



# Advanced nitrogen removal from landfill leachate via Anammox system based on Sequencing Biofilm Batch Reactor (SBBR): Effective protection of biofilm



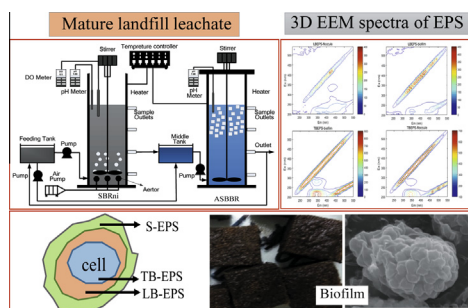
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## HIGHLIGHTS

- Anammox was enriched in carriers to enhance ability against adverse environment.
- As influent TN was 3000 mg/L, effluent TN was below 20 mg/L with TN removal of 95%.
- The ratio of  $\text{NO}_3^-:\text{NH}_4^+$  fell below 0.1 in Anammox process treating real leachate.
- TB-EPS of biofilm and floccule were 46% and 28%, respectively, helped form biofilm.
- Anammox gene ratio of biofilm increased to 13.28%, yet floccule decreased to 3.88%.

## GRAPHICAL ABSTRACT



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## ABSTRACT

High levels of organics negatively affect Anammox for treating landfill leachate. To enhance the ability of Anammox to survive against adverse environments, a lab-scale two-stage Anammox system using a Sequencing Biofilm Batch Reactor was applied to treat mature landfill leachate under 35 °C. Over 107 days, with influent total nitrogen (TN) and chemical oxygen demand (COD) concentrations of  $3000 \pm 100$  and  $3000 \pm 100$  mg/L, effluent TN was below 20 mg/L. For extracellular polymeric substance (EPS) of Anammox, slime-EPS and loosely-bound-EPS of floccules were both higher than biofilm, while tight-bound-EPS of biofilm was significantly higher, contributing to biofilm formation. Quantitative microbial analysis showed that as influent COD increased, Anammox gene ratios of biofilm increased from 1.34% to 13.28%; the gene ratios of floccule first increased, then decreased to 3.88%. This indicated that Anammox and heterotrophic bacteria could coexist because of the biofilm, leading to stable nitrogen removal performance, even when organics were present.

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## 1. Introduction

Landfill leachate is a kind of wastewater, containing large amounts of organics and inorganics, with a particularly high ammonia concentration (Renou et al., 2008). Landfill leachate

treatments are usually classified as physical/chemical and biological treatments (Hu et al., 2016; Renou et al., 2008). The physical/chemical treatment (e.g. coagulation-flocculation) is usually used as the pre-treatment or advanced treatment step due to its high cost and secondary pollution (Zhang et al., 2016). Biological processes, based on active sludge, are usually used to remove nitrogen and organics from the leachate due to their simplicity and cost-effectiveness (Zhu et al., 2013). For early-age or middle-age

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**Table 1**  
Experimental procedure.

Experimental phases	Influent composition of ASBBR	Substances concentrations of ASBBR influence (mg/L)		
		NH <sub>4</sub> <sup>+</sup>	NO <sub>2</sub> <sup>-</sup>	COD
Phase 1	Synthetic wastewater	500–1000	600–1400	–
Phase 2	The mixture of synthetic wastewater and effluent of SBRni	300–600	400–800	100–200
Phase 3	The mixture of raw mature landfill leachate and effluent of SBRni	200–350	250–450	500–1000
Phase 4	The mixture of raw mature landfill leachate and effluent of SBRni	250	300	800–1000

leachate, a conventional nitrification/denitrification process or modified process (such as the process coupling of two-stage up-flow anaerobic sludge bed (UASB) and anoxic/oxic reactor (A/O), or the endogenous denitrification process), is used to remove nitrogen and organics because of the high carbon to nitrogen (C/N) ratio (Peng et al., 2008; Miao et al., 2015). However, it is difficult to achieve high nitrogen removal from mature leachate with a low C/N ratio (usually <3), because the conventional nitrification/denitrification process requires additional external carbon sources. This requirement makes the treatment systems complicated and expensive (Kulikowska and Klimiuk, 2008).

Anaerobic ammonium oxidation (Anammox), is a novel cost-effective process with great potential (Mulder et al., 1995; Kartal et al., 2010). Compared with the conventional nitrification/denitrification process, Anammox does not need external carbon sources, and the power consumption for Anammox can be reduced substantially (Shi et al., 2013). Therefore, it can effectively treat mature landfill leachate (Anfruns et al., 2013). However, Anammox cannot work alone, because it requires nitrite substrate supplied by nitrification. As such, Anammox is usually combined with nitrification to treat wastewater. Nevertheless, some factors adversely affect Anammox, including high nitrite and organic concentrations.

For example, Strous et al. (1999) demonstrated that Anammox was inhibited by nitrite concentrations up to 100 mg/L. Thus, it is important to avoid feeding in a short time for Anammox process

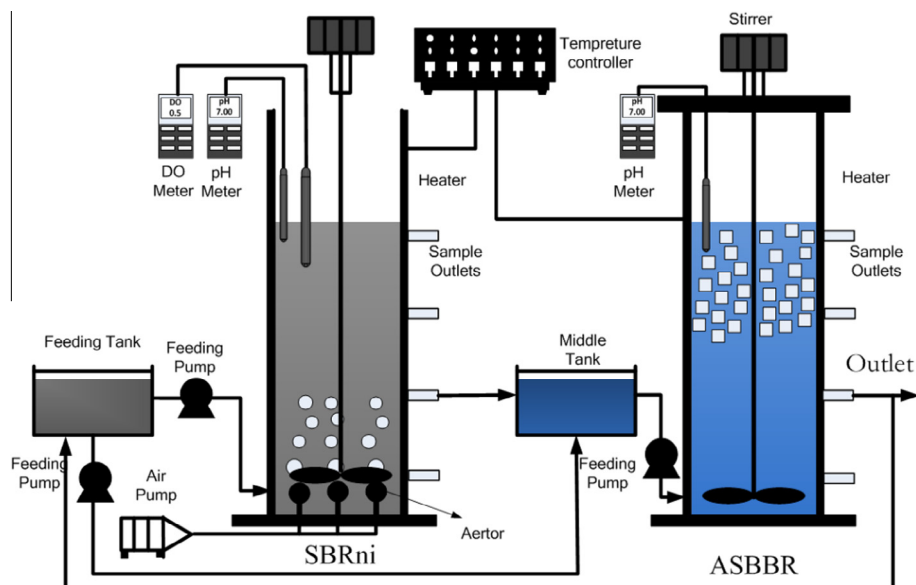
treating the wastewater with high substrate content. Further, organics can significantly influence Anammox (Tang et al., 2010). Molinuevo et al. (2009) observed that a COD concentration up to 292 mg/L could completely inhibit Anammox. Ni et al., 2012a,b studied artificial wastewater, finding that a low COD concentration did not affect nitrogen removal; however, a high COD concentration did suppress Anammox activity and reduced the bacteria population when the COD exceeded 400 mg/L. Miao et al. (2014) used a three-stage Anammox system to treat landfill leachate, finding that Anammox was not significantly inhibited when the biodegradable COD was less than 150 mg/L. Therefore, applying Anammox requires controlling the influent COD concentration. Furthermore, for Anammox to be effective in treating real wastewater, the system must make full use of the organics in the wastewater and enhance nitrogen removal performance through the synergy between Anammox and denitrification.

Because Anammox bacteria grow slowly, it is important to enrich the Anammox bacteria during the start-up of the Anammox system. Compared with the activated sludge process, the biofilm technology associated with Anammox covers a smaller footprint, and has more microbial populations (Van Hulle et al., 2010), a stronger impact resistance (Nicoletta et al., 2000; Han et al., 2012) (such as oxygen and high nitrite concentration), and a more stable ecological system when treating wastewater. In addition, biofilm systems are more efficient and more widely applicable in nitrogen removal systems than activated sludge (Tsushima et al., 2007; van der Star et al., 2007). Therefore, in this study a two-stage Sequencing Biofilm Batch Reactor (SBBR) system coupled with biofilm was applied to treat mature landfill leachate and achieve a fast start-up. The extracellular polymeric substances (EPS) and microbial population of the Anammox SBR during different phases were measured to assess microbiological changes.

## 2. Materials and methods

### 2.1. Experimental setup and operational procedure

The two-stage SBBR system used in this study, was comprised of a nitrification SBR (SBRni) and Anammox SBBR (ASBBR). The system was built using polymethyl methacrylate with a total working volume capacity of 20 L, divided into equal parts of 10 L each for



**Fig. 1.** Schematic diagram of two-stage SBBR process treating mature landfill leachate.

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