Start-up of simultaneous nitrification, anammox and denitrification-sequential batch reactor (SNAD-SBR) for treating anaerobic palm oil mill (POME) Digester Effluent

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Abstract. Simultaneous Nitrification, Anammox, and Denitrification (SNAD) which was discovered around 2008 has become a promising alternative to conventional nitrogen treatment process. The increasing number of researches on SNAD can be seen lately from lab-scale experiment to full-scale operation around the world. However, due to complexity of mutual relationship between three different bacteria, more researches are needed to be done to have better understanding of SNAD on real-scale application. This study is aimed to examine the performance of SNAD on treating effluent from anaerobic digester of palm oil mill effluent. The study will discuss cultivation of anammox, completion of SNAD process, and the feasibility of SNAD on effluent from anaerobic digester. The result of this study will help in explore further application of SNAD besides treating nitrogen-rich wastewater.

1. Introduction
In wastewater stream, both nitrogen and carbon should be removed before discharge into water bodies to prevent pollution which could harm aquatic life and destroy ecology balance. The main nitrogen compound in wastewater is ammonium, which is usually oxidized by ammonium-oxidizing bacteria (AOB) aerobically into nitrite, and oxidized further by nitrite-oxidizing bacteria (NOB) into nitrate. Nitrite and nitrate can be reduced into nitrogen gas (N₂) by denitrifying bacteria (DNB) using organic matter as carbon source [1]. Thus, oxygen and organic carbon is needed to complete the process of nitrification and denitrification. However, nitrogen-rich wastewater is usually coupled with low organic carbon content. Therefore, it is not sustainable in term of cost and treatment efficiency to treat low carbon-to-nitrogen (C/N) ratio wastewater with conventional nitrification and denitrification process [2]. Anammox process was discovered and believed to be a sustainable alternative due to its low energy requirement [3]. In anammox reaction, ammonia act as electron donor and nitrite act as electron accepter and produce nitrogen gas and nitrate. Absence of oxygen in anammox process save the cost for aeration, which solve the problem of high energy consumption in conventional treatment method. At present, most researches targeting nitrogen-rich wastewater are mainly based on anammox
process, with the well-known process namely completely autotrophic nitrogen-removal over nitrite (CANON) process[4] and single reactor system for high activity ammonium removal over nitrite (SHARON) process[5]. However, these processes are not practical for real wastewater because of absence in nitrite, thus SNAD was discovered to complete the overall treatment process without the necessity to add in nitrite into wastewater.

\[
0.5\text{NH}_4^+ + \text{HCO}_3^- + 0.75\text{O}_2 \rightarrow 0.5\text{NO}_2^- + \text{CO}_2 + 1.5\text{H}_2\text{O} \quad (1)
\]

\[
\text{NH}_4^+ + 1.32\text{NO}_2^- + 0.066\text{HCO}_3^- + 0.13\text{H}^+ \rightarrow 1.02\text{N}_2 + 0.26\text{NO}_3^- + 0.066\text{CH}_2\text{O}_{0.5}\text{N}_{0.15} + 2.03\text{H}_2\text{O} \quad (2)
\]

\[
\text{NO}_3^- + 1.08\text{CH}_3\text{OH} + 0.24\text{H}_2\text{CO}_3 \rightarrow 0.47\text{N}_2 + \text{HCO}_3^- + 0.056\text{C}_5\text{H}_7\text{O}_2\text{N} + 1.68\text{H}_2\text{O} \quad (3)
\]

SNAD process shows the possibility of treating ammonium from wastewater without additional source of oxygen and organic matter. The processes can be happened in single reactor, which are partial nitrification, anammox, and denitrification. In SNAD, partial amount of ammonium is converted into nitrite by AOB under limited oxygen environment as in equation (1). Then, remaining ammonium and nitrite are converted into nitrogen gas and small amount of nitrate by anammox bacteria as shown in equation (2). The last process, denitrification convert the nitrate into nitrogen gas by DNB by consuming organic carbon as energy source as illustrated in equation (3). These processes work simultaneously under same environment make the treatment of ammonium become more sustainable and cost-effective (figure 1).

![Figure 1. Interaction of bacteria in SNAD process [6]](Image)

In this study, SNAD in sequencing batch reactor (SBR) was used to remove ammoniacal-nitrogen from effluent of anaerobic digester treating POME. First, SNAD is developed from scratch by cultivating anammox from local leachate collection pond. Then it is mixed with heterotrophic DNB targeted to remove remaining nitrate. Finally, the SNAD is feed with real wastewater to examine its feasibility and sustainability. The results will provide a better understanding on application of SNAD in real wastewater treatment plant replacing conventional nitrification and denitrification process.
2. Materials and methods
Tertiary treatment of effluent from anaerobic digestion is carried out by using SNAD process instead of aerobic digestion to remove both ammonium and organic carbon. The treatment efficiency, interaction between processes, and stability of the system is examined.

2.1. Reactor configuration
Single reactor with approximately 1.5 L of effective volume is used (figure 2). Stirrer with 100 RPM was installed in the reactor, with pH and DO meter attached for daily monitoring. Temperature of the reactor was regulated by water jacket, to be maintained at around 35°C. Anammox seed was obtained from National Chiao Tung University Taiwan, which was frozen to restrain its reactivity. The seed was reactivated by feeding in diluted and conditioned landfill leachate. Additional seed also been cultivated from the same leachate wastewater, with sludge taken from the collection pond. The ammonia concentration of the leachate was 675 mg/L, while the nitrate was 4.7 mg/L with a pH of 7.98. The anammox was allowed to acclimate without further feeding for 30 days before synthetic wastewater is used in later stage. The operation of reactor was based on sequencing batch reactor (SBR). Each 24-hour cycle have assigned time for mixing, decanting, and idling. The decanting time was 30 minutes to prevent disposal of biomass with a slow pumping rate. The idle time was to allow biomass to settle before making sample collection. This is reported to have better removal efficiency [7] and more detailed analysis on the reaction.

![Figure 2. Schematic diagram of SBR used](image)

2.2. Analytical methods
Analysis of solids test including total solid (TS), suspended solid (SS), and volatile suspended solid (VSS) were done in accordance of Standard Methods for the Examinations of Water and Wastewater [8]. COD, NO₂⁻-N, NO₃⁻-N, and NH₄⁺-N were measured using HACH method, in which method 8000 for COD; 8505 for NO₂⁻; 8171 for NO₃⁻; and 10031 for NH₄⁺-N. Dissolved oxygen (DO) and pH was measured using YSI Pro1020 measuring system. Hach DRB200 was used as digester and Hach DR900 multiparameter portable colorimeter was used as measurement device.

2.3. Operational phases
The operation of reactor was based on sequencing batch reactor (SBR). Each 24-hours cycle was assigned time for feeding, mixing, decanting, and idling. The feeding and decanting time was 30
minutes to prevent disposal of biomass with a slow pumping rate. The idle time was to allow biomass to settle before making sample collection. The reactor was operated with two stages, mainly acclimatisation stage and start-up stage. Synthetic wastewater was used in acclimatisation stage, and modified anaerobic effluent was used during start-up stage. NH$_4^+$-N was supplied through (NH$_4$)$_2$SO$_4$, while COD is supplied through glucose.

3. Results and discussion

3.1. Acclimatisation stage

![Figure 3. Concentration of COD, ammonium, nitrate, and nitrite against time of the SNAD-SBR during first two stages](image)

**Figure 3.** Concentration of COD, ammonium, nitrate, and nitrite against time of the SNAD-SBR during first two stages

![Figure 4. DO and pH profile of SNAD-SBR during acclimatisation stage](image)

**Figure 4.** DO and pH profile of SNAD-SBR during acclimatisation stage
Anammox seed sludge was unfreeze slowly with cold water and later with lukewarm water before putting into reactor. Around 100 g of seed sludge was used. After four days staying stagnant in the reactor, the SNAD-SBR was activated with leachate from 5th to 30th day. This is to acclimate the anammox bacteria to the new environment including DO, pH, temperature, and wastewater characteristic. The leachate used was without any conditioning except pH to allow anammox bacteria to uptake and acclimate to previous environment. Figure 3 shows the removal of ammonia and COD was started from 40th day. SNAD was activated and performed first process as equation (1) which is partial nitrification, where partial amount of ammoniacal nitrogen was converted into nitrite. This can be seen from the increasing concentration of nitrite until it reached a maximum value of 34.2 mg NO$_2$-N. At the same time, anammox was activated when there was enough nitrite in the system according to equation (2). This was occurred when the concentration of ammoniacal nitrogen was reducing to the lowest of 2 mg/L NH$_4^+$-N. The result was slightly better compared to work of Casagrande et. al.[9], where they required 70 to 110 days to fully activated anammox process from swine wastewater treatment system. However, due to insufficient feed concentration of ammonium, the anammox bacteria had once again stop temporarily while denitrification as in equation (3) took place to reduce COD in the SNAD-SBR. This can be seen from the drastic reduction of COD concentration from 45th day to 60th day, in which approximately 600 mg/L COD was reduced half to around 300 mg COD/L. After that the COD reduction became slow which might be due to utilisation of available nutrients in the reactor. Accumulation of nitrate from 70th day showed that denitrifying process was performed slower and COD concentration was removed in a gradual trend. The result and time taken for the activation and acclimation stage is similar to the work of Xu et. al.[10], where they reached stable stage of SNAD process after 50 days, not taking account of 240 days of anammox cultivation from their real scale treatment plant. This showing it is easier and faster to start-up a reactor using seed sludge obtained from a healthy source than pure cultivation from treatment plant. Figure 4 shows the pH and DO profile of the SNAD-SBR. The pH was maintained in the range of pH 6-7. However, the pH was drop to a low of 5.81 which was unfavourable for SNAD-SBR, due to increment of DO in the system. The problem was found out to be improper sealing of the reactor thus allowed ambient air to enter when the concentration of oxygen in the reactor was low. Since denitrification and anammox are the two processes produce alkalinity to the reactor to maintain optimum pH [11], the spike of DO was causing the mentioned anaerobic processes to be slowed down and therefore fluctuation of pH occurred. Once the problem was rectified, the performance of SNAD-SBR returned to normal and pH was back to the optimum range.

4. Conclusion
SNAD is novel process derived from well-known anammox process which is believed to be better alternative to conventional nitrification and denitrification process. SNAD possess the advantage of anammox which is high nitrogen removal efficiency and low energy consumption, without the needed to supply additional organic carbon source for denitrification. Essential substrates are produced by each process and provides benefits for the other. In this study, SNAD-SBR was successfully activated and acclimated to treat anaerobic digester effluent in 100 days, starting from dormant state of anammox seed. It took 40 days to activate anammox process, and subsequently another 40 days to activate denitrification process. The removal rate of ammonium and COD by the reactor was 94% and 44% respectively by 100th day. This shows that SNAD can acclimate to different wastewater rather than most researches solely based on nitrogen-rich wastewater such as leachate and sewage. Besides, it provides an alternative to replace aerobic system in POME treatment system that has high energy consumption and high sludge production rate. More studies are required to examine the feasibility of SNAD treating other wastewater in long term operation, to accelerate the application of SNAD in different field and type of wastewater.
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