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

Tran Vu Phuong[1], Nguyen Thi Xuan My[2], Pham Nhat Truong[3], Cao Ngoc Diep[4]

Department of Microbiology Biotechnology, Can Tho University, Vietnam

Abstract— *Landfill leacheate 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 landfill leachate treatment has been found to be a cost-effective practive, a viable technology in terms of environmental protection and sustainability, especially in the developing-countries. For optimum struvite crystallization from landfill leachate, the Mg:PO_4 molar ratio as (1.2:1) was used, the pH of reaction was adjusted to 9 and the sample was stirred continously 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 1076 mg/L to 1.5 mg/L and orthophosphate concentration decreased noticeably from 24.91 mg/L to 7.6 mg/L.*

Keywords— ammonium, bacteria, landfill leachate, orthophosphate, pH, struvite precipitation.

I. INTRODUCTION

Wastewater is usually hazardous to human populations and the environment and must be treated prior to disposal into streams, lakes, seas, and land surfaces [1]. Obligatory anaerobic treatment of domestic and agro-industrial wastewater releases large amounts of phosphorus and nitrogen into wastewater. These nutrients are directly responsible for eutrophication (extraordinary growth of algae as a result of excess nutrients in water bodies) of rivers, lakes, and seas worldwide [2, 3]. Consequently, disposal of wastewaters produces a constant threat to dwindling fresh water on a global scale [4].

Landfill leachate treatment is an important issue of the waste management system in municipal areas [5, 6, 7]. Their quantity and quality depends on a number of factors: the type of deposited wastes as well as the age of the landfill and the phase of waste decomposition. The leachates withdrawn from landfill in methanogenic phase (methanogenic leachates) are characterized by high nitrogen load and large amount of refractory organic compounds with a high chemical oxygen demand/biochemical oxygen demand (COD/BOD) ratio [7]. Landfill leachate that is collected and removed from a landfill must be managed in a suitable manner. This involves some type of treatment process whether on or off-site. The various methods and technologies tested, applied and proposed for the treatment of landfill leachate range from the recirculation of leachate through the landfill to the more sophisticated combination of physical, chemical and biological processes [8, 9, 10, 11,12]. Magnesium ammonium phosphate (MAP) precipitation is a physical/chemical technique [7, 5, 13], which produces $MgNH_4PO_4.6H_2O$ by the precipitation of magnesium, ammonium and phosphate under alkaline conditions. This precipitation method is advantageous due to its ability to effectively precipitate NH_4-N from wastewaters forming easily settleable insoluble compounds, which have a potential as a binding material in cement [14].

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

The most promising compound for recovery from wastewater plants is magnesium ammonium phosphate hexahydrate ($MgNH_4PO_4.6H_2O$), commonly known as struvite, which precipitates spontaneously in some wastewater processes [15, 16, 17]. If formation and collection are controlled and cost-effective, struvite might have potential in the fertilizer market. Struvite precipitates spontaneously in wastewater treatment environments where high concentrations of soluble phosphorus and ammonium are present. Additional essential conditions are low concentration of suspended solids and pH above 7.5. Precipitation of struvite requires that its components are available simultaneously in the wastewater in the molecular ratio 1(Mg^{2+}):1(NH_4^{+}):1(PO_4^{3}). Normally, municipal wastewater and several other wastewaters tend to be rich in ammonium, but deficient in magnesium, so supplementation of magnesium is required, and this helps to increase solution pH [18, 19, 20, 21, 22] . Similarly, addition of magnesium chloride or bittern, a low-cost magnesium, forced precipitation of phosphorus and reduced the concentration of soluble phosphorus in swine waste within a 10-min reaction time [23, 24] . The pH can also be elevated by adding NaOH, an expensive process [16], by air stripping [25], where aeration of wastewater removes CO_2 and pH increases in the process [26] or by ion using phosphate-selective sorbents [27] . This process was viewed as enhanced biological phosphorus removal (EBPR) in wastewater treatment systems [28, 29, 30].

This study investigates the following steps: (1) struvite pretreatment of raw landfill leachate, (2) applying nitrogen removal bacteria and poly-P bacteria to remove N and P out leachate. With 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 landfill leachate, (2) to apply nitrogen removal bacteria and poly-P bacteria to enhance the waste disposal process.

II. MATERIAL AND METHOD

2.1 Raw landfill leachate

The composition of raw landfill leachate used in this study (collected from Kinh Cung landfill, Phung Hiep dist., Hau Giang province, Vietnam) was presented in Table 1.

TABLE 1
THE MAJOR COMPOSITION OF RAW PIGGERY WASTEWATER

Content	Concentration
pH	8.79
TSS (mg/L)	890 (very dark)
TN (mg/L)	392
TP (mg/L)	23.3
NH_4^+ (mg/L)	1076
PO_4^{3-} (mg/L)	24.9

2.2 Chemicals

Magnesium sulphate hexahydrate ($MgSO_4.7H_2O$) and calcium dihydrogen phosphate $Ca(H_2PO_4).2H_2O$ 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.

2.3 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 $Ca(H_2PO_4).2H_2O$ 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 landfill leachate 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 $Ca(H_2PO_4)2.H_2O$

2.4 Effects of pH, ratio of mol Mg and PO4 and stirring time on struvite crystallization

Exp.1. 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 landfill leachate

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 landfill leachate.

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 landfill leachate.

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

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

2.5 Application of nitrogen removal bacteria and poly-P bacteria in landfill leachate treatment

After experiment 3, the supernatants (of landfill leachate) were analysed ammonia and orthophosphate concentration, and they were then added nitrogen removal bacteria (*Pseudomonas stutzeri* D3b strain) [31] and poly-P bacteria (*Kurthia* sp. TGT013L strain) [32] (0.5%), glucose (5 g/L), aerotion with different times (6, 12, 18 and 24/24 h). The experiment was completely randomized design with 3 replications, the experiment constited of 6 treatments as follows:

1. T1: control [without bacteria and aeration],
2. T2: landfill leachate [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 containter containing 1L landfill leachate

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

2.6 Analytical methods

NH_4^+-N (Colometric method or Phenol nitroprusside method) [33], COD, BOD, Orthophosphate (Colormetric method) and pH (pH meter) were determined by Advanced Analyses Laboratory, Can Tho University, Viet Nam.

III. RESULTS AND DISCUSSIONS

3.1 Effect of pH on NH_4^+-N and PO_4_P removal during struvite precipitation

In table 2 showed that the optimization of struvite precipitation for pretreatment of the raw landfill leachate 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*	12.43	1.0 mol Mg : 1 mol PO_4	22.52
pH=8	20.96	1.2 mol Mg : 1 mol PO_4	**26.34**
pH=9	**21.17**	1.5 mol Mg : 1 mol PO_4	21.30
pH=10	20.02	1 mol Mg : 1.2 mol PO_4	22.45
LSD.01	**1.49**	1.5 mol Mg : 1 mol PO_4	26.29
C.V (%)	**2.92**	**LSD.01**	**0.98**
		C.V (%)	**1.20**

After 40 minute stiring, 1.080 g struvite was formed from 50 ml landfill leacheate and this stirring time was the best in comparison with others or the optimal stirring time for struvie formation even through there was no difference between eight treatment significantly (Figure 1). The combination between ($MgSO_4.7H_2O$) and $Ca(H_2PO_4).2H_2O$ with NH_4 in landfill leachate happened slowly from 2 minute to 40 minute furthermore at 40 minute, ammonium concentration reduced lowest (Figure 4).

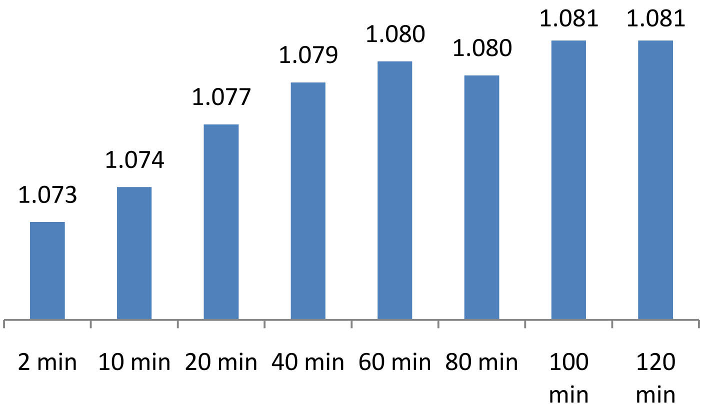


FIGURE 1. Effects of stirring time (min) on struvite formation (g/50 mL landfill leacheate)

Over 70.0% of ammonium and orthophosphate were removed at pH 9, with a residual concentration of 278 mg/L NH_4-N and 5.96 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 landfill leachate under investigation.

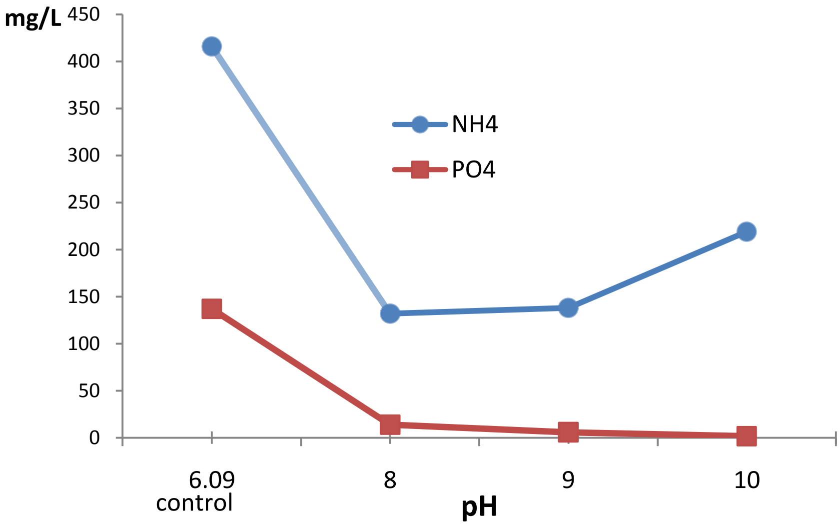


FIGURE 2. Effect of pH on NH_4^+-N and PO_4^{3-} removal during struvite formation

Similarly, molar ratio Mg:PO4 (1:1.2) was the best mol molecule to remove ammonium and orthophosphate in landfill leachate (Fugure 3).

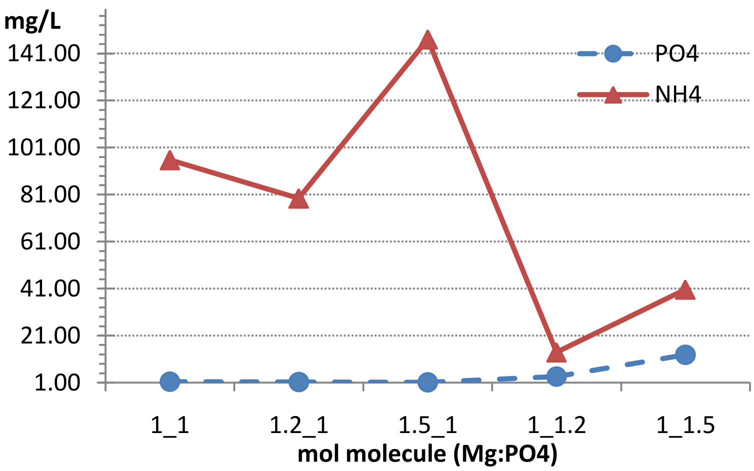


FIGURE 3. Effect of mol Mg:PO_4 on NH_4^+-N and PO_4^{3-} removal during struvite formation

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 lowest ammonium and orthophosphate in landfill leachate.

In experiment 3, the landfill leachate 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 the time but orthophosphate concentration in landfill leachate reduced to 40 minute and after that orthophsphate concentration increased to 120 minute, however struvite formation at all the times was not difference (Figure 1).

3.2 Effects of nitrogen removal bacteria and poly-P bacteria in landfill leachate treatment

In experiment 1-L, application of nitrogen removal bacteria (D3b strain) and poly-P bacteria (TGT013L strain) into landfill leachate 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).

Similarly, orthophosphate concentration in landfill leachate induced from day 1 to day 4 after that PO_4 concentration reduced perhaps aeration increased PO_4 level in landfill leachate, and PO_4 concentration of treatment 3 (NT3) decreased at day 5, day 6. Herewith day 3 reduced ammonium concentration (Figure 5), day 3 was chosen to low PO_4 concentration (Figure 6) for experiment 5L.

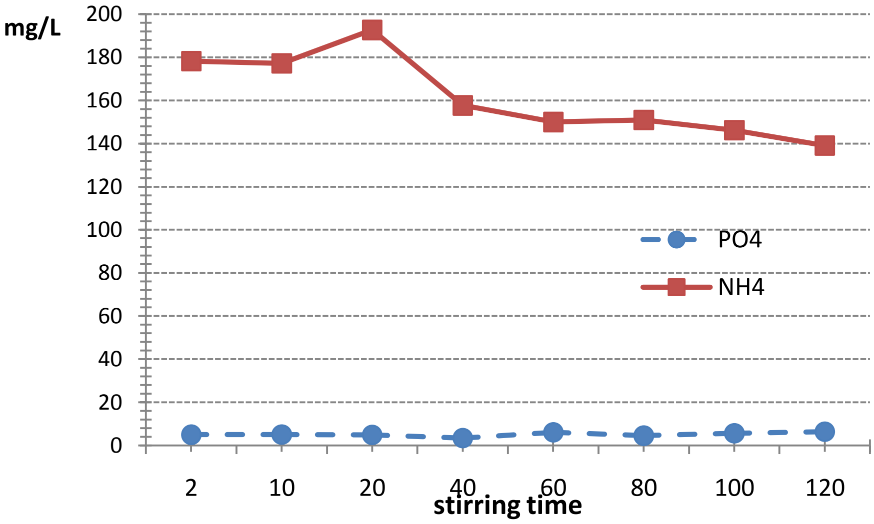
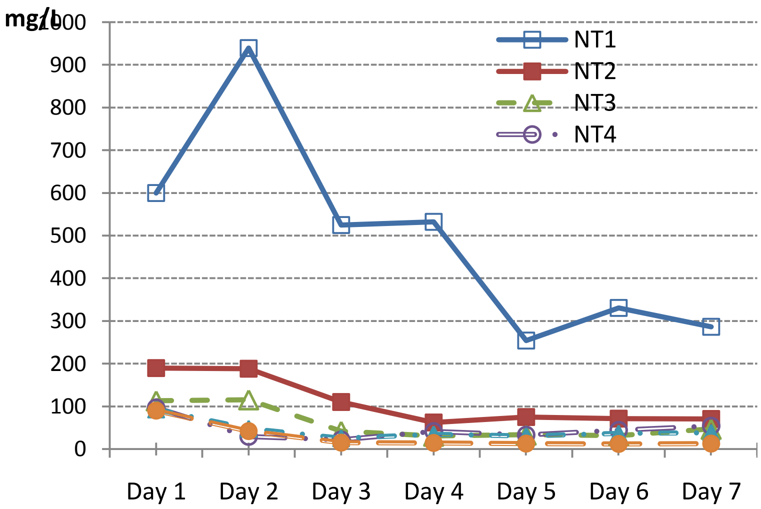


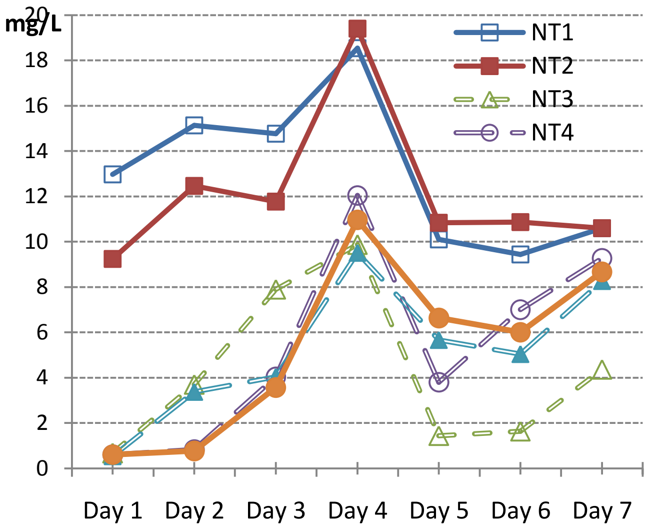
FIGURE 4. Effect of stirring time on NH_4^+-N and PO_4^{3-} removal concentration



NT1: control
NT2: landfill leacheate (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 landfill leachate

From the above results, in the experiment 5L, the experiment only two treatments: control (landfill leachate) without treatment and landfill leacheate applied Mg:PO_4 (1:1.2:1), pH 9, and stiring time in 40 minutes. Supernatant was appplied nitrogen removal bacteria and poly-P bacteria plus 5 glucose/L and aeration 12/24h in 3 days and the results as follows: 362.6 mg/L ammonia reduced to 1.5 mg/L (reached to A table, QC40:2011/BTNMT) and 9.6 mg/L PO_4^{3-} reduced to 7.6 mg/L (B table is 6 mg/L) however wastewater was irrigated in pond with *Lemma* sp., after 3 days ammonium and orthophosphate concentration in landfill leachate disappeared.



NT1: control
NT2: landfill leacheate (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 6. Effects of nitrogen removal bacteria and poly-P bacteria on orthophosphate concentration in landfill leachate

From the above results, in the experiment 5L, the experiment only two treatments: control (landfill leachate) without treatment and landfill leacheate applied Mg:PO_4 (1:1.2:1), pH 9, and stiring time in 40 minutes. Supernatant was appplied nitrogen removal bacteria and poly-P bacteria plus 5 glucose/L and aeration 12/24h in 3 days and the results as follows: 362.6 mg/L ammonia reduced to 1.5 mg/L (reached to A table, QC40:2011/BTNMT) and 9.6 mg/L PO_4^{3-} reduced to 7.6 mg/L (B table is 6 mg/L) however wastewater was irrigated in pond with *Lemma* sp., after 3 days ammonium and orthophosphate concentration in landfill leachate disappeared.

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 [34]. 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 [35][34][16][36]. 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)[37]. The addition of chemicals to the wastewaters would be needed to provide an equimolecular condition of PO_4 and Mg. Yetilmezsoy and Zengin [35] conducted a series of experiments to see the effect of Mg, NH_4 and PO_4 ratio on struvite precipitation and nitrogen removal efficiency.

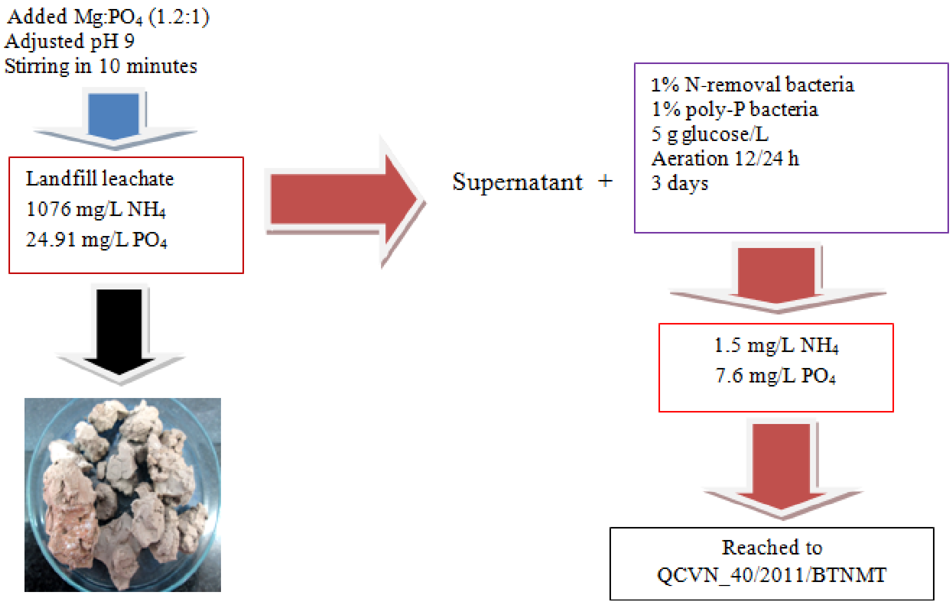
Yetilmezsoy and Zengin [35] stated that a sufficient aeration time should be provided to achieve high removal efficiencies. They obtained about 93.4% NH_4-N removal with an aeration rate of 0.6 L min^{-1} within a period of 24 h. They also found the highest NH_4-N removal (95.3%) in 12 h reaction time with an aeration rate of 10 L min^{-1}. Lei *et al*. [38]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*. [39][40]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 treatement effectively [31]. 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

[41]. 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 [28, 29, 30]. Khoi *et al*. [32] 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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