Optimization of Hetrotrophic Denitrification Using Glycerol as a Sustainable External Carbon Substrate

Sultan Salamah [1,*], Andrew Randall [2]

[1] Department of Civil Engineering, Taibah University, Madinah, KSA
[2] Department of Civil, Environmental and Construction Engineering, UCD, USA; andrew.randall@ucf.edu
* Correspondence: szain@taibahu.edu.sa; Tel.: +966-583-866-619

Abstract: Nutrients removal from residential and industrial wastewaters are essential for environmental and public health protection. Removal of nutrients from wastewater can be achieved chemically or biologically. Biological nutrient removal (BNR) is 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 5-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 5-stage Bardenpho™ BNR systems and reduce the chemical cost. The experiments were carried out using two 5-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 acids (VFAs) 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.

PACS: J0101

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 5-stage Bardenpho™ [1].

However, all of the BNR processes requires 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 requirement [4, 5].

Many studies suggested that the driving force of EBPR is volatile fatty acids (VFAs) which can be produced through 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 nitrogen and phosphorus was achieved [9]. Another study to invistigate 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].

Altirnative carbon sources are 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 potinially 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]. So, using Glycerol as a cheap external carbon substrate to enhance biological nutrient removal can positively impact sustainability rating of both wastewater treatment plants and biodiesel plants.

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

Some studies concluded that direct glycerol addition was 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, Tayà [16] studied the effect of anaerobic and aerobic reactors HRT on the EBPR with direct addition of glycerol. The study concluded that optimum HRT for anaerobic and aerobic zones was 4 and 3.5 hours 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 5-stage Bardenpho™ BNR system using glycerol as a carbon source and to determine if fermented glycerol or direct glycerol were suitable external substrates for heterotrophic denitrification and EBPR.

Two identical 5-stage Bardenpho™ pilot plants were used and named Pilot A and Pilot B. Both pilots were coupled with a side-stream prefermenters that flows to the second anoxic zone. Both pilots were also operated identically except for the location were 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 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 VFAs loading allowed the denitrification and EBPR to accur. The PF in Pilot B received the glycerol dose to be fermented to VFAs 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 duple 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 ratio of 0.26 and 0.83 in PFA and PFB (with glycerol) respectively. Butyric acid in PFA was bellow detection limit and in PFB was about 27%.

2.2. Denitrification

In general, both systems achieved complete denitrification (mg-N/L < 1). The PF effluent entering the AXII caused a slight increase in ammonia (NH_3) concentration but was oxidized in the second aerobic reactor (AEII). The nitrate recycle (NARCY) caused an increase in the Nitrate + Nitrite (NOx) concentration. In the first anoxic reactor (AXI), Pilot A did not achieve complete denitrification (1.5 mg NOx-N/L) but Pilot B did (0.7 mg NOx-N/L). Pilot A had a lower specific denitrification rate (DNR) (0.046 gNOx-N/g VSS-d) than Pilot B (0.054 gNOx-N/g VSS-d). Total nitrogen (TN) removal efficiency in Pilot B was slightly higher than Pilot A with 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 releaseand SOP release/VFA in Pilot B could imply that claims about direct glycerol addition is harder for the phosphorus accumulating bacteria (PAOs) to accumulate than VFAs is not true. However, bot 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 91% in Pilot B. The observed yield in Pilot A was about 24% higher that Pilot B even though both were operated at 10 day SRT.

3. Discussion

The two identical 5-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 acclamation 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 VFAs 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 VFAs concentration when compared with fermentation of primary solids alone. This support 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 that adding glycerol directly will result in higher specific denitrification rate [3, 13, 14]. But, 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 5-stage Bardenpho™ systems with a side-stream prefermenter. Since both pilots had a compleat denitrification, the acual capacity of the systems are still unknown and this is a potinial for future research.

4. Materials and Methods

4.1. Source of Materials

A 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 gallon primary solids was collected from Glendale Wastewater Treatment Plant (Lakeland, Florida) and stored at 4°C. A Fisher Scientific (Tampa, FL) 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 Iron Bridge Wastewater Reclamation Facility (Oviedo, Florida) for the whole duration of the expermint. Each pilot is consisted of a 5-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 the PF reactors were mixed using a stable 50 rpm mixers. The aerobic I and aerobic II were mixed and areated using adjustable air pumps with stone disks. The secondary clarifier was fitted with a 1.1 rpm skimmer. A 200% nitrate recycle (NARCY) and 50% return activated sludge (RAS) recycles 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. Fig1, Table1 and Table2 shows the experimental schematics, some design and operational information, and combined influent characteristics.

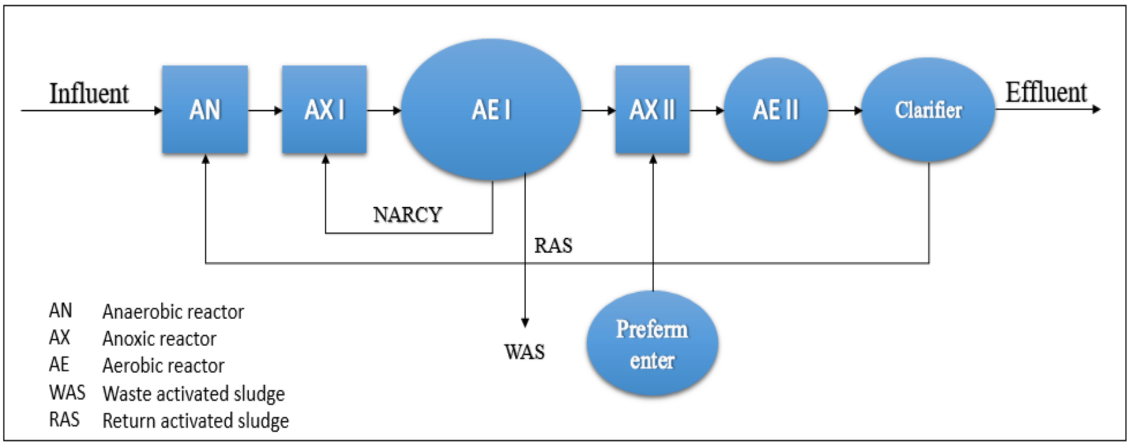

Figure 1. Experimental Schematics for Pilot A and Pilot B.

Table 1. Design and operational volume and HRT.

Reactor	Volume (V)	HRT (hours)
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	mg-N/L	53.5	53.3
NOx		*0	*0

NH3		39.0	36.6
TP	mg-P/L	6.0	5.8
SOP		4.7	4.3
TSS	mg/L	287	270
s-COD		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
** influential + PF effluent

4.3. Analytical Techniques

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

Acknowledgments: The authors wish to thank the City of Orlando's Wastewater Division and the Staff at the Iron Bridge Regional Water Reclamation Facility and the city of Lakeland, Florida, Glendale Wastewater Treatment Plant. This work was funded by SAKAL LLC, a United States subsidiary of Grupo Lakas SA and Taibah University, KSA.

Author Contributions: Both S.S. and A.R. had equal contribution in this study and were involved in reading the literature, formulating the methodology, analyzing the data, and writing the paper.

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
NH3 :	Ammonia
NOx:	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

References

1. Metcalf&Eddy, *Wastewater engineering: treatment and resource recovery*. 2014, New York: McGraw-Hill Education.
2. Wu, C.-Y., et al., Effect of carbon source on biological nitrogen and phosphorus removal in an anaerobic-anoxic-oxic (A2O) process. *Journal of Environmental Engineering*, **2010**. *136*(11): p. 1248-1254.

3. Bernat, K., D. Kulikowska, and M. Godlewski, Crude glycerol as a carbon source at a low COD/N ratio provides efficient and stable denitritation. *Desalination and Water Treatment*, **2016**. *57*(42): p. 19632-19641.
4. Shen, N. and Y. Zhou, Enhanced biological phosphorus removal with different carbon sources. *Applied Microbiology and Biotechnology*, **2016**. *100*(11): p. 4735-4745.
5. Ahmed, Z., et al., Biological nitrogen and phosphorus removal and changes in microbial community structure in a membrane bioreactor: Effect of different carbon sources. *Water Research*, **2008**. *42*(1–2): p. 198-210.
6. Lopez-Vazquez, C.M., et al., Modeling the PAO–GAO competition: Effects of carbon source, pH and temperature. *Water Research*, **2009**. *43*(2): p. 450-462.
7. Oehmen, A., et al., Anaerobic metabolism of propionate by polyphosphate-accumulating organisms in enhanced biological phosphorus removal systems. *Biotechnology and Bioengineering*, **2005**. *91*(1): p. 43-53.
8. Chen, Y., A.A. Randall, and T. McCue, The efficiency of enhanced biological phosphorus removal from real wastewater affected by different ratios of acetic to propionic acid. *Water Research*, **2004**. *38*(1): p. 27-36.
9. Merzouki, M., et al., Effect of prefermentation on denitrifying phosphorus removal in slaughterhouse wastewater. *Bioresource Technology*, **2005**. *96*(12): p. 1317-1322.
10. Eguchi, S., S. Kagawa, and S. Okamoto, Environmental and economic performance of a biodiesel plant using waste cooking oil. *Journal of Cleaner Production*, **2015**. *101*: p. 245-250.
11. Demirbas, A., *Biodiesel*. 2008, London, UK: Springer.
12. Grabińska-ńoniewska, A., T. Słomczyński, and Z. Kańska, Denitrification studies with glycerol as a carbon source. *Water Research*, **1985**. *19*(12): p. 1471-1477.
13. Torà, J.A., et al., Denitritation of a high-strength nitrite wastewater in a sequencing batch reactor using different organic carbon sources. *Chemical Engineering Journal*, **2011**. *172*(2–3): p. 994-998.
14. Bodík, I., et al., Biodiesel waste as source of organic carbon for municipal WWTP denitrification. *Bioresource Technology*, **2009**. *100*(8): p. 2452-2456.
15. Yuan, Q., et al., Enhancing biological phosphorus removal with glycerol. *Water Science and Technology*, **2010**. *61*(7): p. 1837-1843.
16. Guerrero, J., et al., Glycerol as a sole carbon source for enhanced biological phosphorus removal. *Water Research*, **2012**. *46*(9): p. 2983-2991.
17. McCue, T., et al., Changes in anoxic denitrification rate resulting from prefermentation of a septic, phosphorus-limited wastewater. *Water Environment Research*, **2004**. *76*(1): p. 23-28.
18. APHA, Standard methods for the examination of water and wastewater. *American Public Health Association, Washington, DC*, **1995**: p. 456.