above experiments

were carried out through destructive sampling. Three batches of experiments were set up. Each batch was set up with nine replicates for each treatment, and one bottle was taken out from the thermostatic oscillator for each round of sampling, which was then discarded after sampling. The average value was taken, and the amount of  $\text{NH}_4^+\text{-N}$  adsorbed onto the biogas slurry was calculated after the blank was deducted according to the difference in  $\text{NH}_4^+\text{-N}$  concentration before and after adsorption.

### 3.3.3 Adsorption thermodynamic experiments

A 2.5 g soil sample was mixed with 50 mL biogas slurry with initial diluted concentrations of  $\text{NH}_4^+\text{-N}$  of 54.25, 88.66, 105.85, 133.71, 178.80, 273.54, and 542.87 mg/L in a polyethylene centrifuge tube. Two drops of toluene were added to inhibit microbial activity, and different concentrations of biogas slurry without soil samples were used as blank controls. The sealing cover of the centrifuge tube was tightened, and samples were mixed with a vortex oscillator and oscillated at 180 r/min on a thermostatic oscillator (set temperature  $25\pm 1^\circ\text{C}$ ) for 1 h. Samples were then incubated in a thermostat at the same temperature for 3 d, oscillating twice a day at an interval of 12 h for 1 h each time. After cultivation, the samples were centrifuged at 3800 r/min for 10 min. The concentration of supernatant  $\text{NH}_4^+\text{-N}$  was measured by an automatic flow analyzer (SKALAR SAN<sup>++</sup>). Each experiment was repeated three times. The amount of  $\text{NH}_4^+\text{-N}$  absorbed by the tested soil was calculated based on the differences between the initial and final  $\text{NH}_4^+\text{-N}$  concentration in the supernatant.

### 3.3.4 Calculation methods

#### 3.3.4.1 Adsorption capacity calculation formula

$$Q = \frac{(C_0 - C_t) \cdot V}{M} \quad (1)$$

In the formula,  $Q$  is the adsorption capacity of  $\text{NH}_4^+\text{-N}$  (mg/kg);  $C_0$  is the initial concentration (mg/L);  $C_t$  is the concentration of the solution at the time of measurement (mg/L);  $V$  is the volume of the solution (mL); and  $M$  is the soil sample weight (g).

#### 3.3.4.2 Adsorption fitting equations

The fitting equations of adsorption kinetics are shown in **Table 3.4**. Fitting equations for adsorption thermodynamics are shown in **Table 3.5**.



Table 3.4 Four models of soil adsorption kinetics.

Model	Equation
Elovich equation	$Q_t = a + b \ln t$ .....(2)
Parabolic equation	diffusion $Q_t = a + bt^{1/2}$ (3)
First-order equation	reaction $\ln(Q_e - Q_t) = \ln Q_e - kt$ .....(4)
ExpAssoc equation	$Q_t = y_0 + A_1(1 - e^{-t/a}) + A_2(1 - e^{-t/b})$ .....(5)

$Q_t$  is the adsorption capacity of  $\text{NH}_4^+\text{-N}$  at time  $t$  (mg/kg);  $Q_e$  is the adsorption capacity of  $\text{NH}_4^+\text{-N}$  at adsorption equilibrium (mg/kg); and  $a, b, k, y_0, A_1,$  and  $A_2$  are constants used to characterize the adsorption coefficient, where their size indicates the adsorption strength.

Table 3.5 Four thermodynamic models.

Model	Equation	Adjustable model parameters
Langmuir	$\frac{C_e}{Q_e} = \frac{1}{Q_m K_l} + \frac{C_e}{Q_m}$ ..... (6)	$K_l$ is a constant used to characterize the adsorption performance of the soil; and $\text{MBC} = Q_m \times K_l$ , representing the maximum buffer capacity of the soil (mg/kg).
Freundlich	$Q_e = K_f C_e^{1/n}$ ..... (7)	$K_f$ is a constant that represents the strength of soil adsorption force; and $1/n$ represents the heterogeneity factor related to adsorption strength or surface heterogeneity, reflecting the nonlinear degree of adsorption.
Platt	$Q = Q_n \cdot \tanh\left(\frac{A \cdot C_e}{Q_m}\right)$ ..... (8)	$A$ is a constant.
Langevin	$Q = a + b \cdot \left(\coth\left(\frac{C_e - k}{s}\right) - \frac{C_e - k}{s}\right)$ .....(9)	$a, b, k,$ and $s$ are constants, $Q_m = a + b$ .

$Q_e$  is the adsorption capacity of  $\text{NH}_4^+\text{-N}$  at adsorption equilibrium (mg/kg);  $C_e$  is the  $\text{NH}_4^+\text{-N}$  concentration of biogas slurry at adsorption equilibrium (mg/L); and  $Q_m$  is the theoretical saturated adsorption capacity of  $\text{NH}_4^+\text{-N}$  per unit soil (mg/kg), representing the capacity factor.  $\text{MBC}$ : maximum buffer capacity of the soil.

### 3.3.5 Data processing

Microsoft Office Excel (2010) software was used for test data processing and table drawing, Origin 2017 software was used for drawing and curve fitting, and the significance of Pearson correlations between variables was tested by IBM SPSS Statistics 13.0 version (IBM Corp., NY, USA).

## 3.4 Results and analysis

### 3.4.1 Adsorption kinetic characteristics

The adsorption rates of three types of soils on biogas slurry ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) are shown in **Table 3.6**. The adsorption process of  $\text{NH}_4^+\text{-N}$  from biogas slurry was a composite kinetic process comprising two stages of initial rapid adsorption followed by slow adsorption. The initial rapid adsorption generally occurred within 0–1 h. The ratio of the rapid adsorption amounts to the total amount of adsorption was

35.24–43.55%. After 1 h, the process entered the slow adsorption stage. The total adsorption amounts in order from highest to least were adsorbed onto silty loam, loam, and sandy loam.

Table 3.6 Biogas slurry ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) adsorption rate of three types of soils.

Soils		Adsorption rate at different stage ( $\text{mg}/(\text{kg}\cdot\text{h})$ )								
		0–0.25 h	0.25–0.5 h	0.5–1 h	1–2 h	2–4 h	4–6 h	6–12 h	12–18 h	18–24 h
Silty loam (Xinbei)		279.20	131.04	41.65	18.30	17.68	9.47	5.47	3.47	5.61
Loam (Jintan)		165.65	34.85	53.84	22.23	19.82	7.94	1.54	1.59	3.80
Sandy loam (Taixing)		136.91	81.92	25.40	21.65	9.81	7.90	3.33	1.98	2.67

### 3.4.2 Adsorption kinetic model fitting

Fig. 3.2A–D shows the fitting results for the adsorption kinetics of  $\text{NH}_4^+\text{-N}$  in the three kinds of soils obtained by the Elovich equation, a parabolic diffusion equation, a first-order reaction equation, and the ExpAssoc equation. The four equations could effectively simulate the dynamic adsorption process. The fit of the ExpAssoc equation with relevant parameters produced a confidence interval for  $R^2$  ( $0.9923 < R^2 < 0.9966$ ) that was better than for the other models (Table 3.7). Therefore, the ExpAssoc equation was used to fit the adsorption kinetics of  $\text{NH}_4^+\text{-N}$  in the three kinds of soils.

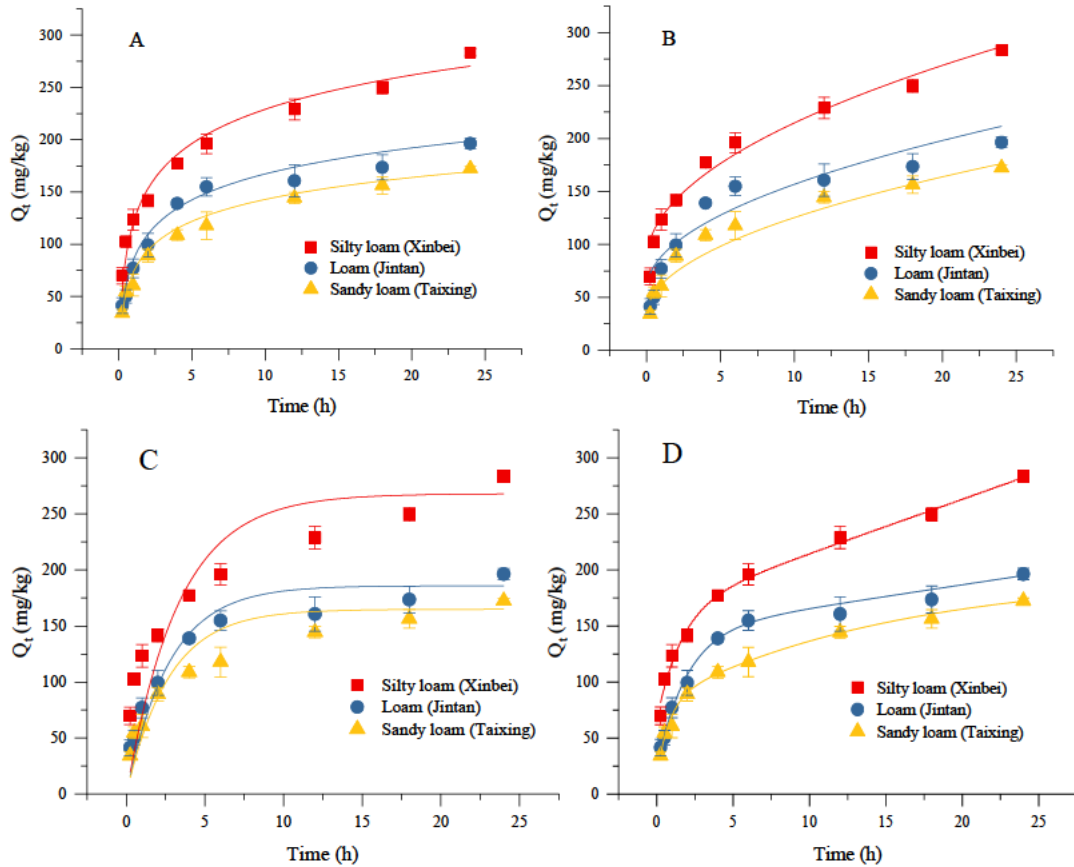


Figure 3.2 Fitting of adsorption kinetics models: (A), (B), (C), and (D) show the fitting results for the adsorption kinetics of  $\text{NH}_4^+\text{-N}$  in the three kinds of soils obtained by the Elovich equation, a parabolic diffusion equation, a first-order reaction equation, and the ExpAssoc equation, respectively.

Table 3.7 Fitted parameters in the adsorption kinetics fitting equations.

Soils	Elovich equation			Parabolic diffusion equation			First-order reaction equation			ExpAssoc equation					
	a	b	R <sup>2</sup>	a	b	R <sup>2</sup>	Q <sub>e</sub>	k	R <sup>2</sup>	y <sub>0</sub>	A <sub>1</sub>	a	A <sub>2</sub>	b	R <sup>2</sup>
Silty loam (Xinbei)	120.	108.	0.9	83.	41.	0.9	267.	0.3	0.7	66.	8.7	1.7	99.7	1.6	0.9
	38	62	7	86	39	7	93	0	8	29	0	9	6	2	9
Loam (Jintan)	84.7	82.4	0.9	57.	31.	0.8	185.	0.3	0.9	23.	1.2	5.9	121.	1.9	0.9
	3	9	8	14	51	1	88	7	2	66	3	2	64	3	9
Sandy loam (Taixing)	73.1	69.6	0.9	32.	29.	0.9	164.	0.3	0.9	15.	65.	0.7	114.	15.	1.0
	7	6	9	74	30	6	82	7	1	73	84	9	37	30	0

### 3.4.3 Adsorption thermodynamic characteristics

The isothermal adsorption curves of the three soils for biogas slurry  $\text{NH}_4^+\text{-N}$  at a temperature of  $25\pm 1^\circ\text{C}$  are shown in Fig. 3.3. With the increase of the initial biogas slurry  $\text{NH}_4^+\text{-N}$  concentration, the adsorption isotherms of three soils on  $\text{NH}_4^+\text{-N}$

increased, primarily due to the increase of initial biogas slurry  $\text{NH}_4^+\text{-N}$  concentration and the increase of the driving force of the soil itself as an adsorbent, an effect that was conducive to the generation of adsorption. When the initial biogas slurry  $\text{NH}_4^+\text{-N}$  concentration was less than 200 mg/L, the  $\text{NH}_4^+\text{-N}$  adsorption capacity of the three soils increased significantly with the increase of initial concentration; when the initial concentration of  $\text{NH}_4^+\text{-N}$  in biogas slurry was greater than 200 mg/L, the  $\text{NH}_4^+\text{-N}$  adsorption capacity increased slowly and tended to balance with the increase of initial concentration. When the initial biogas slurry  $\text{NH}_4^+\text{-N}$  concentration was low, the adsorption sites of the soil itself were not completely occupied. As the initial biogas slurry  $\text{NH}_4^+\text{-N}$  concentration increased, the unused adsorption sites were gradually occupied and thus were unable to be used, ultimately reaching a saturated state so that the soil and the  $\text{NH}_4^+\text{-N}$  solution tended to balance, and the soil adsorption capacity of  $\text{NH}_4^+\text{-N}$  reached its maximum value.

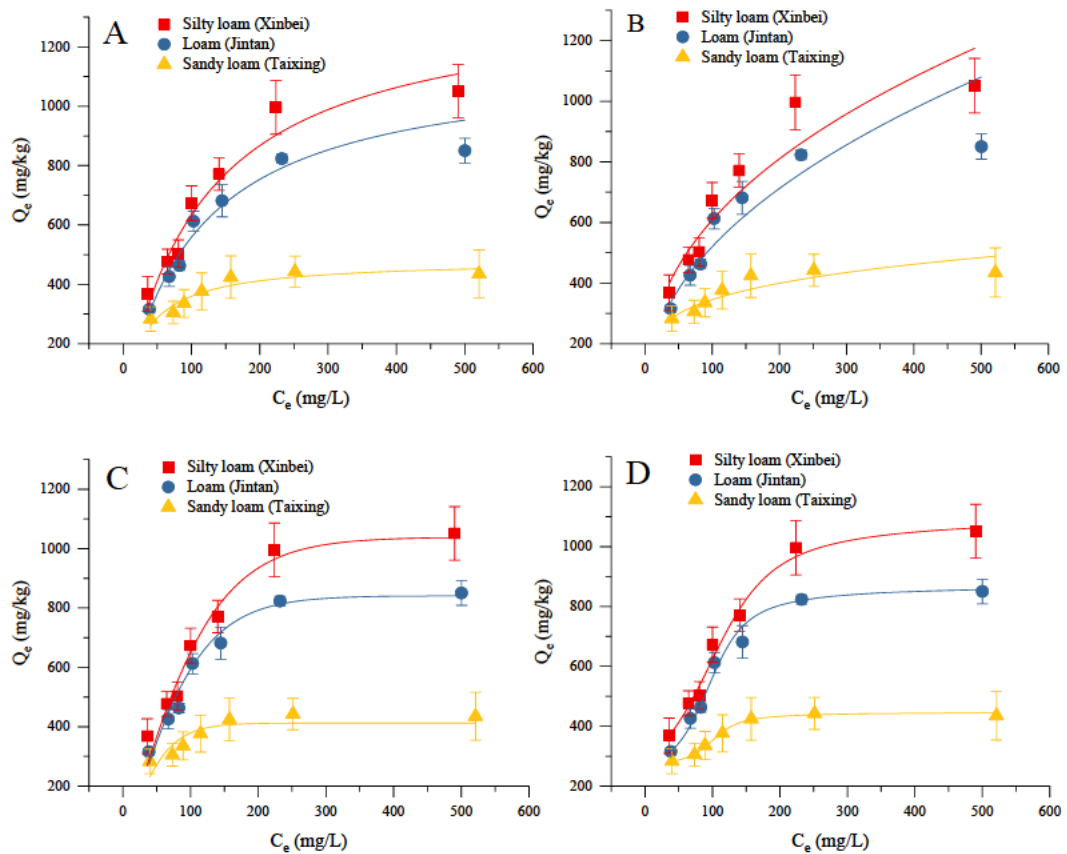


Figure 3.3 Fitting effects of adsorption thermodynamic models: (A), (B), (C), and (D) show the fitting results for the adsorption thermodynamics of  $\text{NH}_4^+\text{-N}$  in the three kinds of soils obtained by

the Langmuir equation, the Freundlich equation, the Plplatt equation, and the Langevin model, respectively, at a temperature of  $25\pm 1^\circ\text{C}$ .

### 3.4.4 Adsorption thermodynamic model fitting

The Langmuir equation and Freundlich equation are classical models used to study the thermodynamic characteristics of soil  $\text{NH}_4^+\text{-N}$  adsorption. The Plplatt equation and the Langevin model were the equations selected in this experiment to achieve better fitting. **Fig. 3.3A–D** shows the fitting results of four equations on the adsorption thermodynamics of biogas slurry  $\text{NH}_4^+\text{-N}$  at a temperature of  $25\pm 1^\circ\text{C}$ . When fitting the theoretical saturated maximum adsorption capacity ( $Q_m$ ) values of biogas slurry  $\text{NH}_4^+\text{-N}$  for the three kinds of soils, the  $Q_m$  value fitted by the Langmuir equation was highest, and the  $Q_m$  value fitted by the Plplatt equation was closer to the measured average value. From the  $R^2$  values (**Table 3.8**), the adsorption thermodynamic behaviour of biogas slurry  $\text{NH}_4^+\text{-N}$  in this experiment was best fitted by the Langevin Model. The optimal theoretical saturated  $Q_m$  values calculated by the sum of the parameters a and b were 1108.55 mg/kg, 874.86 mg/kg, and 448.35 mg/kg for silty loam, loam, and sandy loam, respectively.

Table 3.8 Related parameters in the adsorption thermodynamic fitting equation.

Soil s	Langmuir equation				Freundlich equation			Plplatt equation			Langevin Model				
	$Q_m$	$K_l$	MB C	$R^2$	$K_f$	1/ n	$R^2$	$Q_m$	A	$R^2$	a	k	b	s	$R^2$
Silt y loa m	1372. 44	0.00 86	11. 79	0.9 5	90.1 3	0.4 1	0.8 9	1038. 41	7.6 4	0.9 4	606. 06	96. 24	502. 49	36. 31	0.9 8
Loa m	1157. 60	0.00 93	10. 80	0.9 8	64.2 5	0.4 5	0.9 3	840.8 5	7.3 9	0.9 6	529. 26	93. 68	345. 60	21. 83	1.0 0
San dy loa m	481.8 5	0.02 94	14. 18	0.8 9	131. 39	0.2 1	0.8 6	412.3 3	6.5 2	0.6 5	352. 64	99. 99	95.7 1	16. 05	1.0 0

### 3.4.5 Correlation between adsorption capacity and soil physical and chemical properties

Correlations between adsorption characteristic parameters and soil properties are shown in **Table 3.9**. The  $Q_m$  of the soil for biogas slurry  $\text{NH}_4^+\text{-N}$  was positively correlated with SOM, TN, available phosphorus (AP), available potassium (AK), cation exchange capacity (CEC), and the proportion of 0.02–0.002 mm particles ( $P < 0.01$ ), while it was negatively correlated with soil pH ( $P < 0.05$ ). The adsorption constant  $K_l$

was negatively correlated with SOM, TN, AP, AK, and the content of particles with the particle size of 0.02–0.002 mm ( $P < 0.01$ ), negatively correlated with soil  $\text{NH}_4^+\text{-N}$  and CEC ( $P < 0.05$ ), and positively correlated with soil pH ( $P < 0.01$ ). The maximum buffer capacity (MBC) of the soil was negatively correlated with SOM,  $\text{NH}_4^+\text{-N}$ , AP, AK, and the content of particles with the particle size of 0.02–0.002 mm ( $P < 0.01$ ), negatively correlated with soil TN ( $P < 0.05$ ), and positively correlated with soil pH ( $P < 0.01$ ).

Table 3.9 Correlation between adsorption characteristic parameters and soil properties ( $r$ ,  $n = 9$ ).

	pH	SOM	TN	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	TP	AP	AK	CEC	EC	Soil particle size distribution (%)			
											2–0.2 m	0.2–0.02 m	<0.002 mm	
$Q_m$	-0.763*	0.993**	0.974**	0.558	0.785*	0.081	0.974**	0.951**	0.896**	0.792*	-0.64	-0.335	0.912**	-0.34
$K_l$	0.877**	-0.952**	-0.917**	-0.713*	-0.644	0.119	-0.909**	-0.994**	-0.791*	-0.654	0.566	0.445	-0.944**	0.377
M	0.983**	-0.808**	-0.751*	-0.898**	-0.371	0.423	-0.732**	-0.979**	-0.559	-0.382	0.402	0.581	-0.917**	0.403

SOM: soil organic matter; TN: total nitrogen; TP: total phosphorus; AP: available phosphorus; AK: available potassium; CEC: cation exchange capacity; EC: electrical conductivity. \*-- $P < 0.05$ ; \*\*-- $P < 0.01$ .

### 3.5 Discussion

#### 3.5.1 Adsorption mechanism

Adsorption can be defined as the accumulation of solutes at the solid–liquid interface. This process includes the transfer of solute molecules from the solution, the removal of solvent molecules from the solid surface, and the process of solute molecules attaching to the solid surface (Stumm, 1992). Farmland soil media are heterogeneous aggregates with complex structures. A large number of organic and inorganic colloids and oxides are interlaced and mixed (Li et al., 2021). There are electric fields and residual force fields on the surface of the media that have extremely high surface energy. They can interact with ions, protons, and molecules in the soil liquid and gas phases, and they have strong adsorption on ammonium nitrogen. There are many kinds of active groups in the media, and all kinds of organic and inorganic groups can interact with ammonium. Therefore, the adsorption behavior of ammonium nitrogen in farmland soil media is complex, and the adsorption behavior in different

types of media is significantly different (Krishnamoorthy and Overstreet, 1950). At present, the adsorption mechanism of ammonium nitrogen in soil media is mainly discussed at the macro and micro levels. Most macroscopic research has focused on distinguishing the different active surfaces of soil media using the differences in the extraction capacity of different extractants (Lumbanraja and Evangelou, 1994; Wang and Alva, 2000). Microscopically, the occurrence of ammonium in the medium can be identified at the molecular level using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and other spectral means (Sherman and Smulovitch, 1970; Saruchi and Kumar, 2020). Generally, the adsorption mechanism cannot be accurately described through only a single means. The present study characterized the adsorption characteristics of the three soils, and conducted a preliminary discussion based on the factors affecting the adsorption (Shen et al., 1997), but did not conduct an in-depth study of the adsorption mechanism. This will require further study.

### **3.5.2 Characteristics of adsorption kinetics**

Soil is an important site for nitrogen circulation and transformation, and the only way for nitrogen to enter groundwater (Li et al., 2021; He et al., 2021). The adsorption of  $\text{NH}_4^+\text{-N}$  in the soil shows corresponding regularity with time change, which is one of the important characteristics of soil chemical reaction kinetics. The study of kinetics can reveal the limiting factors and control conditions that affect the adsorption rate. Commonly used adsorption kinetics equations include first-order reaction equations, second-order reaction equations, the Elovich equation, the parabolic diffusion equation, and the ExpAssoc equation. The Elovich equation reflects not only a simple adsorption process but also a complex process involving soil expansion, the activation and deactivation of adsorption sites, and surface diffusion (Sparks and Jardine, 1984). In the present experiment, the coefficient  $R^2$  for the three types of soil adsorption data was the highest for the Elovich equation, indicating that adsorption was a heterogeneous diffusion process. Further analyzing the restrictive factors of the process revealed that the goodness of fit of the parabolic diffusion equation ( $0.81 < R^2 < 0.97$ ) was higher than that of the first-order reaction equation ( $0.78 < R^2 < 0.92$ ) (**Table 3.7**), indicating

that the chemical adsorption process was not the rate-limiting step of the process, while the intra-particle diffusion was the main rate-limiting step. Due to the differences in soil media and environmental conditions, the relationship between adsorption capacity and time often differed during the adsorption process. Previous studies have shown that different kinetics models or the same model have different fits with different soils. In the present study, the best fitting models for the soil adsorption of  $\text{NH}_4^+\text{-N}$  were the first-order reaction equation and the Elovich equation (Xue et al., 1996). The adsorption of  $\text{NH}_4^+\text{-N}$  in silty sand, sandy silt, silt, and silty clay of four typical soils primarily occurred during 0–2 h, and the adsorption kinetics conformed to the second-order reaction equation (Tian, 2011). However, the fitting effect of the adsorption kinetics curve with the ExpAssoc equation was the best (Zhao et al., 2013). The results of this study were consistent with those of Zhao et al. (2013).

### **3.5.3 Adsorption thermodynamic characteristics**

The adsorption of  $\text{NH}_4^+\text{-N}$  by soil is a dynamic equilibrium process. Under the same constant temperature, the curve of the adsorption amount ( $Q$ ) with the equilibrium concentration ( $C$ ) of the solution is normally referred to as the adsorption isotherm, and the corresponding mathematical expression is called the adsorption isothermal formula. This equation reflects the specific relationship between the adsorbent and the adsorption capacity, as well as the influence of different  $\text{NH}_4^+\text{-N}$  concentrations on the ability of the soil to adsorb  $\text{NH}_4^+\text{-N}$ . At present, the Langmuir equation, the Freundlich equation, the Henry equation, and the Temkin equation are commonly used to describe the adsorption of  $\text{NH}_4^+\text{-N}$  in soil. The Langmuir equation assumes that the medium surface is uniform and the adsorption is performed by single molecules; this model can be used to calculate the corresponding maximum saturated adsorption capacity, adsorption coefficient, and MBC, as well as to evaluate and predict the adsorption of  $\text{NH}_4^+\text{-N}$  in the soil. In this experiment, the Langmuir equation had a high degree of fit, indicating that the isothermal adsorption of  $\text{NH}_4^+\text{-N}$  in the three soils was mainly monolayer adsorption, while multi-molecular layer adsorption was of secondary importance. The Freundlich equation is often used to describe the adsorption of non-uniform surfaces,



but the maximum saturated adsorption capacity cannot be calculated. The fitting parameter  $1/n$  of the Freundlich equation in this study was between 0 and 1, indicating that the isothermal adsorption of  $\text{NH}_4^+\text{-N}$  by the three kinds of soils was relatively efficient. The Henry equation is suitable for low concentrations and weak adsorption, while the Temkin equation is only suitable for chemical adsorption. As the latter two models had poor fits to the experimental data, the fitting of these two equations was not presented in this paper. At the same time, in order to further improve the application and evaluation of the fitting results for the maximum saturated adsorption capacity, the analysis screened out the Platt equation whose fitting  $Q_m$  value was close to the measured average value, and the Langmuir model whose fitting curve was more optimal. Although the adaptability of the assumptions that the adsorption model established to soil adsorption characteristics of  $\text{NH}_4^+\text{-N}$  was worthy of further validation, the law summarized by the analysis of the experimental data was basically consistent with the adsorption characteristics reflected by the empirical adsorption isotherm. Therefore, it was effective to use empirical adsorption isotherms to quantitatively describe the thermodynamic behavior of the soil adsorption of  $\text{NH}_4^+\text{-N}$ .

#### **3.5.4 Soil factors affecting adsorption**

The  $\text{NH}_4^+\text{-N}$   $Q_m$  of soil is affected by soil texture, environmental temperature and humidity, artificial fertilization, and crop rotation. The  $Q_m$  obtained by the Langmuir equation reflected the maximum saturated adsorption capacity of  $\text{NH}_4^+\text{-N}$  in the soil, and the number of soil adsorption sites. Li et al. (2009) found that  $Q_m$  was significantly negatively correlated with soil pH and CEC, while it was significantly positively correlated with soil C:N. Research by Cong et al. (2017) showed that  $Q_m$  was significantly positively correlated with soil pH and CEC, while it was significantly negatively correlated with SOM and TN content. Wang et al. (2015) reported that the greater the organic matter content, the greater the  $\text{NH}_4^+\text{-N}$  adsorption capacity of albic soil. Xue et al. (1996) found that the adsorption capacity of  $\text{NH}_4^+\text{-N}$  increased with the increase of soil CEC and soil clay content to varying degrees. The results of the present study showed that the  $Q_m$  value was significantly negatively correlated with soil pH,

consistent with the results of Li et al. (2009). A possible reason was that the biogas slurry was alkaline, and the pH values of weakly acidic soils in Xinbei and Jintan were neutralized, resulting in the release of adsorption sites originally occupied by  $H^+$  and thereby reducing the competition between  $H^+$  and  $NH_4^+$  for adsorption sites. There was a significant positive correlation between the  $Q_m$  value and CEC, consistent with the results of Cong et al. (2017), Xue et al. (1996), and Shen et al. (1997).  $Q_m$  was positively correlated with SOM, consistent with the results of Li et al. (2009) and Wang et al. (2015). This might be because organic matter had a large number of different functional groups (Liu et al., 2010), a higher CEC, and a larger specific surface area (Acosta et al., 2016; Khorram et al., 2015; Kumar, et al., 2016); these were all factors that could increase the adsorption capacity of  $NH_4^+$ -N through surface complexation, ion exchange, and surface precipitation. These results have been verified in many studies aimed at improving soil adsorption of  $NH_4^+$  by biochar (Yao et al., 2012).

Jiang (2004) showed that the finer the soil particles, the lower the percentage of sand powder with a particle size of  $\geq 0.01$  mm, while the higher the percentage of clay particles with a particle size of  $< 0.005$  mm, the stronger the adsorption. Xue et al. (1996) reported that  $NH_4^+$ -N adsorption capacity was mainly affected by clay content, with clay  $>$  loam  $>$  sandy soil. Cong et al. (2017) showed that the  $NH_4^+$ -N adsorption capacity of soil was in the order of light clay  $>$  light loam. The results of this study showed that the  $Q_m$  value of silty loam  $>$  loam  $>$  sandy loam was consistent with the above research results. Wan et al. (2004) found that there was a very significant positive correlation between fixed ammonium and clay content  $< 0.01$  mm, with clay content of 0.005–0.01 mm, and with clay content of 0.001–0.005 mm, but there was no significant correlation with clay content  $< 0.001$  mm. The results of this study showed that there was a significant positive correlation between the  $Q_m$  value and the content of particles with particle size of 0.02–0.002 mm in the soil. The same trend was observed in the results of Wan et al. (2004). Therefore, it is speculated that the increase of soil fixed ammonium content is a characteristic that can be further verified based on the fact that the fixed ammonium soil content increased with the increase of ammonium ion concentration (Juang et al., 2001; Liang and MacKenzie, 1994). In addition, there was

a very significant positive correlation between the  $Q_m$  value and soil TN content, as well as a significant correlation with soil nitrate nitrogen. This may be the reason for the positive and negative electrical adsorption, and may be responsible for maintaining the balance between soil new ammonium nitrogen and nitrate nitrogen. The  $Q_m$  value was positively correlated with the soil AK. This was because active  $K^+$  ions could provide adsorption sites and solid interlayer localization for  $NH_4^+$  ions in external soil, which was in line with cation exchange theory (Kittrick, 1966; Rich and Black, 1964). The  $Q_m$  value was positively correlated with soil AP in this experiment. There were two possible reasons: the positive and negative charge adsorption, and the combination of  $NH_4^+$  ions and  $PO_4^{3-}$  ions to form  $[(NH_4) PO_4]^{2-}$ , subsequently forming complex precipitates  $Mg(NH_4)[PO_4] \cdot 6H_2O$  with the  $Mg^{2+}$  and  $Ca^{2+}$  plasma in the soil, thereby increasing the adsorption of  $NH_4^+$ -N. The correlation between the Langmuir equation adsorption constant  $K_l$  value, the MBC value, and soil property-related indicators, and the correlation between the  $Q_m$  value and soil property-related indicators were opposite (Cong et al., 2017; Li et al., 2009), indicating that the adsorption strength and the adsorption capacity were complementary. In other words, when the adsorption capacity is high, the adsorption strength is low. In addition, the fitted  $Q_m$  values of the  $NH_4^+$ -N adsorption of the soils in this study were lower than the fitted value of a single chemical ammonium salt solution, thus indicating that the rich complex components of the biogas slurry interfered with the adsorption of ammonium nitrogen in the soil as described in Zhao et al. (2013). While a preliminary demonstration has been achieved in the present research, the specific mechanism of action requires further study.

### 3.6 Conclusions

The soil adsorption of biogas slurry  $NH_4^+$ -N predominantly occurred within 0–1 h, and the adsorption capacity within 0–1 h accounted for 35.24–43.55% of the total adsorption. The ExpAssoc equation produced a good fit for the adsorption kinetic behaviour. The optimal theoretical saturated adsorption capacity ( $Q_m$ ) values fitted by the Langevin model were 1108.55 mg/kg, 874.86 mg/kg, and 448.35 mg/kg for silty loam, loam, and sandy loam, respectively. The  $Q_m$  was significantly positively

correlated with SOM, TN, AP, AK, CEC, and particle content of 0.02–0.002 mm, but significantly negatively correlated with soil pH.

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## CHAPTER 4: AGING CHARACTERISTICS AND FATE

### ANALYSIS OF LIQUID DIGESTATE AMMONIUM NITROGEN DISPOSAL IN FARMLAND SOIL

This chapter has been published as detailed below:

Wang Z, Sun G, Zhang L, Zhou W, Sheng J, Ye X, Olaniran AO, Kana EBG, Shao H. 2022. Aging characteristics and fate analysis of liquid digestate ammonium nitrogen disposal in farmland soil. *Water* 14(16):2487. <https://doi.org/10.3390/w14162487>.

#### 4.1 Abstract

Water environment safety is the focus of engineering measures to eliminate liquid digestate in farmland. It is of great significance to study the aging characteristics of soil absorbing and fate of liquid digestate ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) to realize safe and efficient disposal. In this paper, simulation experiments of digesting  $\text{NH}_4^+\text{-N}$  (with an application of 0, 120, 180, and 300  $\text{kg}/\text{hm}^2$ ) by static soil column are carried out to study disposal efficiency, migration and transformation characteristics, and fate proportion of  $\text{NH}_4^+\text{-N}$  in saturated water content soil. The result showed that after 3 days of application, the overlying water  $\text{NH}_4^+\text{-N}$  concentration decreased by 63.5–80.7%, and the reduction rate of total  $\text{NH}_4^+\text{-N}$  was 65.8–82.3%. After 4 days, the  $\text{NH}_4^+\text{-N}$  concentration of pore water in the 0–10 cm soil layer reached the peak value. After 7 days, the  $\text{NH}_4^+\text{-N}$  concentration adsorbed by the 0–10 cm soil layer reached the peak value. After 15 days, the overlying water  $\text{NH}_4^+\text{-N}$  concentration decreased by 97.0–98.7%, the reduction rate was 97.9–99.2%, and the proportion of  $\text{NH}_4^+\text{-N}$  absorbed in the 0–10 cm soil layer accounted for 63.5–76.3%. The disposal is mainly based on soil sorption and pore water migration. A duration of 0–3 days is the rapid disposal period, and 15 days is the completion period of safe digestion.

**Keywords:** *waste biomass utilization; liquid digestate; ammonium nitrogen; sorption; migration; transformation*

## 4.2 Introduction

To reduce the environmental pollution of the livestock and poultry breeding industry (Wu et al., 2014), in recent years, the Chinese government has continued to strengthen the construction of biogas projects in livestock and poultry farms, and has continuously promoted the transformation and upgrading of rural biogas projects in combination with the green development of agriculture and the action of replacing chemical fertilizers with organic fertilizers (Song et al., 2021; Li et al., 2022; Zhang et al., 2021b), using the anaerobic fermentation process to dispose of aquaculture excrement, in order to realize the harmless treatment and resource reuse of aquaculture manure (Nasir et al., 2012; Lu et al., 2021). By the end of the year 2020, 128,976 small and medium-sized biogas projects and 10,122 large-scale biogas projects have been built nationwide (Zou et al., 2020). Liquid digestate is a by-product of biogas engineering, accounting for more than 90% of the total fermentation residue (Dong et al., 2021). According to estimates, China produces 1.12 billion tons of liquid digestate annually (Zou et al., 2020). Due to the large amount of liquid digestate produced, high storage and transportation costs, difficult treatment to meet standards, and low commercialization value, there are serious secondary pollution environmental risks (Liang et al., 2013). The treatment and utilization of liquid digestate have become the focus and difficulty of domestic and foreign research (Jiang et al., 2011c; Wang et al., 2014a; Nkoa, 2014; Hagos et al., 2017; He et al., 2017; Fang et al., 2015). The use of farmland soil, crops, and microorganisms living in the soil to absorb liquid digestate is a widely recognized green treatment method (Wang et al., 2016; Manici et al., 2021), but the amount of farmland consumption cannot exceed the limit of land carrying capacity (Feng and Li, 2018); otherwise, it will cause serious pollution to the surrounding soil and water bodies (Monlau et al., 2015; Ternoeven-Urselmans et al., 2009). High ammonia nitrogen ( $\text{NH}_4^+\text{-N}$ ) concentration in liquid digestate components is the primary risk factor for environmental pollution (Wang et al., 2016; Zhang et al., 2021a; Zhang et al., 2020). Therefore, it is of great significance to study the aging characteristics of farmland soil to absorb liquid digestate and the fate of  $\text{NH}_4^+\text{-N}$  to

realize safe and efficient disposal of liquid digestate in farmland.

According to the soil nitrogen transport theory (Wang et al., 2014b; Tan et al., 2015), after the liquid digestate is applied to the farmland,  $\text{NH}_4^+\text{-N}$  in the unsaturated water content soil completes vertical and horizontal transport with water in convection mode, while the saturated water content soil completes migration from the high-concentration area to the low-concentration area by diffusion infiltration. During the migration process,  $\text{NH}_4^+\text{-N}$  will be rapidly adsorbed and gradually nitrified into nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ) (Pote et al., 2001). When local surface runoff is generated,  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  not adsorbed by soil particles will be lost and leached with water at the same time, thus polluting the surrounding water sources (Lovejoy et al., 1997; Velthof et al., 2005; Paul et al., 1998). Liquid digestate contains relatively more available nitrogen. Therefore, it has been proposed that liquid digestate application will lead to more nitrogen leaching loss than manure application. However, after a two-and-a-half-year corn field experiment, there was no significant difference in nitrogen leaching amount between digestate application and manure and chemical fertilizer application (Svoboda et al., 2013). When liquid digestate is applied by spraying and deepening in the slack season in autumn, there is no risk of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  leaching. However, when liquid digestate is applied by injection, there is still a potential risk of  $\text{NH}_4^+\text{-N}$  leaching even when the nitrogen dosage of liquid digestate is  $90 \text{ kg}/(\text{hm}^2\cdot\text{d})$  (Liu et al., 2021). The study on disposal of liquid digestate in paddy fields shows that the concentration of  $\text{NH}_4^+\text{-N}$  in field surface water decreases rapidly 1–4 days after application (Wang et al., 2016; Jiang et al., 2011a). After 8 days of application, the  $\text{NH}_4^+\text{-N}$  concentration in field surface water can basically reach the level of the blank control field. The concentration of  $\text{NH}_4^+\text{-N}$  in groundwater is always lower than that of chemical fertilizer treatment, and does not increase with the increase in liquid digestate application amount (Shi, 2010); meanwhile, the content of  $\text{NO}_3^-\text{-N}$  in field surface water and groundwater will not increase significantly (Chen et al., 2013). The increase in ammonia volatilization is considered to be the main negative impact of liquid digestate application on the farmland environment (Jiang et al., 2011a; Huijsmans et al.,

2001; Nicholson et al., 2017). After liquid digestate application, the ammonia volatilization is higher than that of the total chemical fertilizer treatment. With the increase in liquid digestate dosage, the ammonia volatilization is increased, and the soil wetting or flooding conditions can reduce the ammonia volatilization (Smith et al., 2000; Hou et al., 2007; Zhou et al., 2009).

The above studies monitored and qualitatively analyzed the changes of nitrogen concentration in farmland water after liquid digestate was applied, but there was a lack of quantitative research on the reduction process of liquid digestate  $\text{NH}_4^+\text{-N}$ . It is a new way to treat liquid digestate by using farmland with saturated water content for disposal, which is different from the fertilizer utilization of liquid digestate. When taking measures to absorb liquid digestate in farmland with saturated water content, farmers are more concerned about the main destination of  $\text{NH}_4^+\text{-N}$  after liquid digestate is applied in farmland and the time required for the discharge of field water quality up to standard. In this paper, the simulation experiment of indoor static soil column is used to study the time-effect of absorbing  $\text{NH}_4^+\text{-N}$  from liquid digestate in saturated water content soil, analyze the migration and transformation characteristics and fate ratio of  $\text{NH}_4^+\text{-N}$  from liquid digestate, and discuss the time required for the discharge of field surface water quality to meet the standard, to provide theoretical basis and technical guidance for the efficient elimination of liquid digestate in farmland under the safety of water environment.

### **4.3 Materials and methods**

#### **4.3.1 Materials**

The tested liquid digestate was taken from Jiangsu Yangyu Ecological Agriculture Co., Ltd. (Taizhou, China), which produces around 120,000 commercial pigs annually, and was recognized by the Ministry of Agriculture and Rural Affairs as a “pig standardization demonstration farm”, a provincial key leading enterprise of agricultural industrialization in Jiangsu Province, and a comprehensive demonstration base of circular ecological agriculture of Jiangsu Academy of Agricultural Sciences (Nanjing,

China). The liquid digestate was generated from liquid manure and sewage through primary anaerobic fermentation in biogas engineering with a continuous stirred-tank reactor (CSTR) and a secondary anaerobic fermentation using a covered lagoon storage process (**Figure 4.1**). Once taken back to the laboratory, the liquid digestate was stored in sealed plastic barrels and was mixed well. The solid and insoluble matter were filtered out of digestate through a 0.25-mm mesh screen and were not used for the experiment. The average properties of liquid digestate measured before this test are: pH value  $8.05 \pm 0.06$ , total nitrogen (TN)  $461.63 \pm 5.39$  mg/L,  $\text{NH}_4^+\text{-N}$   $409.12 \pm 6.75$  mg/L,  $\text{NO}_3^-\text{-N}$   $31.56 \pm 0.08$  mg/L, total phosphorus (TP)  $17.72 \pm 0.14$  mg/L, total potassium (TK)  $279 \pm 2.74$  mg/L, chemical oxygen demand (COD)  $470.11 \pm 7.85$  mg/L, electrical conductivity (EC)  $3.81 \pm 0.01$  mS/cm, and total solid (TS)  $1.63 \pm 0.01$  g/L.

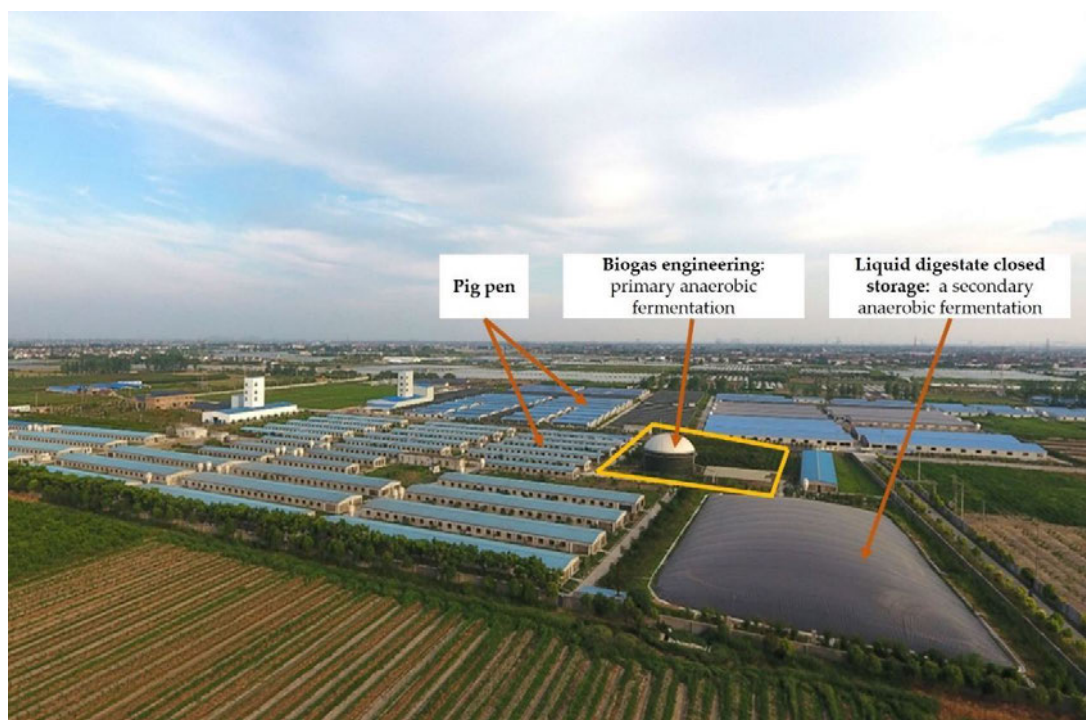


Figure 4.1 Biogas engineering and liquid digestate storage facilities in pig farms.

The experimental soil was collected from the Xinbei District of Changzhou City in the Yangtze River Basin of China. It was 0–20 cm topsoil of permanent basic farmland, and its texture was silty loam. The soil was dried naturally, while stones, plant roots, and other sundries found in the soil were removed. The soil was then crushed with a round wooden stick, sieved through a 2 mm aperture mesh screen, and finally fully mixed into a clean plastic storage box for future use. The basic physical and

chemical properties of the soil are: soil organic matter (SOM)  $29.09 \pm 0.39$  g/kg, pH value  $6.45 \pm 0.04$ , TN  $1.16 \pm 0.17$  g/kg,  $\text{NH}_4^+\text{-N}$   $8.93 \pm 0.57$  mg/kg,  $\text{NO}_3^-\text{-N}$   $56.97 \pm 0.43$  mg/kg, TP  $0.57 \pm 0.02$  g/kg, available phosphorus (AP)  $13.10 \pm 1.47$  mg/kg, available potassium (AK)  $122.91 \pm 13.21$  mg/kg, cation exchange capacity (CEC)  $16.27 \pm 0.49$  cmol/kg, and EC  $492.67 \pm 19.14$   $\mu\text{S}/\text{cm}$ . The soil particle group is composed of 30.5% particles with a particle size of 2–0.05 mm, 52.9% particles with a particle size of 0.05–0.002 mm, and 16.5% particles with a particle size less than 0.002 mm.

#### 4.3.2 Static soil column fabrication

An indoor static soil column was used to simulate the experiment (**Figure 4.2**). The manufacturing method for the soil column is as follows: firstly, a flat-bottom glass tube with an inner diameter of 6.0 cm and a height of 30.0 cm is customized, and the cross-sectional surface area of the test tube is  $28.26 \text{ cm}^2$ . Use a 1% electronic balance to accurately weigh 600 g of the prepared soil into a flat-bottomed tube, shake the tube to make the soil solid (the soil depth is about 20 cm), and then add 369.9 mL of deionized water (the data is the sum of the saturated water content and pore water content of the soil used in the actual test) so that the water content of the soil column reaches the maximum saturated state, and stand for use after standing overnight.

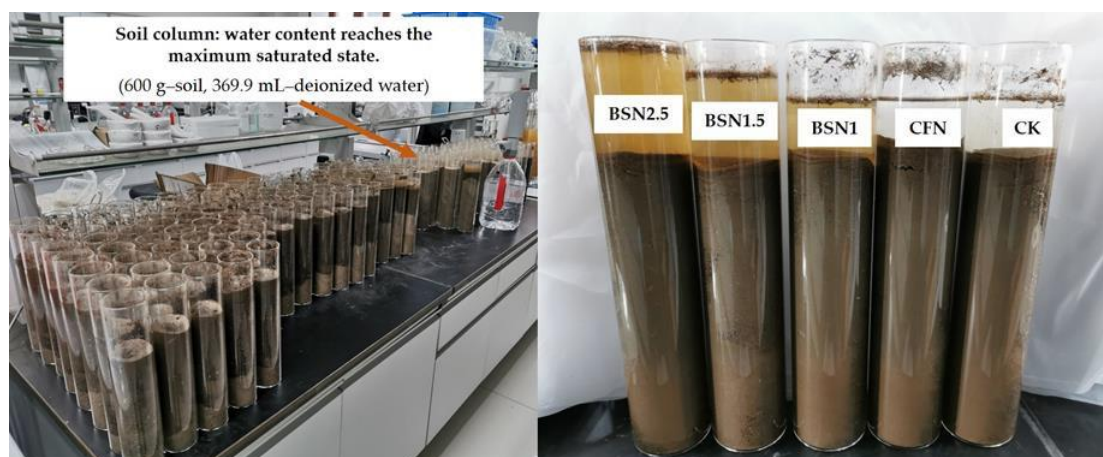


Figure 4.2 Physical photos of static soil column.

### 4.3.3 Design and setting

There are 5 treatments in the experiment as follows:

Treatment ①: Apply chemical fertilizer  $\text{NH}_4^+\text{-N}$  120 kg/hm<sup>2</sup>. The amount refers to the customary nitrogen fertilizer amount of farmers in the rice panicle fertilizer stage of saturated water content paddy fields, which is recorded as: CFN1. Weigh the pure analytical reagent  $\text{NH}_4\text{Cl}$  and add it into deionized water, then prepare 409 mg/L  $\text{NH}_4^+\text{-N}$  solution with the same concentration as the liquid digestate. Measure 82.9 mL of solution, and add it to the soil column surface.

Treatment ②: Apply liquid digestate  $\text{NH}_4^+\text{-N}$  120 kg/hm<sup>2</sup>, which is 1 times the amount of chemical fertilizer  $\text{NH}_4^+\text{-N}$  in Treatment ①, denoted as: BSN1. Measure 82.9 mL of liquid digestate, and add it to the surface of the soil column.

Treatment ③: Apply liquid digestate  $\text{NH}_4^+\text{-N}$  180 kg/hm<sup>2</sup>, which is 1.5 times the amount of chemical fertilizer  $\text{NH}_4^+\text{-N}$  in Treatment ①, referring to the accustomed nitrogen fertilizer dosage of farmers in the rice base-tiller fertilizer period of saturated water content paddy fields, denoted as: BSN1.5. Measure 124.4 mL of liquid digestate, and add it to the surface of the soil column.

Treatment ④: Apply liquid digestate  $\text{NH}_4^+\text{-N}$  300 kg/hm<sup>2</sup>, which is 2.5 times the amount of chemical fertilizer  $\text{NH}_4^+\text{-N}$  in Treatment ①, with reference to the total nitrogen fertilizer dosage used by farmers in the rice season in paddy fields with saturated water content, recorded as: BSN2.5. Measure 207.3 mL of liquid digestate, and add it to the surface of the soil column.

Treatment ⑤ : No fertilization control, keep the same amount of water as Treatment ①, denoted as CK. Measure 82.9 mL of deionized water, and add it to the soil column surface.

Thirty replicates were set up for each treatment, for a total of 150 soil pillars. Take destructive sampling, take 3 repeated soil columns each time, and discard the soil columns after the measurement.

### 4.3.4 Sampling and analysis

At 0, 1, 2, 3, 4, 5, 7, 9, 12, and 15 days after the application of liquid digestate, the



overlying water was taken from the soil column, and the concentrations of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N in the overlying water were measured. After removing the overlying water, excavate 0–10 cm topsoil in the soil column, centrifuge at 4000 r/min for 5 min, and take the supernatant (soil pore water) to measure  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N concentrations. The soil after centrifugation (Soil Sample 1) was retained, and the soil water content, soil water-soluble  $\text{NH}_4^+$ -N content (Kowalenko and Yu, 1996), and soil ion-exchanged  $\text{NH}_4^+$ -N content (Steffens and Sparks, 1997) were determined.

Determination method of soil water-soluble  $\text{NH}_4^+$ -N content (Zhang, 2017): Take 8.00 g of the centrifuged soil (Soil Sample 1) sample, put it into a centrifuge tube, add 40 mL of deionized water according to the solid–liquid ratio of 1:5, and tighten the sealing cap of the centrifuge tube. Mix thoroughly, shake at 160 r/min for 30 min at 25 °C with a thermostatic oscillator, then centrifuge at 4000 r/min for 20 min, collect the supernatant, and repeat the above operation twice for the soil samples in the centrifuge tube. The supernatants collected three times were mixed for the determination of soil water-soluble  $\text{NH}_4^+$ -N content. Retain the centrifuge tube and the soil in the tube (Soil Sample 2).

Determination method of ion-exchanged  $\text{NH}_4^+$ -N content (Zhang, 2017): Add 40 mL of KCl solution with a concentration of 0.5 mol/L to the centrifuge tube where the soil (Soil Sample 2) after extraction of water-soluble  $\text{NH}_4^+$ -N is located, tighten the sealing cap of the centrifuge tube, and mix well. At 25 °C, shake at 160 r/min for 60 min with a thermostatic oscillator, then centrifuge at 4000 r/min for 10 min, collect the supernatant, and repeat the above operation twice for the soil samples in the centrifuge tube. The supernatants collected three times were mixed and used to determine the ion-exchanged  $\text{NH}_4^+$ -N content of the soil.

The  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N contents of all water quality in this experiment were determined by a SKALAR SAN++ full-automatic flow analyzer (Skalar Analytical B.V. Products, Breda, the Netherlands). Daily water evaporation loss of the soil column was measured by using a 1% electronic balance to weigh and calculate the difference with the subtraction method.

### 4.3.5 Calculation formula

Water evaporation loss of soil column:

$$V_t = \frac{(m_0 - m_t)}{\rho} \quad (1)$$

In the Formula (1):  $V_t$  is liquid digestate evaporation loss of overburden water in t day (mL);  $m_0$  is overall mass of soil column on Day 0 (within 8 h) after liquid digestate is applied (g);  $m_t$  is the overall mass of soil column on t-day (g);  $\rho$  is density of water (g/mL).

Reduction rate of liquid digestate  $\text{NH}_4^+\text{-N}$  in overlying water:

$$R(\%) = \frac{M - C_t \cdot (V_0 - V_t)}{M} \times 100 \quad (2)$$

In Formula (2):  $M$  is total application amount of  $\text{NH}_4^+\text{-N}$  (mg);  $C_t$  is  $\text{NH}_4^+\text{-N}$  concentration in overlying water on t-day (mg/L);  $V_0$  is initial application volume of liquid digestate (L);  $V_t$  is liquid digestate evaporation loss on t-day (L).

Fate of liquid digestate  $\text{NH}_4^+\text{-N}$ :

$$F(\%) = \frac{C_t \cdot V}{M} \times 100 = \frac{\omega_t \cdot m}{M} \times 100 \quad (3)$$

In Formula (3):  $M$  is total application amount of  $\text{NH}_4^+\text{-N}$  (mg);  $C_t$  is  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  concentration in overlying water or in soil pore water on t-day (mg/L);  $V$  is residual volume of overlying water or pore water volume (L);  $\omega_t$  is the concentration of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  adsorbed by the soil on t-day (mg/kg);  $m$  is soil mass (kg).

### 4.3.6 Data analysis

Microsoft Office Excel (2016) software was used for the summary, analysis, and graphing of experimental data, and IBM SPSS Statistics (22) software was used for one-way ANOVA and Duncan's method for analysis of variance and multiple comparisons ( $\alpha = 0.05$ ). Data in the graph are mean  $\pm$  standard deviation.

## 4.4 Results

### 4.4.1 Variation characteristics of $\text{NH}_4^+\text{-N}$ concentration and $\text{NH}_4^+\text{-N}$ reduction rate in overlying water

The dynamic change process of liquid digestate  $\text{NH}_4^+\text{-N}$  consumption and the reduction rate in overlying water with saturated water content farmland soil are shown in **Figure 4.3**. After liquid digestate was applied to the soil surface with saturated water content, the concentration of  $\text{NH}_4^+\text{-N}$  in the overlying water decreased gradually with the extension of time, and the reduction rate of the total amount of  $\text{NH}_4^+\text{-N}$  gradually increased. Among them, 0–3 days is the rapid digestion period. During this period, the concentration of  $\text{NH}_4^+\text{-N}$  in the overlying water drops rapidly, the decline range is 63.5–80.7% (**Figure 4.3a**), and the total reduction rate of  $\text{NH}_4^+\text{-N}$  reaches 65.8–82.3% (**Figure 4.3b**).

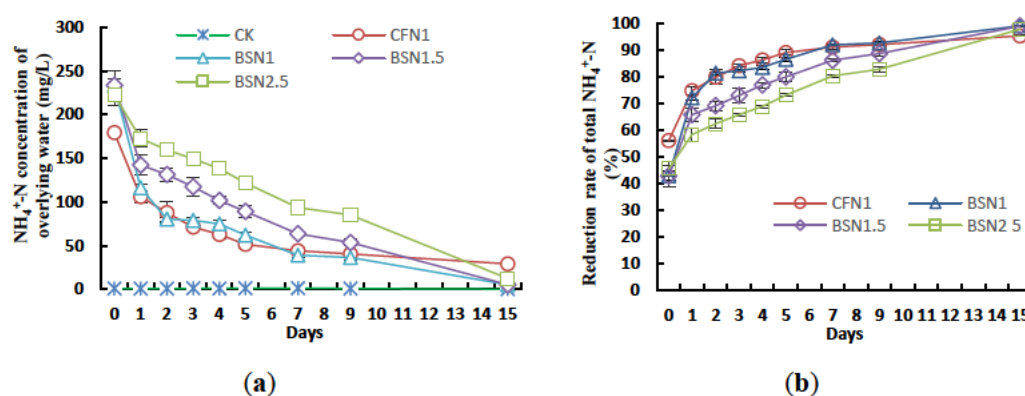


Figure 4.3 Digestion characteristics (a) and reduction rate (b) of liquid digestate  $\text{NH}_4^+\text{-N}$  in the overlying water.

Compared with CFN1 treatment, under the condition of equal  $\text{NH}_4^+\text{-N}$ , the  $\text{NH}_4^+\text{-N}$  concentration in overlying water of BSN1 treatment increased significantly ( $p < 0.05$ ) and then decreased rapidly after 0 days of liquid digestate application (the sampling time was within 8 h after liquid digestate application). On the third day after application, the  $\text{NH}_4^+\text{-N}$  concentration of the overlying water decreased to 78.88 mg/L, which was lower than the discharge concentration of 80 mg/L, specified in the emission standard of pollutants for the livestock and poultry breeding industry (GB18596-2001) (General Administration of Environmental Protection of the People's Republic of China, 2003), and lower than that of the CFN1 treatment, but the difference was not significant. The

$\text{NH}_4^+\text{-N}$  concentration rebounded slightly after application for 3–7 days and remained lower than that of CFN1 treatment after application for 7 days. After 15 days, the  $\text{NH}_4^+\text{-N}$  concentration in the overlying water of BSN1 treatment decreased to 5.16 mg/L, which was significantly lower than that of CFN1 treatment ( $p < 0.05$ ). The  $\text{NH}_4^+\text{-N}$  concentration decreased by 98.7%, and the total reduction rate of  $\text{NH}_4^+\text{-N}$  reached 99.2%. However, with high ammonium nitrogen treatment of BSN1.5 and BSN2.5, the  $\text{NH}_4^+\text{-N}$  concentration in the overlying water was significantly higher than that of CFN1 treatment from 0 to 9 days after application, but after 15 days, the  $\text{NH}_4^+\text{-N}$  concentration decreased to 5.18 mg/L and 12.32 mg/L, respectively, which were significantly lower than that of CFN1 treatment ( $p < 0.05$ ). The  $\text{NH}_4^+\text{-N}$  concentration of the two treatments decreased by 98.7% and 97.0%, and the total reduction rate of  $\text{NH}_4^+\text{-N}$  was 99.0% and 97.9%.

#### **4.4.2 Migration and soil sorption characteristics of liquid digestate $\text{NH}_4^+\text{-N}$**

**Figure 4.4a** shows the change of  $\text{NH}_4^+\text{-N}$  concentration in soil pore water in the 0–10 cm soil layer. After the application of liquid digestate, the  $\text{NH}_4^+\text{-N}$  in the overlying water diffused and migrated downward, and the  $\text{NH}_4^+\text{-N}$  concentration in soil pore water increased rapidly. The larger the amount of liquid digestate applied, the higher the  $\text{NH}_4^+\text{-N}$  concentration in soil pore water in the 0–10 cm soil layer. On the fourth day after application, the  $\text{NH}_4^+\text{-N}$  content in the pore water reached a peak value and then decreased slowly. The  $\text{NH}_4^+\text{-N}$  concentration of BSN1, BSN1.5, and BSN2.5 treatments were 24.41 mg/L, 27.40 mg/L, and 28.91 mg/L, which were significantly higher than those of CFN1 treatment by 16.4%, 30.7%, and 37.9%, respectively ( $p < 0.05$ ).

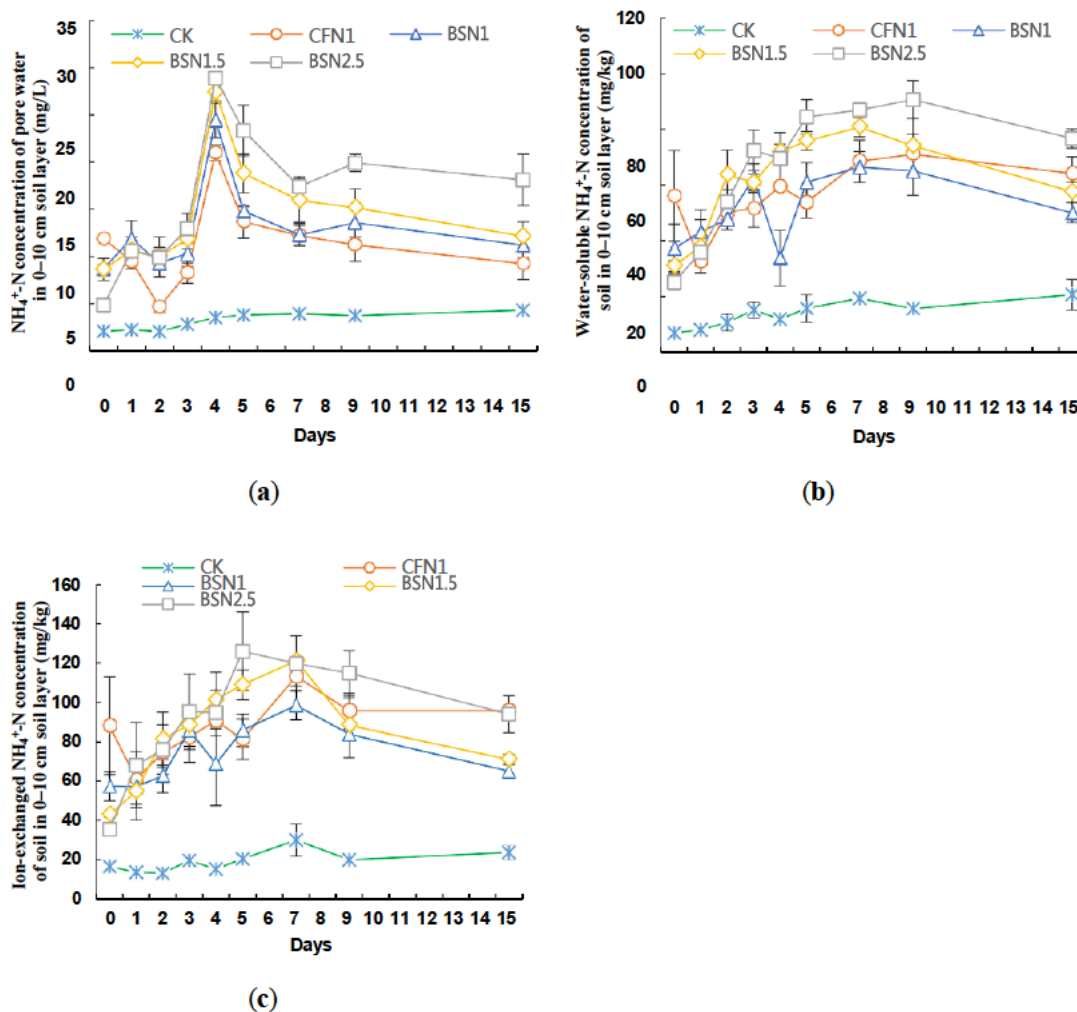


Figure 4.4 Migration of  $\text{NH}_4^+\text{-N}$  in soil pore water (a) and changes of  $\text{NH}_4^+\text{-N}$  adsorbed by soil (b,c).

**Figure 4b,c** shows the changes of soil water-soluble  $\text{NH}_4^+\text{-N}$  and ion-exchanged  $\text{NH}_4^+\text{-N}$  in the 0–10 cm soil layer. After the liquid digestate was applied, the increase in  $\text{NH}_4^+\text{-N}$  concentration in soil pore water created a good environment for soil particles to adsorb  $\text{NH}_4^+\text{-N}$ . The soil water-soluble  $\text{NH}_4^+\text{-N}$  concentration and ion-exchange  $\text{NH}_4^+\text{-N}$  concentration increased first and then decreased, and the more liquid digestate applied, the higher the soil water-soluble  $\text{NH}_4^+\text{-N}$  concentration and the ion-exchanged  $\text{NH}_4^+\text{-N}$  concentration. Under the condition of equal  $\text{NH}_4^+\text{-N}$  content, the soil water-soluble  $\text{NH}_4^+\text{-N}$  concentration and ion-exchange  $\text{NH}_4^+\text{-N}$  concentration of BSN1 treatment reached the peak value on the seventh day after liquid digestate application, but they were 3.0% and 13.1% lower than those of CFN1 treatment respectively, and the difference between treatments was not significant. With the high ammonium nitrogen content BSN1.5 treatment, on the seventh day, the soil water-soluble  $\text{NH}_4^+\text{-N}$

concentration and ion-exchange  $\text{NH}_4^+\text{-N}$  concentration reached the peak, which were significantly higher than that of CFN1 treatment by 17.9% and 7.0%, respectively ( $p < 0.05$ ). The concentration of ion-exchange  $\text{NH}_4^+\text{-N}$  in BSN2.5 treatment reached the peak on the fifth day, but the concentration of water-soluble  $\text{NH}_4^+\text{-N}$  reached the peak on the ninth day, which was significantly higher than that in BSN1 treatment. After the  $\text{NH}_4^+\text{-N}$  sorption (Pourret et al., 2022) reached the peak, desorption and transformation gradually appeared. On the 15th day, the concentration of water-soluble  $\text{NH}_4^+\text{-N}$  and the concentration of ion-exchange  $\text{NH}_4^+\text{-N}$  in BSN1 and BSN1.5 treatments were lower than that in CFN1 treatment, and the difference of ion-exchange  $\text{NH}_4^+\text{-N}$  concentration reached a significant level ( $p < 0.05$ ).

#### **4.4.3 Characteristics of liquid digestate $\text{NH}_4^+\text{-N}$ converted to $\text{NO}_3^-\text{-N}$**

The concentration changes of liquid digestate  $\text{NH}_4^+\text{-N}$  converted to  $\text{NO}_3^-\text{-N}$  was shown in **Figure 4.5**. The change trends of  $\text{NO}_3^-\text{-N}$  in overlying water (**Figure 4.5a**), pore water (**Figure 4.5b**), and soil water-soluble (**Figure 4.5c**) are basically the same.  $\text{NO}_3^-\text{-N}$  concentrations were consistently low and there were no significant differences between treatments. Since the seventh day, the  $\text{NO}_3^-\text{-N}$  concentrations in overlying water, pore water, and soil water-soluble  $\text{NO}_3^-\text{-N}$  of treatments BSN1, BSN1.5, and BSN2.5 all increased significantly compared with those of CFN1 and CK treatments ( $p < 0.05$ ). The greater the amount of liquid digestate application, the greater the increase in  $\text{NO}_3^-\text{-N}$ .

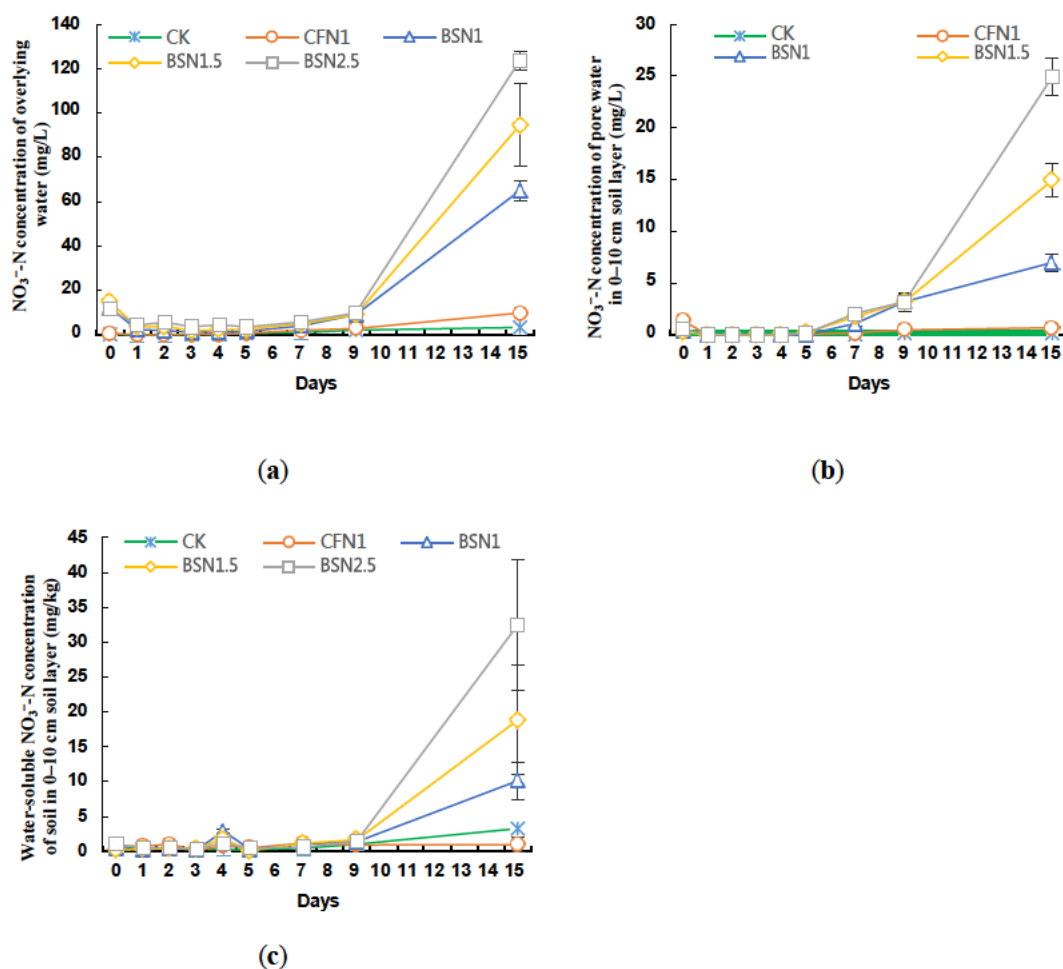


Figure 4.5 Concentration changes of  $\text{NO}_3^-$ -N in overlying water (a), pore water (b), and soil water-soluble (c).

#### 4.4.4 Fate analysis of farmland absorbing liquid digestate $\text{NH}_4^+$ -N

Figure 4.6 is the analysis of the fate of  $\text{NH}_4^+$ -N in BSN1, BSN1.5, and BSN2.5 treatments on the 15th day of liquid digestate application. The disposal of liquid digestate  $\text{NH}_4^+$ -N is mainly based on soil particle sorption and conversion, but with the increase in liquid digestate application, the proportion of  $\text{NH}_4^+$ -N absorbed by soil decreases. The proportions of nitrogen (sum of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N) in soil sorption and pore water storage in the 0–10 cm soil layer of treatments BSN1, BSN1.5, and BSN2.5 accounted for 76.3%, 67.1%, and 63.5% of the total applied  $\text{NH}_4^+$ -N, respectively. The residual proportions of overlying water were 0.9%, 1.1%, and 2.4%, respectively, and the proportions of other destinations (including migration to deeper soil layers, transformation, and volatilization loss of overlying water, etc.) were 22.8%, 31.8%, and 34.1%, respectively. Among them, BSN1, BSN1.5, and BSN2.5 treatments

accounted for 62.8%, 49.7%, and 44.7% of the total applied  $\text{NH}_4^+\text{-N}$  by soil sorption in the 0–10 cm soil layer, respectively, and the  $\text{NH}_4^+\text{-N}$  adsorbed by soil ion-exchange state was greater than that adsorbed by water-soluble state. The proportion of nitrogen contained in pore water is 7.5%, 8.3%, and 8.4%, which are higher than the corresponding residual amount of overlying water, indicating that the diffusion and migration of liquid digestate  $\text{NH}_4^+\text{-N}$  from overlying water to interstitial water in the 0–10 cm soil layer has been completed after 15 days of liquid digestate application, and the more application, the more migration. The proportion of water-soluble  $\text{NO}_3^-\text{-N}$  in soil accounts for 6.1%, 9.2%, and 10.3%, indicating that the liquid digestate  $\text{NH}_4^+\text{-N}$  has been transformed into  $\text{NO}_3^-\text{-N}$  in the 0–10 cm soil layer after 15 days of application, and the more amount of liquid digestate applied, the greater the quantity of the transformation.



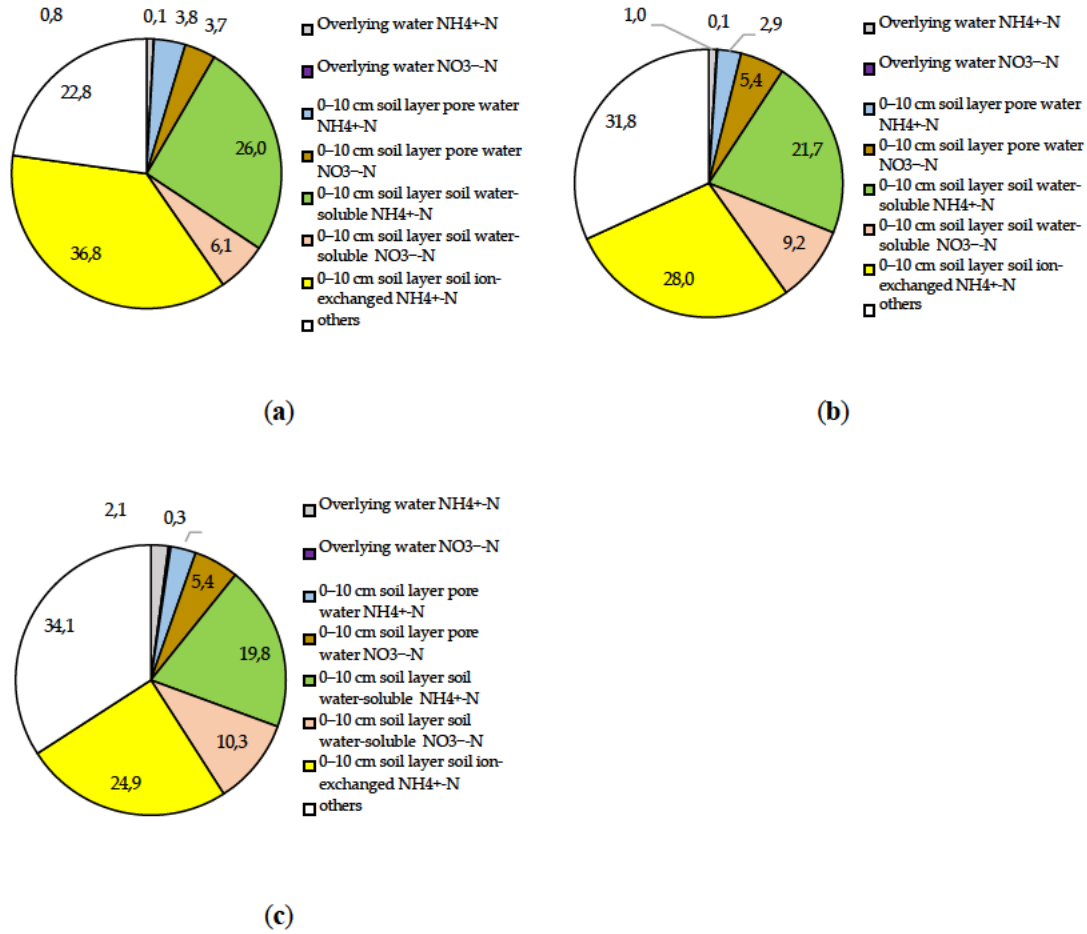


Figure 4.6 Fate analysis of liquid digestate  $\text{NH}_4^+\text{-N}$  in treatments of BSN1 (a), BSN1.5 (b), and BSN2.5 (c).

## 4.5 Discussion

### 4.5.1 Time node of liquid digestate $\text{NH}_4^+\text{-N}$ removal in farmland

Saturated water content farmland has the self-regulation function of the soil-microbial complex system and the comprehensive purification ability of pollutants, and has great liquid digestate disposal potential. The safe bearing capacity of farmland soil and the frequency of digestion (Shi, 2010; Jiang et al., 2011b) are important parameters for determining the area configuration of liquid digestate in land-consumption farms in the combined planting and breeding system. Research on the engineering measures for disposal of liquid digestate in paddy fields shows that the  $\text{NH}_4^+\text{-N}$  concentration in field water is affected by the digested amount of liquid digestate. At the beginning of application, it increased with the increase in liquid digestate and decreased significantly

with time. The  $\text{NH}_4^+\text{-N}$  concentration decreased by 47.52%–85.27% after 3 days of liquid digestate application (Wang et al., 2016), and the concentration of  $\text{NH}_4^+\text{-N}$  in field water is stably lower than the emission concentration of 80 mg/L specified in the emission standard of pollutants for the livestock and poultry breeding industry (GB18596-2001). It can be stably lower than 40 mg/L after 5 days of application (Jiang et al., 2011a). The results of this study are slightly different from the above reports in the digestion time of  $\text{NH}_4^+\text{-N}$  in liquid digestate. After 3 days of application, only the  $\text{NH}_4^+\text{-N}$  concentration in the overlying water of BSN1 treatment decreased to less than 80 mg/L. After 7 days of application, BSN1 and BSN1.5 treatments can stabilize below 80 mg/L, of which BSN1 treatment is lower than 40 mg/L. The reason might be that the static soil column used in this study is a soil-microbial composite system, which lacks the participation of farmland plants, so the digestion speed and aging are slightly delayed.

In addition, by monitoring the change of  $\text{NH}_4^+\text{-N}$  concentration in field water, we can predict the water environment pollution risk and water quality standard discharge time node of farmland disposal liquid digestate engineering measures (Wang et al., 2016; Jiang et al., 2011a; Wang et al., 2015a; Wang et al., 2015b). However, it is impossible to distinguish whether the main reason for the decrease in  $\text{NH}_4^+\text{-N}$  concentration is farmland digestion or farmland irrigation water dilution, which has been questioned in production practice. This study further quantifies the reduction rate of  $\text{NH}_4^+\text{-N}$  in the liquid digestate. From the perspective of reduction of  $\text{NH}_4^+\text{-N}$  input, it is verified again that the first three days after liquid digestate application is a rapid reduction period, during which the prohibition of runoff plays an important role in preventing environmental pollution of surrounding water bodies. After 15 days of application, under the condition of an equal amount of  $\text{NH}_4^+\text{-N}$ , the total amount of  $\text{NH}_4^+\text{-N}$  in the overlying water of liquid digestate treatment decreased by 99.2%, significantly lower than that of fertilizer  $\text{NH}_4^+\text{-N}$  treatment, which proved that the purpose of disposal liquid digestate  $\text{NH}_4^+\text{-N}$  had been achieved, and 15 days could be used as the time node for the end of the first digestion cycle.

#### **4.5.2 Migration and transformation characteristics of $\text{NH}_4^+\text{-N}$ in farmland consuming liquid digestate**

In this study, under the condition of applying the same amount of  $\text{NH}_4^+\text{-N}$ , the  $\text{NH}_4^+\text{-N}$  concentration in the overlying water of the liquid digestate treatment was lower than that of the chemical fertilizer  $\text{NH}_4^+\text{-N}$  treatment on the third day, but it rebounded from 4 to 7 days, mainly due to the  $\text{NH}_4^+\text{-N}$  accounts for 88.6% of the total nitrogen in the liquid digestate, and other nitrogen-containing organic substances in the liquid digestate components are oxidized and decomposed by microorganisms, which increases the  $\text{NH}_4^+\text{-N}$  content of the overlying water. The concentration of  $\text{NH}_4^+\text{-N}$  in overlying water treated with liquid digestate for 0 days (the sampling time in this study is within 8 h after application) is significantly higher than that of fertilizer  $\text{NH}_4^+\text{-N}$  treatment, but the concentration of  $\text{NH}_4^+\text{-N}$  in pore water and the concentration of  $\text{NH}_4^+\text{-N}$  adsorbed by soil are lower than that of fertilizer  $\text{NH}_4^+\text{-N}$  treatment. This is because there is a competitive and mutually exclusive relationship between other cations (Song et al., 2021) and  $\text{NH}_4^+\text{-N}$  ions in liquid digestate, thus delaying the molecular diffusion rate of  $\text{NH}_4^+\text{-N}$  in pore water, it also reduces the dominant sorption of  $\text{NH}_4^+\text{-N}$  on soil particles (Zhao et al., 2013). The abnormal value of BSN1 treatment on the fourth day of application may be related to the operation error of the destructive test, and the value at this point can be regarded as the missing value. After 7 days of application, the  $\text{NO}_3^-\text{-N}$  concentration in overlying water, pore water, and soil with the liquid digestate  $\text{NH}_4^+\text{-N}$  treatment was significantly higher than that of the fertilizer  $\text{NH}_4^+\text{-N}$  treatment, which may be that the organic active substances in the liquid digestate components promoted the reproduction of nitrifying microorganisms (Chen et al., 2020), thus promoting the conversion of  $\text{NH}_4^+\text{-N}$  to  $\text{NO}_3^-\text{-N}$ .

#### **4.5.3 Fate of farmland disposal liquid digestate $\text{NH}_4^+\text{-N}$**

Using farmland to consume liquid digestate is a widely recognized and effective treatment method, and the environmental pollution risk related to this measure has always been the focus of attention (Lovejoy et al., 1997; Velthof et al., 2005; Paul et al., 1998). The total nitrogen in liquid digestate is mainly  $\text{NH}_4^+\text{-N}$ . There is no clear report

on the final whereabouts of  $\text{NH}_4^+\text{-N}$  when a large amount of liquid digestate is applied to farmland. Some scholars believe that liquid digestate  $\text{NH}_4^+\text{-N}$  might enter the underlying soil through leaching and then pollute the groundwater (Wang et al., 2015b), but many experimental studies have shown that the application of liquid digestate in dryland (Svoboda et al., 2013), and in paddy fields (Wang et al., 2016; Chen et al., 2013) has not significantly increased the  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  in groundwater. The results of this study showed that the concentration of  $\text{NH}_4^+\text{-N}$  in the pore water of 0–10 cm soil layer reached the peak value after 4 days of liquid digestate application, and the  $\text{NH}_4^+\text{-N}$  sorption by soil particles reached the peak after 7 days, indicating that the  $\text{NH}_4^+\text{-N}$  in overlying water was gradually migrating to the soil layer over time. The proportion of liquid digestate nitrogen (including  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  transformed from  $\text{NH}_4^+\text{-N}$ ) absorbed by soil and contained in pore water in the 0–10 cm soil layer accounted for 76.3% of the total  $\text{NH}_4^+\text{-N}$  after being applied for 15 days, indicating that the disposal of liquid digestate  $\text{NH}_4^+\text{-N}$  in farmland was mainly soil sorption and transformation. However, with the increase in liquid digestate application, the proportion of  $\text{NH}_4^+\text{-N}$  adsorbed by soil in the 0–10 cm soil layer decreases, which is due to the limit value of soil sorption capacity of 1108.55 mg/kg (Wang et al., 2022). When the sorption limit value is exceeded,  $\text{NH}_4^+\text{-N}$  will migrate to the 10–20 cm soil layer. Only when the amount of  $\text{NH}_4^+\text{-N}$  applied exceeds the sorption limit value of 0–20 cm cultivated soil layer, there will be the risk of polluting groundwater.

Ammonia volatilization loss was once considered as one of the main ways to reduce  $\text{NH}_4^+\text{-N}$  in field water (Jiang et al., 2011a; Huijismans et al., 2001), but the ammonia volatilization process is very complex and affected by many factors, so it is very difficult to accurately estimate its loss under natural conditions. Some studies have shown that soil wetting or flooding conditions can reduce ammonia volatilization (Hou et al., 2007; Zhou et al., 2009). The standing test of liquid digestate showed that the removal rate of ammonium nitrogen was only 53% under natural conditions for 100 days (Shi, 2010). In this study, the other fate of liquid digestate  $\text{NH}_4^+\text{-N}$  in the BSN1, BSN1.5, and BSN2.5 treatments accounts for 22.8%, 31.8%, and 34.1%, including the

migration of  $\text{NH}_4^+\text{-N}$  to deeper soil layer (Wang et al., 2014b; Tan et al., 2015), transformation (Pote et al., 2001) and ammonia volatilization loss of overlying water. Some scholars believe that the key period of ammonia volatilization is within 7 days after liquid digestate application. The ammonia volatilization loss rates of 1 N liquid digestate, 2 N liquid digestate, 4 N liquid digestate, and 1 N chemical fertilizer treatment are 18.8%, 14.3%, 9.9%, and 6.6%, respectively (Shi, 2013). In this experiment, the other directions of liquid digestate  $\text{NH}_4^+\text{-N}$  were not subdivided, so the proportion of ammonia volatilization loss in other directions could not be determined. Therefore, it cannot be proved that ammonia volatilization loss is one of the main ways to reduce liquid digestate  $\text{NH}_4^+\text{-N}$  in overlying water. However, at the beginning of liquid digestate application, the concentration of  $\text{NH}_4^+\text{-N}$  in overlying water maintained a high level, decreased rapidly within 7 days, and gradually transformed into  $\text{NO}_3^-\text{-N}$  after 7 days. Therefore, it is speculated that if ammonia volatilization really exists, then the critical period of ammonia volatilization should be within 7 days, but the proportion of ammonia volatilization loss will not be too high.

#### **4.6 Conclusions**

The use of saturated water content farmland soil for disposal of liquid digestate ammonium nitrogen is mainly based on soil sorption and pore water migration. With the extension of time, the ammonium nitrogen concentration in the overlying water gradually decreases, and the reduction rate of the total ammonium nitrogen gradually increases. However, the reduction speed and rate showed a downward trend with the increase in the application amount of the ammonium nitrogen in the liquid digestate. The application of 0–3 d is the rapid consumption period for preventing and controlling the pollution of surrounding water bodies, and the application of 15 d is the completion period of one-time safety consumption.

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## CHAPTER 5: ASSESSMENT OF BIOGAS SLURRY PRETREATMENT ON SOIL PROPERTIES, SOIL MICROFLORA AND GROWTH OF *CAPSICUM* SPP.

### 5.1 Abstract

This study reports the effects of pretreated biogas slurry on farm soil properties, microflora and growth of *Capsicum* spp. The split zone approach was adopted for the experiment, with untreated soil as the control (CK). This was investigated under various process conditions. The saturated water content, clean water (W) (495 m<sup>3</sup>/hm<sup>2</sup>), low biogas slurry (LBS) (495 m<sup>3</sup>/hm<sup>2</sup>) and high biogas slurry (HBS) (990 m<sup>3</sup>/hm<sup>2</sup>) were used in the soil pretreatment. In addition, the four treatments (CKM, WM, LBSM, HBSM) were set up with film mulching as the sub zone. The responses of soil properties, microorganisms and *Capsicum* spp. growth to biogas slurry pretreated soil were determined. Biogas slurry pretreatment of protected soil increases the soil total nitrogen (0.15–0.32 g/kg), total phosphorus (0.13–0.75 g/kg), available phosphorus (102.62–190.68 mg/kg), available potassium (78.94–140.31 mg/kg), organic carbon content (0.67–3.32 g/kg) and soil pH value, while the population, diversity and distribution of soil bacteria, fungi, actinomycetes, and fusarium were significantly affected. Interestingly, soil ammonium nitrogen, soil pH, and soil nitrate nitrogen were highly correlated to the population of bacteria and fungi present in the pretreated soil. The soil with biogas slurry pretreatment of 495 m<sup>3</sup>/hm<sup>2</sup> favoured the growth, seedlings survival rate, flowering rate and fruit-bearing rate of *Capsicum* spp. and significantly reduced the rate of rigid seedlings. The application of biogas slurry to pretreat protected soil has achieved the multi-goals of biogas slurry valorization, soil biofertilization, preventing and controlling soil-borne microbial diseases. These findings are of significant importance for safe and environmental-friendly application of biogas slurry for soil pretreatment.

**Keywords:** liquid digestate, biogas slurry, ammonium nitrogen, soil microorganisms, *Capsicum*

## 5.2 Introduction

The extensive application of acidic or physiologically acidic fertilizers, pesticides and high-intensity single planting mode in protected vegetable cultivation can easily cause continuous cropping obstacles such as soil quality degradation, soil-borne diseases, soil acidification, secondary salinization and nutrient imbalance (Pérez-Brandán et al., 2014). These usually result in a sharp decline in vegetable yield, sustainable vegetable cultivation and seriously affect the economic income of farmers (Zhu et al., 2013). It is reported that the losses caused by pathogenic bacteria in vegetable industry around the world are up to US \$1 billion every year (Lamour et al., 2012). Research on the effective methods of preventing and controlling soil degradation as well as soil-borne diseases in protected lands has become imperative and important for promoting sustainable agricultural development.

Soil-borne diseases are caused by plant pathogenic fungi, bacteria, nematodes and viruses in the soil. The microbes can be proliferate when the conditions are suitable, infecting the roots and stems of crops (Cai and Huang, 2016). Among them, the root rot disease of protected land vegetables caused by pathogenic fungi is more serious, and the yield can be reduced by more than 60%. Additionally, soil acidification provides a suitable living environment for the growth of pathogenic fungi. The accumulation of phenolic acid allelochemicals in soil affects the permeability of root cell membranes, which is the main reason for the autotoxic effect of continuous cropping of vegetables, hence, providing pathogenic fungi with carbon source and energy for growth (Xie et al., 2014). Efforts have been made to mitigate soil-borne diseases such as Reductive Soil Disinfestation (RSD). The RSD method has proven to be a very strong method of "curing" the infection of soil-borne pathogens (Butler et al., 2012; Momma et al., 2013; Cai et al., 2015). The core method is to apply a large amount of easily decomposed organic materials, irrigate and mulch to prevent air from diffusing into the soil, creating a strong soil reduction state in a short time, and thus, killing soil-borne pathogens.

As a by-product of biogas production, biogas slurry is weakly alkaline, this can be

used to alleviate soil acidification. Also, biogas slurry is rich in organic carbon and nutrient elements, which can provide rich carbon sources and energy for microbial growth. Hence, the application of biogas slurry to soil could significantly influence the population, community structure and activity of soil microorganisms (Tao et al., 2014). Biogas slurry treatment has been found to increase the population of bacteria such as *Pseudomonas fluorescens* and *Trichoderma*, fungi, actinomycetes, in the rhizosphere soil as well as improve the diversity index of soil bacteria and fungi (Cao et al., 2013). In another related study, Sui et al. (2016), showed that the population of soil actinomycetes and fungi increased by 72.4% and 61.6%, respectively, while the number of soil bacteria decreased by 18.4% when biogas slurry drip irrigation was used in solar greenhouse. Moreover, biogas slurry has a certain inhibitory effect on a variety of crop pathogenic fungi (Tao et al., 2011). Li et al. (2013), reported a significant inhibitory effect on *Rhizoctonia solani*, *Fusarium nivalea*, *F. oxysporum*, and *Fusarium solani*, with an inhibition rate of 70%, 40%, 68%, and 70%, respectively when biogas slurry was used for the treatment of farmland. Similarly, biogas slurry of 21 different large-scale biogas projects in Jiangsu Province of China had different degrees of inhibitory effect on the growth of strawberry Fusarium wilt when applied to farmland. In addition, the stage of obtaining the biogas slurry had a significant effect on its antibacterial effect (Ma et al., 2011). Although, there have been different application of biogas slurry on farmland as potential biofertilizers and pesticides, there is a scarcity of knowledge on the use of biogas slurry to pretreat degraded protected soil. Similarly, there is a dearth of information on preventing and controlling soil-borne diseases of protected farmland using biogas slurry. Knowledge of multiple potential usage of biogas slurry, the impact of biogas slurry on soil and its operating mechanism provides a theoretical basis and technical approach to biogas slurry farmland application, thereby improving resource utilization of biogas slurry.

Furthermore, studies have shown that the application of biogas slurry can improve the physical and chemical properties of farmland (Möller, 2015; Niyungeko et al., 2020; Tang et al., 2021) while effectively valorizing the biogas slurry (Chen et al., 2015).

Treatment of farmland with biogas slurry has a direct positive effect on improving soil organic matter, soil structure, and fertility (Xu et al., 2013; Liu et al., 2018; Yu et al., 2018). Overall, pretreatment of farmland with biogas slurry have multiply benefits to the soil and cultivated crops. Thus, it is imperative to study the dual benefits of biogas slurry on soil physiochemical properties and growth performance of cultivated crop.

Hence, the present study assessed the application of biogas slurry to pretreat the degraded protected soil to achieve the dual goals of preventing and controlling soil-borne pathogens in the protected farmland. Also, the efficiency of biogas slurry absorption per unit area of farmland was evaluated through field experiments. The changes in soil properties, soil microbial community and the growth of *Capsicum* spp. following the application of biogas slurry was also analysed, based on the RSD method.

### **5.3 Materials and methods**

#### **5.3.1 Study site and soil properties**

The experiment was carried out in a *Capsicum* spp. greenhouse in Sihe Township, Sihong County, China. *Capsicum* spp. was planted continuously for more than 5 years in the greenhouse. The basic soil properties of the greenhouse after the harvest of the previous crop were organic carbon (OC)  $8.92 \pm 0.61$  g/kg, pH  $7.40 \pm 0.02$ , total nitrogen (TN)  $1.49 \pm 0.15$  g/kg, ammonium nitrogen ( $\text{NH}_4^+$ -N)  $49.37 \pm 1.67$  mg/kg, nitrate nitrogen ( $\text{NO}_3^-$ -N)  $351.87 \pm 13.81$  mg/kg, total phosphorus (TP)  $1.09 \pm 0.02$  g/kg, available phosphorus (AP)  $21.15 \pm 2.78$  mg/kg, available potassium (AK)  $242.35 \pm 11.12$  mg/kg. Soil culturable microbial indicators: bacteria  $(3.20 \pm 0.19) \times 10^6$  cfu/g, fungi  $(1.00 \pm 0.06) \times 10^4$  cfu/g, actinomycetes  $(2.84 \pm 0.09) \times 10^6$  cfu/g, fusarium  $(1.08 \pm 0.14) \times 10^3$  cfu/g.

#### **Biogas slurry properties**

The biogas slurry tested was obtained from anaerobic digester using pig farm manure and sewage from adjacent farm. The average properties of biogas slurry are TN  $628.34 \pm 103.22$  mg/L,  $\text{NH}_4^+$ -N  $519.89 \pm 96.83$  mg/L,  $\text{NO}_3^-$ -N  $65.64 \pm 8.58$  mg/L, TP  $339.72 \pm 96.13$  mg/L, total potassium (TK)  $423.47 \pm 81.67$  mg/L, COD  $1060 \pm 35$  mg/L, pH  $8.14 \pm 0.31$ . The experimental *Capsicum* spp. variety was Moxiu No. 8.

### 5.3.2 Design and setting

A split-plot experimental design was adopted in the application of the biogas slurry. Four main farmland plots were implemented: the blank control without any treatment (CK); the clean water treatment-W (applying clean water of 495 m<sup>3</sup>/hm<sup>2</sup>), the low concentration biogas slurry treatment-LBS (applying biogas slurry of 495 m<sup>3</sup>/hm<sup>2</sup>) and the high concentration biogas slurry treatment-HBS (applying 990 m<sup>3</sup>/hm<sup>2</sup> of biogas slurry). The W, LBS and HBS treatments were brought to soil saturated water content by watering 450 m<sup>3</sup>/hm<sup>2</sup> a day to prior applying the biogas slurry on the farmland. Two additional subplots were set up, one with the black mulch and another without the black mulch. Thus, a total of eight treatments namely CK, W, LBS, HBS, CKM, WM, LBSM and HBSM were implemented. Each experimental treatment was repeated three times. The area of each plot is 35m<sup>2</sup> (length 7m, width 5m), underground depth of 80cm, reserved height above ground of 20cm with black PE impermeable membrane buried around the plot. The biogas slurry was extracted by a mud pump, and then applied using a flow meter.

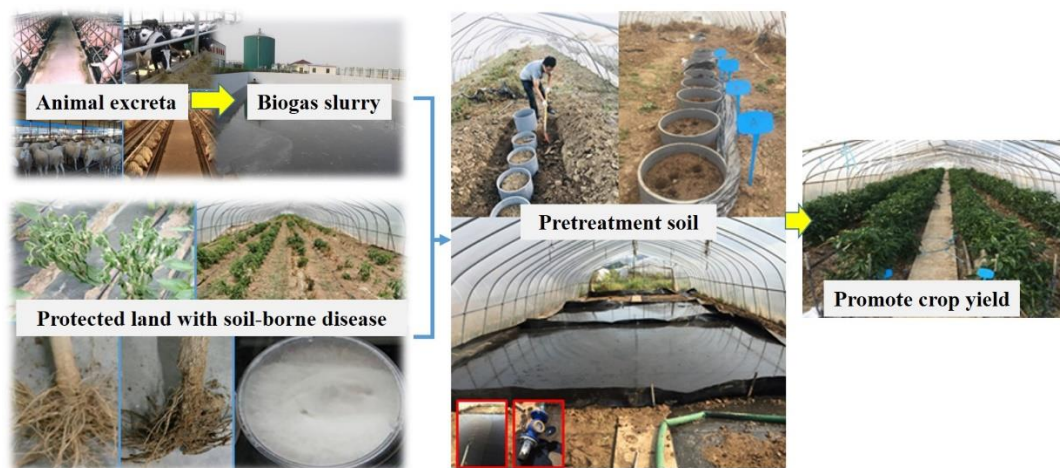


Figure 5.1 Graphical abstract.

After application of biogas slurry, the facility greenhouse was closed for 20 days, then the covering film was removed, and the greenhouse was further kept ventilated for 7 days, after which the land was ploughed and aired for another 14 days. The *Capsicum* spp. seedlings were transplanted after 51 days seedling period using local farmer transplanting density (approximate 21,975 plants per ha). During the *Capsicum* spp.



planting, 45% of nitrogen and potassium fertilizer was used as base fertilizer, 30% as topdressing at flowering stage, and 25% as topdressing at fruit stage. The daily management of pest control, weeding and water irrigation was carried out using conventional methods.

### 5.3.3 Sampling and analysis

#### 5.3.3.1 Determination content

Soil samples were taken at 5, 10, 15 and 20 days after biogas slurry application to determine the soil properties. The soil properties analysed were TN, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, TP, AP, AK, OC, pH, soil culturable bacteria, fungi, actinomycetes and fusarium population. Also, microbial community diversity using 16S rDNA V3+V4 area/fungal ITS rDNA ITS2 area sequencing was carried out.

The growth of *Capsicum* spp. in each treatment was measured 50 days after transplanting. Data on the total number of transplanted *Capsicum* spp., seedlings survival rate, rate of hardened seedlings, rate of flowering plants, rate of fruit-bearing plants, and plant height were obtained using Equations 1 to 4

$$\text{Survival rate seedlings (\%)} = \frac{\text{Survival seedling number}}{\text{Total number of transplanted}} \times 100 \quad (1)$$

$$\text{Rigid seedlings rate (\%)} = \frac{\text{Hardened seedlings number}}{\text{Survival seedlings number}} \times 100 \quad (2)$$

$$\text{Flowering plants rate (\%)} = \frac{\text{Flowering plants number}}{\text{Survival seedlings number}} \times 100 \quad (3)$$

$$\text{Fruit – bearings rate (\%)} = \frac{\text{Fruit–bearing plants number}}{\text{Survival seedlings number}} \times 100 \quad (4)$$

#### 5.3.3.2 Soil sample collection methods

The multi-point sampling method was used for the soil sampling analysis. The soil was collected from 0–10 cm soil layer with an undisturbed soil drill. The fresh soil sample was spread on clean paper and mixed evenly. Then 30g of finely crushed fresh soil was stored in a ziplock PE bag at 4 °C thereafter used to enumerate microorganisms

present in the soil. Another 5g of fine fresh soil was wrapped in an Aluminum foil then stored in liquid nitrogen for the determination of microbial community diversity. The remaining soil sample was dried, sieved and used for the determination soil properties.

### **5.3.3.3 Soil properties determination**

The chemical properties of the soil were determined using standard methods for chemical analysis of soil and agriculture (Lu, 2000). First, the soil sample was digested with concentrated sulfuric acid and the content of TN and TP were determined with an automatic flow analyzer (SKALAR SAN++, Holland). Then the contents of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  in the soil were extracted with 2mol/L KCl solution (ratio of 2mol/L KCl solution to the soil was 5:1), and determined with SKALAR SAN++ (Holland) analyzer. Moreover, AP content was obtained by sodium bicarbonate leaching-molybdenum antimony anti-spectrophotometry (HJ 704-2014), while AK content was determined by ammonium acetate extraction flame photometer method (NY/T 889-2004) (Gao et al., 2020). The OC was determined using the potassium dichromate volumetric method (Cesarano et al., 2017). Lastly, the pH value was measured by Thunder Magnetic PHS-3C pH meter (Sadaf et al., 2017; Yao et al., 2022).

### **5.3.3.4 Soil microbial community determination**

The gradient dilution plate coating counting method was used to determine the culturable bacteria, fungi, actinomycetes, and fusarium. The beef extract peptone agar medium, Martin's medium, modified Komada's No. 1 medium, and modified Komada's selective media were employed for the culturable microorganisms (Yao et al., 2016). This was achieved by weighing 10g of soil into 90ml of sterile water, incubated for 1h at 28 °C constant temperature oscillation. Then it was diluted according to 10 times gradient and coated with specific culture medium at corresponding dilution times (coating was carried out 330ul per plate). This was repeated three times, and enumerated after 2–5 days of incubation (Zhou, 2015).

Soil microbial community diversity was determined using high-throughput sequencing. The specific steps are as follows:

①**DNA extraction and quality control.** The FastDNA® SPIN Kit (Mpbio, USA)

was used to extract the total microbial DNA in the soil (Miller et al., 1999). The specific experimental steps were carried out according to the manufacturing instructions. 1% agarose gel electrophoresis was used to detect the degradation and impurities of the extracted DNA samples. Nano Drop nucleic acid protein analyzer was used to detect the sample concentration and total amount of DNA, and the PCR pre-amplification to test whether the sample is qualified.

**②16S/ITS rDNA library preparation.** Ten nanogram (10ng) of DNA template was used to carry out PCR amplification of target region; bacterial 16S rDNA V3+V4 region amplification primers were 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), fungal ITS rDNA ITS2 region amplification primers are ITS3F (5'-GCATCGATGAAGAACGCAGC-3 ') and ITS4R (5'-TCCTCCGCTTATTGATATGC-3') (Essel et al., 2018; Wang et al., 2021). The PCR amplification was divided into two steps: first, specific primers to amplify the target fragment with the EX *Taq* enzyme of TaKaRa to ensure amplification efficiency and accuracy. The target fragment was purified and recovered by Novozen AMPure XP magnetic beads, and then the recovered product was used as a template for secondary PCR amplification. The connectors, sequencing primers and barcodes required for sequencing on the Illumina platform were added to both ends of the target fragment. After the library was constructed using Qubit2.0 Fluorometer for preliminary quantification and diluting the library to 1ng/μL, Agilent2100 was used to detect the insert size of the library. After the desired insert size was obtained, a Thermo 2720 fluorescence quantitative PCR instrument StepOnePlus (Thermo-Life Company) was used to perform a QPCR to accurately quantify the effective concentration of the library.

**③Computer sequencing:** Paired-end sequencing was performed on the Illumina HiSeq platform for qualified libraries using the PE250 sequencing strategy.

**④Sequencing analysis:** The data filtering is completed by removing low-quality bases, Ns, and linker contamination sequences to obtain credible target sequences for subsequent analysis.

The corresponding Read1 and Read2 (Read1 and Read2 refer to sequence fragments obtained from 5 'and 3' directions respectively) sequenced at both ends were

spliced using the sequence splicing method PEAR (Zhang et al., 2014). Then, the spliced sequence was analyzed using software QIIME version 1.8.0, including OTUs extraction for OTUs analysis and alpha diversity analysis (Caporaso et al., 2010; Edgar 2010; Vasileiadis et al., 2012; Quast et al., 2013; Adler et al., 2013). And the canonical correspondence analysis (CCA) was performed using the Canoco windows 4.5 to find the most significant environmental variables.

### 5.3.4 Data analysis

Using Office Excel 2016 software and OriginPro 2017 were used analyze and map soil traits, soil microorganisms and *Capsicum* spp. plant growth data. One-way ANOVA and Duncan's method were further used for analysis of variance and multiple comparisons ( $\alpha=0.05$ ) using IBM SPSS Statistics (22).

## 5.4 Results and analysis

### 5.4.1 Effect of biogas slurry pretreatment on soil properties

The impact of biogas slurry on soil properties is shown in **Figure 5.2**. After 20 days of biogas slurry treatment of the soil, compared with CK treatment, LBS, HBS, LBSM treatment increased in TN content by 25.3%, 11.7%, 19.0%, respectively (Fig. 5.2A). The HBS, LBSM and HBSM treatments significantly increased the content of  $\text{NH}_4^+\text{-N}$  by 24.6%, 33.1% and 86.8%, respectively while LBS significantly reduced the content of ammonium nitrogen by 14.2% (Fig. 5.2B). On the other hand, all the treatments significantly reduced  $\text{NO}_3^-\text{-N}$  content with LBS, HBS, LBSM, HBSM and WM treatments reduced  $\text{NO}_3^-\text{-N}$  content by 47.8%, 52.2%, 51.3%, 55.9% and 84%, respectively (Fig. 5.2C). Moreover, the LBS, HBS, LBSM and HBSM treatments increased the TP content by 79.2%, 55.8%, 65.2% and 14.2%. LBS, HBS and LBSM treatments increased the TP content significantly ( $P<0.05$ ) (Fig. 5.2D). Similarly, the LBS, HBS, LBSM and HBSM treatments increased the content of available phosphorus and potassium in the treated soil. The content of available phosphorus increased by 1378.5%, 1355.9%, 778.6% and 741.9% (Fig. 5.2E), while, the content of available

potassium increased by 87.7%, 84.8%, 61.0% and 108.4% respectively (Fig. 5.2F). Likewise, the LBS, HBS, LBSM and HBSM treated soil increased in soil organic carbon content by 38.7%, 27.4%, 40.3% and 8.1% (organic carbon content in LBS, HBS and LBSM treatments was observed to increased significantly ( $P<0.05$ )) (Fig. 5.2G). Also, the pH value in the HBS, LBSM and HBSM treated soil increased significantly by 0.8%, 2.2% and 2.5% respectively (Fig. 5.2H).

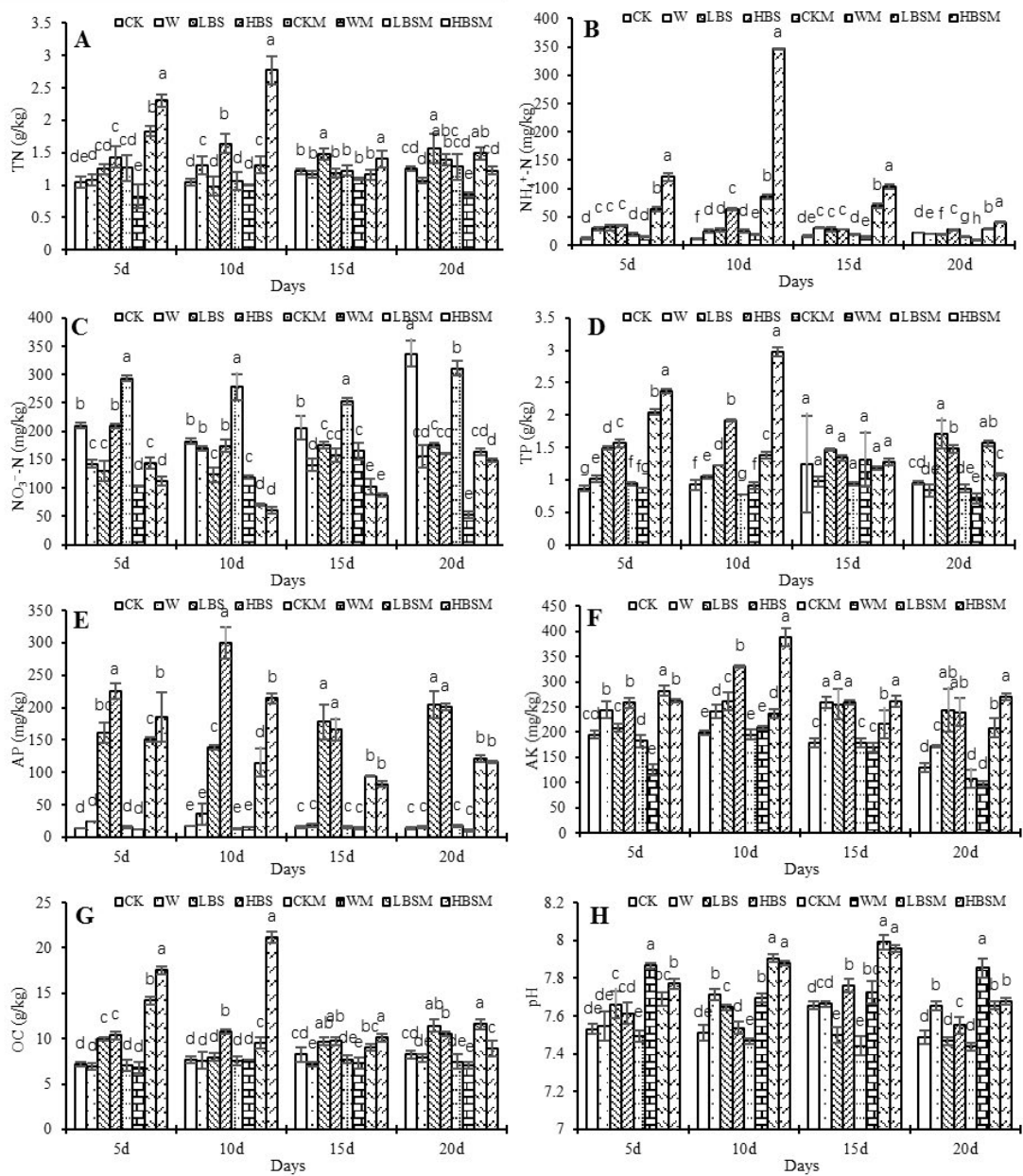


Figure 5.2 Changing trend of soil properties following biogas slurry pretreatment.

## 5.4.2 Effect of biogas slurry pretreatment on soil microorganisms

### 5.4.2.1 Soil culturable microorganisms

Shown in **Figure 5.3** is the impact of different pretreatment measures on culturable bacteria, fungi, actinomycetes and fusarium. The results obtained showed that the soil pretreatment for 15 days significantly reduced the number of culturable bacteria in soil (Fig. 5.3A). There was an increase in the population of culturable bacteria in soil after the biogas slurry application when compared with the control (CK treatment) while, the difference in the population of culturable bacteria between treatments decreases with the increase in pretreatment time. After the cultivation period of 20 days, the number of culturable bacteria in LBSM treatment reduced compared to CK treatment (33.3%). Similarly, the fungi load of culturable fungi in the soil increased first and then decreased after 20 days of pretreatment (Fig. 5.3B). On the other hand, comparing LBS and HBS treatment to CK treatment, LBS and HBS treatment increased the number of culturable fungi by 130.1% and 30.1%, while LBSM and HBSM treatment resulted in the decreased in the fungi load of culturable fungi by 41.0% and 13.0%, respectively, without observable significant difference ( $P < 0.05$ ).

Moreover, in Fig 5.3C, a gradual increased in the actinomycetes population was observed, although the cultivation period was extended there was no substantial effect on the population of actinomycetes. On the 20th day, the number of actinomycetes decreased by 31.1%, 54.5%, 42.6% and 52.9% for LBS, HBS, LBSM and HBSM treatment, respectively. The reduction in actinomycetes was found to be significant within treatment and in comparison, with control ( $P < 0.05$ ).

Furthermore, biogas slurry pretreatment of farmland reduced the population of cultivable fusarium in the soil (Fig. 5.3D). The LBS treated soil showed fusarium population initially increase ( $1.19 \times 10^2$  cfu/g at day 5,  $7.90 \times 10^2$  cfu/g at day 10, and  $1.02 \times 10^3$  cfu/g at day 15) and then decreased ( $1.20 \times 10^2$  cfu/g at day 20) as the treatment period extended. LBSM treatment showed a decreasing trend in fusarium population while, HBS and HBSM treated soil showed an initial decrease in fusarium

population, then increasing to later decrease. When these are compared to CK treatment, LBS, HBS, LBSM and HBSM treatment significantly decreased the number of cultivable fusarium in soil by 16.4%, 48.4%, 77.9% and 70.5% respectively after the 15th day of treatment. On the 20th day, the population of cultivable fusarium in each treatment decreased less than that in CK treatment, but there was no significant difference in the population of cultivable fusarium between LBS, LBSM and CK treatment.

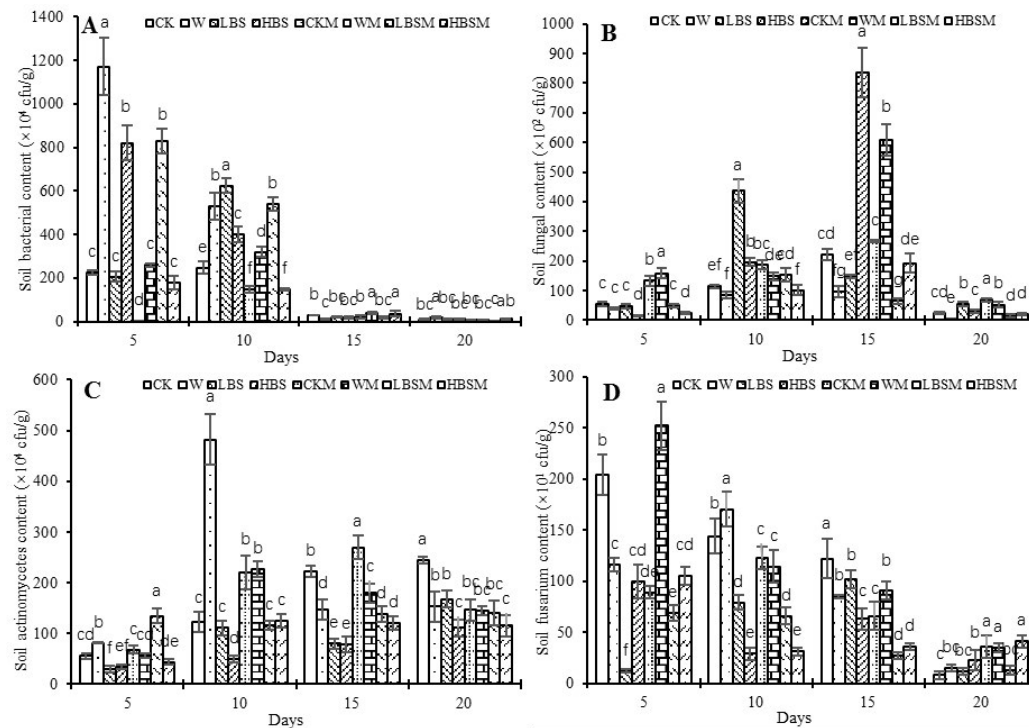


Figure 5.3 Profile of culturable bacteria, fungi, actinomycetes and fusarium in biogas slurry pretreated soil.

#### 5.4.2.2 Changes in soil microbial community diversity

**Figure 5.4** shows the OTU cluster analysis and annotation results of high-throughput sequencing of bacterial 16S and fungal ITS in different soil treatments. Compared with CK treatment, the number of soil bacterial OTU in BS (LBS+HBS) and BSM (LBSM+HBSM) treatment decreased by 12.9% and 49.3% respectively. Moreover, the population of fungi OTU in BS (LBS+HBS) treatment decreased by 4.2%, but the number of fungi OTU in BSM (LBSM+HBSM) treatment increased by 14.4%. The OUT-annotation results showed that the number of bacteria and fungi in each treatment

changed to varying degrees in terms of kingdom, phylum, class, order, family, genus and species. **Table 5.1** represent the  $\alpha$  diversity analysis of the sample. The Chao1 index of bacteria and fungi in the BS treatment compared with the CK treatment decreased by 14.5% and 5.4%, respectively, while the BSM treatment decreased by 46.8% and 34.5%. This is an indication that BS and BSM treatment reduced the population of species in soil bacterial and fungal communities. Similarly, the Shannon index and Simpson index of bacteria and fungi in BS and BSM treatments were lower than those in CK treatments, indicating that BS and BSM treatments led to the decrease in the diversity of bacteria and fungi communities in soil. Also, BS and BSM treatments resulted in the uniformity of species distribution in microbial communities.

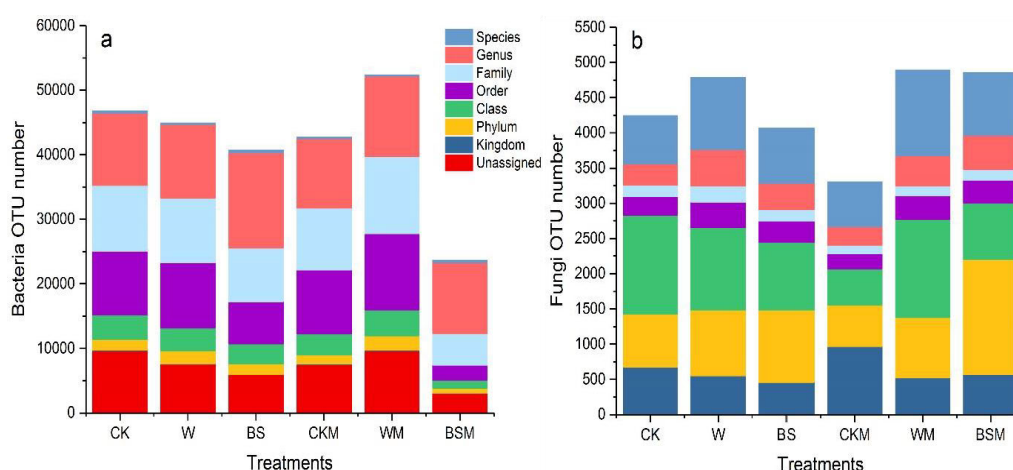


Figure 5.4 OTU cluster analysis and annotation results of soil bacteria and fungi in pretreated soil.

Table 5.1 Change in  $\alpha$  diversity indices of bacteria and fungi under different treatments

Type	Sample name	Shannon	Simpson	Chao1	Goods coverage
Bacteria	CK	11.14±0.61ab	0.99±0.00a	19238.07±5150.93ab	0.93±0.03ab
	W	11.44±0.34ab	1.00±0.00a	19913.37±5801.58ab	0.93±0.03ab
	BS	10.51±0.27b	0.99±0.00a	16453.05±2047.63ab	0.94±0.01a
	CKM	11.30±0.73ab	1.00±0.00a	31899.50±23013.83a	0.88±0.05bc
	WM	11.91±0.28a	1.00±0.00a	27582.11±7239.36a	0.87±0.03c
	BSM	8.11±1.43c	0.95±0.05b	10236.63±3830.54b	0.95±0.02a
Fungi	CK	6.59±0.14a	0.97±0.00a	1956.38±514.75ab	0.99±0.00a
	W	6.09±0.24a	0.95±0.02a	1630.31±387.41b	1.00±0.00a
	BS	6.17±0.85a	0.96±0.02a	1851.14±606.25ab	1.00±0.00a
	CKM	6.54±0.16a	0.97±0.00a	2494.80±756.80a	0.99±0.00a
	WM	6.23±0.43a	0.95±0.02a	1868.95±146.70ab	1.00±0.00a
	BSM	3.45±2.82b	0.55±0.39b	1281.76±118.88b	1.00±0.00a



### 5.4.2.3 Canonical correspondence analysis (CCA)

The CCA analysis shows that the content of  $\text{NH}_4^+\text{-N}$  in soil was the main contributory factor to the observed inhibition of the growth of bacteria without mulching (Figure 5.5a/b), while the soil pH was mainly responsible for the inhibition of the growth of bacteria and fungi in soil with mulching (Figure 5.5c/d). In addition, soil  $\text{NO}_3^-\text{-N}$  was positively correlated with the proliferation of bacteria and fungi, and soil OC was positively correlated with the proliferation of fungi, but negatively correlated with the reproduction of bacteria.

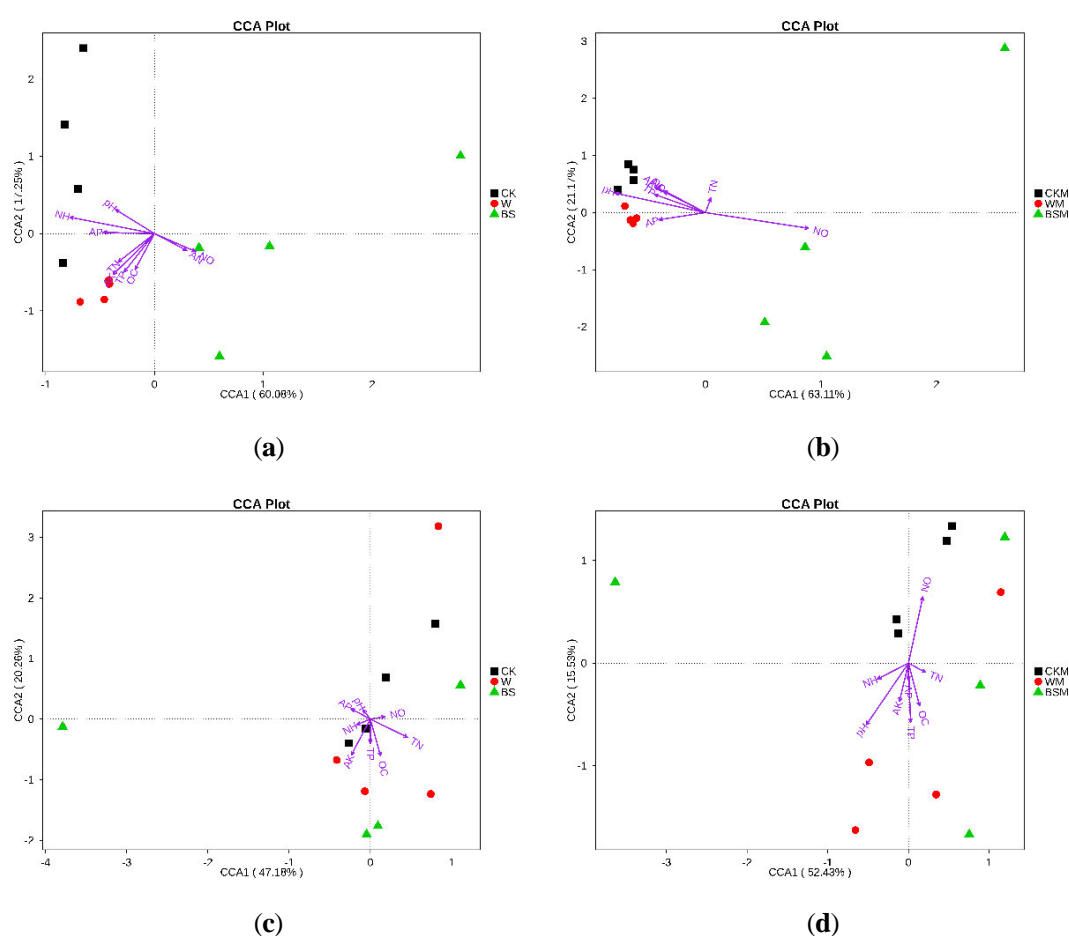


Figure 5.5 Canonical correspondence analysis (CCA) between soil bacteria (a, b), fungi (c, d) and soil properties

### 5.4.3 Effect of biogas slurry pretreatment on the growth of *Capsicum* spp.

Figure 5.6 shows the growth performance of *Capsicum* spp. on biogas slurry treated soil after 50 days. The survival rate of *Capsicum* spp. pretreated with biogas slurry LBS, HBS, LBSM and HBSM was significantly increased ( $P < 0.05$ ). Comparing the LBS,

HBS, LBSM and HBSM with CK treatment, the survival rate of *Capsicum* spp. was increased by 71.3%, 76.2%, 76.2% and 76.2% respectively. Similarly, comparing these treatments with CKM treatment, an increase of 46.7%, 50.9%, 50.9% and 50.9%, respectively was obtained. When these treatments were also compared with W treatment, the growth performance increased by 1.2%, 4.1%, 4.1% and 4.1% respectively (Fig. 5.6a). The rate of rigid seedlings in WM treatment was the highest, reaching 32.5%, followed by W treatment, which was 29.0%. Both treatments were significantly higher than that obtained from CK, CKM, LBS, HBS, LBSM, HBSM ( $P<0.05$ ). Moreover, comparing CK treatment to LBS, HBS, LBSM and HBSM treatment, the rate of rigid seedlings was observed to reduce by 63.2%, 52.6%, 42.1% and 84.2% respectively. Similarly, when W treatment was compared with LBS, HBS, LBSM and HBSM treatment the rate of rigid seedlings was significantly ( $P<0.05$ ) reduced by 90.4%, 87.7%, 84.9% and 95.9% respectively ( $P<0.05$ ) (Fig. 5.6b). Furthermore, the soil pretreated with biogas slurry significantly increased the plant height of *Capsicum* spp. plants and when this was compared with CK treatment, the plant height of *Capsicum* spp. plants treated with LBS, HBS, LBSM and HBSM increased by 113.1%, 83.6%, 96.7% and 50.8% respectively (Fig. 5.6c). Also, population of flowering *Capsicum* spp. and the percentage of fruit-bearing *Capsicum* spp. were substantially improved. Comparing CK treatment to other treatments (LBS, HBS, LBSM and HBSM), an increase (98.8%, 82.9%, 86.5% and 81.7%) in the population of flowering *Capsicum* spp. was observed. This was 35.82, 30.08, 31.38 and 29.65 times higher than CK treatments, and 1.64, 1.37, 1.43 and 1.35 times higher than W treatments, respectively (Fig. 5.6d). The highest (47.8%) population of fruit-bearing *Capsicum* spp. was observed with LBS treatment. This was 47.8%, 17.32, 2.31, 19.66 and 5.92 times of CK, W, CKM and WM, respectively (Fig. 5.6e).

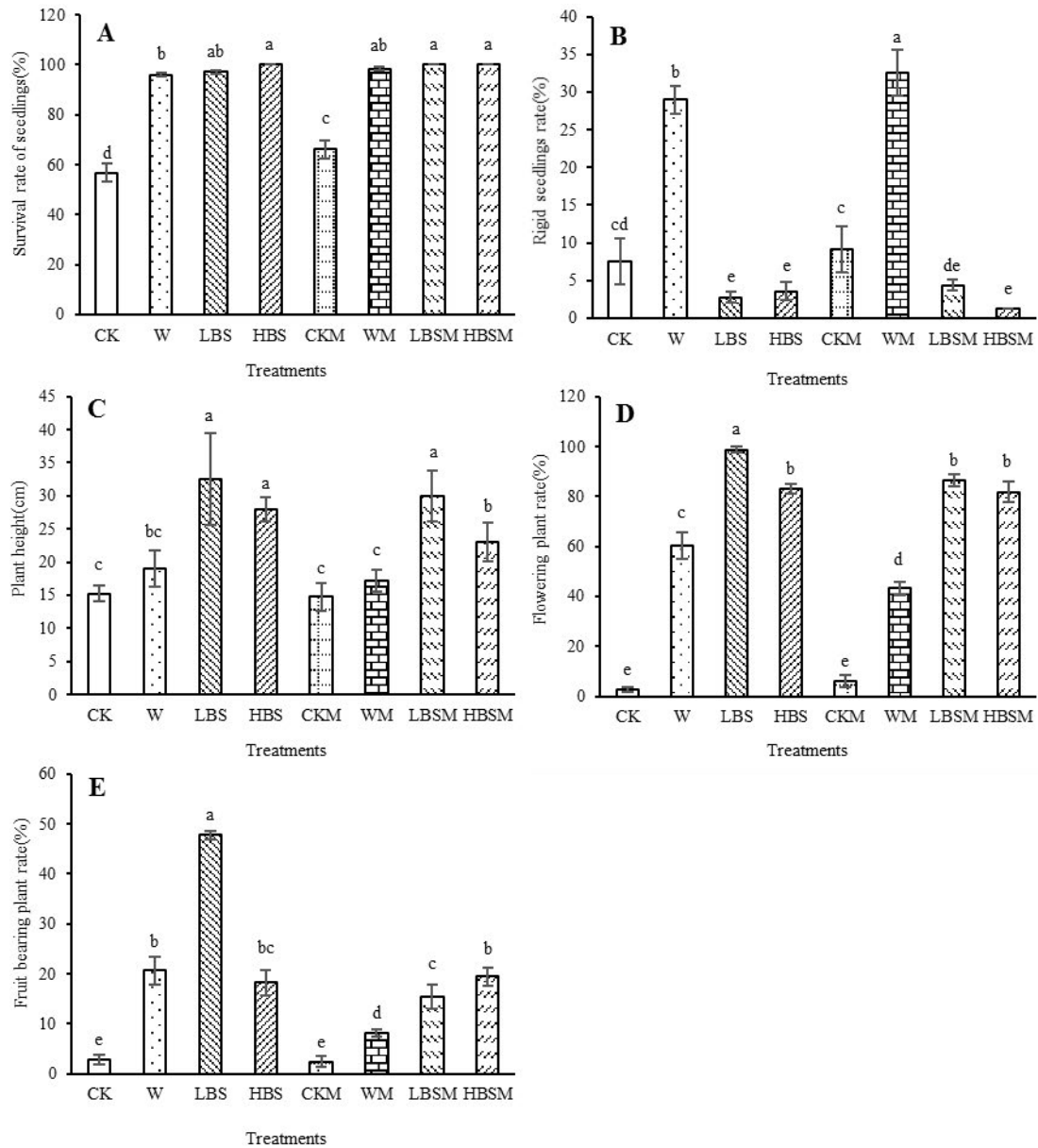


Figure 5.6 Growth of *Capsicum* spp. under different biogas slurry pretreatments.

## 5.5 Discussion

### 5.5.1 Effect of biogas slurry pretreatment on soil properties

This study shows that biogas slurry pretreatment of protected soil improved the physical and chemical properties of the soil (Niyungeko et al., 2020; Tang et al., 2021). Effective adjustment in the proportion of each nutrient content such as soil organic carbon in the soil (Yan et al., 2019), and the pH value (Xu et al., 2019). It has a direct positive effect on soil fertility (Yu et al., 2018). Studies have shown that under similar

nitrogen condition, the TN,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , AP, AK and pH of soil increased to varying degrees after soil was treated with pig manure biogas slurry to soil, while the OC content of the soil was not significantly impacted (Li et al., 2021). Similarly, Lai et al. (2018), reported controlling the concentration of swine manure biogas slurry applied in three years within the range of  $546.25\text{--}626.00\times 10^3\text{ kg/hm}^2$  significantly increase soil available potassium, phosphorus, and alkaline hydrolyzable nitrogen as well as reducing the risk of soil acidification. In another related study, the TN,  $\text{NH}_4\text{-N}$ , TP, AP, AK and OC content of paddy field treated with continuous application of biogas slurry for four years were significantly higher than those of no biogas slurry application (Dong et al., 2021). The authors also observed an increase in the soil organic matter content which was directly proportional to the amount of biogas slurry applied (Liu et al., 2021). The effects of biogas slurry pretreatment on soil TN, TP, AP, AK, and OC in this study were consistent with the results of previous studies. Soil  $\text{NH}_4^+\text{-N}$  content decreased in low-volume biogas slurry pretreatment (LBS treatment), which may be related to soil microbial activity, while the relative increase in soil TN was higher in the same treatment. The reduction of  $\text{NO}_3^-\text{-N}$  content in the treatments may be ascribed to the downward leaching of  $\text{NO}_3^-\text{-N}$  in surface soil caused by irrigation that keeps the soil saturated before pretreatment. The pH value of soil treated with LBS decreased slightly, indicating that low application of biogas slurry could not prevent soil acidification, while high application of biogas slurry fully neutralized soil acids (Xie et al., 2014), as well as inhibited the growth and proliferation of acidobacteria, thus, reducing soil acidification (Wang et al., 2021).

### **5.5.2 Effect of biogas slurry pretreatment on soil microorganisms**

Appropriate application of biogas slurry can promote the growth and enrichment of soil dominant bacteria, microbial alpha diversity (Lv et al., 2011; Abubaker et al., 2013; Cao et al., 2013; Xu et al., 2013; Wentzel and Joergensen, 2016), and help prevent and control soil-borne diseases of crops (Cao et al., 2016; Li et al., 2021). Studies showed that the application of biogas slurry could increase the culturable number of soil bacteria (Feng et al., 2014; Chai et al., 2019), fungi (Tang et al., 2021) and

actinomycetes (Yu et al., 2010) to a certain extent. Although nitrogen input is the key factor to increase soil microbial nitrogen energy (Möller, 2015), a large amount of ammonium nitrogen in biogas slurry may play a role in inhibiting microbial growth in the short term. Some studies found that the population of bacteria in the soil decreased (Cao et al., 2013), the main fungi in the soil decreased by 55.03% (Yang et al., 2017), and *Fusarium oxysporum* decreased significantly (Cao et al., 2015) after the application of biogas slurry. The relative abundance of actinomycetes increased with the increase of biogas slurry concentration, but the further increase of biogas slurry concentration would inhibit the growth of actinomycetes (Xu et al., 2019).

Furthermore, when the biogas slurry was applied at 180 m<sup>3</sup>/hm<sup>2</sup>, the richness and diversity of bacteria were increased, when the Chao1 index and Shannon index were high, which the diversity of fungi decreased with low Chao1 index and Shannon index (Wang et al., 2021). In this study, the biogas slurry pretreatment of protected soil showed reduction in culturable number of soil microorganisms, decrease in the richness and diversity of soil microorganisms, and altered the soil dominant microorganisms. These alterations in the microbial compositions through biogas slurry treatment is not only related to the complex factors of closed greenhouse, high temperature, mulching film, soil moisture and other supporting technologies, but also closely related to the change of soil properties.

### **5.5.3 Effect of biogas slurry pretreatment on the growth of crops**

Biogas slurry pretreatment measures can promote the growth and development of subsequent crops through fertilization and elimination of soil borne pathogenic microorganisms, with no risk of affecting crop growth. On the other hand, high NH<sub>4</sub><sup>+</sup>-N (Zhang et al., 2021), electrical conductivity (Tigini et al., 2016), and chemical oxygen demand (Wang et al., 2019; Gao et al., 2019) in biogas slurry can result in plant roots' adversity stress leading to plant growth retardation. In this study, the plant height and flowering rate of *Capsicum* spp. with low amount of biogas slurry (LBS) were better than those of high amount of biogas slurry (HBS and HBSM), which buttresses the aforementioned observations. The application of biogas slurry can significantly reduce

plant disease index (Wang et al., 2021). For instance, a study by Cao et al. (2016), showed watermelon fusarium wilt was significantly inhibited, and disease index was reduced by 36.4%. Moreover, in this study, the survival rates of *Capsicum* spp. with clean water (W, WM) and biogas slurry (LBS, HBS, LBSM, HBSM) were higher than that of the blank control (CK, CKM). This is an indication that soil moisture played an important role in improving the survival rate of *Capsicum* spp. plants. Also, the rigid seedling rate of clean water treatment (W, WM) is significantly higher than those of biogas slurry treatment (LBS, HBS, LBSM, HBSM). This substantiates the role of biogas slurry in promoting the growth of crops in biogas slurry pre-treatment soil. This might be related to the nutrient richness of biogas slurry and its potential in the reduction of soil-borne pathogens, but more in-depth research might be needed to substantiate and elucidate this.

## 5.6 Conclusions

This study has demonstrated the potential of biogas slurry pretreatment of protected soil to improve soil fertility, alleviating continuous cropping obstacles and promoting plant growth for high productivity. Soil pretreatment with biogas slurry dosage of 495 m<sup>3</sup>/hm<sup>2</sup> and 990 m<sup>3</sup>/hm<sup>2</sup> increased soil total nitrogen, total phosphorus, available phosphorus, available potassium, organic carbon and soil pH, while inhibiting the growth of soil bacteria, fungi, actinomycetes and fusarium. It also reduced the soil microbial flora as well as the evenness of species distribution. Moreover, the soil ammonium nitrogen, soil pH, and soil nitrate nitrogen were closely correlated to the growth and proliferation of soil bacteria and fungi. Interestingly, biogas slurry dosage of 495 m<sup>3</sup>/hm<sup>2</sup> improved the growth of *Capsicum* spp., which significantly improved the survival rate of *Capsicum* spp. seedlings, the plant height, the flowering rate and the fruit-bearing rate. In addition, the rate of rigid seedlings was substantially reduced. These findings demonstrate that biogas slurry which is usually disposed unfriendly to the environment can be an excellent sustainable source for improving soil fertility and crop productivity.

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## CHAPTER 6: GENERAL DISCUSSION AND CONCLUSION

### 6.1 Research in perspective

In the present study, the coefficient  $R^2$  for the three types of soil adsorption data was the highest for the Elovich equation, an indication that the adsorption was a heterogeneous diffusion process. Further probing into the restrictive factors of the process revealed that the goodness of fit of the parabolic diffusion equation ( $0.81 < R^2 < 0.97$ ) was higher than that of the first-order reaction equation ( $0.78 < R^2 < 0.92$ ) (**Table 3.7**). The high  $R^2$  for the parabolic diffusion equation suggests that the chemical adsorption process was not the rate-limiting step of the process, while the intra-particle diffusion was the main rate-limiting step. At present, the adsorption mechanism of ammonium nitrogen in soil media is mainly reported at the macro and micro levels. Most macroscopic research has focused on distinguishing the different active surfaces of soil media using the differences in the extraction capacity of different extractants (Wang and Alva, 2000). Microscopically, the occurrence of ammonium in the medium can be identified at the molecular level using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and other spectral means (Saruchi and Kumar, 2020). Generally, the adsorption mechanism cannot be accurately described using a single approach. Hence, this study used different kinetic approaches to lay a groundwork for research into the ammonium adsorption mechanism. The adsorption characteristics of the three soils (silty loam, loam, and sandy loam), provided a baseline data on the factors affecting the adsorption process (Shen et al., 1997). Since, soil is an important unit for nitrogen circulation and transformation (He et al., 2021). The adsorption of  $\text{NH}_4^+\text{-N}$  in the soil shows corresponding regularity with time change, which is one of the important characteristics of soil chemical reaction kinetics. Due to the differences in soil media and environmental conditions, the relationship between adsorption capacity and time often differed during the adsorption process. Previous studies have shown that different kinetics models or the same model have different fits with different soils (Xue et al., 1996). In the present study, the best fitting models for

the soil adsorption of  $\text{NH}_4^+\text{-N}$  were the first-order reaction equation and the Elovich equation. The adsorption of  $\text{NH}_4^+\text{-N}$  in silty sand, sandy silt, silt, and silty clay of four typical soils primarily occurred during 0–2 h, and the adsorption kinetics conformed to the second-order reaction equation (Tian, 2011).

Moreover, model equations were used to reflect the relationship between the soil adsorbent and the adsorption capacity, as well as the influence of different  $\text{NH}_4^+\text{-N}$  concentrations on the ability of the soil to adsorb  $\text{NH}_4^+\text{-N}$ . In the current study, the Langmuir equation had a high degree of fit, indicating that the isothermal adsorption of  $\text{NH}_4^+\text{-N}$  in the three soils was mainly monolayer adsorption, while multi-molecular layer adsorption was of secondary importance. The fitting parameter  $1/n$  of the Freundlich equation in this study was between 0 and 1. This shows that the isothermal adsorption of  $\text{NH}_4^+\text{-N}$  by the three kinds of soils was relatively efficient. At the same time, in order to further improve the application and evaluation of the fitting results, for the maximum saturated adsorption capacity, the analysis screened out the Platt equation that has fitting  $Q_m$  value that was close to the measured average. However the adaptability of the model assumptions to the soil adsorption characteristics of  $\text{NH}_4^+\text{-N}$  was worthy of further validation. The validation results were basically consistent with the adsorption characteristics reflected by the empirical adsorption isotherm. Therefore, it was effective to use empirical adsorption isotherms to quantitatively describe the thermodynamic behavior of the soil adsorption of  $\text{NH}_4^+\text{-N}$ .

Furthermore, the  $\text{NH}_4^+\text{-N}$   $Q_m$  of soil is affected by soil texture, environmental temperature and humidity, artificial fertilization, and crop rotation. The  $Q_m$  obtained by the Langmuir equation reflected the maximum saturated adsorption capacity of  $\text{NH}_4^+\text{-N}$  in the soil at the prevailing experimental conditions. The observations in this study showed that the  $Q_m$  value was significantly negatively correlated with the soil pH. A possible reason was that the biogas slurry was alkaline, and the pH values of weakly acidic soils in Xinbei and Jintan were neutralized, resulting in the release of adsorption sites originally occupied by  $\text{H}^+$  and thereby reducing the competition between  $\text{H}^+$  and  $\text{NH}_4^+$  for adsorption sites. Moreover, there was a significant positive correlation



between the  $Q_m$  value and cation exchange capacity. Also, the  $Q_m$  was positively correlated with soil organic matter. This might be ascribed to the large number of different functional groups present in the organic matter and high cation exchange capacity. These factors could increase the adsorption capacity of  $\text{NH}_4^+\text{-N}$  through surface complexation, ion exchange, and surface precipitation (Acosta et al., 2016). These results on soil adsorption of  $\text{NH}_4^+$  are consistent with many studies in literature (Yao et al., 2012). For instance, Li et al. (2009) found that  $Q_m$  was significantly negatively correlated with soil pH and cation exchange capacity, while it was significantly positively correlated with soil carbon-nitrogen (C:N) ratio. Similarly, Cong et al. (2017) showed that  $Q_m$  was significantly positively correlated with soil pH and cation exchange capacity, while it was significantly negatively correlated with soil organic matter and total nitrogen content. Likewise, Wang et al. (2015) reported that the greater the organic matter content, the greater the  $\text{NH}_4^+\text{-N}$  adsorption capacity of albic soil. In other related study, Xue et al. (1996) found that the adsorption capacity of  $\text{NH}_4^+\text{-N}$  increased with the increase of soil cation exchange capacity and soil clay content to varying degrees.

The  $Q_m$  value of silty loam > loam > sandy loam was consistent with the other reports. For instance, Xue et al. (1996) reported that  $\text{NH}_4^+\text{-N}$  adsorption capacity was mainly affected by clay content, with clay > loam > sandy soil. Similarly, Cong et al. (2017) showed that the  $\text{NH}_4^+\text{-N}$  adsorption capacity of soil was in the order of light clay > light loam. Moreover, there was a significant positive correlation between the  $Q_m$  value, the content of the soil and the particle size of the soil. Likewise, Wan et al. (2004) found that there was a very significant positive correlation between fixed ammonium and clay content < 0.01 mm, with clay content of 0.005–0.01 mm, and with clay content of 0.001–0.005 mm, but there was no significant correlation with clay content < 0.001 mm. In addition, there was a very significant positive correlation between the  $Q_m$  value and the soil TN content, as well as a significant correlation with soil nitrate nitrogen. This may be the reason for the positive and negative electrical adsorption and may be responsible for maintaining the balance between soil ammonium

nitrogen and nitrate nitrogen. The  $Q_m$  value was also positively correlated with the soil available potassium and available phosphorus. This was because active  $K^+$  ions could provide adsorption sites and interlayer solid localization for  $NH_4^+$  ions in external soil, which was in line with cation exchange theory (Kittrick, 1966). There were two possible reasons: the positive and negative charge adsorption, and the combination of  $NH_4^+$  ions and  $PO_4^{3-}$  ions to form  $[(NH_4) PO_4]^{2-}$ , subsequently forming complex precipitates  $Mg(NH_4)[PO_4] \cdot 6H_2O$  with the  $Mg^{2+}$  and  $Ca^{2+}$  in the soil, thereby increasing the adsorption of  $NH_4^+$ -N. The correlation between the Langmuir equation adsorption constant  $K_1$  value, the MBC value, and the correlation between the  $Q_m$  value and soil property-related indicators were opposite (Cong et al., 2017). This indicates that the adsorption strength and the adsorption capacity were complementary. In other words, when the adsorption capacity is high, the adsorption strength is low. In addition, the fitted  $Q_m$  values of the  $NH_4^+$ -N adsorption of the soils in this study were lower than the fitted value of a single chemical ammonium salt solution, thus signifying the rich complex components of the biogas slurry interfering with the adsorption of ammonium nitrogen in the soil (Zhao et al., 2013). The soil adsorption of biogas slurry  $NH_4^+$ -N predominantly occurred within 0–1 h, and the adsorption capacity within 0–1 h accounted for 35.24–43.55% of the total adsorption. The ExpAssoc equation produced a good fit for the adsorption kinetic behavior. The optimal theoretical saturated adsorption capacity ( $Q_m$ ) values fitted by the Langevin model were 1108.55 mg/kg, 874.86 mg/kg, and 448.35 mg/kg for silty loam, loam, and sandy loam, respectively. The  $Q_m$  was significantly positively correlated with soil organic matter, total nitrogen, available phosphorus, available potassium, cation exchange capacity, and particle content of 0.02–0.002 mm, but significantly negatively correlated with soil pH.

The safe bearing capacity of farmland soil and the frequency of digestion (Jiang et al., 2011b) are important parameters for determining the area configuration of biogas slurry in land-consumption farms in the combined planting and breeding system. Previous report showed that the disposal of biogas slurry in paddy fields revealed that  $NH_4^+$ -N concentration in field water was affected by the digested amount of biogas

slurry. The report also showed that  $\text{NH}_4^+\text{-N}$  concentration increased with the increase in biogas slurry and decreased significantly with time. The  $\text{NH}_4^+\text{-N}$  concentration decreased by 47.52%–85.27% after 3 days of biogas slurry application (Wang et al., 2016), and the concentration of  $\text{NH}_4^+\text{-N}$  in field water is stably lower than the discharge concentration of 80 mg/L specified in the discharge standard of pollutants for the livestock and poultry breeding industry (GB18596-2001). It can be stably lower than 40 mg/L after 5 days of application (Jiang et al., 2011a). The results of this study were slightly different from the above reports in the digestion time of  $\text{NH}_4^+\text{-N}$  in biogas slurry. After 3 days of application, only the  $\text{NH}_4^+\text{-N}$  concentration in the overlying water of BSN1 treatment decreased to less than 80 mg/L. After 7 days of application, BSN1 and BSN1.5 treatments stabilized below 80 mg/L, of which BSN1 treatment is lower than 40 mg/L. The reason might be that the static soil column used in this study is a soil-microbial composite system, which lacks the participation of farmland plants, so the digestion speed and aging are slightly delayed.

In addition, by monitoring the change of  $\text{NH}_4^+\text{-N}$  concentration in field water, we can predict the water environment pollution risk and water quality standard discharge time node of farmland disposal biogas slurry (Wang et al., 2016). However, it is impossible to distinguish whether the main reason for the decrease in  $\text{NH}_4^+\text{-N}$  concentration is farmland digestion or farmland irrigation water dilution, which has been questioned in production practice. This study further quantifies the reduction rate of  $\text{NH}_4^+\text{-N}$  in the biogas slurry. From the perspective of reduction of  $\text{NH}_4^+\text{-N}$  input, it was demonstrated again that the first three (3) days after biogas slurry application was the rapid reduction period during which the prohibition of runoff plays an important role in preventing environmental pollution of surrounding water bodies. After 15 days of application, under the condition of an equal amount of  $\text{NH}_4^+\text{-N}$ , the total amount of  $\text{NH}_4^+\text{-N}$  in the overlying water of biogas slurry treatment decreased by 99.2%. This was significantly lower than that of the fertilizer  $\text{NH}_4^+\text{-N}$  treatment, which underscore the use of disposal biogas slurry  $\text{NH}_4^+\text{-N}$ . Thus, 15 days could be used as the time node for the end of the first digestion cycle.

Furthermore, under the same application condition with the same amount of  $\text{NH}_4^+$ -N, the  $\text{NH}_4^+$ -N concentration in the overlying water of the biogas slurry treatment was lower than that of the chemical fertilizer  $\text{NH}_4^+$ -N treatment on the third day. But it rebounded from 4 to 7 days, mainly due to the  $\text{NH}_4^+$ -N accounts for 88.6% of the total nitrogen in the biogas slurry. And other nitrogen-containing organic substances in the biogas slurry components are oxidized and decomposed by microorganisms, which increases the  $\text{NH}_4^+$ -N content of the overlying water. The concentration of  $\text{NH}_4^+$ -N in overlying water treated with biogas slurry for 0 days (the sampling time in this study is within 8 h after application) is significantly higher than that of fertilizer  $\text{NH}_4^+$ -N treatment, but the concentration of  $\text{NH}_4^+$ -N in pore water and the concentration of  $\text{NH}_4^+$ -N adsorbed by soil are lower than that of fertilizer  $\text{NH}_4^+$ -N treatment. This is because there is a competitive and mutually exclusive relationship between other cations (Song et al., 2021) and  $\text{NH}_4^+$ -N ions in biogas slurry, thus delaying the molecular diffusion rate of  $\text{NH}_4^+$ -N in pore water, it also reduces the dominant sorption of  $\text{NH}_4^+$ -N on soil particles (Zhao et al., 2013). The abnormal value of BSN1 treatment on the fourth day of application might be due to the operational error of the destructive test, and the value at this point can be regarded as the missing value. After 7 days of application, the  $\text{NO}_3^-$ -N concentration in overlying water, pore water, and soil with the biogas slurry  $\text{NH}_4^+$ -N treatment was significantly higher than that of the fertilizer  $\text{NH}_4^+$ -N treatment, which might be that the organic active substances in the biogas slurry components promoted the reproduction of nitrifying microorganisms (Chen et al., 2020), thus promoting the conversion of  $\text{NH}_4^+$ -N to  $\text{NO}_3^-$ -N. The concentration of  $\text{NH}_4^+$ -N in the pore water of 0–10 cm soil layer reached the peak value after 4 days of biogas slurry application, and the  $\text{NH}_4^+$ -N sorption by soil particles reached the peak after 7 days, indicating that the  $\text{NH}_4^+$ -N in overlying water was gradually migrating to the soil layer over time. The proportion of biogas slurry nitrogen absorbed by soil and contained in pore water in the 0–10 cm soil layer accounted for 76.3% of the total  $\text{NH}_4^+$ -N after 15 days application, signifying that the disposal of biogas slurry  $\text{NH}_4^+$ -N in farmland was mainly soil sorption and transformation. However, with the increase in

biogas slurry application, the proportion of  $\text{NH}_4^+\text{-N}$  adsorbed by soil in the 0–10 cm soil layer decreases, which is due to the limit value of soil sorption capacity of 1108.55 mg/kg (Wang et al., 2022). When the sorption limit value is exceeded,  $\text{NH}_4^+\text{-N}$  will migrate to the 10–20 cm soil layer. Only when the amount of  $\text{NH}_4^+\text{-N}$  applied exceeds the sorption limit value of 0–20 cm cultivated soil layer, there will be the risk of polluting groundwater. Ammonia volatilization loss was once considered as one of the main ways to reduce  $\text{NH}_4^+\text{-N}$  in field water (Huijsmans et al., 2001), but the ammonia volatilization process is very complex and affected by many factors, so it is very difficult to accurately estimate its loss under natural conditions. Some studies have shown that soil wetting or flooding conditions can reduce ammonia volatilization (Zhou et al., 2009). The test of biogas slurry showed that the removal rate of ammonium nitrogen was only 53% under natural conditions for 100 days (Shi, 2010). In this study, the other fate of biogas slurry  $\text{NH}_4^+\text{-N}$  in the BSN1, BSN1.5 and BSN2.5 treatments accounts for 22.8%, 31.8%, and 34.1%, respectively, including the migration of  $\text{NH}_4^+\text{-N}$  to deeper soil layer, transformation and ammonia volatilization loss of overlying water (Tan et al., 2015). Some scholars believe that the key period of ammonia volatilization is within 7 days after biogas slurry application. The ammonia volatilization loss rates of 1 N biogas slurry, 2 N biogas slurry, 4 N biogas slurry, and 1 N chemical fertilizer treatment are 18.8%, 14.3%, 9.9%, and 6.6%, respectively (Shi, 2010). In this study, the other directions of biogas slurry  $\text{NH}_4^+\text{-N}$  were not subdivided, therefore, the proportion of ammonia volatilization loss in other directions could not be determined. Therefore, it cannot be proved that ammonia volatilization loss is one of the main ways to reduce biogas slurry  $\text{NH}_4^+\text{-N}$  in overlying water. However, at the beginning of biogas slurry application, the concentration of  $\text{NH}_4^+\text{-N}$  in overlying water maintained a high level, decreased rapidly within 7 days, and gradually transformed into  $\text{NO}_3^-\text{-N}$  after 7 days.

The use of saturated water content farmland soil for disposal of biogas slurry ammonium nitrogen is mainly based on soil sorption and pore water migration. With the extension of time, the ammonium nitrogen concentration in the overlying water

gradually decreases, and the reduction rate of the total ammonium nitrogen gradually increases. Nevertheless, the reduction speed and reduction rate showed a downward trend with the increase in the application amount of the ammonium nitrogen in the biogas slurry. The application of 0–3 d is the rapid consumption period for preventing and controlling the pollution of surrounding water bodies, and the application of 15 d is the completion period of one-time safety consumption.

The results of the biogas slurry pretreatment of protected soil improved the physical and chemical properties of the soil (Tang et al., 2021). Effective positive adjustment in the proportion of each nutrient content such as soil organic carbon in the soil (Yan et al., 2019) and the pH value (Xu et al., 2019) were observed, with a direct positive effect on soil fertility (Yu et al., 2018). Studies have shown that under similar nitrogen condition, the total nitrogen,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , available phosphorus, available potassium and pH of soil increased to varying degrees after soil was treated with pig manure biogas slurry to soil, while the organic carbon content of the soil was not significantly impacted (Li et al., 2021). Soil  $\text{NH}_4^+\text{-N}$  content decreased in low- volume biogas slurry pretreatment (LBS treatment), which may be related to soil microbial activity, while the relative increase in soil total nitrogen was higher in the same treatment. The reduction of  $\text{NO}_3^-\text{-N}$  content in the treatments may be ascribed to the downward leaching of  $\text{NO}_3^-\text{-N}$  in surface soil caused by irrigation that keeps the soil saturated before the biogas slurry pretreatment of the soil. The pH of the soil treated with LBS decreased slightly, suggesting that low application of biogas slurry could not prevent soil acidification, while high application of biogas slurry fully neutralized soil acids (Xie et al., 2014), as well as inhibited the growth and proliferation of acidobacteria, thus, reducing soil acidification (Wang et al., 2021).

The biogas slurry pretreatment of protected soil showed significant reduction in culturable number of soil microorganisms, as well as a decrease in the richness and diversity of soil microorganisms. These could alter the soil dominant microorganisms. Alterations in the soil microbial compositions through biogas slurry treatment is not only related to the complex factors of closed greenhouse, high temperature, mulching

film, soil moisture and other supporting factors, but also closely related to the changes in soil properties. The CCA analysis shows that the content of  $\text{NH}_4^+$ -N in soil was the main contributory factor to the inhibition of the growth of bacteria without mulching (**Figure 5.4a/b**), while the soil pH was mainly responsible for the inhibition of the growth of bacteria and fungi in soil with mulching (**Figure 5.4c/d**). In addition, soil  $\text{NO}_3^-$ -N was positively correlated with the proliferation of bacteria and fungi, while soil organic carbon was positively correlated with the proliferation of fungi, but negatively correlated with the reproduction of bacteria. Appropriate application of biogas slurry can promote the growth and enrichment of soil dominant bacteria (microbial alpha diversity) (Wentzel and Joergensen, 2016), and thus, help prevent and control soil-borne diseases of crops (Li et al., 2021). Although nitrogen input is the key factor to increase soil microbial nitrogen energy (Möller, 2015), a large amount of ammonium nitrogen in biogas slurry could play a role in inhibiting microbial growth in the short-term. Some studies reported that the population of bacteria, fungi and *Fusarium oxysporum* in the soil decreased significantly after the application of biogas slurry (Cao et al., 2013; Cao et al., 2015; Yang et al., 2017). The increase in biogas slurry concentration till optimal concentration resulted in the relative increase of actinomycetes, while further increment in the concentration of biogas slurry inhibits the growth of actinomycetes (Xu et al., 2019). Furthermore, when the biogas slurry was applied at  $180 \text{ m}^3/\text{hm}^2$ , with high Chao1 and Shannon index the richness and diversity of bacteria increased, while the diversity of fungi decreased with low Chao1 index and Shannon index (Wang et al., 2021).

Biogas slurry pretreatment of soil can promote the growth and development of subsequent crops through fertilization and elimination of soil-borne pathogenic microorganisms, with no risk of affecting crop growth. On the other hand, high  $\text{NH}_4^+$ -N (Zhang et al., 2021), electrical conductivity (Tigini et al., 2016), and chemical oxygen demand (Wang et al., 2019) in biogas slurry can result in plant roots' adversity stress leading to plant growth retardation. In this study, the plant height and flowering rate of *Capsicum* spp. with low amount of biogas slurry (LBS) were better than those with high

amount of biogas slurry (HBS and HBSM), which buttresses the aforementioned observations. Appropriate application of biogas slurry to the soil can significantly reduce plant disease index (Wang et al., 2021). For instance, a study by Cao et al. (2016), showed watermelon fusarium wilt was significantly inhibited, and disease index was reduced by 36.4%. Moreover, in this study, the survival rates of *Capsicum* spp. with clean water (W, WM) and biogas slurry (LBS, HBS, LBSM, HBSM) were higher than that of the blank control (CK, CKM). This is an indication that soil moisture played an important role in improving the survival rate of *Capsicum* spp. plants. Also, the rigid seedling rate of clean water treatment (W, WM) is significantly higher than those of biogas slurry treatment (LBS, HBS, LBSM, HBSM). This substantiates the role of biogas slurry in promoting the growth of crops in biogas slurry pretreatment soil. This might be related to the nutrient richness of biogas slurry and its potential in the reduction of soil-borne pathogens, but more in-depth research might be needed to substantiate and elucidate this. Soil pretreatment with biogas slurry dosage of 495 m<sup>3</sup>/hm<sup>2</sup> and 990 m<sup>3</sup>/hm<sup>2</sup> increased soil total nitrogen, total phosphorus, available phosphorus, available potassium, organic carbon and soil pH. But both of them inhibited the growth of soil bacteria, fungi, actinomycetes and fusarium, and reduced the soil microbial flora as well as the evenness of species distribution. Moreover, with the soil ammonium nitrogen, soil pH, and soil nitrate nitrogen were closely related to the growth and proliferation of soil bacteria and fungi. Interestingly, biogas slurry dosage of 495 m<sup>3</sup>/hm<sup>2</sup> improved the growth of *Capsicum* spp., which significantly improve the survival rate of *Capsicum* spp. seedlings, the plant height, the flowering rate, the fruit-bearing rate and the reduced the rigid seedling rate substantially.

## 6.2 Concluding Remarks

The need for safe digestion or treatment of biogas slurry has received serious attention and requires an urgent solution. This will help to reduce unfriendly environmental disposal of livestock and poultry waste pollution in China. The use of biogas slurry as fertilizer meets the requirements for sustainable environmental development. Present field application of biogas slurry discharged from intensive



large-scale livestock and poultry farms as well as biogas projects have exceeded the bearing capacity of adjacent farmland. Also, the maximum farmland fertilizer requirement and agricultural seasonality have been greatly affected. It is therefore necessary to further explore alternative treatment options for this biogas slurry as well as improve biogas slurry waste efficient utilization. Also, there is a significant knowledge gap on the adsorption mechanism and kinetics of biogas slurry ammonium nitrogen in the soil. Hence, this study uses waste biogas slurry pretreatment continuous cropping obstacle soil in facilities protected land to diversify biogas slurry application. And uses semi-pilot field trials to understand the synergistic combination measures. It needed to achieve the multiple goals of efficient biogas slurry digestion per unit area of farmland, improvement of soil fertility and productivity as well as the prevention and control of soil-borne diseases in facility protected land.

Three typical farmland ploughing layer soils (silty loam, loam and sandy loam) were selected for the adsorption kinetics and thermodynamic studies. The adsorption characteristics of the three types of soils on biogas slurry ammonium nitrogen were analyzed, and the optimal model was selected to fit the maximum adsorption value to explore the relationship between soil physicochemical properties and adsorption capacity. The theoretical saturated adsorption capacity of biogas slurry ammonium nitrogen were 1038.41–1372.44 mg/kg in silty loam, 840.85–1157.60 mg/kg in loam, and 412.33–481.85 mg/kg in sandy loam. The optimal values were 1108.55 mg/kg, 874.86 mg/kg, and 448.35 mg/kg for silty loam, loam, and sandy loam, respectively. The value was significantly positively correlated with soil organic matter, total nitrogen, available phosphorus, available potassium, cation exchange capacity, and particle content of 0.02–0.002 mm, but significantly negatively correlated with soil pH. The maximum adsorption capacity of three types of soil (silty loam = 1108.55 mg/kg, loam = 874.86 mg/kg, sandy loam = 448.35 mg/kg) to ammonium nitrogen in biogas slurry were determined, providing the safe dosage/concentration for biogas slurry pretreatment of protected soil.

The disposal of biogas slurry ammonium nitrogen in farmland soil with saturated

water content is mainly based on soil sorption and pore water migration. A duration of 0–3 days was established as rapid disposal period with the overlying water ammonium nitrogen concentration decrease of 63.5–80.7%, while the reduction rate of total ammonium nitrogen was 65.8–82.3%. Also, 15 days was established as the completion period for safe digestion of the biogas slurry, resulting in 97.0–98.7% decrease in the overlying water ammonium nitrogen concentration and a , the reduction rate of 97.9–99.2%, while the proportion of ammonium nitrogen absorbed in the 0–10 cm soil layer accounted for 63.5–76.3%.

Moreover, through the static soil column simulation experiment, the speed and efficiency of biogas slurry ammonium nitrogen disposed by the saturated water content soil were studied. Also, evaluated was the migration, transformation characteristics and the fate ratio of the biogas slurry ammonium nitrogen alongside the time point of the discharge when the field surface water quality reached the standard. The safe cycle (15 days) of absorbing biogas slurry in saturated water content soil and the final fate of ammonium nitrogen in biogas slurry were established, to provide theoretical support for the safe consumption of biogas slurry pretreatment protected soil.

Semi-pilot split-plot studies on the application of waste biogas slurry were carried out to understand the impacts of biogas slurry on soil properties, microorganisms and crop growth on the field (facility protected soil). The use of biogas slurry to pretreat facility protected soil noticeably increased the soil total nitrogen, total phosphorus, available phosphorus, available potassium, organic carbon content and soil pH. During biogas slurry pretreatment of the soil, soil ammonium nitrogen, soil pH, and soil nitrate nitrogen were closely related to the growth and development of soil bacteria and fungi. The effect of biogas slurry pretreatment on the growth of crops was elucidated, and the response effect on soil microorganisms and soil properties to biogas slurry pretreatment were determined. The relationship of soil properties, microorganisms and *Capsicum* spp. growth to biogas slurry pretreated soil were dose-based responses. The biogas slurry soil pretreatment with a dosage of 495 m<sup>3</sup>/hm<sup>2</sup> was most beneficial to the growth of the *Capsicum* spp., which significantly improved the survival rate of *Capsicum* spp.

seedlings, the flowering plants rate, the fruit-bearing plants rate and the plant height, and substantially reduce the rate of rigid seedlings. The soil with biogas slurry pretreatment of 495 m<sup>3</sup>/hm<sup>2</sup> favoured the health and growth of *Capsicum* spp. by preventing and controlling soil-borne microbial diseases.

Moreover, the use of biogas slurry to pretreat protected soil increases the soil total nitrogen, total phosphorus, available phosphorus, available potassium, organic carbon content and soil pH value, while the population, diversity and distribution of soil microbes were significantly influenced. Interestingly, soil ammonium nitrogen, soil pH, and soil nitrate nitrogen were highly correlated to the population of bacteria and fungi present in the pretreated soil.

### **6.3 Recommendations**

The safe application of biogas slurry to pretreat continuous cropping obstacle soil in protected land still is critical. Findings from this study has provided baseline data on the adsorption kinetics and thermodynamic of biogas slurry ammonium in the soil, significant impacts of biogas slurry application on soil physiochemical properties, soil microorganisms, and *Capsicum* spp. health and growth. A semi-plot field application of waste biogas slurry was also demonstrated. In the future, based on the environmental safety requirements, there are many biogas slurry contents and their implications in the environments that need to be further studied. Such studies should include:

- i characterization of adsorption, migration, and transformation of different components of biogas slurry in a specific soil type as well as in different soil types. Similarly, the complex and varied components of biogas slurry, soil texture and plough layer properties, crop types and continuous cropping conditions need to be considered;
- ii analysis of the correlation coefficient between soil properties and the adsorption process of biogas slurry components; and,
- iii determination of the interaction between the components of biogas slurry during adsorption, migration and transformation.
- iv assessment of the inhibitory effect of biogas slurry on specific soil pathogens.

Using biogas slurry to pretreat continuous cropping obstacle soil in protected land

involves the intersection of resources, environment, and soil microorganisms. An in-depth study of biogas slurry pretreatment of continuous cropping obstacle soil in protected land is also important to provide the interlink among different disciplines such as agronomy, microbiology, soil science and chemistry.

In addition, it is particularly necessary to develop corresponding matching combination technologies according to local conditions. The effect of high temperature, high humidity, greenhouse sealing, film mulching, and other factors on the biogas slurry pretreatment also need to be studied.

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