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Physicochemical and Biological Characteristics of Shrimp Pond Sludge in the Thua Thien Hue Province, Vietnam

Nguyen Quang Lich[1,*], Le Dai Vuong[1], Te Minh Son[2], Ve Quoc Linh[3]

[1]School of Engineering and Technology - Hue University, 01 Dien Bien Phu, Hue 530000, Vietnam
[2]University of Sciences, Hue University,77 Nguyen Hue, Hue 530000, Vietnam
[3]University of Agriculture and Forestry, Hue University, 102 Phung Hung, Hue 530000, Vietnam

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KEYWORDS

Microorganisms

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ABSTRACT

This study has been carried out to analyze the physical and biological indicators of shrimp pond sludge samples obtained from the Phu Vang and Phu Loc districts of the Thua Thien Hue Province, Vietnam. All standard methodologies have been used to analyze the selected parameters like pH, organic carbon, total nitrogen, total phosphate, and microbial density. The results of the study revealed that the sludge was characterized by a neutral to alkaline pH (6.9 - 7.5), and the total organic carbon content was in the range of 103.8–173.5 mg/kg. The sludge was rich in organic matter (17.8–29.9%), total nitrogen (13.5–32.5 g/kg), and total phosphate (7.9–20.1 g/kg). Further, in the case of the microbial density of pathogenic microorganisms, the density of total bacteria, *coliform*, *E.coli*, *Salmonella* spp., *Vibrio* spp., and *Clostridium* spp. was also estimated at two opposing weather conditions (spring, February to March; summer, June to July). The microbial community increased rapidly during the cool spring months. The total bacterial levels were recorded as 8.77 log10 CFU/mL in the Phu Loc district and 9.11 log10 CFU/mL in the Phu Vang district. The levels decreased during the hot summer months, and the level of total bacteria, *Coliform, E.coli, Salmonella* spp., and *Vibrio* was reported 2.57, 1.49, 1.06, 0.56, and l2.54 log10 CFU/mL respectively from the Phu Loc district of Vietnam. The results obtained using the anaerobic decomposition model showed that on the 60th day, the amount of CH_4 generated at the high output value for the Phu Vang district was 22385 ppm. The results reported here revealed that CH_4 gas can be potentially produced from shrimp waste sludge in this province.

* Corresponding author

E-mail: nguyenquanglich@hueuni.edu.vn (Nguyen Quang Lich)

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1 Introduction

Whiteleg shrimp (*Litopenaeus vannamei*) farming is a profitable industry for Vietnamese farmers (Van Nguyen et al. 2021). Currently, Whiteleg shrimp are widely grown in Vietnam. Shrimp farming is concentrated mainly in the Phong Dien, Phu Vang, Quang Dien, and Phu Loc districts within the Thua Thien Hue province. In general, the farm areas are in the range of 5–30 hectares. In some areas, the areas of the shrimp farms are in the range of 50–100 hectares. However, the development of aquaculture also leads to many difficulties. Unregulated construction of farms, use of non-standard disease-preventing chemicals, and direct discharge of water and sludge from ponds into waterways are some important issues that are directly associated with aquaculture development and caused server environmental pollution (Lich et al. 2022). It is highly profitable to farm Whiteleg shrimps as these shrimps have high protein value and are in demand for human consumption. Many reports have indicated that Whiteleg shrimp is sensitive to disease factors as well as environmental changes, and these factors lead to financial risks for farmers. The severity of diseases in shrimp has increased with the development of shrimp farming.

Recently Janecko et al. (2021) and Kumar et al. (2021) reported an association of the bacterial pathogen *Vibrio* spp. with Asian and Mexican shrimp aquaculture which causes an acute hepatopancreatic necrosis disease (AHPND). This pathogen causes an annual loss amounting to USD 1 billion and shrimp mortality exceeds 70% (Han et al. 2017). The occurrence of this disease is associated with various factors, including pond-water pollution. According to the assessment of the Ministry of Agriculture and Rural Development of Vietnam, wastewater and waste from intensive farming facilities contain excess feed, feces, other excreta, drug residues, chemicals, and garbage and it is increasing with each passing year.

Further, the sewage sludge formed during the aquaculture process contains decomposing residual food sources, chemicals, antibiotics, minerals, diatomite, dolomite, sulfur deposits, and toxic Fe^{2+}, Fe^{3+}, Al^{3+}, SO_4^{2-} species, and this mud layer is 0.1–0.3 m-thick. Toxic decomposition products, such as H_2S, NH_3, CH_4, and mercaptan, are formed during anaerobic digestion, and these are discharged into the surrounding environment, affecting the quality of farmed aquatic products. The sludge obtained under conditions of industrial shrimp farming contains Si (27,842 mg/kg), Ca (13,256 mg/kg), K (5,642 mg/kg), Fe (11,210 mg/kg), H_2S (8.3 mg/kg), NH_3 (36.1 mg/kg), NO_3 (0.3 mg/kg), NO_2 (0.1 mg/kg), and PO_4 (1.8 mg/kg), and taken together, they amount to 29.5% of the total mass. The total organic carbon (TOC) content for the bottom sludge of the Catfish pond (pH range: 4.37–5.39) is in the range of 1.56–1.89%, and it contains approximately 24% nitrogen and 24% phosphorus. Among these, nitrogen and phosphorus cause serious environmental pollution that needs to be treated effectively (Anh et al. 2010). However, most shrimp farming facilities are not equipped with an effective sludge treatment system.

The risk of infection attributable to pathogenic microorganisms is also a concern in the ponds if the ponds are not handled carefully after each cycle of shrimp culture. Alfiansah et al. (2018) studied the microbiome density in an industrial shrimp pond system through 16s rRNA sequencing and reported that the dominating microbial groups in shrimp ponds sediment are *Gammaproteobacteria*, *Alphaproteobacteria*, *Flavobacteria*, *Bacillus* spp., and *Actinobacteria*, belonging to the Halomonas and Psychrobacter groups. In this context, microorganisms can be divided into two main groups' i.e. organic compound decomposers and pathogenic microorganisms.

To date, a comprehensive study on microbial contamination in shrimp ponds in the Thua Thien Hue province has not been conducted. Therefore, the current study has been carried out to analyze the physical and biological indicators of shrimp pond sludge samples obtained from the Phu Vang and Phu Loc districts of the Thua Thien Hue Province, Vietnam. These samples were collected at two different times in a year (spring and summer), and an analysis of changes in the population of pathogenic microorganisms and the physical and biological characteristics of the sludge samples were carried out. The results provide an assessment of the contamination level of disease-causing microbial populations, which helps in mapping out strategies to prevent and treat pollution.

2 Materials and methods

2.1 Study site and sample collection

A total of 12 bottom sludge samples were collected from Whiteleg shrimp ponds located at Phu Loc (6 samples) and Phu Vang (6 samples) in different months (February and March, representing spring, and June and July, representing summer) (Figure 1). These samples were collected using a sterilized bucket and were transferred to a 2 L sterilized screw-capped bottle. Subsequently, the samples were transported to the laboratory in an icebox within 4 hrs after collection for microbiological analysis. In addition, on-site analysis of temperature, electrical conductivity, redox potential, and sludge sampling period was carried out. The sampling buckets were sterilized and flushed with water for 10 min before sampling. All samples were transported and stored at 4 °C and processed within 24 hrs.

2.2 Physical and organic characterization of the sludge

The pH, total organic carbon (TOC), organic matter (OM), total phosphate (TP), and total nitro (TN) of the collected sludge were determined by following standard methods.

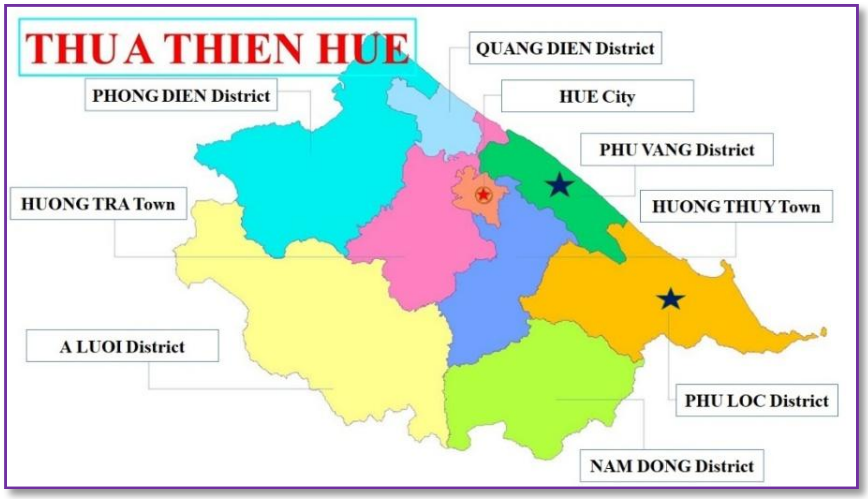

Figure 1 Map of the study area (sampling locations are denoted by blue stars)

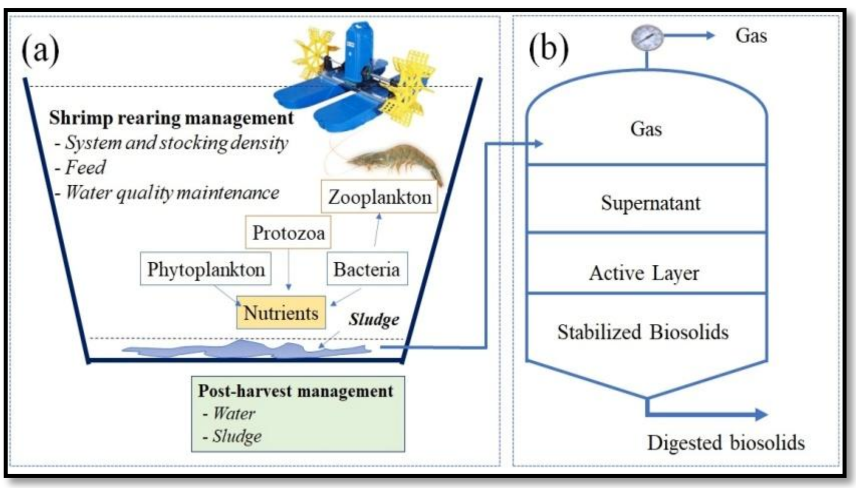

Figure 2 (a) Model of shrimp farming, and (b) model of the CH_4 gas generation process in the shrimp pond sludge

2.3 Isolation and identification of microbial strains

The compositions of the solid wastes obtained from the collected samples are shown in Tables 1 and 2. To isolate the microbial content, 1 mL of the waste obtained from shrimp pond sludge was mixed with 9 mL of sterile distilled water. The sample was then serially diluted to 10^{-4}. One hundred microliters of the sample from the last dilution were placed on the surface of casein agar in a Petri dish (1% casein and 2% agar), and the prepared sample was incubated at 35 °C for 72 hrs (Lich et al. 2022). The bacterial strains with strong protease activity were identified by molecular techniques.

The rate of flow of biogas and accumulated gas content, including CH_4 and H_2S, was also determined by using the gas chromatography (GC, GC-14 Gas Chromatograph, Shimadzu, Japan) technique. The machine was fitted with a flame ionization detector (FID), and the sample was quantified against a standard.

2.4 Statistical analysis

All experiments were performed in triplicate. Absorbances were recorded three times, and the average values were used for statistical analysis. Data were analyzed using Statgraphics 19, and the results are presented as mean ± standard deviation (SD). The difference between means was assessed by ANOVA, and Duncan's test was used to compare data from different samples. $P< 0.05$ was considered to be statistically significant.

3 Results and Discussion

Shrimp pond farming in Thua Thien Hue Province proceeds over several phases: pond preparation, shrimp fry selection, shrimp rearing management, and post-harvest management (Figures 2a and 2b). Environmental factors shape the structure and function of microbial communities, and research has shown that the succession of microbial communities was influenced by combinations of

Table 1 Characteristics of sludge obtained from different areas in the Thua Thien Hue district (spring season)

Name of sampling point	pH	TOC (mg/kg)	OM (%)	TP (g/kg)	TN (g/kg)
Phu Loc	6.9 ± 0.2	130.0 ± 6.7	22.4 ± 1.1	20.1 ± 0.2	32.5 ± 1.6
Phu Vang	7.3 ± 0.1	173.5 ± 5.3	29.9 ± 0.9	7.9 ± 0.1	24.1 ± 0.9

Table 2 Characteristics of sludge obtained fromdifferent areas of theThuaThien Hue district (summer season)

Name of sampling point	pH	TOC (mg/kg)	OM (%)	TP (g/kg)	TN (g/kg)
Phu Loc	7.5 ± 0.1	103.8 ± 4.2	17.8 ± 0.7	12.7 ± 0.1	16.6 ± 1.2
Phu Vang	7.5 ± 0.1	107.8 ± 1.7	18.6 ± 0.3	17.4 ± 0.2	13.5 ± 0.8

available organic matter, TN, TP, chemical oxygen demand, pH, and feed sources of the ponds (Alfiansah et al., 2018; Liu et al., 2020). Recently, researchers have studied the impact of pH on the cultured shrimp digestion process. The changes in intestinal activities were reported with the changes in the pH of the pond water. It was reported that the suitable pH for Whiteleg shrimp farming ranged from 7.8 to 8.5. The characteristics of the sludge collected from different areas of Thua Thien Hue during the spring and summer seasons are presented in Tables 1 and 2. The results of the study suggested that the pH of the sludge obtained from the two districts was different, and the pH values varied with the seasons. In spring, the mean pH of the sewage sludge collected from the Phu Vang district was 7.3, while the pH of the sludge samples collected from the Phu Loc district was 6.9. A small increase in the pH value was reported during summer, and the pH of the sewage sludge collected from the Phu Vang and Phu Loc districts was reported to be 7.5. The National technical regulation QCVN 02–19:2014/BNNPTNT was outlined by the Ministry of Agriculture and Rural Development of Vietnam for brackish shrimp farm conditions to ensure veterinary hygiene, environmental protection, and food safety. It has been reported that the optimal pH ranges between 5.5 and 9. During aquaculture, the pH of the sludge can change easily, which directly affects the physical, chemical, and biological conditions of the water environment of the pond. The change in pH also affects the cultured shrimp. The pH of the system can be controlled by controlling the number of algae and the amount of carbon oxide (CO_2) released by shrimps during respiration. The amount of CO_2 produced depends on the density and weight of the cultured shrimp. The respiration process of shrimps reduces the pH, as, during respiration, organisms consume dissolved oxygen and release carbon dioxide. If oxygen is deficient in shrimps, the sludge at the bottom of the pond is acidified. In addition, under anaerobic conditions, the sludge undergoes anaerobic fermentation, resulting in a reduction in the sulfate (SO_4^{2-}) content and the production of hydrogen sulfide (H_2S), which is toxic to shrimps. Similarly, Hung et al. (2015) reported the average pH of the sludge samples collected from Ha Noi, Vietnam, was in the range of neutral to alkaline (7.04–7.4). In contrast to this, Selamawit and Agizew (2022) reported that the pH values of the sludge samples obtained from wastewater collected from the sewerage system and exchange stations were 5.1 and 6.9, respectively. Sudiartha et al. (2022) reported that the pH of the aerated sludge sample was 7.09, and this value increases as the temperature increases. The pH increases to 7.64 at 42 °C. In contrast, the pH of anaerobically stabilized sludge varies between 6.7 and 7.8 (Michalska et al. 2022). The findings of the current study are consistent with previously reported results (Table 2). An increase in the pH was reported during the summer months, which can be potentially attributed to water heating in the presence of sunlight. This results in an electrochemical reaction that splits water into OH^- and H^+. As the concentration of the H^+ ions in the pond increases, the pH also increases. As the pH increases with temperature, polymerization and condensation times associated with the reduction of acid content increases (Gascó et al. 2005).

The TOC content corresponding to the sludge samples collected from different areas of Thua Thien Hue during the spring and summer months ranged from 103.8 ±4.2 to 173.5 ±5.3 mg/kg (Tables 1 and 2). As shown in figure 3a, the TOC corresponding to the sludge sample collected from the Phu Vang district is higher than that collected from Phu Loc. The TOC corresponding to these samples is higher in spring than in summer. The maximum average TOC was recorded for the sludge sample collected from the Phu Vang district during spring (173.5 ±5.3 mg/kg), and the minimum value was recorded for the sludge sample collected from the Phu Loc district in the summer months (103.8 ±4.2 mg/kg). Further, figure 3b showed the organic matter (OM) content of the sludges obtained from the two districts in the spring and summer seasons. The OM content does not greatly vary between the different shrimp farming areas in the Thua Thien Hue province in the summer, and it was reported in the range of 17.8 ±0.7–18.6 ±0.3%. However, the OM value corresponding to the Phu Vang district increased to 29.9 ±0.9%in spring. These results are consistent with the study reported by Azzouz et al. (2017). Nutrients are mineralized during the decomposition of OM and the level of C, N, and cation exchange increases with the increase of the OM. Chemical properties such as pH, electrical conductivity, and redox potential of the soil vary with seasons (Clapp et al. 1986). Azzouz et al. (2017) recorded the value of OM in the sewage sludge collected from the Guarchia wastewater treatment plant, in Benghazi, Libya, and reported that the maximum OM value was reached in February (24.20%) and the minimum was recorded in March (17.83%). Silva et al. (2018) reported that the OM obtained during the acidogenic stage was efficiently used to produce CH_4. Recent studies have suggested that the OM content in sewage sludge can reach 29 to 39% (Michalska et al. 2022).

The TP content in shrimp pond sludge samples is presented in Figure 3c. The minimum TP concentration was recorded from the sample collected from the Phu Vang district in the spring (7.9 ±0.1g/kg)), and the maximum TP was also recorded during the spring from the two locations of the Phu Loc district (20.1 ±0.2 g/kg). The variations in the TP between districts can be potentially attributed to the differences in the farming method, farming time, pond size, the density of shrimp farming, and the shrimp farming habits in the two areas. The accumulation of excess food and shrimp feces at the bottom of the pond increased the phosphorus content in the sludge. Burzyńska (2019) suggested that the maximum amount of dissolved phosphorus in the sludge solution is present in the top layer. Phosphorous from the top layer may migrate with surface runoff, increasing the risk of eutrophication. Figure 3d shows that the average TN content in the collected sludge samples did not differ significantly between seasons. The maximum average TN content (32.5 ±1.6 g/kg) was found for the sludge sample collected from Phu Loc in spring, while the minimum (13.5 ±0.8 g/kg) was recorded for the sample collected from Phu Vang in the summer. These values are below the maximum level specified in Vietnam's technical regulations on industrial wastewater. The factor influencing the release of P and N from sludge and the migration of these nutrients into the sludge-water environment affects the mineralization process associated with the OM in sludge (Burzyńska 2019). Scientists have suggested that shrimps incorporate 24 to 37% of nitrogen and 11 to 20% of phosphorus from the feed into their bodies. The presence of leftovers will result in changes in pH, dissolved oxygen in the water and pond sediment, and the extent of proliferation of bacteria and plankton (Alfiansah et al. 2018; Liu et al. 2020).

Shrimp intended for export must meet the heavy-metal content and bacteriological standards of the importing countries. The microbial density of pathogenic microorganisms in the selected ponds was also studied under two different sets of weather i.e. cold season/spring (Figure 4) and the hot season/summer (Figure 5) for two different locations of the Thua Thien Hue province to elucidate the presence of microorganisms in the collected sludges.

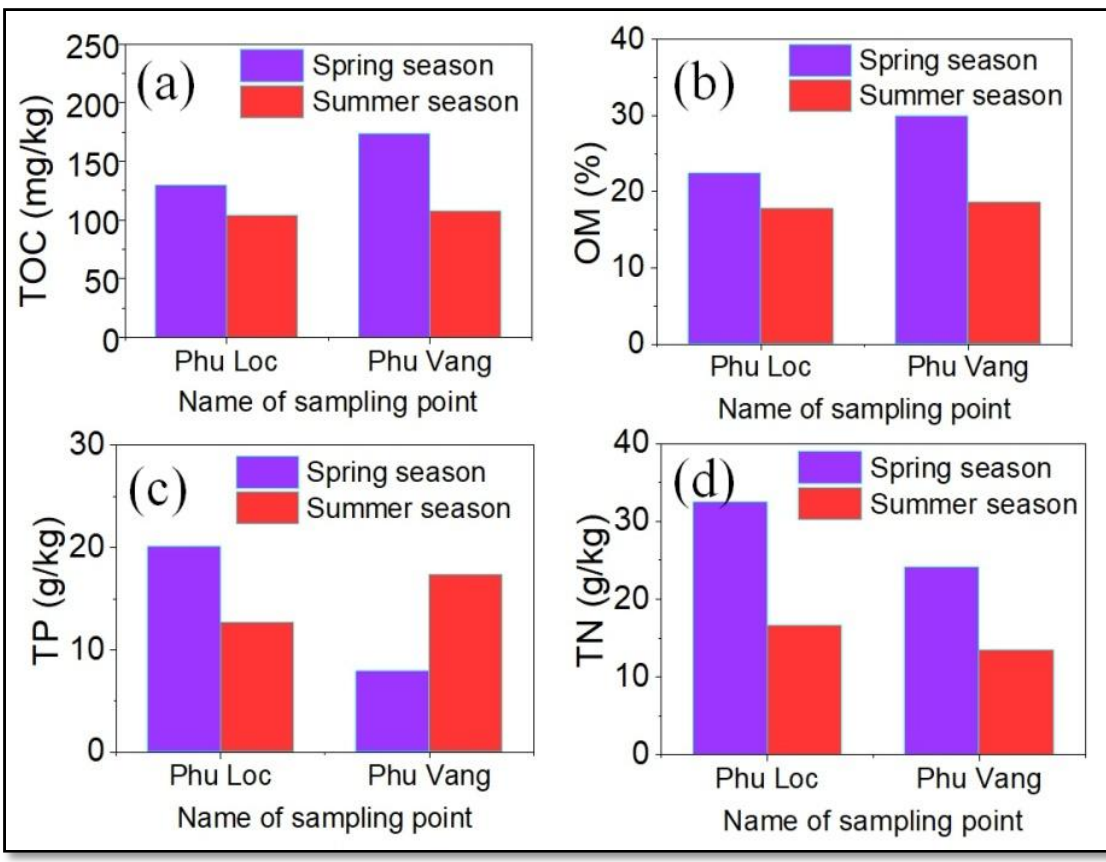

Figure 3 Physicochemical characteristics of sludge samples obtained from different locations of Thua Thien Hue

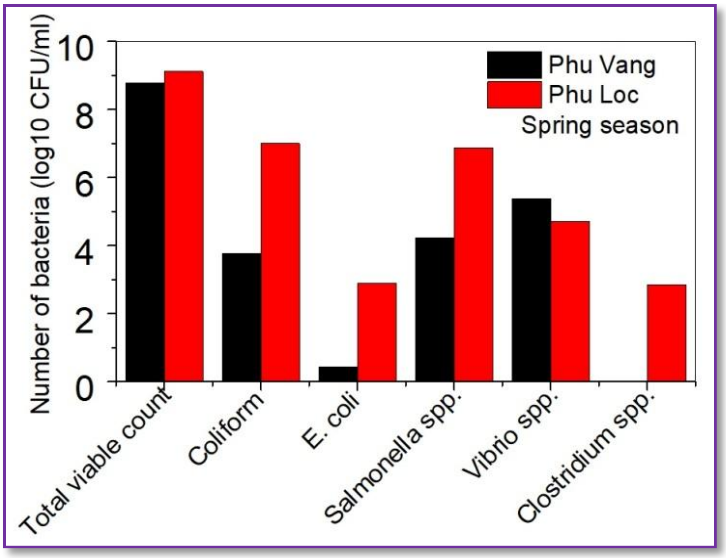

Figure 4 Microbial density in pond sludge samples obtained from different areas of the Thua Thien Hue district (spring season)

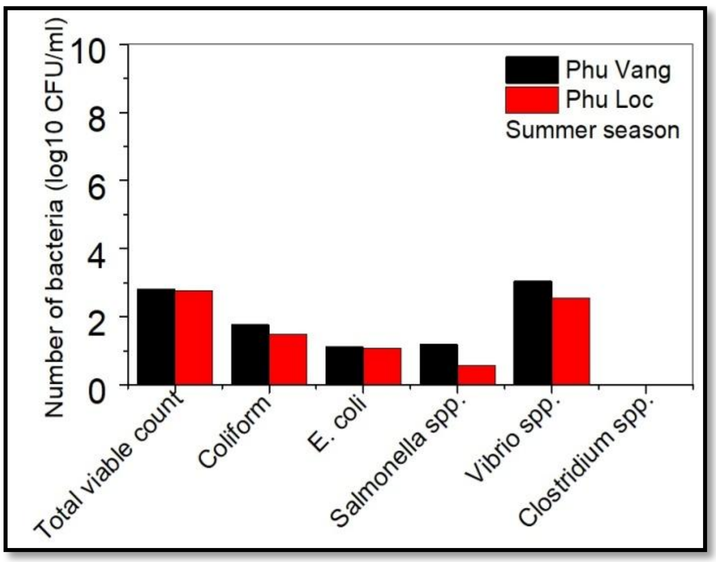

Figure 5 Microbial counts in pond sludge samples obtained from different areas of the Thua Thien Hue district (summer season)

Results presented in Figure 4 revealed the counts of total bacteria, *Coliform*, *E.coli*, *Salmonella* spp., and *Vibrio* spp. in the sludge of shrimp ponds in spring. Further, the presence of *Clostridium* spp. was reported in sludge samples collected from the Phu Loc District, while it was absent in the samples collected from the ponds of the Phu Vang District. The analyzed results showed that the number of bacteria detected in the sludge obtained from both districts in spring is quite high, and values ranged from 8.8 to 9.11 log 10CFU/mL. The bacterial content in the sludge samples collected from the Phu Loc districts tended to be higher than that of the Phu Vang districts (Figure 4). The relatively high counts of *Coliform* (6.99 log10 CFU/mL), *Salmonella* spp. (6.78 log10 CFU/mL), and *Vibrio* spp. (4.7 log10 CFU/mL) in the ponds of the Phu Loc District, in particular, indicate the need for water treatment methods for these bacteria in the Phu Loc District. Further, *Salmonella* spp. and *Vibrio* spp. are recognized as important food-borne pathogens, and most importing countries do not accept their presence in raw frozen shrimp (Yen et al. 2020). Thus, the farm owners need to carefully treat the pond bottom so that pathogens have no opportunity to enter the shrimp. In summer, the *total bacteria*, *Coliform*, *E.coli*, *Salmonella* spp., and *Vibrio* spp. counts decreased, while *Clostridium* spp. disappeared altogether (Figure 5). In general, for a given location, the incidence of bacteria recorded for the sludge samples obtained from shrimp ponds during spring was higher than those recorded during the summer seasons (Figure 6).

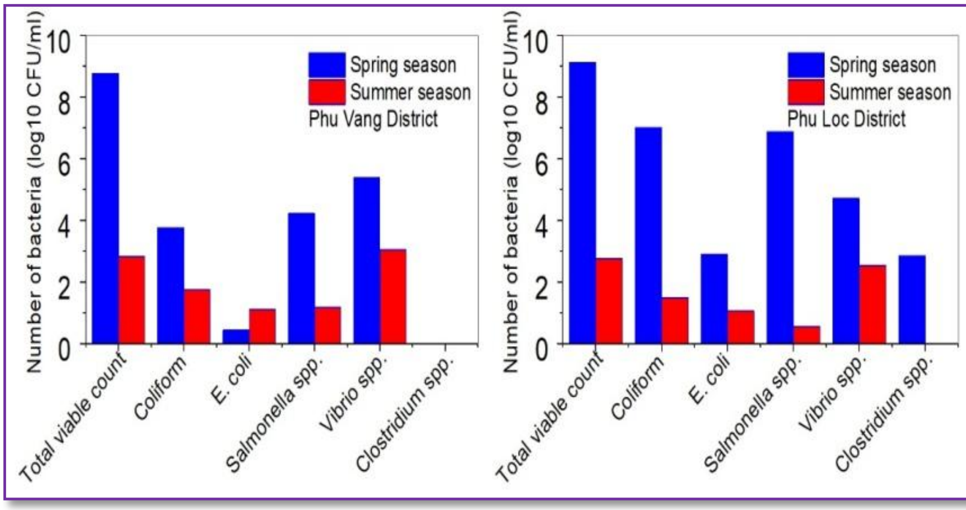

Figure 6 Microbial counts in pond sludge samples obtained from (a) Phu Vang and (b) Phu Loc

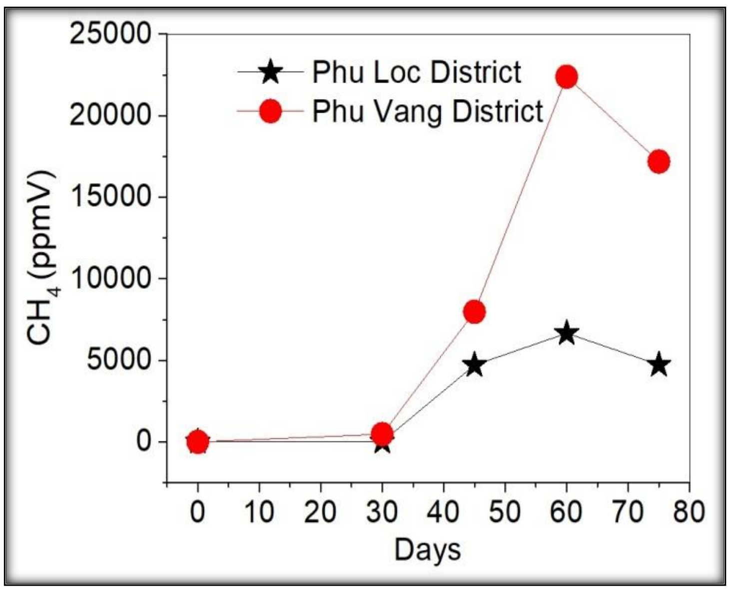

Figure 7 CH_4 production across 75 days of anaerobic digestion using shrimp pond sludge samples obtained from the Phu Vang and Phu Loc districts

In "Industry 4.0", renewable resources will play a crucial role. Biogas from waste, residues, and energy crops can be used as a replacement for fossil fuels in power and heat production. Sewage sludge can be treated using biochemical, thermochemical, or mechanical methods to generate biogas. Recently, researchers have suggested that sludge has a lot of potential for gas production (Selamawit and Agizew 2022). According to a review report, the sludge of shrimp ponds contains particulate and dissolved organics such as microorganisms, leftover shrimp food, shrimp manure, and other organic substances. Thus, it can be potentially used for generating gas. This is confirmed by the use of an anaerobic digester that was used to investigate the biodegradability of sludge through the conversion of biomass energy into biogas (Figure 7).

The amount of CH_4 produced gradually increased with the increase of the reaction time for the shrimp pond sludge samples collected from both the Phu Vang and Phu Loc Districts. After the 30^{th} day of sludge incubation, the amount of CH_4 gas increased sharply, and it reached its maximum value on the 60^{th} day. Following this, the CH_4 gas content began to decrease. For Phu Loc, on the 60^{th} day of the experiment, the amount of CH_4 generated from the sludge was 6664 ppm, and it shows an increase of 70 times compared to the 95 ppm recorded on the 30^{th} day. The amount of CH_4 gas generated from the shrimp pond sludge samples collected from Phu Vang was even higher, and it reached 22385 ppm on the 60^{th} day of the experiment. Through theoretical and experimental studies Schaum et al. (2015) reported that at 37 °C, the content of dissolved methane in digested sludge was approximately 22 ppm. According to studies, OMs decompose, and the grain size reduces within an anaerobic digester system. This improves the solubilization of organic waste, and the increased bacterial activity results in the release of hydrolytic enzymes, which significantly increases the extent of biogas production realized (Selamawit and Agizew 2022). It can be predicted that the CH_4 yield depends on substrate origin and composition. It also depends on the operating conditions (temperature and strains of bacteria). The fact that the extent of CH_4 gas production peaked on the 60^{th} day of sludge incubation revealed that this is the best condition for gas generation. However, when the OM content in the sludge samples was completely decomposed, the amount of CH_4 gas produced began to decrease. Similar results were reported by Sudiartha et al. (2022), who investigated CH_4 production in a two-stage anaerobic digestion system by co-digesting food waste, sewage sludge, and glycerol. According to another study, an increase in CH_4 gas production was accompanied by an increase in the *Methanoculleus* content at 48 °C (Sudiartha et al. 2022). It has also been reported that sludge can be used to produce CH4 gas effectively. Silva et al. (2018) investigated the process of CH_4 production using a mixture of food waste, anaerobic sewage sludge, and glycerol. The maximum yield of CH_4 obtained from this mixture was 342.0 mL CH_4·g. The results herein revealed that in addition to economic benefits, shrimp farming promotes CH_4 production under anaerobic digestion conditions. These results allow prospects of the production of CH_4 gas from shrimp waste sludge in Thua Thien Hue province. In addition, an effective method that can improve the production of CH_4 gas from shrimp sludge using *Stenotrophomonas rhizophila* MT1 bacterial isolated from sludge samples of shrimp ponds (Lich et al. 2022) which can be a topic that will open the door of future research.

Conclusions

Results of the current study revealed the physical and biological indicators of shrimp pond sludge samples taken from the Phu Vang and Phu Loc districts of the Thua Thien Hue Province, Vietnam. The results can be concluded that the sludge samples of the study area are characterized by neutral to alkaline pH (6.9–7.5), the TOC, OM, TN, and TP ranged from 103.8 to 173.5 mg/kg, 17.8 to 29.9%, 13.5 to 32.5 g/kg, and 7.9 to 20.1 g/kg respectively. These parameters vary differently between regions and seasons. Further, the microbial density of pathogenic microorganisms such as total bacteria, *Coliform*, *E.coli*, *Salmonella* spp., *Vibrio* spp., and *Clostridium* spp. was also determined in the collected sludge sample under the conditions of two opposing sets of weather (cool spring and hot summer season). The microbial count was found to increase rapidly during spring and reached 8.77 log10 CFU/mL in the Phu Loc district, and this value was recorded at 9.11 log 10CFU/mL for the Phu Vang district. This microbial count was decreased during the summer seasons. During the summer months, the total bacterial, *Coliform, E.coli, Salmonella* spp., and *Vibrio* count was recorded 2.57, 1.49 1.06, 0.56, and 12.54 log10 CFU/mL respectively in the Phu Loc district. Later on, the *Clostridium* population fell to zero. Results from experiments carried out with an anaerobic decomposition model system showed that on the 60^{th} day of the experiment, the amount of CH_4 generated at the highest output value was 22385 ppm and 6664 ppm for the Phu Vang and Phu Loc regions, respectively. These results revealed that CH_4 gas can be potentially produced from shrimp waste sludge obtained from the Thua Thien Hue province.

Acknowledgments

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Conflicts of interest

All authors declare no conflicts of interest.

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