Vietnam Journal of Science and Technology ***60*** *(6) (2022) 1005-1013*

doi:10.15625/2525-2518/16672

ISOLATION AND IDENTIFICATION OF MICROORGANISMS CAUSING WATER-BASED PAINT AND WALL PAINT DESTRUCTION

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Received: 29 October 2021; Accepted for publication: 21 December 2021

Abstract. From samples of moldy wall paint and damaged water-based paint, we isolated and quantified the density of microorganisms present in the samples and determined the morphology of the isolates; thereby, identifying the genera of the bacteria and mold strains. The results showed that the density of the strains collected from the wall paint samples was much higher than that of the water-based paint samples (106 CFU/mL and 104 CFU/mL, respectively). In the wall paint samples, only mold colonies were observed, not bacterial colonies, while in the water-based paint samples both mold and bacterial colonies appeared, although mold colonies were still predominant. Based on the observation of colony formation and microscopic morphology of molds, we classified six mold strains into five genera: *Aspergillus* sp., *Cladosporum* sp., *Acremoium* sp., *Chaetomium* sp., and *Fusarium* sp. The frequency of strains belonging to the genus *Aspergillus* sp. accounted for the majority in both wall and water-based paint samples. Among the three bacterial strains isolated, we identified two bacterial strains as G (-) and one strain as G (+).

Keywords: bacteria, biocorrosion, biodegradation, paint destruction, mold.

Classification numbers: 2.5.2, 2.5.3.

1. INTRODUCTION

Biocorrosion or biodegradation is recognized as an important type of corrosion leading to significant economic loss in many industries and services. Biological corrosion is difficult to distinguish from normal corrosion cases, with some authors estimating that about 20 % of all corrosion cases are biological corrosion [1]. Microorganisms can interact with materials/surfaces in the environment in a variety of ways that are complex and difficult to assess and predict under standard corrosion models. The phenomenon of microbiological corrosion is a relatively difficult problem, especially in countries with hot and humid tropical climate like Viet Nam. The hot and humid climate is an ideal environment for microorganisms to grow, causing damages to the coatings and/or materials of buildings, machinery and equipment when installed and operated.

Biodegradation leads to a deterioration of the protective and decorative properties of coatings. The growth of microorganisms on and inside the paint can cause the paint to peel and discolor [1].

Among the types of coatings, water-based paints are the most susceptible to biodegradation. Cellulose ethers used as thickeners in water-based paints are often attacked by enzymes produced by fungi and bacteria. They enter the paint at the manufacturing stage along with contaminated components [1]. Orehek *et al*. studied the biodegradation of carboxymethyl cellulose by *B. subtilis* NCIB 3610 in waterborne coatings. The viscosity of the liquid decreased from 10 to 1.4 mPas and the concentration of reducing sugar increased eighteen times. The strain *B. subtilis* NCIB 3610 was also shown to be able to synthesize cellulose that breaks chemical bonds in carboxymethyl cellulose [2].

Obidi *et al*. studied the microbial contamination of paint products. Fresh paint samples were taken and isolated strains of bacteria: *B. brevis, L. brevis, E. coli* and the fungus *A. niger and P. citrinum*. Follow-up over a period of 10 months, the results showed that the population of isolated microorganisms increased significantly. For bacteria, the population increased from $1.6.10^1$ CFU/mL in fresh samples to $4.7.10^5$ CFU/mL after 10 months. The corresponding figures for fungi are $1.0.10^1$ and $5.5.10^3$ CFU/mL, respectively [3]. In addition, the bacterial strain *P. aeruginosa,* which is the causative organism, was also isolated from a water-based paint sample [4].

Giacomucci *et al*. [5] studied the biodegradation of nitrocellulose paints by the bacteria *D. desulfuricans*. The study measured changes in nitrate, nitrite and ammonia concentrations in the culture medium. To determine chemical changes in the paint, infrared spectroscopy was performed, and colorimetric tests were also carried out to identify color changes caused by microorganisms. Microscopic measurements show that bacteria can adhere to the painted surface and alter its adhesive properties. The study determined that *D. desulfuricans* is capable of degrading nitrocellulose used as binders in products as mentioned [5].

Ishfaq *et al*. [6] studied the biodegradation of coatings by fungi and bacteria. The results showed that fungi such as *Aspergillus* sp., *Phanerochaete* sp. and *Rhizopus* sp. were able to grow on agar culture medium containing paint**.** The study also used *Bacillus* sp. and *Pseudomonas* sp. The results showed that, under some conditions, selected species of fungi and bacteria were able to adhere to painted surfaces and cause paint degradation. Other related studies indicate that molds are more capable of destroying paint coatings than bacteria [7 - 10].

In Viet Nam, studies on biodegradation of coatings by microbial strains are very limited. Or, studies have approached the problem only in terms of the physical chemistry of the material rather than the biological nature of this destruction process. One of the scientists who has approached the problem systematically and delved into the biological nature of the process is Dr. Dang Vu Hong Mien. Scientific reports and monographs of Dr. Dang have fully systematized the potential microbial species causing the destruction of coatings, natural organic materials (rattan, bamboo) or inorganic materials (metal, glass, optics, etc.) under tropical conditions in Viet Nam [12]. However, the studies were carried out a long time ago (1970s - 1980s), the techniques and methods are outdated, and the concepts and methods of classification and identification of bacteria are also outdated. The organisms have changed significantly since that time. Therefore, it is necessary to have systematic studies, following the above studies according to more up-to-date and modern methods and knowledge.

2. MATERIALS AND METHODS

2.1. Isolation and quantification

Paint samples: There were 2 types of paint samples studied: 1. Water-based paint samples showing signs of deterioration (odor due to decomposition), 2. paint samples on walls that were peeling, discolored and showing signs of damage by microorganisms. These paint samples were collected to perform the microbial isolation process.

Microbial isolation nutrient medium: Two nutrient media, PDA (peptone dextrose agar) and MPA (meat peptone agar), were used to isolate microorganisms from damaged paint samples and stained wall paint samples. The ingredients were taken according to the recipe and were wet sterilized (using an autoclave) at 121 ^{o}C, 1 atm for 20 minutes. The medium was then divided into petri dishes and inclined agar tubes.

Isolation and quantification from water-based paint samples: Take 1mL of a damaged water-based paint sample and dilute it with 99 mL of sterile saline to make a 10^{-2} dilution, then dilute to 10^{-5}, 10^{-6}, and 10^{-7}. Apply 100 µL of the final dilution to PDA and MPA nutrient medium. Incubate the plates at 35 °C and observe daily for microbial growth.

Isolation and quantification from discolored wall paint samples: Use a sterile cotton swab to wipe the surface of the wall paint sample (1 cm^2). Sampling swabs were soaked in 1 mL of sterile saline and vortex for 2 minutes. Carry out further dilution to concentrations of 10^{-4}, 10^{-5}, 10^{-6} and on the agar surface of 2 media PDA and MPA as described above.

2.2. Purification and preservation

Microbial strains isolated from the two samples were purified and preserved for further analysis. The strains were purified by descending whisker culture on PDA and MPA agar, after individual colonies were separated. Reduced cultures were repeated in 3 cycles to ensure that the strains obtained were pure strains, which were then stored in inclined agar tubes at 4 ^{o}C.

2.3. Morphological observation of isolated strains

Gram stain for bacteria: Fix the bacterial sample with an alcohol lamp flame, use crystal violet to stain the sample for 1 min. Rinse with water for up to 5 seconds. Add Lugol solution (1 % iodine, 2 % KI) for 1 min, wash with alcohol for 10 seconds. Coat the sample with 95 % ethanol for approximately 1 minute. Further stain with fuchsin for one minute, then rinse with water and allow to dry.

Specimen microscopic observations of molds: The observation of the microstructure of the molds was carried out by placing the specimen slide on the mold culture PDA medium. Lamin (4 pieces) was surface disinfected and obliquely inserted at an angle of 30^{o} into the PDA medium. The fungi were inoculated in the center of the agar plate and cultivated for 5 - 7 days for the growth of mycelium on the lamin. The slide was then removed from the medium and made as a microscope slide. Based on the structure of microorganisms, molds can be classified into genera (genus), according to the literature on morphological taxonomy of fungi.

3. RESULTS AND DISCUSSION

3.1. Isolation and quantification

Wall paint samples are very susceptible to mold when exposed to moisture. Mold will grow on the paint layer and secrete some organic acids that destroy the paint film. The pigments produced by the mold filaments grow together with the fungal spores, causing the wall paint to become blackened, unsightly and peeling off the paint layer (Figure 1). In our study, samples

were taken directly from the stained paint area, where mold spores were concentrated, for isolation and quantification.

In the 3 samples of wall paint that we collected, the isolation results showed that the average density of the sample was from 10^6 to 10^7 CFU/mL of wall paint, in which only mold appeared, very few bacterial colonies. There were four forms of mold colony morphology appearing in wall paint samples with different frequencies. Among the mold colonies, black mold appeared with the highest frequency in isolates (92.3 %), followed by gray mold colonies (4.5 %) and green mold (2.1 %), the rest were yellow mold colonies (1.1 %) (Table 1).

Figure 1. Mold growth and destruction of paint on walls, and mold sampling locations.

The water-based paint sample was damaged by microorganisms, causing the paint color to darken, the viscosity was significantly reduced and causing the characteristic odor of the microbial decomposition process. Microbiological contamination of water-based paints also caused water to be separated from other components of the paint without forming a homogeneous system, producing a layer of water on the paint surface (Figure 2). From the damaged paint sample, the process of isolation and quantification was conducted.

Figure 2. Normal water-based paint samples (left) and paint samples damaged by microorganisms (right).

In the damaged water-based paint samples, the microbial density was smaller than that of the wall mold samples (range: 10^4 - 10^5 CFU/mL). In the water-based paint samples, the results showed that mold colonies still dominated with two main forms of colony morphology, however, some bacterial colonies also appeared with different morphologies (Table 1).

Table 1. Morphology and occurrence frequency of colonies isolated from wall and water-based paint samples.

Sample	Type	Colonial appearance	Occurrence frequency (%)
Wall paint	Mold	Black (ST1)	92.3
		Gray (ST2)	4.5
		Green (ST3)	2.1
		Yellow (ST4)	1.1
	Bacteria	Not detected	0
Water-based paint	Mold	Black (SN1)	50.4
		Yellow (SN2)	26.3
	Bacteria	Creamy, shine (VK1)	18.1
		Clear, shine (VK2)	3.3
		Light yellow, rough (VK3)	1.9

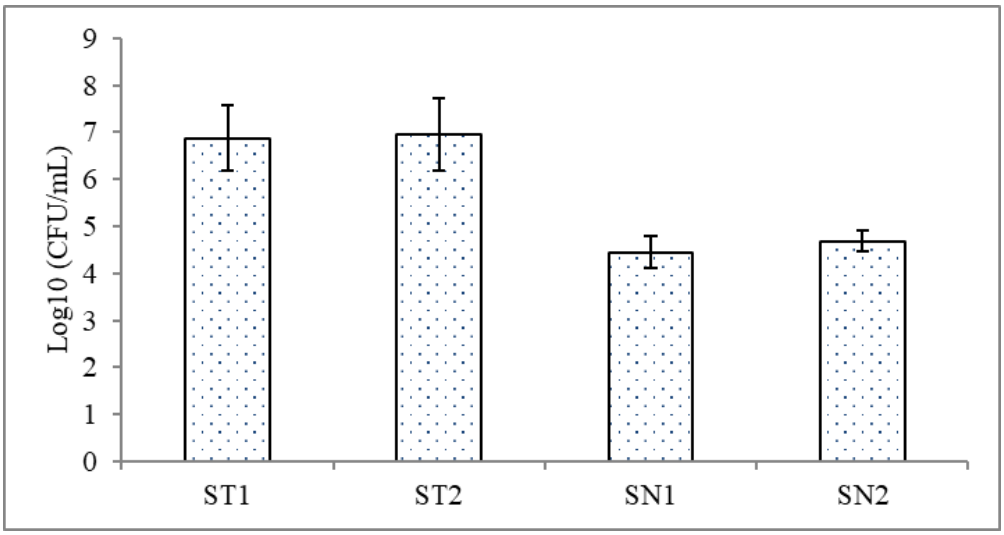

Figure 3. Microbial density of wall paint and water-based paint samples.
"ST" stands for wall paint; "SN" for water-based paint.

3.2. Classification based on microscopic morphology

The isolates of bacteria and molds were used for microscopy. For bacteria, we only described the morphology and determined the Gram type for each strain. For mold strains, we classified them into genera (genus) of each strain based on colony characteristics and microscopic morphology.

3.3.1. Morphology and gram staining of bacterial strains

After gram staining, in 3 bacterial strains isolated in water-based paint samples, there were two strains of G- (VK1 and VK2) and one strain of G+ (VK3).

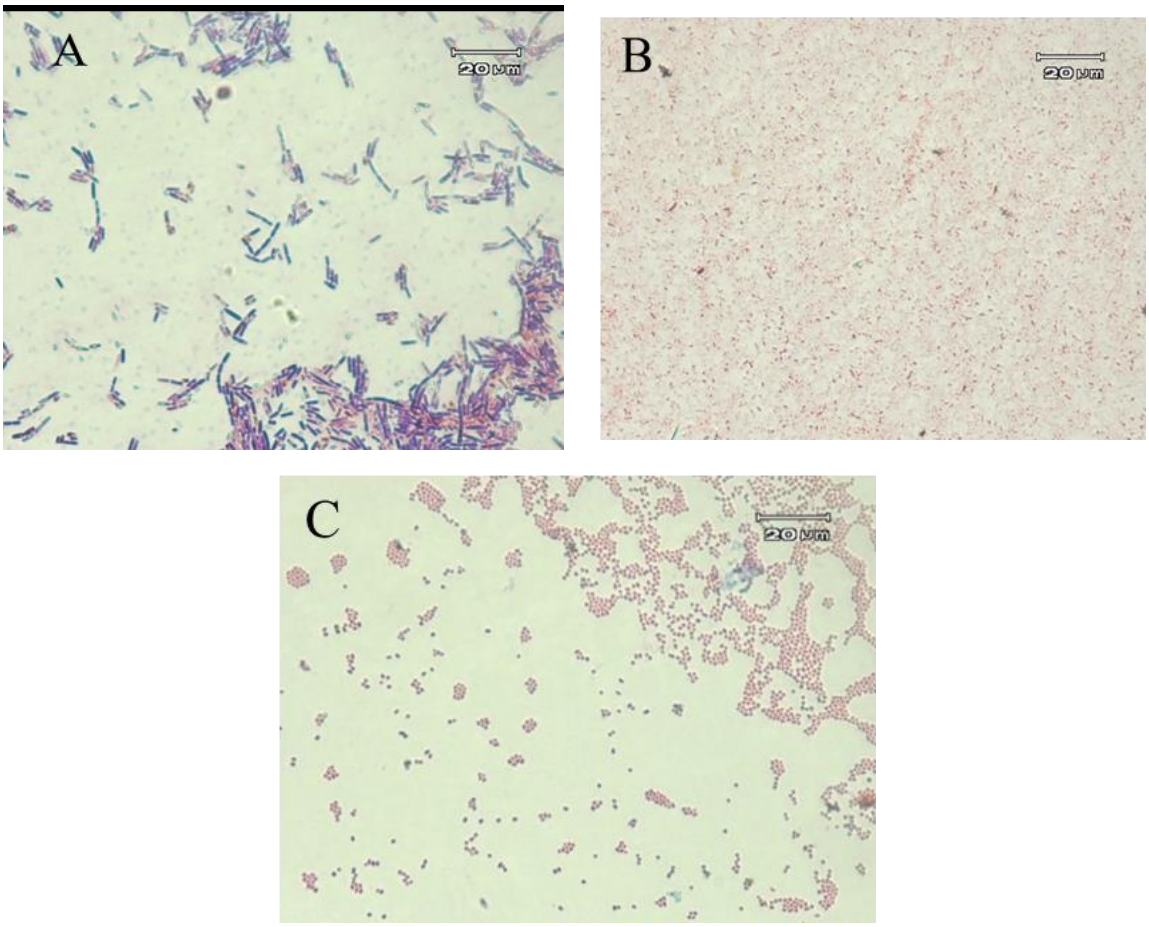

Figure 4. Gram staining results of bacterial strains isolated from water-based paint samples.

Strain VK1: a G (-) bacterium, the cells were spherical, often clumps of cells together, sometimes with individual cells or in pairs, or pairs of 3 cells (Figure 4C).

Strain VK2: a G (-) bacterium, the cells were oval in shape, the individual cells did not clump together like strain VK1 (Figure 4B).

Strain VK3: a G (+) bacterium, the cells were rod-shaped and were linked together in a chain (Figure 4A).

3.3.2. Microscopic images of mold isolates

To classify mold strains based on colonial morphology and microscopic structure, we conducted observations and analysis according to the classification guideline described in "Vietnamese mold system" by Dang (2001) [12] and "Dictionary of fungi" by Kirk *et al*. (2008) [13]. The taxonomy, morphology, and growth cycle of the fungi were described and classified into genera of molds, most likely belonging to a group with similar characteristics.

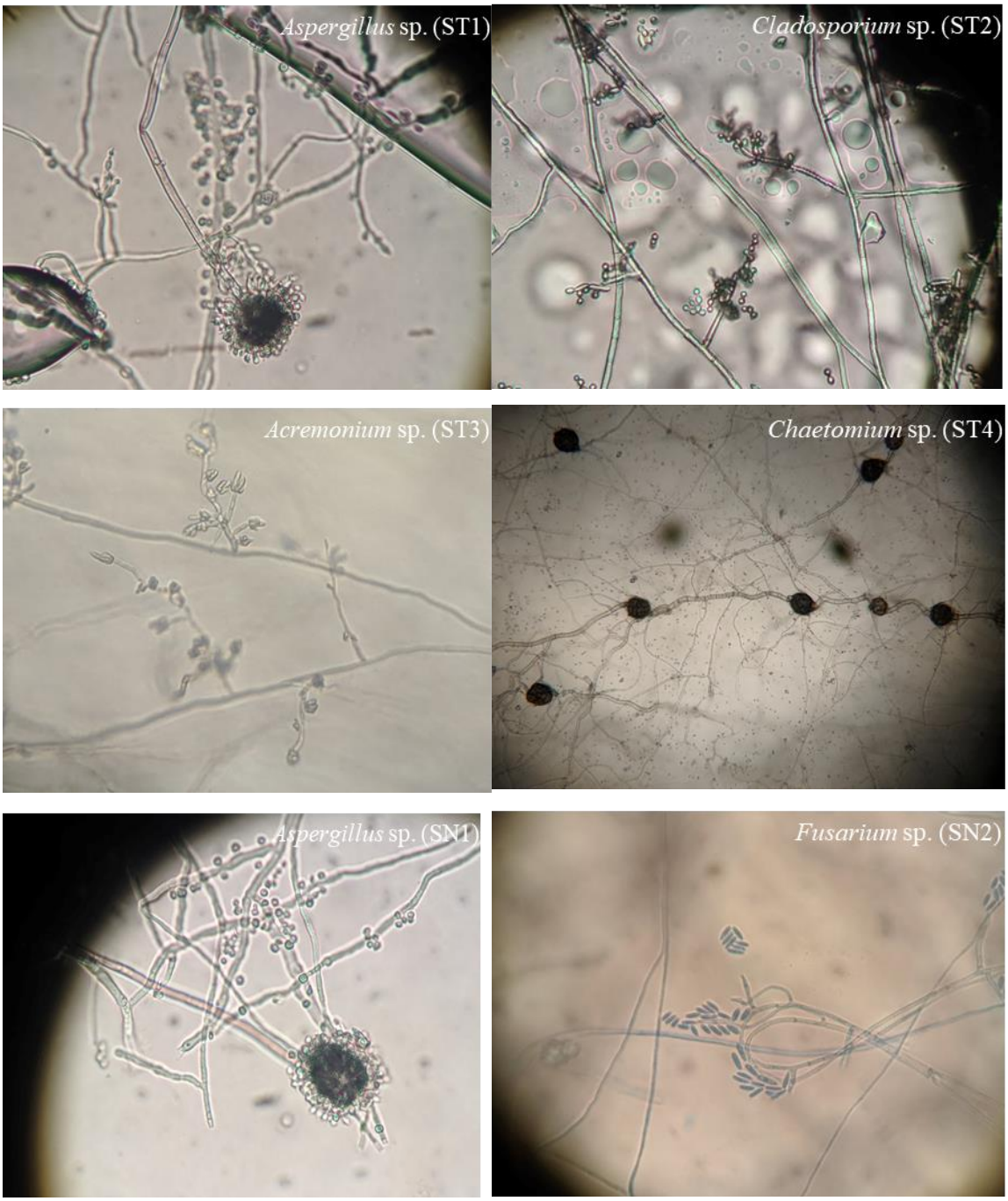

Figure 5. Microscopic morphology of isolated mold strains.

ST1: Colonies grew slow, the diameter after seven days was 3.3 cm with black color. The spores formed were petiolate, radial, and black. The spore-producing area grew all over the face of the bladder. Based on the colony morphology and microstructure, it can be concluded that strain ST1 belongs to the genus *Aspergillus* sp.

ST2: Colonies grew fast, the diameter after seven days was 7.5 cm with gray color. The hyphae produced many black, round-bottomed oval-shaped fruiting bodies with curly mycelium. The spores were elliptical with two slightly pointed ends. The strain was isolated from wall paint samples. Based on the colony morphology and microstructure, it can be concluded that strain ST2 belongs to the genus *Chaetomium* sp.

ST3: Colonies grew fast, the diameter after seven days was 6.3 cm with green color. The mycelium was colorless, much branched, and the septum was sparse. The corpus callosum grew from the phylum or bundles of hyphae. The spores were elongated, slightly curved, and adhered to the top of the flask to form a false head. The strain was isolated from wall paint samples. Based on the colony morphology and microstructure, it can be concluded that strain ST3 belongs to the genus *Acremonium* sp.

ST4: Colonies grew slow, the diameter after seven days was 2.5 - 3 cm with dark yellow color. The sporophyte was short branched. The spores were elliptic, ovoid with long chains and spiky spore walls. Based on the colony morphology and microstructure, it can be concluded that strain ST4 belongs to the genus *Cladosporium* sp.

SN1: Colonies grew slow, the diameter after seven days was 4 cm with black color. The gaseous hyphae grew from the substrate and were spherical, radiating. The spore-producing area grew all over the face of the bladder. Based on the colony morphology and microstructure, it can be concluded that strain SN1 belongs to the genus *Aspergillus* sp.

SN2: Colonies grew at an average rate, the diameter after seven days was 4.4 cm with light yellow color. The spores had a slightly curved back and a long abdomen with many small cells, and were cylindrical, pear-shaped. Based on the colony morphology and microstructure, it can be concluded that strain SN2 belongs to the genus *Fusarium* sp.

4. CONCLUSIONS

From the two types of damaged paint samples, the analysis results on the density and types of microorganisms are summarized as follows:

- Six mold strains and two bacterial strains were isolated and identified from the damaged paint samples.
- The wall paint sample had a microbial density of 10^7 CFU/mL, while the microbial density of the water-based paint sample was 10^4 CFU/mL.
- The six mold strains were classified into different genera in order of occurrence from highest to lowest: *Aspergillus* sp., *Cladosporum* sp., *Acremoium* sp., *Chaetomium* sp., and *Fusarium* sp.
- The bacterial isolates were two Gram (-) and one Gram (+) strains.

Acknowledgements. The research was financially supported by the Vietnam Academy of Science and Technology (VAST) from the Superior Researcher Program (Grant number: NVCC 13.10/21-21). The Institute for Tropical Technology (ITT) was acknowledged for supporting research facilities.

CRediT authorship contribution statement. T.T.T. Van: Funding acquisition. B.V. Cuong and N.T. Huy: Planning and Investigation. V.Q.Thai and T.D Dung: Methodology. C.T. Hong, V.T.K. Anh and N.T. Xuyen: Formal analysis. T.T.T. Van and T.D. Lam: Supervision.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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