REMOVAL OF FLOUROQUINOLONE ANTIMICROBIALS (CIPROFLOXACIN AND NORFLOXACIN) FROM SHRIMP POND SEDIMENT DURING COMPOSTING

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ABSTRACT

This study aimed to investigate the removal of ciprofloxacin and norfloxacin from shrimp pond sediment during composting process with different antimicrobial concentrations. The results showed that after 40 days of composting, temperature varied from 25 to 55 ^{o}C. The highest temperature was on the day 20th. The pH values reduced along with composting time from weak base to neutral. During the composting processes, the removal efficiency of norfloxacin found in the range of 32.5–87.5 % depends on the initial antimicrobials concentrations. For ciprofloxacin, after 20 days of composting, the removal efficiency obtained was around 55 and 45 % and pH was 8.5 and 5.0. Salinity significantly affected ciprofloxacin removal that created remarkably efficiency reduction of ciprofloxacin in salty water as compared to fresh- and brackish water. However, no differences in removal efficiency of norfloxacin were observed between fresh and brackish water. The composted fertilizer was dark brown in color and odorless, contained 15.7-18.8 % C; 2.05 – 2.15 % N; and C/N ratio was 7.5–10.95. This indicated that compost fertilizer was completely decomposed.

Keywords: antimicrobial, ciprofloxacin, composting norfloxacin, shrimp pond sediment.

1. INTRODUCTION

In Viet Nam, shrimp farming has been developing rapidly in terms of faming scale, stocking density, and production. However, farmers frequently use antibiotics to prevent and eliminate diseases in their shrimps, which might lead to the increase of antibiotic residues in shrimp, and the accumulation of the used antibiotics in pond sediment. Therefore, they might have harmful impacts on the environment and human health if they are not treated properly. Recently, inappropriate planing of shrimp farming in many coastal areas has resulted in the reduction of water quality and post larvaes. Farmers use a variety of antibiotics by mixing with aquafeed to prevent disease and cure for affected shrimp. Antibiotics are mixed food used to feed the shrimp The misuse of antibiotics in terms of types, time, dosage can affect the quality of shrimp and lead to the existence of antibiotic residues in shrimp pond water and sediment. In a study of D.T.H. Anh *et al.* [1], the residues of Ciprofloxacin (CPFX) and Norfloxacin (NRFX) in water, sediment and shrimp samples from Nam Dinh province were determined. The results showed that only CPFX was found in water and sediment and ranged from 0.06 - 0.35 μg/L to 0.22 - 0.40 μg/g, respectively. Data from the beginning of the shrimp culture indicated that CPFX had been used to disinfect water and the environment [1]. A study of L. X. Tuan and Yukihiro Munekage. [2] found several antibiotics that were used in shrimp ponds such as Norfloxacin (NRFX), Trimpethoprim (TMP), Sulfamethoxazole (SMX), Oxolinic acid (OXLA). Tran T. K. Chi *et al.* [3] reported that 20 different antimicrobial products which are readily available at a local pharmacy were used for disease prevention and treatment in shrimp and fish culture. As the fact that, the pond sludge has been utilized for composting and soil remediating and may caused the risk for plants and environment [4, 5]. In this study, the removal of ciprofloxacin and norfloxacin from shrimp pond sediment during composting was investigated in composting mixture experiments with different antimicrobials concentration in shrimp pond sediments.

2. MATERIALS AND METHODS

2.1. Materials

(1) Shrimp pond sediment: from shrimp ponds in Duc Giang (Yen Dung-Bac Giang); (2) Sawdust: from wood processing factory in Thach Cam (Thach Thanh-Thanh Hoa) (3) EM bioproducts contained 80-100 useful microorganisms, including photosynthetic bacteria, lactic acid bacteria, bacteria, yeast. Total microorganisms of over 10^8 CFU/g are produced by Center of Research and Application of Biotechnology and Environment.

2.2. Methods

a) Composting process: Input materials included dry sediment (62 % moisture) and sawdust that were well mixed and then were added with EM. Water was added with sufficient amount to ensure the moisture of the mixture of 60-65 %. The mixture was composted in sealed and closed styrofoam boxes and put in the dry place with sufficient light to optimize the temperature during the processes. The composting experiments were conducted in 6 styrofoam boxes (54×38×30 cm).

Table 1. Experimental formula for norfloxacin decomposition experiments.

Formula	Pond sediment weight (kg)	Sawdust weight (kg)	EM weight (g)	NRFX concentration (ppm)
TN1	5	1	10	0
TN2	5	1	10	1.28
TN3	5	1	10	2.86
TN4	5	1	10	4.28

Two experimental series were designed to determine the decomposition of norfloxacin and ciprofloxacin and the impact of salinity and pH (Table 1 and 2). Salinity was adjusted by adding seawater and NaCl solution. The compost piles were turned manually once a week.

2.3. Analytical methods

Temperature, pH, and moisture were measured every 5-days. Moisture was determined by weight method the mixture was dryed at 105 ^{o}C until constant weight (after 2-3 hours). The total organic carbon, total nitrogen, disolvable phosphorus were analyzed according to TCVN 9294: 2012; TCVN 8557: 2010; TCVN 8661: 2011. The concentrations of norfloxacin and ciprofloxacin were analyzed in Center for Training, Consultancy and Technology Transfer, VAST using LC/MS/MS Thermo TSQ Quantum Access –USA (UPLC-Accela coupled with MSMS TSQ Quantum Access): column Hypersil Gold C18, 3 µm, 150×2.1 mm –Thermo.

Table 2. Experimental formula for ciprofloxacin decomposition experiments.

Formula	Pond sediment weight (kg)	Sawdust weight (kg)	EM weight (g)	CPFX concentration (ppm)	Salinity (ppt)	pH
TN5	10	2	20	4.17	0	5
TN6	10	2	20	4.17	0	8.3
TN7	10	2	20	4.17	0	7.3
TN8	10	2	20	4.52	0	8.3
TN9	10	2	20	4.52	4.15	8.3
TN10	10	2	20	4.52	32.83	8.3

3. RESULTS AND DISCUSSIONS

3.1. Characteristics of shrimp pond sediment

The analysis results of chemical and physical characteristics of the pond sediment (Table 3) indicated an alkaline pH value (8.3), an average organic carbon value (by Chiurin rating scale), a good level of total nitrogen (by Kyuma rating scale). Particularly in the sediment, dissolvable phosphorus was in the rich group (by P Oslen rating scale).

Table 3. Chemical and physical compounds in the pond sediment.

Component	Unit	Values	Component	Unit	Value
C	%	2.363	Cd	mg/kg	0.01
N_{ts}	%	0.192	Ni	mg/kg	0.76
$P_{Dissolvable}$	mg/kg	183	pH		8.3
Cu	mg/kg	1.54	Moisture	%	62
Zn	mg/kg	11.98	Salinity	ppt	0
Pb	mg/kg	<0.01	Ratio C/N		12.3

In addition, the content of some heavy metals in the pond sediment ranged from 0.01 to 11.98 mg/kg, which was at an allowable range for agriculture soil according to QCVN 03: 2008/BTNMT. In particularly, Pb and Cd were very low, reaching a concentration of 0.01 mg/kg.

According to Mathur [6], the effectiveness of composting was achieved when C/N ratio ranged from 20 to 40. If the C/N ratio was very low, the nitrogen component might be lost in the form of NH_3 then reduce the quality of compost. If the C/N ratio was too high, the composting rate would reduce accordingly. In this study, the pond sediment has C/N ratio of 12.3 so it was not optimal condition for composting. Therefore, sawdust was added to provide sufficient carbon quantity for compositing.

3.2. Variation of temperature, pH and moisture during the composting

Temperature: The temperature was regularly monitored with a frequency of 5 days during the composting process. The results are displayed in Figure 1.

Under the composting processes, microorganisms grew up, decomposed organic matter and generated heat, so that the temperature in the pile also changed with the growth stage of the microorganisms. During the compositing processes, temperature of the compost piles increased rapidly during the first 20 days, from 25 to 55 °C, and then decreased to 35-40 °C. The highest temperature was observed at day 20. These results are in accordance with reports by Ryckeboer *et al.* [7] in which during organic decomposition under incompletely anaerobic or aerobic conditions, the temperature was 45-55 °C on day 3, and reached 70 °C on day 9, then remained consitantly until day 22. An increase in temperature was due to strong organic degradation of microorganisms, then it began to decrease and dropped to the ambient temperature.

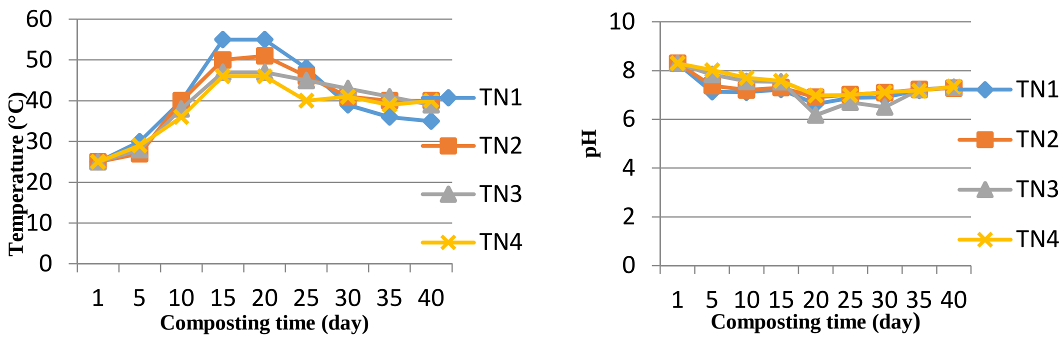

Figure 1. The change of temperature and pH by composting time.

On the other hand, the temperature variation of the experiments having formulas TN1, TN2, TN3, TN4 corresponded to four concentrations of antibiotics, NRFX: 0; 1.28; 2.86 and 4.28 ppm illustrated that in the first 15 days, the highest increase in the temperature was recorded in TN1 (30 °C), followed by TN2 (25 °C), TN3 (22 °C), TN4 (21 °C) and the temperature in TN1 reached the highest value (55°C). This result shows that the higher the concentration of antibiotic in the pond sediment was, the slower the decomposition of organic matter by the microorganisms would be, which might lead to a slow rise in the temperature and vice versa. This is due to the presence of antibiotics in the pile that prevented or killed some microorganisms by the antibiotics. Therefore, it leads to the decrease in the number of microorganisms or the slow growth. However, the temperature after 40 days was much higher than the ambient temperature (27 °C), which indicated that the microbial activities were still ongoing, or the organic decomposing process was not finished. The decomposition process reached the optimal value when the temperature of the compost piles tended to be equal or approximately with the ambient temperature [8].

pH: The pH values of the piles during the composting processes are presented in Figure 2. Results showed that pH of the piles after 40 days ranged from 6.2 to 8.3, which is considered as neutral value. During the first 5 days and from day 15 to day 20, the pH values decreased from 8 to 6. The reason was that in these two stages, the microorganism began to rapidly decompose the organic substances, which produced the organic acids and reduced the pH. In addition, higher variation of pH in the TN1 (zero antibiotic concentration) was observed as compared to that in TN2, TN3, TN4. This suggested that the activity of the microorganisms in the TN1 was stronger than in the other treatments. Because the antibiotics concentrations in TN2, TN3, and TN4 were higher than that of TN1, it prevented the growth of some microorganisms in the pile, so that the decomposing process was reduced.

Moisture: The moisture can affect growth rate and the metabolism of microorganisms. The results showed that the moisture of the piles fluctuated largely during 40 days of composting, in the range of 50.8–77.0 %. This moisture was suitable for microorganisms to grow. In general, moisture tended to decrease by composting times.

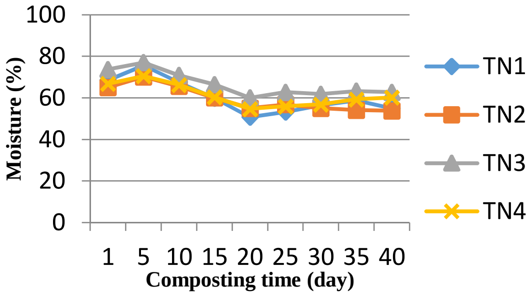

Figure 2: The variation in moisture by the composting times.

The observation conducted in the experiments with different antibiotics concentrations showed that the moisture increased in the first five days, TN1 (upto 7 %), TN2 (upto 5.1 %), TN3 (upto 3.3 %), and TN4 (up to 3.7 %). The main cause is that, during this period, the microorganisms began to adapt to the environmental conditions and started decomposing the organic matter that produced water and CO_2, so the moisture was increased.

3.3. Decomposition of Norfloxacin (NRFX) antibiotic during composting

The experiments were conducted at different initial concentrations of Norfloxacin (Table 1) of NRFX antibiotic concentrations of piles over time were shown in Figure 3.

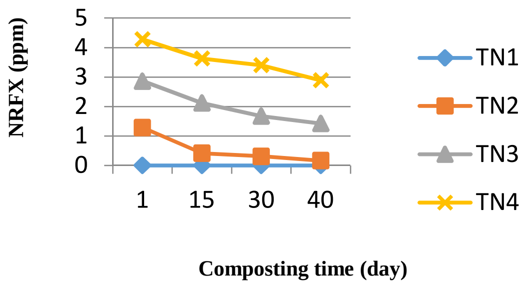

Figure 3. The variation in moisture by the composting times.

The results showed that the concentrations of NRFX antibiotic during 40 days of composting tended to decrease sharply. During the first 15 days, there was a large decrease in the concentration of antibiotics in all three experiments such as: TN2 (0.87 ppm), TN3 (0.74 ppm), TN4 (0.65 ppm). This finding can be explained by pointing out that this was the time when microorganisms grew sharply, and some bacteria were able to produce enzymes that degrade antibiotics. The reduction level of antibiotics in TN2, TN3, TN4 after 40 days of composting corresponded to 87.5, 50.3, and 32.5 %, respectively. Thus, the results indicated that under the same initial environmental conditions, the higher the antibiotic concentrations in the piles were, the slower the ability to break down the antibiotic was. Similar to the findings in study of Nemati et al. [9], this situation indicates that antibiotic depletion was closely related to the amount of microorganisms living in the piles, the better the organism grows, the faster the antibiotics were removed and vice versa.

3.4. Effects of salinity and pH on ciprofloxacin (CPFX) during composting process

a) Effect of salinity: Salinity and ciprofloxacin concentrations were monitored by taking samples on day 1, 7, 14, and 20 of composting processes. The results are shown in Figure 4.

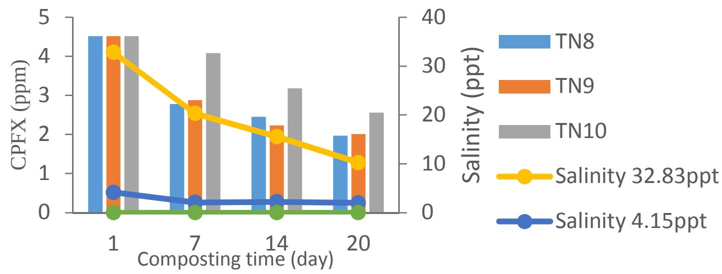

Figure 4. The variation in salinity and CPFX during composting processes.

Salinity tended to decrease rapidly during the first 20 days. The pile with salinity 32.83 ppt decreased more rapidly than that with salinity of 4.15 ppt. For the first 7 days, the reduction in salinity of the two piles was the fastest, from 4.15 to 2.08 ppt and from 32.83 to 20.3 ppt. Reduction of antibiotic concentrations in the compost piles was the same and dropped sharply in the first 7 days. CPFX concentrations reduced by 1.74; 1.64 and 0.44 ppm at salinity 0; 4.15 and 32.83 ppt, respectively. The findings indicate that high salinity will slow down the decomposition of CPFX. CPFX removal efficiency of the experiments reached 56, 55 and 43 % with initial CPFX content of 0; 4.15 and 32.83 ppt, respectively.

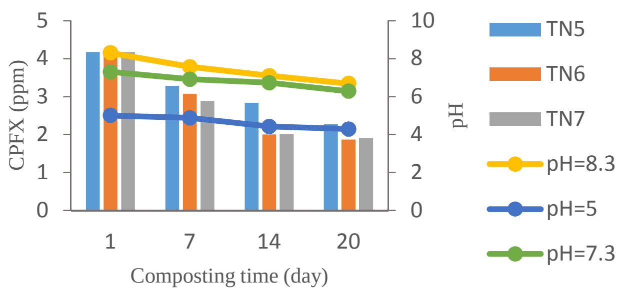

Figure 5. The variation of pH and CPFX during the composting process.

b) Effect of pH: During the first 20 days, pH of all piles tended to decrease. pH value of the experiments at pH at 8.3, 5, and 7.3 dropped by 1.6, 0.7, and 1.0, respectively (Figure 5). In contrast, the piles with pH at 5 obtained the slowest rate. The main cause for that situation is that the microorganism grew rapidly in the pH ranging from 5.5 to 8.5. At these values, the microorganisms broke down the most organic matters and produced a lot of organic acids, so the pH dropped sharply.

In addition, CPFX concentrations at a wide range of pH values tended to decrease. The decline in pH value was the highest during the first week of observation such as pH = 8.3 (reduced by 1.28 ppm), pH = 5 (reduced by 0.89 ppm), and pH = 7.3 (reduced by 1.1 ppm). The abilities to remove antibiotic in case of pH = 8.3; 5 and 7.3 were 54; 45 and 55 %, respectively.

3.5. Characterisation of compost

After 40 days of composting, the compost mixture is dark brown and odorless. Moisture of the mixture significantly decreased in comparison with the original, the height of the compost pile went down from 2 to 3 cm. HC ranged from 15.7 to 18.8 %, (Table 4) which did not meet organic fertilizer quality standards (≥ 20 %), however these meet quality standard for mineral-organic compost. Also, nitrogen content and C/N ratio both meet mineral-organic fertilizer quality standards regulated by the Ministry of Agriculture and Rural Development.

Table 4. Compost characteristics after 40 days.

Parameters	TN1	TN2	TN3	TN4	Mineral-organic compost quality standard (TT 41/2014 MARD)
HC (%)	15.7	16.3	18.3	18.8	≥ 15
N_{ts} (%)	2.1	2.01	2.15	2.05	≥ 2
C/N Ratio	7.5	8.1	8.5	9.17	< 12 (organic compost)

4. CONCLUSIONS

The shrimp pond sediment has high moisture (about 80 %) and low concentration of some heavy metals. During the composting processes, the temperature varied and ranged from 25 to 55 °C, peaked at day 20th. pH varied from 6.2 to 8.3. The moisture of the piles fluctuated remarkably during 40 days, from 50.8 to 77 %. After 40 days of composting, NRFX concentrations significantly reduced to the range of 32.5 - 87.5 %. The decline of CPFX concentrations after 20 days decreased differently depending on the initial salinity and pH. The removal efficiency of CPFX antibiotics were found to be better in alkaline pH, reached 54, 45 and 55 % with pH of 8.3; 5.0; 7.3, respectively.

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