Treatment of piggery wastewater through struvite precipitation and nitrogen removal bacteria and poly-phosphate bacteria (in-pots experiment)

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Abstract— *Piggery wastewater is a type of wastewater which contains large amounts of nitrogen and phosphorus, therefore it needed to be treated before releasing to directly to the environment. The combination between struvite precipitation and nitrogen removal and poly-P bacteria into wastewater for piggery wastewater treatment has been found to be a cost-effective practice, a iable technology in terms of environmental protection and sustainability, especially in the developing-countries. For optimum struvite crystallization from piggery wastewater, the Mg:PO4 molar ratio as (1.2:1) was used, the pH of reaction was adjusted to 9 and the sample was stirred continuously during 40 minutes. The supernatant sample was then added 1% nitrogen removal bacteria (Pseudomonas stutzeri D3b strain) and 1% poly-P bacteria (Kurthia sp. TGT1013L strain), 5 g glucose/L and aeration 12/24h during 3 days, ammonium concentration reduced significantly from 1271 mg/L to 1.2 mg/L and orthophosphate concentration decreased noticeably from 24.91 mg/L to 16.1 mg/L.*

***Keywords—* *ammonium, bacteria, orthophosphate, pH, piggery wastewater, struvite precipitation*.**

I. INTRODUCTION

Wastewaters contain a high amount of organic matter, nitrogen and phosphorus [1], a considerable amount of Mg [2], different macro and micro elements [3], and heavy metals [4,5,6] due to which it is considered as one of the major polluting agents discharged into the environment. The livestock waste stream is therefore, very rich in phosphorus. A significant amount of nitrogen comes out through excreta as a residue of protein supplement as well as dead animals. Improper management of livestock waste creates a nuisance and obnoxious environment which also greatly affect on public health. Some forms of nitrogen (ammonia, nitrite, and nitrate) and phosphorus (orthophosphate and monophosphate) produce toxicity in the water and affect on aquatic life. It is obligatory to remove nitrogen and phosphorus from wastewaters before discharging it into the water stream to create an eco-friendly, pollution-free environment. So, many countries are paying attention to water pollution resulted from wastewaters, and have tightened legislation and discharging standards.

Great efforts have been done by researchers for the removal of nitrogen from wastewater through biological nitrification and denitrification [7], ammonia-stripping [8], electrochemical conversion [9], ion exchange [6][10], microwave irradiation 11] and struvite precipitation [12].

Wastewater that contains high concentration of N, and P is an effective source of struvite recovery. In recent years, struvite has been recovered from different types of wastewaters, such as swine wastewater [1][2][13][14], calf manure wastewater 15], leather tanning wastewater [16], sewage sludge [17], dairy wastewater [18], wasted sludge [19], digester supernatant [20][21], industrial wastewater [22], municipal land fill leachate [23], lagoon wastewater [24], poultry manure wastewater [25], agro-industrial wastes [26], slaughterhouse wastewater [27], anaerobic digester effluents [28], synthetic wastewater [29], slurry type swine wastewater [23], animal manure [30], urine [31] and fertilizer plant wastewater [32]. Recently, struvite has been produced from wastewaters using microbial fuel cells by Ichihashi and Hirooka [33]. Struvite is a crystalline substance consisting of magnesium, ammonium and phosphorus in equal molar concentrations ($MgNH_4PO_4$.$6H_2O$). The crystals form in an alkaline condition according to the reaction shown below [34].

$$Mg_2^{+} + NH_4^{+} + H_2PO_4 + 6H_2O \longrightarrow MgNH_4PO_4^{-} + 6H_2O + 2H_2$$

This study investigates the following steps: (1) struvite pretreatment of raw piggery wastewater (after biogas), (2) applying nitrogen removal bacteria and poly-P bacteria to remove N and P out wastewater. The objectives of this study were: (1) to investigate the effects of pH and molar ratios for magnesium, and phosphate ions on ammonia N and phosphate P removal from raw piggery wastewater, (2) to apply nitrogen removal bacteria and poly-P bacteria to enhance the waste disposal process.

II. MATERIAL AND METHOD

The composition of raw piggery wastewater used in this study (collected from Pig farm, Binh Minh dist., Vinh Long province, Vietnam) was presented in Table 1.

TABLE 1
THE MAJOR COMPOSITION OF RAW PIGGERY WASTEWATER

Content	Concentration
pH	7.8
TSS (mg/L)	332
NH_4^+ (mg/L)	1271
PO_4^{3-} (mg/L) BOD_5 (mg/L)	50 640

2.1 Chemicals

Magnesium sulphate octahydrate ($MgSO_4.7H_2O$) and Calcium dihydrogen phosphate $Ca(HPO_4)2.H_2O$ were employed as magnesium and phosphate sources, respectively. pH was adjusted using 10 and 6 M sodium hydroxide, as well as concentrated sulfuric acid (98%). All chemicals used in the study are analytical grade.

2.2 Struvite precipitation

The initial precipitation experiments for optimization were carried out in 1-L beakers. Precipitation was initiated with the addition of the desired amount of $MgSO_4.7H_2O$ and $Ca(HPO_4)2.H_2O$ at stoichiometric ratios with pH adjusted by using NaOH. Mixing with the use of magnetic stirrer was continued until a steady pH. During MAP reaction, the pH of samples was adjusted to desired values by adding gradually 6N or 10N NaOH. Subsequent to this, the mixtures were allowed to settle for 30 min and the supernatant samples were collected for ammonium and orthophosphates analyses. Chemicals were added into piggery wastewater to receive struvite, applying to equation as follows:

$$m = (n - a) \times M$$

with

- m is weigh of chemicals adding into landfill leachate ($MgSO_4.7H_2O$ and $Ca(H_2PO_4)2.H_2O$)
- n (nmol) of mol NH_4^+ in 1 litre landfill leachate
- a (nmol) of mol Mg^{2+} (or PO_4^{3-}) in 1 litre landfill leachate
- M is block molecule of $MgSO_4.7H_2O$ and $Ca(H_2PO_4)2.H_2O$

2.3 Effects of pH, ratio of mol Mg and PO_4 and stirring time on struvite crystallization

Exp1. The pH treatments were estimated at 8, 9, 10 while control was not adjusted pH. The Mg^{2+}:NH_4^+:PO_4^{3-} molar ratio was controlled at 1:1:1 . Each treatment replicated 3 times and the volume of reaction was 0.5 L piggery wastewater.

Exp.2. Based on the result of preliminary test (Exp. 1), the subsequence was then carried out at the optimum pH. Five ratios of mol (Mg^{2+}:NH_4^+:PO_4^{3-}) ratio of 1:1:1, 1.2:1:1, 1.5:1:1, 1:1:1.2 and 1:1:1.5 were estimated with three replications, each replication was 1 beaker 1-L containing 0.5 L piggery wastewater.

Exp. 3. Optimal stiring time (0, 10, 20, 40, 60, 80, 100 and 120 min) was conducted with three replications, and each replication was a beaker 1-L containing 0.05 L piggery wastewater.

The objective of these three experiments was not only the highest struvite precipitation but also removed ammonium and orthophosphate out of piggery wastewater.

Piggery wastewater samples after precipitation process above were filtered through filter paper (Ø11 cm, Hangzhou Special Paper Industry Co. Ltd, Zhejiand, China) and the supernatant samples were analysed ammonium and orthophosphate concentration at Advanced Lab., Can Tho University, Vietnam.

2.4 Application of nitrogen removal bacteria and poly-P bacteria in piggery wastewater treatment

After experiment 3, the supernatants (of piggery wastewater) were analysed ammonia and orthophosphate concentration, and they were then added nitrogen removal bacteria (*Pseudomonas stutzeri* D3b strain) [35] and poly-P bacteria (*Kurthia* sp. TGT013L strain) [36] (1.0%), glucose (5 g/L), aeration with different times (6, 12, 18 and 24/24 h). The experiment was completely randomized design with 3 replications, the experiment consisted of 6 treatments as follows:

1. T1: control [without bacteria and aeration],
2. T2: piggery wastewater [after withdrawal struvite] without bacteria and aeration,
3. T3: T2 applied D3b + TGT13L + aeration 6/24h
4. T4: T2 applied D3b + TGT13L + aeration 12/24h
5. T5: T2 applied D3b + TGT13L + aeration 18/24h
6. T6: T2 applied D3b + TGT13L + aeration 24/24h

Each treatment was one 2-L plastic container containing 1L piggery wastewater

The result from the above experiment was done with 10-L bigger plastic container containing 5 litres piggery wastewater and the experiment with only two treatments as control and optimal treatment were conducted with 3 replications.

2.5 Analytical methods

NH_4^+-N (Colometric method or Phenol nitroprusside method) [37], COD, BOD, Orthophosphate (Colormetric method) and pH (pH meter) were determined by Advanced Analyses Laboratory, Can Tho University, Viet Nam.

III. RESULTS AND DISCUSSIONS

In Table 2 showed that the optimization of struvite precipitation for pretreatment of the raw piggerry wastewater was pH 9 (21.17 g/L) and at pH 9 obtained at the theoretical stoichiometric ratio of Mg^{2+}:NH_4^+:PO_4^{3-} ratio of 1.2:1:1 the highest struvite precititation (26.34 g/L).

TABLE 2
EFFECTS OF pH AND MG:PO_4 ON AMOUNT OF STRUVITE CRYSTALLIZATION

Treatment	g/L	Treatment	g/L
Control*	19.57	1 mol Mg : 1 mol PO_4	32.71
pH=8	31.57	1.2 mol Mg : 1 mol PO_4	34.47
pH=9	**32.03**	1.5 mol Mg : 1 mol PO_4	35.20
pH=10	29.97	1 mol Mg : 1.2 mol PO_4	37.47
LSD.01	**1.70**	1 mol Mg : 1.5 mol PO_4	**44.37**
C.V (%)	**2.20**	**LSD.01**	**0.88**
		C.V (%)	**0.89**

After 20 minute stiring, 1.773 g struvite was formed from 50 ml piggery wastewater and this stirring time was the best in comparison with others or the optimal stirring time for struvie formation (Figure 1)

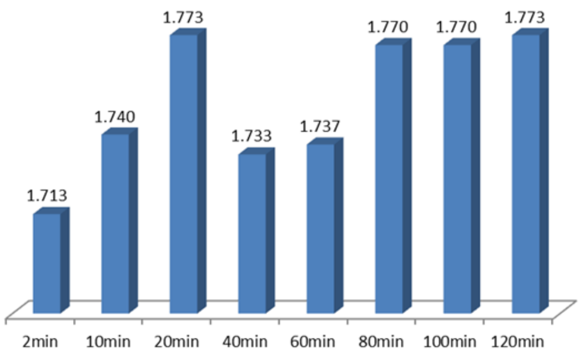


FIGURE 1. EFFECTS OF STIRRING TIME (min) ON STRUVITE FORMATION (g/50 mL PIGGERY WASTEWATER)

Over 80.0% of ammonium (509 mg/L NH_4-N) and orthophosphate (169 mg/L) were removed at pH 9, with a residual concentration of 126.2 mg/L NH_4-N and 42.1 mg/L PO_4_P as depicted in Figure 2, thus indicated that pH 9 was the most suitable for struvite formation for the raw piggery wastewater under investigation. Similarly, molar ratio Mg:PO4 (1:1.2) was the best molar molecule to remove ammonium and orthophosphate in piggery wastewater (Figure 3).

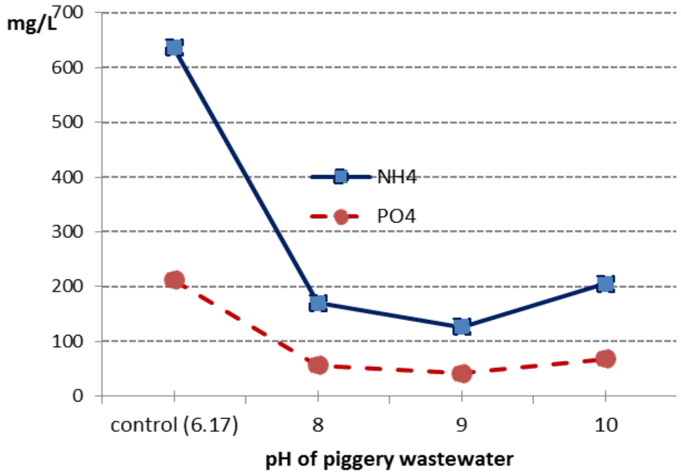


FIGURE 2. EFFECT OF pH ON NH_4^+-N and PO_4^{3-} REMOVAL DURING STRUVITE FORMATION

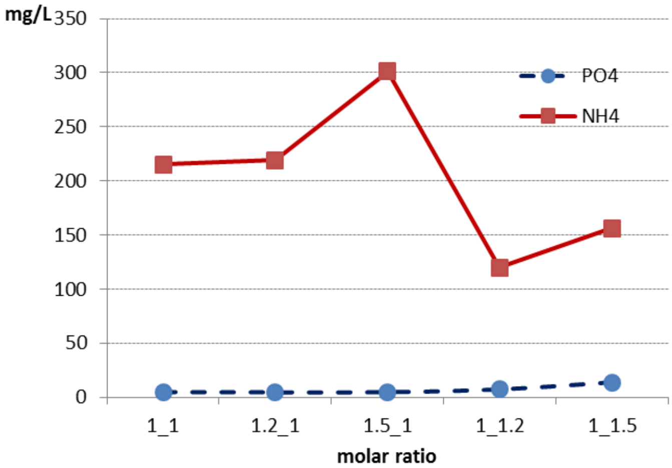


FIGURE 3. EFFECT OF MOL Mg:PO_4 on NH_4^+-N and PO_4^{3-} REMOVAL DURING STRUVITE FORMATION

From the above results (Table 2, Figure 2, Figure 3), pH 9, mol Mg:PO_4 (1:1.2:1) were chosen the optimal conditions for sturvite formation and the low ammonium and orthophosphate in piggery wastewater.

In experiment 3, the piggery wastewater was adjusted at pH 9 and mol Mg:PO_4 (1:1.2:1), the stiring time or aeration began, the result from Figure 4 showed that ammonium concentration reduced to 10-min and ammonium concentration increased to 70 mg/L at 40-min, after the this time ammonium reduced to the time (to 120-min) but orthophosphate concentration in piggery wastewater enhanced from 7.93 mg/L to 9.96 mg/L at 40-min and orthophosphate concentration reduced slowly to 120-min. At 100-min and 120-min, ammonium concentration in piggery wastewater reduced the lowest (3.93 and 1.41 mg/L NH_4-N) perhaps ammonium in wastewater was escaped to the air by the stirrer however struvite formation at all the times was not difference (Figure 1). To save energy for electricity, 10-min was choose in this experiment because at this time low ammonium and orthophosphate concentration in wastewater.

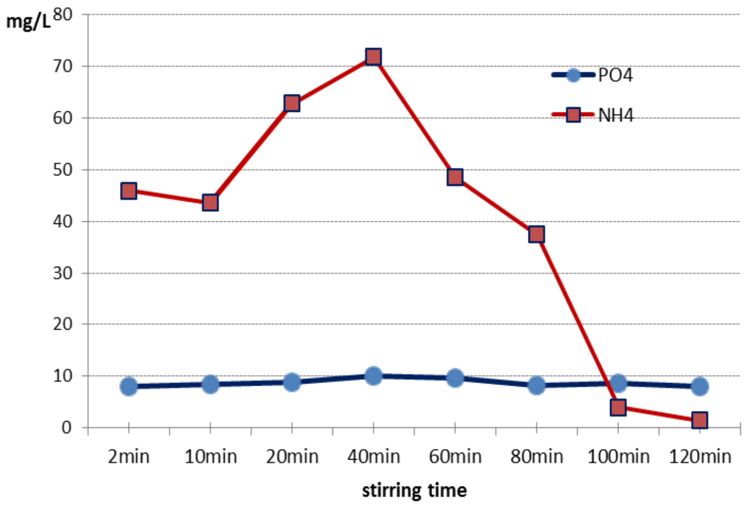
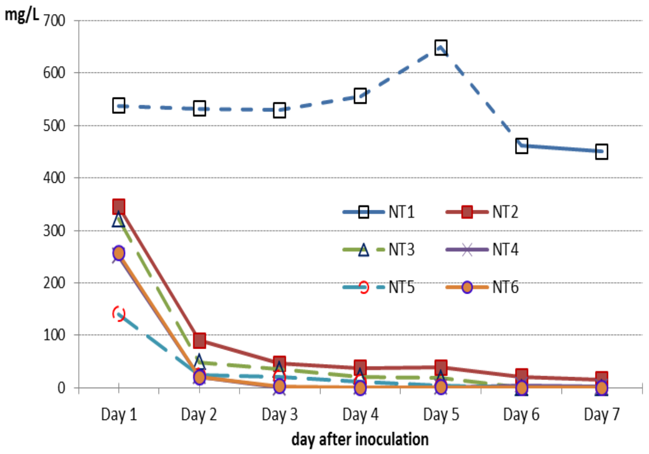


FIGURE 4. EFFECT OF STIRRING TIME ON NH_4^+-N and PO_4^{3-} REMOVAL CONCENTRATION

3.1 Effects of nitrogen removal bacteria and poly-P bacteria in piggery wastewater treatment

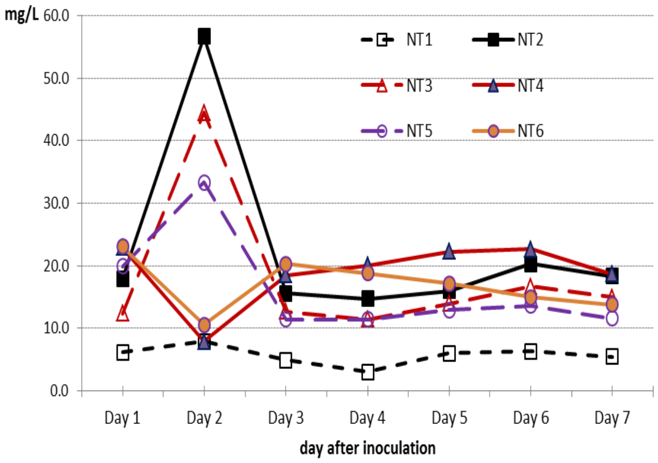
In experiment 1-L, application of nitrogen removal bacteria (D3b strain) and poly-P bacteria (TGT013L strain) into piggery wastewater reduced ammonium concentration to the time however aeration 12/24 h (NT4) reduced ammonium concentration at day 3 and saved energy (only aeration 12/24 h compared to 18/24 or 24/24 h) (Figure 5).



NT1: control
NT2: piggery wastewater (evicted struvite)
NT3: NT2 + N removed and poly-P bacteria + 6 g glucose/l + aeration 6/24 h
NT4: NT3 but aeration 12/24 h
NT5: NT3 but aeration 18/24 h
NT6: NT3 but aeration 24/24 h

FIGURE 5. EFFECTS OF NITROGEN REMOVAL BACTERIA AND POLY-P BACTERIA ON AMMONIUM CONCENTRATION IN PIGGERY WASTEWATER

However, orthophosphate concentration in piggery wastewater of treatments (NT4 and NT6) reduced from day 1 to day 3 after that PO_4 concentration induced to 18 and 20 mg/L PO_4/L perhaps aeration increased PO_4 level in piggery wastewater, and PO_4 concentration of treatment 2 (NT2), NT3, and NT4 increased in day 2 after that decreased from day 3 to day 6, especially control treatment had orthophosphate concentration lower during 6 days, this demonstrated that aeration supported to increasing PO_4 in wastewater. Herewith day 3 reduced ammonium concentration (Figure 5), day 3 was chosen to low PO_4 concentration (Figure 6) for experiment 5L.



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NT4: NT3 but aeration 12/24 h
NT5: NT3 but aeration 18/24 h
NT6: NT3 but aeration 24/24 h

FIGURE 6. EFFECTS OF NITROGEN REMOVAL BACTERIA AND POLY-P BACTERIA ON ORTHOPHOSPHATE CONCENTRATION IN PIGGERY WASTEWATER

Struvite crystallization process is an effective eco-friendly process that removes and recovers P and N from wastewaters. The hazardous elements in wastewaters (mainly NH_4 and PO_4) might be converted to a valuable resource through this process. So, the optimization of Mg molar ratio, pH level, aeration rate, reaction time and temperature would enhance the quality as well as production.

The pH plays an important role during the struvite precipitation process. Struvite or MAP can be precipitated at a wide range of pH (7.0–11.5), but the suitable pH ranges between 7.5 to 9.0 [38]. Efficiency of MAP precipitation depends on the concentration and molar ratios of $Mg_2^{+,}$ NH_+^{4} , & PO_4^{3-} , pH, aeration rate, temperature, and presence of Ca^{2+} in the reacting media [25][38][39][40].

It is found that a wide range of PO_4 and Mg ratio was applied for struvite precipitation, but in most cases, the effective ratio was 1:1 or 1:1.2 (Rahman *et al*., 2011)[12]. The addition of chemicals to the wastewaters would be needed to provide an equimolecular condition of PO_4 and Mg. Yetilmezsoy and Zengin [25] conducted a series of experiments to see the effect of Mg, NH_4 and PO_4 ratio on struvite precipitation and nitrogen removal efficiency.

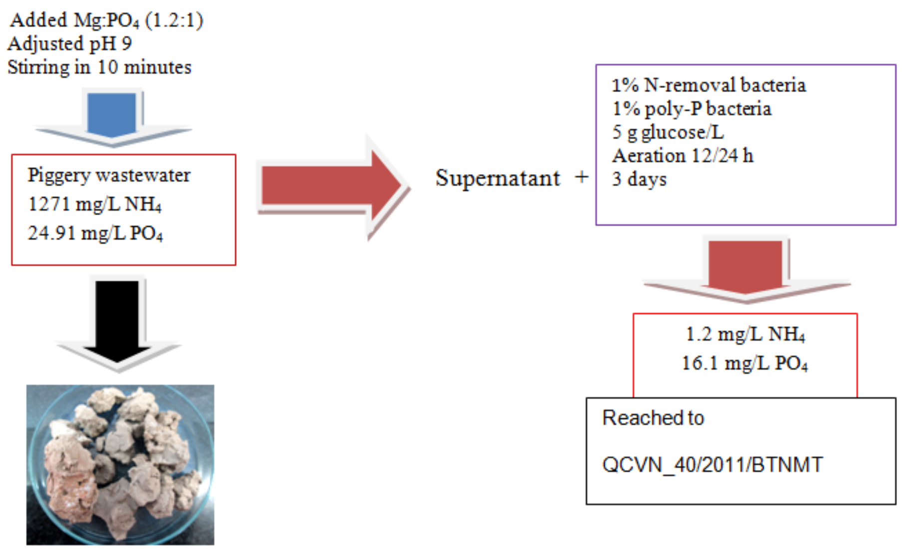
Air flow plays an important role in the removal of NH_4-N from the solution. Air flow agitates the solution and creates a removal pathway for dissolved ammonia to volatilize from the solution. Moreover, air flow dilutes the concentration of gasphase NH_4-N and increase the driving force for dissolving NH_4-N to separate the gaseous phase. So, there is an increasing tendency for ammonia volatilization with increasing airflow rate. Yetilmezsoy and Zengin [25] stated that a sufficient aeration time should be provided to achieve high removal efficiencies. They obtained about 93.4% NH_4-N removal with an aeration rate of 0.6 L min^{-1} within a period of 24 h. They also found the highest NH_4-N removal (95.3%) in 12 h reaction time with an aeration rate of 10 L min^{-1}. Lei *et al*. [41]found about 60.2% ammonia removal with an aeration rate of 0.6 L min 1 in a reaction time of four hours. On the contrary, they achieved the same removal efficiency without aeration in a period of 24 h. Liu *et al*. [5][6]found that struvite formation is proportional to the aeration rate and reached a plateau at around 0.73 L min^{-1}.

Pseudomonas stutzeri strain D3b was isolated from in wastewater of catfish fish-ponds in the Mekong Delta and its application for wastewater treatment effectively [35]. Application of *Pseudomonas stutzeri* D3b strain and *Acinetobacter lwoffii* TN7 strain to remove ammonia in wastewater of biowaste was carried out to evaluate their ability of ammonia removal at different concentrations with and without aeration condition in laboratory; The results showed that these species had ammonia removal ability effectively at both 50 mg/l and 100 mg/l ammonia. *Pseudomonas stutzeri* strain D3b and *Acinetobacter lwoffii* strain TN7 are the best bacterial species to remove ammonia. Besides that, both of species removed ammonia in aerobic condition better than anaerobic condition. In three days, the ammonia removal efficiency of *Pseudomonas stutzeri* D3b were 97.2% and 98.57% and *Acinetobacter lwoffii* TN7 were 96.32% and 98.31% in 50 mg/l and 100 mg/l ammonia concentrations in wastewater of biowaste, respectively [42]. Polyphosphate accumulating organisms (PAOs) is known as the microorganisms to absorb free phosphate in the environment and assimilate them as intracellular polyphosphate (poly-P) particles. This process was viewed as enhanced biological phosphorus removal (EBPR) in wastewater treatment systems [43][44][45]. Khoi *et al*. [36] applied *Kurthia* sp. TGT013L to remove orthophosphate in wastewater effectively.

IV. CONCLUSION

Production of struvite from wastewaters will reduce the hazard of eutrophication in the water bodies by removing N and P. Production of struvite from wastewater and its utilization as fertilizer would partially help to reduce global warming and thus, it would be an effective eco-friendly fertilizer.

Treatment of piggery wastewater consisting of struvite eviction and removal of nitrogen and phosphate using nitrogen removal bacteria and poly-P bacteria were high effectiveness and low cost with process as follows:



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