Optimization of Heterotrophic Denitrification Using Glycerol as a Sustainable External Carbon Substrate [†]

Sultan Salamah ^{1,*} and Andrew Randall ²

¹ Department of Civil Engineering, Taibah University, Madinah 42353, Saudi Arabia

² Department of Civil, Environmental and Construction Engineering, UCF, Orlando, FL 32816, USA; andrew.randall@ucf.edu

* Correspondence: szain@taibahu.edu.sa; Tel.: +966-583-866-619

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Abstract: Nutrients' removal from residential and industrial wastewaters is essential for environmental and public health protection. Removal of nutrients from wastewater can be achieved chemically or biologically. Biological nutrient removal (BNR) uses a series of anaerobic, anoxic, and aerobic zones to provide conditions for the biomass to uptake the nitrogen and phosphorus species and comes in different configurations such as A/O, A2O, and five-stage Bardenpho™. However, BNR systems require a sufficient carbon source which most wastewaters lack. The goal of this study is to use a sustainable carbon source to optimize the five-stage Bardenpho™ BNR systems and reduce the chemical cost. The experiments were carried out using two five-stage Bardenpho™ BNR systems coupled with side-stream prefermenters. Glycerol, a biodiesel by-product, was used as a sustainable carbon source by direct addition or after fermentation. The results from both systems were beneficial to the BNR system and resulted in similar effluent quality. Both systems achieved complete denitrification and excellent phosphorus removal (82–89%). Co-fermentation of glycerol and primary solids resulted in a significant increase in the volatile fatty acid (VFA) loading beyond the estimated results, but did not correlate to better behavior between the two pilots since both systems achieved complete denitrification.

Keywords: biological nutrient removal; denitrification; fermentation; glycerol; enhanced biological phosphorus removal; volatile fatty acids

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1. Introduction

Eutrophication and other environmental problems can be caused by municipal and industrial wastewater with poor nutrient removal. Nutrient removal can be achieved through chemical or biological processes. Biological nutrient removal (BNR) systems are widely used to control eutrophication by removing the nitrogen and phosphorus species from wastewater. Many BNR system configurations are widely used and well established such as A/O, A2O, University of Cape Town (UCT), and five-stage Bardenpho™ [1].

However, all of the BNR processes require a sufficient carbon source to allow for complete denitrification (removal of nitrogen forms) and enhanced biological phosphorus removal (EBPR), which most wastewaters lack [1–3]. Methanol, propionate, and acetate are usually used as an external carbon source to meet the system requirements [4,5].

Many studies have suggested that the driving force of EBPR is volatile fatty acids (VFAs), which can be produced through the fermentation process [6–8]. For example, a sequencing batch reactor

(SBR) experiment failed to provide biological nutrient removal due to the lack of VFAs. However, after adding prefermenters to the SBR system, 99% removal of nitrogen and phosphorus was achieved [9]. Another study to investigate the effect of prefermentation on wastewater nutrient removal was done on a pilot-scale UCT process in Singapore. The results showed better denitrification after starting the process prefermentation [4].

Alternative carbon sources have been studied as a carbon substrate to optimize nutrient removal in wastewater. Glycerol is a biodiesel major by-product that can be fermented to VFAs and potentially used as a carbon source. Biodiesel is considered a sustainable clean source of energy [10]. Unfortunately, glycerol, a major biodiesel by-product, is considered one of the primary limiting factors of full-scale biodiesel plants due to the handling and disposal cost [11]. Thus, using Glycerol as a cheap external carbon substrate to enhance biological nutrient removal can positively impact the sustainability rating of both wastewater treatment plants and biodiesel plants.

One of the first studies to use glycerol as a direct denitrification carbon substrate compared glycerol to the widely used methanol. The study concluded that glycerol achieved similar efficiency with a lower carbon/nitrogen ratio than methanol [12]. Many other studies showed that glycerol increased the specific denitrification rate (SDR) [3,13,14].

Some studies have concluded that direct glycerol addition is more favorable than fermented glycerol with regards to EBPR, but other studies showed the opposite is true [15,16]. The uncertainty in the literature about EBPR when adding glycerol directly could be a result of insufficient hydraulic retention time (HRT) in the anaerobic and aerobic zone. Guerrero and Tayà [16] studied the effect of anaerobic and aerobic reactors HRT on EBPR with direct addition of glycerol. The study concluded that optimum HRT for anaerobic and aerobic zones was 4 and 3.5 h respectively. The four hours anaerobic will cause partial degradation of glycerol to VFAs, which can be used by the polyphosphate accumulating organisms (PAOs) in the aerobic zone to remove phosphorus.

This study aims to optimize the five-stage Bardenpho™ BNR system using glycerol as a carbon source and to determine if fermented glycerol and/or direct glycerol addition were suitable external substrates for heterotrophic denitrification and EBPR.

Two identical five-stage Bardenpho™ pilot plants were used and named Pilot A and Pilot B. Both pilots were coupled with a side-stream prefermenter that flowed to the second anoxic zone. Both pilots were also operated identically, except for the location where the glycerol was added. In pilot A, the glycerol was added directly to the second anoxic zone. In pilot B, the glycerol was added to the prefermenter. The results concluded that glycerol is a suitable carbon substrate for both denitrification and EBPR when added directly or when fermented before adding.

2. Results

2.1. Prefermenters (PF)

Both systems were operated with a side-stream PF. The effluent of the PF was pumped into the second anoxic reactor (AXII) at a 2 L/day flowrate. Both Pilot A and Pilot B were not able to achieve equilibrium until the PF reactor was linked to the system. The amount of PF VFA loading allowed denitrification and EBPR to occur. The PF in Pilot B received the glycerol dose to be fermented to VFA before entering the system. Glycerol was added to the AXII directly in Pilot A to study the effect of direct addition. The prefermenter with co-fermentation of primary solids and glycerol had almost double the amount of total VFAs than the prefermenter with no glycerol, with 2469 and 1219 average mg COD/L respectively. Acetic acid to propionic acid ratios were 0.26 and 0.83 in PFA and PFB (with glycerol), respectively. Butyric acid in PFA was below the detection limit and in PFB was about 27%.

2.2. Denitrification

In general, both systems achieved complete denitrification ($\text{mg-N/L} < 1$). The PF effluent entering the AXII caused a slight increase in ammonia (NH_3) concentration, but this was oxidized in the second aerobic reactor (AEII). The nitrate recycle (NARCY) caused an increase in the nitrate + nitrite (NO_x) concentration. In the first anoxic reactor (AXI), Pilot A did not achieve complete

denitrification (1.5 mg NO_x-N/L) but Pilot B did (0.7 mg NO_x-N/L). Pilot A had a lower specific denitrification rate (DNR) (0.046 gNO_x-N/g VSS-d) than Pilot B (0.054 gNO_x-N/g VSS-d). Total nitrogen (TN) removal efficiency in Pilot B was slightly higher than in Pilot A, at 95% and 92% respectively.

2.3. EBPR

EBPR was functional in both Pilot A (direct glycerol addition) and Pilot B (fermented glycerol). Average soluble ortho-phosphate (SOP) removal in Pilot A and Pilot B was 82% and 89%, respectively. Pilot A and Pilot B had a similar P uptake/release ratio of 1.19. The SOP release/VFA in Pilot A and Pilot B was 0.48 and 0.27 (mg/L-P)/(mg-COD/L), respectively. The lower SOP release and SOP release/VFA in Pilot B could imply that claims about direct glycerol addition being harder for phosphorus-accumulating bacteria (PAOs) to accumulate than VFAs are not true. However, both pilots had similar SOP removal efficiency and did not show any sign of deterioration or failure during the time of the experiment.

2.4. Other Parameters

The effluent total-COD, soluble COD, total suspended solids (TSS), and pH for both systems were very close at an average of 43, 34, 8.0, and 7.7 respectively. No sign of significant pH fluctuation or solids accumulation was observed for the duration of the experiment. Average COD removal in Pilot A was 92%, and it was 91% in Pilot B. The observed yield in Pilot A was about 24% higher than in Pilot B, even though both were operated at 10-day SRT.

3. Discussion

The two identical five-stage Bardenpho™ pilot plants A and B were designed to test the effect of glycerol direct addition and after fermentation in the prefermenters on the overall behavior of the activated sludge system. In the acclimation stage, both pilots failed to reach sufficient denitrification and EBPR, and that was due to insufficient VFAs in the raw wastewater. This is almost the exact observation in many of the studies in the literature [2,3]. Adding the side-stream prefermenters increased the VFA loading and allowed biological nutrient removal to be carried out, similar to the finding of some previous studies [9,17].

Co-fermentation of glycerol and primary solids resulted in a significant increase in VFA concentration when compared with fermentation of primary solids alone. This supports that glycerol can be easily fermented to VFAs [10]. The non-complete denitrification in the AXI of Pilot A (direct glycerol) and the lower DNR could partially contradict the previous finding in the literature that adding glycerol directly will result in higher specific denitrification rate [3,13,14]. However, the results are not conclusive, since both pilots achieved complete denitrification in the AXII. With regards to EBPR, having slightly higher SOP release and 25% higher SOP release/VFA ratio does not favor the idea of VFAs being easier to convert to polycyclic aromatic hydrocarbons (PHAs) by the PAOs than pure glycerol [15,16].

Both locations where glycerol was added (direct to AXII vs. PF) resulted in sufficient EBPR and complete denitrification, which means that using glycerol directly or after fermentation has a minimal effect on the overall behavior of the five-stage Bardenpho™ system with a side-stream prefermenter. Since both pilots had a complete denitrification, the actual capacities of the systems are still unknown, and this creates potential for future research.

4. Materials and Methods

4.1. Source of Materials

Screened (1/4 inch-mesh) 400-L raw wastewater was collected from Iron Bridge Wastewater Reclamation Facility (Oviedo, Florida) on a daily basis. A weekly screened (1/4 inch-mesh) 10 gallons of primary solids was collected from Glendale Wastewater Treatment Plant (Lakeland, Florida) and

stored at 4 °C. Fisher Scientific (Tampa, FL, USA) glycerol (99.5% pure) was used in this study to minimize the effect of crude glycerol impurities.

4.2. The Pilot Plant

The pilots (A and B) were constructed and kept at the Iron Bridge Wastewater Reclamation Facility (Oviedo, Florida) for the whole duration of the experiment. Each pilot is consisted of a five-stage Bardenpho™ BNR pilot system (anaerobic, anoxic I, aerobic I, anoxic II, and aerobic II), a 10-L side stream prefermenter (PF), a 3.1-L secondary clarifier, and A 100-L effluent tank. Both pilots received influent from a 400-L influent tank that was cleaned and filled on a daily basis with the screened raw wastewater. Anaerobic, anoxic I, anoxic II, and PF reactors were mixed using a stable 50-rpm mixers. The aerobic I and aerobic II were mixed and aerated using adjustable air pumps with stone disks. The secondary clarifier was fitted with a 1.1-rpm skimmer. Both a 200% nitrate recycle (NARCY) and 50% return activated sludge (RAS) cycles were maintained for the duration of the experiment using flexible tubes and peristaltic pumps. Waste activated sludge (WAS) was maintained for a 10-day solid retention time (SRT) in both pilots.

In Pilot A, a 4270 mg COD/day glycerol dose was pumped to the second anoxic reactor (AXII), while the same glycerol dose was pumped to the prefermenter in Pilot B. Eight comprehensive sampling events were done in 120 days including one-month acclimation period. Figure 1 and Tables 1 and 2 show the experimental schematics, some design and operational information, and combined influent characteristics.

Figure 1. Experimental schematics for Pilot A and Pilot B.

Table 1. Design and operational volume and HRT.

Reactor	Volume (V)	HRT (h)
AN	3.6	1.6
AX I	5.9	2.6
AE I	18	7.8
AX II	3.3	1.4
AE II	0.8	0.3
Total	31.6	13.7
Clarifier	3.1	1.4
PF	10	5

AN = anaerobic; AX =anoxic; AE = aerobic; PF = prefermenter.

Table 2. Combined influent characteristics.

		Combined Influent **	
		Pilot A	Pilot B
TN		53.5	53.3
NO _x	mg-N/L	* 0	* 0
NH ₃		39.0	36.6
TP		6.0	5.8
SOP	mg-P/L	4.7	4.3
TSS		287	270
s-COD	mg/L	265	226
TCOD		393	400
VFA	mg-COD/L	44.0	88.3
DO	mg/L	0.08	
PH		7.5 ± 0.3	

* below detection limit; ** influent + PF effluent.

4.3. Analytical Techniques

Samples were collected on a weekly basis from all the reactors (main-stream, side-stream, clarifier, influent tank, and effluent tank). In each location, the sample was collected in two 50 mL clean bottles. One bottle was filtered immediately on site with a glass fiber filter, and the other was not filtered. All chemical analysis was performed in accordance with the Standard Methods for the Examination of Water and Wastewater [18]. VFA samples were analyzed using a gas chromatograph.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

BNR	Biological nutrient removal
EBPR	Enhanced biological phosphorus removal
HRT	Hydraulic retention time
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
NARCY	Nitrate recycle
NH ₃	Ammonia
NO _x	Nitrate + nitrite
PAOs	Polyphosphate accumulating organisms
RAS	Return activated sludge
SDR	Specific denitrification rate
SOP	Ortho-Phosphorus
SRT	Solid retention time
VFA	Volatile fatty acid

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