A Single-Process System for Cost-Effective and Efficient Removal of Organic Carbon and Nitrogen Content from Highly Polluted Streams

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Abstract

Organic carbon and nitrogen content in low-strength wastewaters can be removed by conventional aerobic processes such as Modified Ludzak Ettinger (MLE), but these treatment methods are not appropriate for treating strong wastewaters. Treatment of highly polluted effluents from slaughterhouse, food processing, brewery, dairy, landfill leachates, etc., which are rich in organic matter and nutrients requires specific considerations. The main removal pathways in the aerobic processes are bacterial assimilation and complete nitrification-denitrification for nitrogen content removal which is accompanied by organic carbon removal during this process. Various biological aerobic treatment processes and operations have been tried in the past to effectively treat strong streams, but high energy requirement and sludge production are serious drawbacks. Anaerobic digestion (AD) is the best alternative to the aerobic processes for treatment of strong wastewaters but biological nutrient removal is not possible, and needs further treatment.

In high-strength wastewater treatment, neither aerobic nor anaerobic treatment methods alone as a single-process can produce treated effluents complying with discharge standards. Therefore, existing conventional technologies for treatment of highly polluted streams are combined systems. These conventional combined systems are a pair of at least two separate processes in series. Most of the combined systems are applying the AD as a head system which is followed by a polishing aerobic process such as conventional activated sludge. Synchronizing two different processes in a series in combined systems is expensive and complicated with intensive operation and maintenance requirements. Hence, in order to prevail these difficulties, it is paramount to develop an efficient and less expensive single-process technology with simple operation and maintenance.

In this thesis, a conventional MLE system as a single-process was modified for treating highly polluted wastewaters, with a performance similar to the combined systems. This modified system is referred to throughout the thesis as SAO/PND (Simultaneous Anaerobic Oxidation/Partial Nitrification–Denitrification). After several unsuccessful modifications were tried, the main successful modifications were increasing the hydraulic retention time (HRT) in the pre-anoxic reactor, and decreasing the solids retention time (SRT) to create favorable conditions for anaerobic oxidation and partial nitrification-denitrification. A laboratory-scale of SAO/PND was used to conduct the experiments in this research. SAO/PND looks like the MLE process regarding the reactor configurations and recycle and return lines. Ammonia concentrations above 150 mg/L can be toxic for the MLE system, but SAO/PND improves the situation so that ammonia concentration is not toxic until close to 290 mg/L. Another issue with MLE process is that it requires high amounts of oxygen and alkalinity which results in high amounts of sludge production. But, SAO/PND produces less sludge, and does not need high amounts of oxygen and alkalinity.

The results showed more than 95% chemical oxygen demand (COD) and 90% total inorganic nitrogen (TIN) removal from synthetic wastewater, respectively, in our laboratory environment. In addition, volumetric design loading rates determined as 11.80 kg COD/(m^3.d) and 0.63 kg TIN/(m^3.d) with synthetic solution as a feed. Furthermore, the results showed high performance of the system in treating dairy and brewery industry effluents. In this modified single-process system, organic carbon was removed through anaerobic oxidation, assimilation, P–uptake by polyphosphate accumulating organisms (PAOs) and denitrifying PAOs (DPAOs), aerobic heterotrophs, and denitrification by DPAOs and denitrifying ordinary heterotrophic organisms (OHOs). Nitrogen content removal mechanisms were assimilation, partial nitrification–denitrification, anaerobic ammonium oxidation (anammox), and small portion uncharacterized processes.

95% and 90% less oxygen requirements along with 60% and 44% less sludge production compared to the conventional aerobic processes and conventional combined systems, respectively, resulted in significant potential cost savings by this modified system. Finally, the applied modified single-process system in this thesis is found as a sustainable, robust, cost-effective, and small footprint process with less intensive operation and maintenance requirements which will yield new insights into the design of treating highly polluted streams in the future.

This thesis is dedicated to my beloved parents **Omranali** and **Marzieh** for all their endless love, support, and encouragement in my life

Acknowledgement

I would like to express my sincere appreciation to the Vice-Dean, Graduate Studies of the Faculty of Engineering at the University of Ottawa, Dr. Liam Peyton. The completion of this thesis would not have been possible without his encouragement and support. Also, I would like to acknowledge all my friends and colleagues in the Department of Civil Engineering at the University of Ottawa for all their help.

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Abbreviations and Nomenclature

A/ASFF	Anoxic/aerobic submerged fixed-film
ABR	Anaerobic baffled reactor
AD	Anaerobic digestion
AG	Aerobic granules
AGS	Aerobic granule sludge
ALR	Ammonium loading rate
Anammox	Anaerobic ammonium oxidation
AnRBC	Anaerobic rotating biological contactor
AOB	Ammonia oxidizing bacteria
A/O SBRs	Anoxic/oxic SBRs
AS	Activated sludge
ASFF	Aerated submerged fixed-film
AVS	Attached volatile solids
BVS	Biomass volatile solids
COD	Chemical oxygen demand
CSTR	Continuous stirred tank reactor
DGAOs	Denitrifying GAOs
DO	Dissolved oxygen
DPAOs	Denitrifying PAOs
EF	Electroflotation
EGSB	Expanded granular sludge bed

FB	Fixed bed
GAOs	Glycogen accumulating organisms
HRT	Hydraulic retention time
IFAS	Integrated fixed film activated sludge
IFMBR	Integrated fixed-film MBR
LSCFB	Liquid-solid circulating fluidized bed bioreactor
MABR	Modified anaerobic baffled reactor
MB	Mass balance
MBBR	Moving-bed biofilm reactor
MFC	Microbial fuel cell
MLE	Modified Ludzak Ettinger
MLSS	Mixed liquor suspended solids
NLR	Nitrogen loading rates
NOB	Nitrite-oxidizing bacteria
OHOs	Ordinary heterotrophic organisms
OLR	Organic loading rate
PAOs	Polyphosphate accumulating organisms
PDMLE	Post-denitrification MLE
PHA	Poly–b–hydroxyalkanoates
PHB	Poly–b–hydroxybutyrate
RAS	Return activated sludge
ROPEC	Robert O. Pickard Environmental Centre
SAO/PND	Simultaneous anaerobic oxidation/partial nitrification-denitrification

SBR	Sequencing batch reactor
SND	Simultaneous nitrification denitrification
SNDPR	Simultaneous nitrification denitrification and phosphorous removal
SRT	Solids retention time
SS	Steady-state
T	Temperature
TAN	Total ammonia nitrogen
TIN	Total inorganic nitrogen
TKN	Total Kjeldahl nitrogen
TN	Total nitrogen
TP	Total phosphate
TSS	Total suspended solids
UASB	Up-flow anaerobic sludge blanket
UASRs	Up-flow anaerobic sponge reactors
UCT	University of Cape Town
VSMBR	Vertical submerged membrane bioreactor
VSS	Volatile suspended solids
WAS	Waste activated sludge
Y_{obs}	Observed yield
F_{TSS}	Flux of total suspended solids
F_{VSS}	Flux of volatile suspended solids
R_{O2}	Oxygen requirement
$WAS_{daily\ TSS}$	Daily waste activated sludge as total suspended solids

$WAS_{daily\ VSS}$	Daily waste activated sludge as volatile suspended solids
VSS/TSS ratio	Volatile suspended solids/total suspended solids ratio
μ_m	Maximum specific growth rate
$\mu_{n,m}$	Nitrification maximum specific growth rate
K_n	Nitrification half-velocity constant
K_d	Decay half-velocity constant
Y_n	Nitrification yield
f_d	Fraction of decay

1. Introduction

1.1. General background

The activated sludge process as a common biological treatment method for organic carbon and nitrogen removals, includes processes for the oxidation of biodegradable carbon and nitrification-denitrification of nitrogen (Li et al., 2014; Seka et al., 2001). Inappropriate removal of organic carbon and nitrogen content can result in oxygen depletion and toxicity in receiving water bodies (Rocher et al., 2019; Rodgers et al., 2006). Efficient and reliable treatment of municipal wastewater which contains 250-800 mg/L chemical oxygen demand (COD), and 12-45 mg/L NH_3-N are mostly by bacteria assimilation, and nitrification-denitrification accompanied by organic carbon removal (Metcalf and Eddy, 2003).

Biological nitrification coupled with denitrification is commonly used in wastewater treatment plants to remove organic carbon and nitrogen. Nitrification is achieved through the aerobic oxidation of ammonia (NH_3) and ammonium (NH_4^+) into nitrite (NO_2^-) by ammonia oxidizing bacteria (AOB), followed by the oxidation of the nitrite into nitrate (NO_3^-) by nitrite-oxidizing bacteria (NOB) (Ward et al., 2011). The former is termed nitritation and the latter nitratation. Denitrification process consists of consecutive reaction steps in which nitrate is reduced to nitrogen gas by denitrifying bacteria using the organic matter of wastewater under anoxic conditions: the reduction of nitrate via nitrite and nitric oxide to nitrous oxide or nitrogen gas (Xu et al., 2014; Guo et al., 2013). However, in the nitrification process, it was reported that ammonia concentrations higher than 150 mg/L NH_3-N would significantly inhibit the activities of both AOB and NOB (An et al., 2007). This nitrification-denitrification involves two separate reactors/processes and the denitrification reactor in post-anoxic processes frequently requires the addition of a supplemental carbon source, such as methanol. To minimize costs and reduce complexity many municipal applications, only the nitrification step is performed. Thus, their effluent contain substantial concentrations of nitrite and nitrate which may serve as nutrients to algae in the receiving waters.

Modified Ludzak Ettinger (MLE) as a single-process treatment method is one of the pre-anoxic processes with the described removal pathways for treating low-strength wastewaters like municipal wastewater (250-800 mg/L COD, and 12-45 mg/L NH_3-N). MLE is a modification of the activated sludge process, it process consists of an anoxic reactor followed by an activated sludge unit with sludge and effluent recycle to the anoxic tank. The activated sludge unit nitrifies the wastewater and the effluent recycle allows for nitrate to be denitrified in the anoxic reactor. The organics in the feed wastewater serve as the carbon source for the denitrification. Favorable conditions to treat the low concentration wastewaters with the mentioned removal pathways have been created because of a short hydraulic retention time (HRT) of the pre-anoxic reactor, and long solids retention time (SRT) of the system. The MLE is an aerobic activated sludge process with high amount of sludge production and oxygen requirements. These are the drawbacks of the aerobic systems, but are justifiable for application of these systems for treating low concentration wastewaters like municipal wastewaters. It should be mentioned that the MLE as a single-process is a less intensive system with respect to the operation and maintenance in comparison to the combined processes (combination of at least two single-processes in series).

Treatment of highly polluted streams (COD and ammonium concentrations above 1300-1500 mg/L and 150 mg/L NH_4^+-N, respectively) needs special considerations. Typical range of organic carbon and nitrogen content for some highly polluted streams reported in literature include: slaughterhouse wastewater (COD=876-9,300 mg/L and ammonium=84-584.2 mg/L NH_4^+-N), dairy wastewater (COD=1,150-9,200 mg/L and ammonium=14-288 mg/L NH_4^+-N), food processing wastewater (COD=1,000-18,000 mg/L and ammonium=53-1,000 mg/L NH_4^+-N), many landfill leachates in advanced countries with appropriate solid waste management systems like most European countries or USA and Canada (COD=1,740-13,800 mg/L and ammonium=72-800 mg/L NH_4^+-N) (Chokshi et al., 2016; Galib et al., 2016; Lu et al., 2015; Giustinianovich et al., 2014; Pan et al., 2014; Wang et al., 2014; Wei et al., 2012; Fongsatitkul et al., 2011; Rodríguez et al., 2011; Eldyasti et al., 2010; Lemaire et al., 2009; Li et al., 2008; Moreira et al., 2008; Renou et al., 2008; Laitinen et al., 2006; Sarkar et al., 2006; Demirel et al., 2005; He at al., 2005; Meknassi et al., 2005; Meknassi et al., 2004; Kennedy et al., 2000). In developing countries without organic waste separation plans, the COD and ammonium concentrations can be above the mentioned concentrations which are not applicable to the modified system in this thesis. It should be mentioned that treatment of leachates with very high COD concentrations (roughly more than 20,000 mg/L) are not the aim of this thesis.

1.2. Statement of problem

Removal of organic matter and nutrients in highly polluted streams by conventional aerobic treatment processes, and various types of these systems have been applied in different studies. However, there are serious drawbacks for using these aerobic processes for treating strong wastewaters, as they consume high amounts of oxygen for COD and nitrogen removal along with the significant amount of sludge production. In general, if the influent COD concentrations are higher than 1300–1500 mg/L, conventional aerobic systems are not recommended, and they should be switched to anaerobic digestion. Also, during the nitrification process in aerobic processes, ammonia concentrations above 150 mg/L would create toxic environment for nitrifiers and result in significant inhibition of nitrification.

Strict anaerobic processes are better alternative to the aerobic processes for treatment of high strength organic wastewaters but nutrients removal is impossible. AD has been successfully applied to treat streams with high concentrations of organic carbon, and this process is also feasible for treatment of low-strength wastewaters, such as domestic wastewaters, particularly under tropical conditions in which the supply of heat is not required. In these systems, the majority of organic carbon will be removed by strict anaerobic conditions in the anaerobic units; meanwhile, ammonia production (ammonification) happens (Grady et al., 1999). Therefore, post treatment is necessary to further polish the remaining organic pollutants and nutrients (Hosseinlou, 2021; Mendes et al., 2016). In addition, operational stability in anaerobic systems is one of the major concerns as these systems are highly sensitive to temperature and operational changes that can cause adverse effects (Metcalf and Eddy, 2014).

In high-strength wastewater treatment, neither aerobic nor anaerobic treatment methods as a single-process can produce treated effluents complying with discharge standards. Therefore, existing conventional technologies for treatment of highly polluted streams are combined systems. These conventional combined systems are a pair of at least two single-processes which are working together in series. Most of the conventional combined systems are applying the AD as a head system which is followed by a polishing aerobic process. AD systems as the head of the combined systems can be processes such as anaerobic ponds, up-flow anaerobic sludge blanket (UASBs), anaerobic contact reactors, anaerobic sequencing batch reactor (SBRs), anaerobic filters, anaerobic expanded granular sludge bed, and anaerobic fixed film reactors. The subsequent aerobic systems in the combined technologies can be treatment methods as follows: activated sludge processes, constructed wetlands, rotating biological contactors, trickling filters, aerobic ponds, aerobic lagoons, moving-bed biofilm reactor (MBBRs), and intermittent sequencing batch reactors (Aziz et al., 2019).

The effluent of AD is suitable feed to a subsequent polishing aerobic system for further removal to meet the effluent discharge standards. In these systems, majority of the COD will be removed by anaerobic processes which accompanies ammonification. Then the following aerobic treatment processes remove the ammonia/ammonium through nitrification-denitrification along with the COD degradation. However, synchronizing two different processes in a series in combined systems is expensive and complicated with intensive operation and maintenance requirements. Hence, in order to prevail these concerns, it is paramount to develop an efficient and less expensive single-process technology with simple operation. A single-process known as SAO/PND in this thesis, produces less sludge, and does not need high amounts of oxygen and alkalinity.

1.3. Research objectives

In performing the literature review (see chapter 2), the author could not find a report on biological treatment processes capable of removing high concentrations of both organic carbon and nitrogen content in a single-process. Because of the simple operation of the MLE process as a single-process, and possible success of this system for treating highly polluted wastewaters, this thesis investigates modifications of the MLE system and the feasibility of these modifications with different and efficient removal pathways for removing organic carbon and nitrogen content from highly polluted streams. Therefore, the main objectives of this research are defined as follows:

- Finding the optimum operating conditions for the modified single-process system (MLE reactor configuration), and assessment of the performance of the modified system regarding organic carbon and nitrogen content removals from synthetic solution as a feed.
- Determination of design loading rates of the COD and total inorganic nitrogen (TIN) for the modified system with highest removal efficiencies and feeding with synthetic wastewater.
- Explanation of organic carbon and nitrogen content removal pathways with more detail.
- Application of the modified single-process system for treating real highly polluted streams such as the effluents of real dairy and brewery industries, and confirmation the applicability of the modified single-process system for treatment of real strong wastewaters.
- Investigation of potential cost savings by this modified single-process system in comparison with two other conventional treatment methods which are: i) conventional aerobic systems; and ii) conventional combined systems consisted of AD process and subsequent polishing aerobic system.

1.4. Research environment

All the experiments that were conducted as part of this research took place first in a lab run by Dr. Sartaj (from 2015-2017) and then in a lab run by Dr. Delatolla (from 2017-2019). I did the experiments and collected the data with direction from them. The first paper, in Chapter 3, was co-written with both professors and I was responsible for all the analysis of the data and the writing of the paper. My analysis and paper was reviewed by them and I incorporated their feedback. The remaining papers in Chapter 4, 5, and 6 were written entirely by me and I was responsible for all analysis performed in those papers. The papers in Chapter 3, 4, 5, and 6 went through peer review and I incorporated the feedback from that peer review.

The four papers, were written based on the data collected from the following four experiments listed below. I would like to acknowledge my collaboration with the following students and lab technicians whom I interacted with and discussed my experiments while they were being performed: Patrick D'Aoust, Ataollah Babakhani, Warsama Ahmed, and Akinwumi Akindele.

Before these experiments were performed, there was a period of investigation that explored possible configurations and different modifications to the basic MLE system until we arrived at an optimized SAO/PND system. There were several failed configurations that were tried before we arrived at these four successful experiments. Different HRTs, Q_r/Q_{in} ratios, MLSS in the reactors, Q_{RAS}/Q_{in} ratios, DO in the aerobic and SRTs were tried to identify optimum SAO/PND system. Optimum operational conditions are presented as a table in Chapter 6.

Main goal of the preliminary experiments was to modify the MLE system for removing both organic carbon and nitrogen content from synthetic solution; however, exploring the biological removal mechanisms were not aimed. In most of the modified scenarios, organic carbon was removed successfully, but overall performance of the system was reported as a failure because of the poor nitrogen removal. Finally it was find out that HRT and SRT were two key parameters for a successful operation in removing organic carbon and nitrogen content by a modified single-process.

1.4.1. Experiment 1

Optimized SAO/PND system was used to carry out the experiments in this phase at room temperature ($25^{O}C$), and only synthetic solution was used as a feed. First, maximum possible applicable organic carbon and nitrogen content concentrations were tried. Second, under steady-state conditions with COD and nitrogen content concentrations around 4100, and 210 mg/L, respectively, and based on mass balance calculations, removal pathways were identified. Ingredients of the synthetic solution were the same as other experiments.

It took around two months to acclimatize the bacterial community to the optimized system in the beginning of the experiments. During this stage of experiments, synthetic solution was used as a feed with constant COD and nitrogen concentrations around 400 mg/L and 20 mg/L, respectively.

1.4.2. Experiment 2

During the second phase of experiments, the SAO/PND system was run under optimum operational conditions, and temperature was $25^{O}C$. First, system was fed with synthetic solution to identify design loading rates without failure system. Afterwards, dairy wastewater was used as a feed to the optimized SAO/PND system with the same reactor configuration. Finally, during complementary experiments, synthetic solution (COD and nitrogen content concentrations close to 4100, and 210 mg/L, respectively) with the same ingredients of other phases was used to identify a comprehensive removal mechanisms.

1.4.3. Experiment 3

This set of experiments were conducted by using the optimized SAO/PND system at $25^{O}C$, and performance of the system was evaluated in treating brewery wastewater. Then, steady-state conditions and synthetic solution with organic carbon concentrations around 4100 mg/L and nitrogen content close to 210 mg/L was applied to identify removal pathways, and help to find the range of each responsible mechanism in this thesis.

1.4.4. Experiment 4

This phase of experiments used only synthetic solution with the same ingredients of other phases. This synthetic solution contained around 4100 mg/L of organic carbon and 210 mg/L nitrogen content. Previously identified SAO/PND system with the same reactor configuration under optimum operational conditions was used and temperature was 25^{O}C.

1.5. Thesis organization and outline

The thesis is laid out in eight chapters in order to facilitate the understanding and presentation of the key findings in this research. Chapters 3 to 6 are 4 published papers in peer review journals and are included here exactly as they were originally published (see links to published papers below). The paper based format in this thesis results in some repetition, since each paper has to stand alone and be self-sufficient. As well, chapter 2 was written before chapters 3, 4, 5, and 6 were submitted to journals for publication so all four papers include some material from chapter 2.

There is also a references section at the end of each of Chapter 1, 2, 3, 4, 5, 6 rather than a single references section at the end of the thesis. As well, in bringing these papers together into a single thesis, the author received significant feedback after the papers were accepted for publication. Detailed notes are included as Appendix A, B, C, and D to comment the publications to acknowledge some weaknesses or limitations and add some clarifications in the context of the thesis.

Chapter 1 provides a brief overview of related background information on organic carbon and nitrogen content removal from low and high-strength wastewaters, and present a general description of the identified problems as well as research objectives.

Chapter 2 presents theoretical and technical background information on the conventional organic carbon and nitrogen content removals. A comprehensive literature review has been carried out on the organic carbon and nitrogen content removals by existing conventional technologies, and different treatment processes in relevant various studies have been overviewed.

Chapter 3 investigates the optimization of a modified system for removing organic carbon and nitrogen content from highly polluted streams in a single-process system by feeding the system with synthetic solution. Performance of the modified single-process system regarding the organic carbon and nitrogen content removals has been monitored, and general removal pathways are described. This chapter is published in a peer review journal as follows:

Daniel Hosseinlou, Majid Sartaj, Robert Delatolla

Simultaneous anaerobic oxidation/partial nitrification–denitrification for cost-effective and efficient removal of organic carbon and nitrogen from highly polluted streams.

Environmental Technology, 2019, 40 (16), 2114–2126.

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Chapter 4 determines the design loading rates of the optimized modified system for COD and TIN components with high performance. In addition, organic carbon and nitrogen content removal pathways are explained with more detail. The modified system was applied for treating real dairy industry effluent to confirm the efficiency of the system under real conditions as well. This chapter is published in a peer review journal as follows:

Daniel Hosseinlou

Determination of design loading rates for simultaneous anaerobic oxidation/partial nitrification–denitrification process and application in treating dairy industry effluent.

Journal of Environmental Chemical Engineering, 2021, 9(3), 1–10.

https://doi.org/10.1016/j.jece.2021.105176

Chapter 5 confirms the applicability of the modified single-process system for treatment of real brewery industry effluent. As the modified system has been claimed as a general solution, this modified system needs more confirmations for high efficiency in different real conditions. This chapter is published in a peer review journal as follows:

Daniel Hosseinlou

Application of an efficient, cost-effective and newly developed single-process SAO/PND technology for treating brewery industry effluent.

South African Journal of Chemical Engineering, 2022, 41, 34–42.

https://doi.org/10.1016/j.sajce.2022.04.003

Chapter 6 investigates potential cost savings by the modified system in comparison with two other conventional systems: i) conventional aerobic processes, and ii) conventional combined systems. Potential capital and operational costs are compared to conclude the cost-effectiveness of the modified single-process system. This chapter is published in a peer review journal as follows:

Daniel Hosseinlou

Significant cost savings using a newly developed single-process technology compared to conventional processes for treating high-strength effluents.

Water and Environment Journal, 2022, 1–11.

https://doi.org/10.1111/wej.12818

Chapter 7 integrates all the results in all phases of this study, and provides a general summary of results which are presented in Chapters 3, 4, 5, and 6.

Chapter 8 summarizes the main conclusions from the different studies undertaken through this thesis, and provides possible recommendations for future extensions of this thesis.

Fig. 1.1 shows the organization of the thesis.

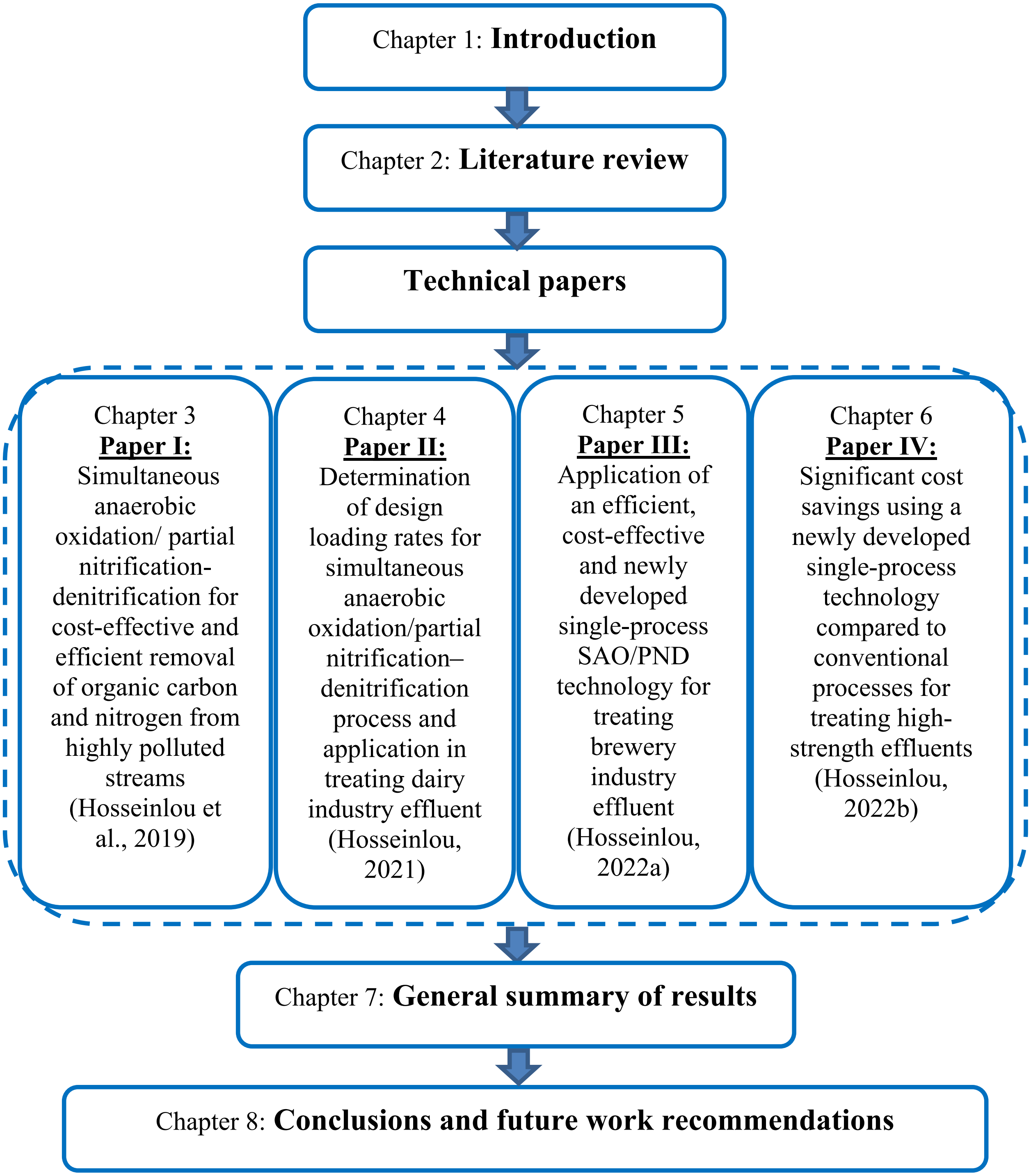

Fig. 1.1. Structure of this thesis and summary of chapters.

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2. Literature Review

In this chapter, biological nitrification and denitrification processes will be explained, and then the organic carbon and nitrogen content removal studies will be presented. These studies have investigated the modifications of the pre-anoxic systems which are considered as single-processes. The main removal pathways in these systems are the nitrification-denitrification process which is accompanied by organic carbon removal. It is tried to include those studies which are targeting higher concentrations of influent organic carbon and nitrogen content (1300-1500 mg/L of COD and 150 mg/L of NH_4^+-N) in these modified pre-anoxic and post-anoxic systems. However, it was not identified studies to apply these modified system for treating highly polluted wastewaters. It is believed that the lack of these researches can be as a result of extraordinary amount of oxygen requirements and sludge productions for treating highly polluted streams with the same removal pathways (nitrification-denitrification process which accompanies organic carbon removal). Then, the combined systems will be studied as a solution for treating highly polluted streams which are consisted of at least two single-processes. Finally, a modified pre-anoxic system will be proposed in this research as an alternative solution for treating highly polluted wastewaters with an efficiency similar to the combined processes but operation similar to the single-process. The possibility of this proposed system will be evaluated in this research.

2.1. Biological Nitrification

The need for nitrification in wastewater treatment comes from water quality concerns over: i) the influence of ammonium on receiving water bodies with respect to DO concentrations and fish toxicity, ii) the need to provide nitrogen removal to control eutrophication, and iii) the need to provide nitrogen control for water-reuse applications including groundwater recharge (Metcalf and Eddy, 2014).

Nitrification is a two-step biological process in which firstly ammonia (NH_3) and ammonium (NH_4^+) is oxidized to nitrite (NO_2^-) and secondly nitrite is oxidized to nitrate (NO_3^-) (Grady et al., 1999). Distribution of ammonia species in liquid depends on pH and temperature, and at normal pH values, ammonia in wastewater is actually in the form of the ammonium cation (NH_4^+) and Fig. 2.1 depicts this fact (Lin et al., 2009). Studies have shown that ammonia is the more toxic with inhibition effects on microbial activities, including nitrification (Metcalf and Eddy, 2014). For vertebrates, the toxicity of ammonia accumulation can be characterized by over-excitation of the nervous system (Walsh et al., 2007).

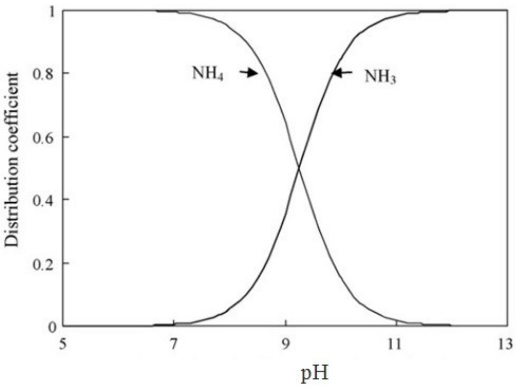


Fig. 2.1. Distribution of NH_3 and NH_4^+ at different pH levels (20°C) (Lin et al., 2009).

As with organic carbon removal, the nitrification process can be fulfilled in both suspended growth and attached growth biological processes. In suspended growth processes, a common approach is to achieve nitrification along with organic carbon removal in the same single-sludge process, consisting of an aeration tank, clarifier, and sludge return system as depicted in Fig. 2.2 (Metcalf and Eddy, 2014).

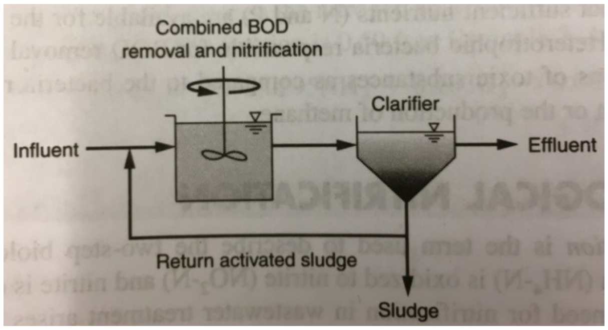


Fig. 2.2. Configuration used for single-sludge suspended growth system for biological nitrification process (Metcalf and Eddy, 2014).

A two-sludge suspended growth process (Fig. 2.3) is preferred in cases where there is a significant potential for toxic and inhibitory substances in the wastewater. The two-sludge system consists of two aeration tanks and two clarifiers in series with the first aeration tank/clarifier unit operated at a short SRT for organic carbon removal. The first unit removes organic carbon and toxic substances, so that nitrification can proceed unhindered in the second. In this process configuration, a portion of the influent wastewater usually has to be bypassed to the second sludge system to provide a sufficient amount of solids for efficient solids flocculation and clarification. Because the nitrifiers are slow growth bacteria in comparison to heterotrophic bacteria, systems designed for nitrification generally have much longer HRTs and SRTs than those for systems designed only for organic carbon removal (Metcalf and Eddy, 2014).

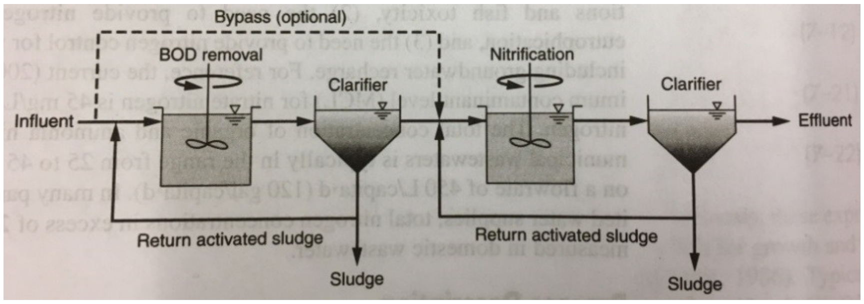


Fig. 2.3. Configuration used for two-sludge suspended growth system for biological nitrification process (Metcalf and Eddy, 2014).

Aerobic autotrophic bacteria are responsible for nitrification in activated sludge, biofilm processes, and in the two-step process (Metcalf and Eddy, 2014). The first stage, the ammonia and ammonium oxidation to nitrite, is accomplished by one group of autotrophic bacteria and the subsequent nitrite to nitrate oxidation is accomplished by another group of autotrophic bacteria. It should be noted that the two groups of autotrophic bacteria are distinctly different. The autotrophic Nitrosomonas bacteria oxidize ammonia and ammonium to nitrite, and then Nitrobacters oxidize nitrite to nitrate. Other autotrophic bacteria genera (prefix with Nitroso-) such as Nitrosococcus, Nitrosospira, Nitrosolobus, and Nitrosorobrio are capable of obtaining energy from the oxidation of ammonia and ammonium to nitrate (Metcalf and Eddy, 2014).

2.2. Biological Denitrification

Biological denitrification is a biological reduction of nitrate to nitric oxide, nitrous oxide, and nitrogen gas (Grady et al., 1999). This process is an integral part of biological nitrogen removal, which involves both nitrification and denitrification. Two basic flow diagrams for activated sludge denitrification process and operating conditions can be utilized (Figs. 2.4 and 2.5). Fig. 2.4 known as the MLE process is the most common process used for biological nitrogen removal in municipal wastewater treatment. MLE system consists of an anoxic tank for denitrification followed by the aeration tank where nitrification occurs. Nitrate produced in the aeration tank is recycled back to the anoxic tank by a recycle line. Organic components in the influent wastewater provide the electron donor for oxidation reduction reactions by using nitrate, and because of this the process is termed substrate denitrification. As a general rule, it has been estimated that for denitrification processes, 4 g of BOD is needed per 1 g of NO_3^--N reduced to provide sufficient amount of electron donors for nitrate removal. Further, because the anoxic reactor precedes the aeration reactor, the process is known as a pre-anoxic denitrification process (Metcalf and Eddy, 2014).

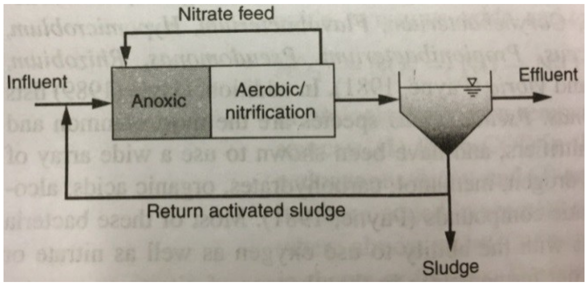


Fig. 2.4. Substrate driven denitrification (pre-anoxic denitrification) (Metcalf and Eddy, 2014).

The second option is post-anoxic denitrification (Fig. 2.5). In the process, denitrification occurs after nitrification and the electron donor source is from endogenous decay. In this process configuration, organic carbon removal has occurred first and is not available to drive the nitrate reduction reaction. When a post-anoxic denitrification process depends solely on endogenous respiration for energy, it has a much slower rate of reaction than for the pre-anoxic processes which use organic carbon form the influent wastewater as its energy source. Therefore, mostly an exogenous carbon source, such as methanol or acetate, is added to post-anoxic processes to provide sufficient organic carbon for nitrate reduction and to increase the rate of denitrification (Metcalf and Eddy, 2014).

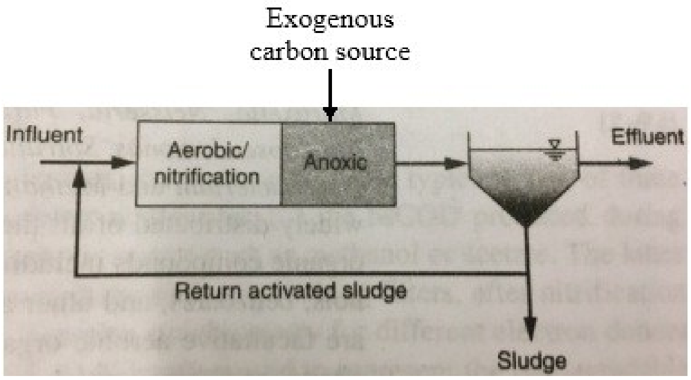


Fig. 2.5. Endogenous driven denitrification process (post-anoxic denitrification) (Metcalf and Eddy, 2014).

The pre-anoxic and post-anoxic denitrification processes described use autotrophs to oxidize ammonia and ammonium to nitrite and nitrate but heterotrophs are responsible for nitrate reduction. However, it should be mentioned that other pathways for biological nitrogen removal exist as well (Metcalf and Eddy, 2014).

A wide range of bacteria has been shown capable of denitrification. Capable heterotrophic denitrifiers include the following genera: Achromobacter, Acinetobacter, Agrobacterium, Alcaligenes, Arthrobacter, Bacillus, Chromobacterium, Corynebacterium, Flavobacterium, Hypomicrobium, Moraxella, Neisseria, Paracoccus, Propionibacterium, Pseudomonas, Rhizobium, Rhodopseudomonas, Spirillum, and Vibrio (Metcalf and Eddy, 2014).

2.3. Organic Carbon and Nitrogen Content Removal Studies

The following studies have investigated the modifications of the pre-anoxic systems in order to remove organic carbon and nitrogen content from different wastewaters. Studies were not identified to apply these modified systems for treating highly polluted wastewaters with respect to the organic carbon and nitrogen content. The lack of these studies can be related to the high amount of oxygen requirements and sludge production. As it is believed that treating highly polluted streams by these modified systems use the same removal pathways of the pre-anoxic systems (nitrification-denitrification process accompanied by the organic carbon removal), which will result in these drawbacks.

Hamoda and Bin-Fahad (2012) conducted a pilot-scale study to investigate the effect of hydraulic loading on nitrogen removal in parallel testing of an aerated submerged fixed-film (ASFF) system and anoxic/aerobic submerged fixed-film (A/ASFF) system. All compartments of the ASFF reactors were aerated, but in the A/ASFF, only the second to fourth compartments were aerated. They used a four-compartment reactor that was packed with Biolace media and operated at loadings of 0.03 to 0.3 g BOD/(g BVS.d), 0.01 to 0.11 g NH_4^+/(g BVS.d), HRTs 0.7 to 8 h, C/N of 6, and 28±2°C (Fig. 2.6). Biomass volatile solids (BVS) is the summation of mixed liquor volatile suspended solids (MLVSS) and attached volatile solids (AVS). The results showed that the system effectively treated municipal wastewater with COD, BOD and ammonium removals of up to 75%, 98%, and 97%, respectively. Also, increasing the loading rates up to 10-fold did not have negative effects on the overall performance of operation modes. According to their literature review, both the ASFF and A/ASFF systems achieved higher specific nitrification rates in comparison with some other studies. In addition, their research showed the presence of active nitrifying microorganisms mainly in the second and third compartments (at all loading rates) and presence of nitrates in the aerobic compartments (second, third, and fourth) confirmed the activity of the nitrifying bacteria. It was observed that the A/ASFF was more stable and efficient at higher loadings, with 60% COD removal and 90%

BOD removal in the anoxic stage. In addition, this study showed that denitrification with organic carbon removal occurred in the A/ASFF system, which eliminated 6.6 g COD (or 3.35 g BOD) per each gram of denitrified NO_3^--N. Hamoda and Bin-Fahad (2012) clarified that COD and BOD were consumed in the anoxic reactor as carbon source by denitrifying bacteria that was the great advantage of implementing the anoxic process for organic carbon removal.

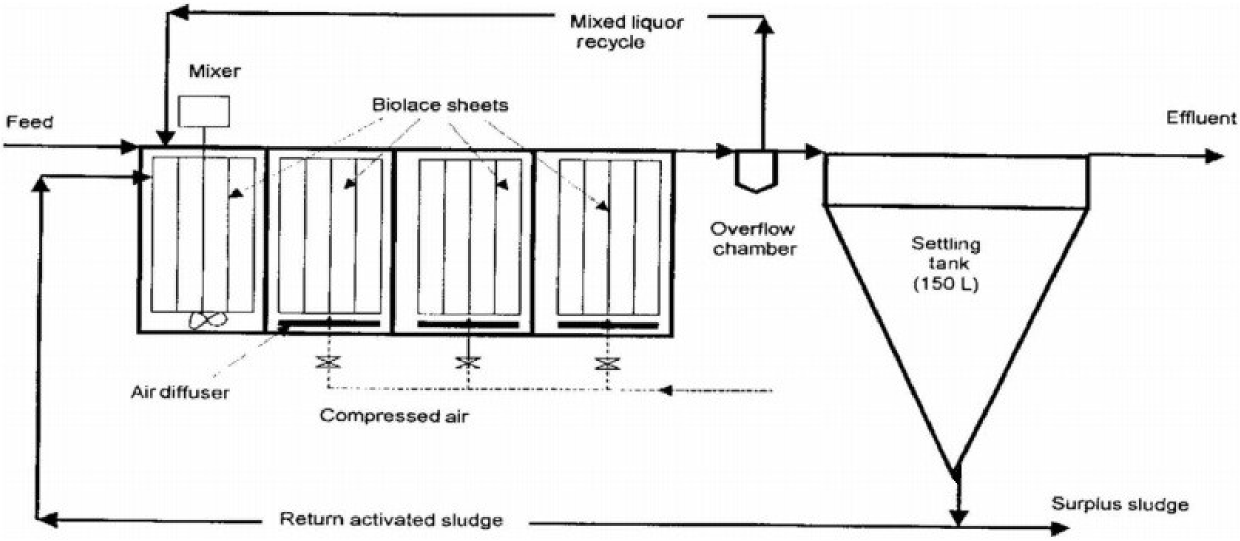


Fig. 2.6. Schematic diagram of the A/ASFF experimental set-up by Hamoda and Bin-Fahad (2012).

A vertical submerged membrane bioreactor (VSMBR) composed of anoxic and oxic zones in one reactor was developed and operated by Chae et al. (2006) to reduce the problems concerning effective removal of pollutants from synthetic wastewater as well as membrane fouling. The influent synthetic wastewater contained 120-300 mg/L COD, 30 mg/L as total nitrogen (TN), and 6 mg/L as total phosphate (TP). As shown in Fig. 2.7, a laboratory-scale VSMBR with anoxic zone (lower layer) and oxic zone (upper layer) was operated. The influent and recycle line form oxic zone were introduced to the anoxic zone through the flow distributors. Complete mixing in the anoxic zone was obtained by using a low-speed mixer. The aerobic zone is separated from the anoxic zone by a horizontal plate with a hole in the center. In the aerobic zone, disk-type diffusers were used to provide air bubbles for oxidation of organic and ammonium and to reduce membrane fouling. This study observed that in the anoxic zone, the supplied organic matter is firstly used for denitrification and phosphorus release. Organic carbon was not effectively consumed in the anoxic zone which caused a decrease in the nitrification in the oxic zone. High organic carbon concentration into the aerobic period had a detrimental effect on the nitrification process because nitrifiers were outgrown by heterotrophs. This study showed that the optimal volume ratio of anoxic zone/oxic zone was 0.6, and the desirable internal recycle rate and HRT (anoxic/oxic together) for effective nutrient removal were 400% and 8h, respectively. During the whole experimental period, COD were removed by higher than 98% but the average removal efficiencies of TN and TP were 75% and 71%, respectively.

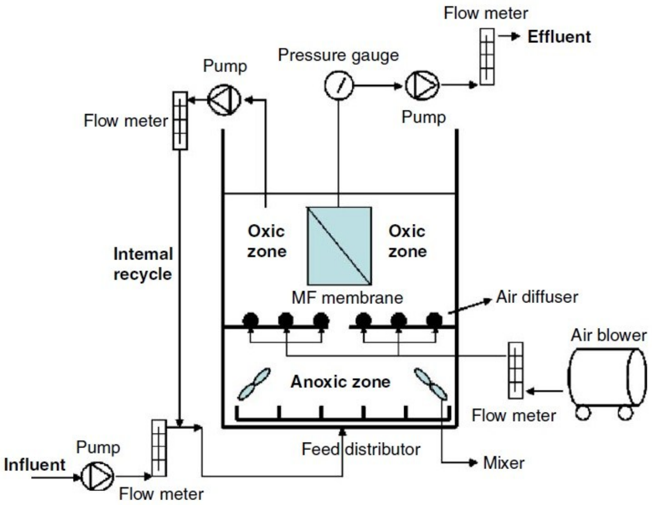


Fig. 2.7. Schematic diagram of the VSMBR system (Chae et al., 2006).

Performance of a submerged membrane bioreactor with mixed liquor recirculation (MLE/MBR) and a membrane bioreactor with the addition of integrated fixed biofilm medium (IFMBR) were compared by Liang et al. (2010). The comparison was based on the organic carbon and nitrogen content removal, biomass characteristics, microbial activity, and nitrifying bacterial community structure at a defined SRT. In addition, the membrane flux and the total membrane resistance of the two types of MBRs at a constant HRT were compared to assess the biofouling in these systems. Two laboratory bench-scale submerged membrane bioreactors in parallel having the same type of membrane module with identical reactor volume were constructed. One MBR was divided into anoxic, aerobic and hollow fiber membrane module by glass baffles to introduce alternating anoxic/aerobic conditions (MLE/MBR). On the other hand, integrated fixed-film MBR (IFMBR) was divided into two chambers: reaction chamber and hollow fiber membrane module. A biofilm support plastic medium was added to the IFMBR with 50% filling volume of the reaction chamber. Both MBRs were operated in parallel with SRT and HRT of 20 d and 1 d, respectively. The influent synthetic wastewater mainly contained COD concentration of 500 mg/L, 51.7 mg/L TN, 30 mg/L NH_4^+-N, and 6 mg/L total P. The results of both the MLE/MBR and IFMBR systems showed excellent COD removal efficiencies (>97.7%). However, the total N removal rates were 67 and 41% for the MLE/MBR and IFMBR, respectively. In comparison to the IFMBR system, the heterotrophic and autotrophic microbial activities were higher in the MLE/MBR system, which was attributed to the alternating anoxic/aerobic processes. Results indicated that the higher nitrifying activities were correlated with more diversity of nitrifying bacterial populations in the MLE/MBR system. In addition, the results showed that metabolic selection via alternating anoxic/aerobic processes has the potential of having higher bacterial activities and improved nutrient removal in MBR systems.

Wang et al. (2015) conducted a research in order to develop a simple anaerobic/aerobic conditions in sequence for simultaneous nitrification denitrification and phosphorous removal (SNDPR) system. They used a SBR to achieve an efficient removal of nutrient and organic carbon treating domestic wastewater at low C/N ratio (≤ 3.5) without external carbon addition. The SNDPR-SBR system (Fig. 2.8) was enriched with phosphorus accumulating organisms (PAOs), denitrifying PAOs (DPAOs), and glycogen accumulating organisms (GAOs) at the ratio of 2:1:1 to achieve this goal. As shown in Fig. 2.8, a laboratory-scale open-mouthed SBR fed with domestic wastewater was used in this study and the system was operated for 120 days under extended anaerobic and short low aerobic conditions in sequence. The short low aerobic stage was used for the concurrence of simultaneous nitrification denitrification (SND) and P uptake, and extended anaerobic stage was used to enhance the utilization of carbon sources and provide sufficient intracellular carbon for SND and P removal at the following short low aerobic stage. The cycle time of the SBR was 6 h, which consisted of 180 min anaerobic reaction, 150 min aerobic reaction, 20 min settling, 5 min decanting phase, and 5 min idle phase. The SBR was operated at room temperature with SRT of 10.9 d, and DO concentration was maintained at 1.0 ± 0.3 mg/L in the aerobic stage. The main characteristics of the wastewater taken from a septic tank in the residential area as feed to the SNDPR-SBR system were: COD 142.4-268.3 mg/L, NH_4^+-N 50.2-69.4 mg/L, NO_2^--N < 1 mg/L, NO_3^--N < 1 mg/L, PO_4^{3-}-P 5.1-7.9 mg/L, TN 68.4-79.2 mg/L, and C/N ratio ≤ 3.5. Results showed that extended anaerobic stage was used to enhance the utilization of the influent carbon at the anaerobic stage, and combined with short low oxygen aerobic stage to achieve a simultaneous C, N, and P removal without external carbon addition and about 65% energy saving for aeration. Extended anaerobic stage achieved a sufficient storage of intracellular carbon mainly polyhydroxybutyrate (PHB) in PAOs and GAOs (40% in PAOs and 60% in GAOs), and provided sufficient carbon sources for the SND and P uptake in the following short low aerobic stage which resulted in 81% COD removal by this system. Nitrification, endogenous denitrification, aerobic and denitrifying phosphorus uptake were achieved at short aerobic stage. Endogenous denitrification by denitrifying GAOs (DGAOs) and DPAOs improved the TN removal efficiency (77.7%) and SND efficiency (49.3%).

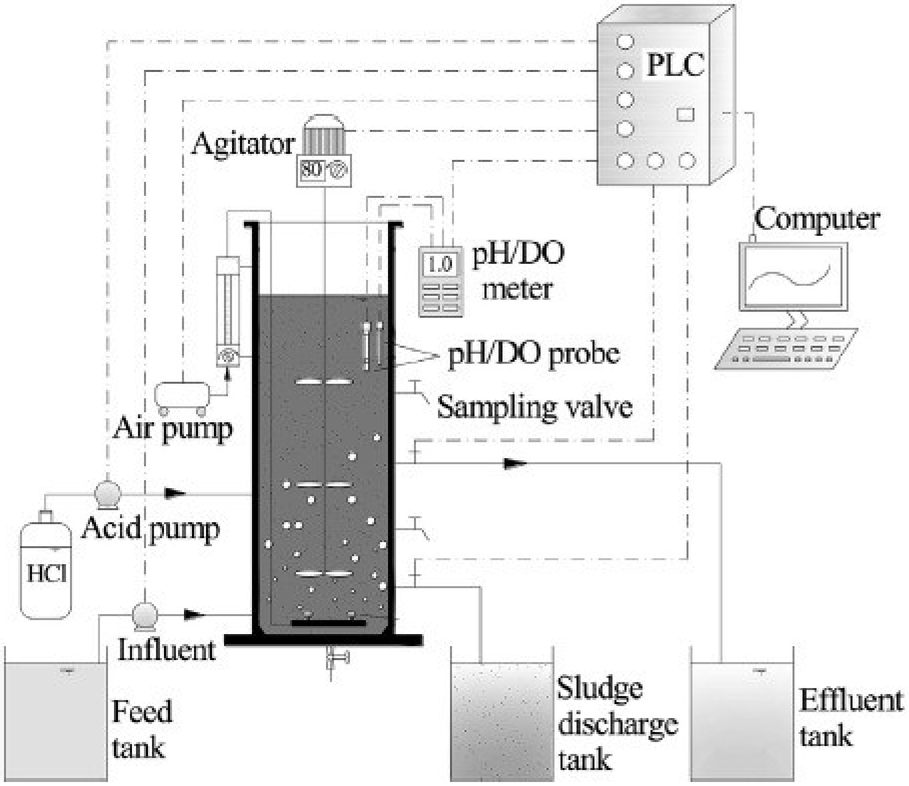


Fig. 2.8. Experimental setup of the SNDPR-SBR system (Wang et al., 2015).

Islam et al. (2009) carried out a research to investigate the impact of the COD/N ratio on the process performance, with particular focus on total nitrogen removal. A laboratory-scale liquid-solid circulating fluidized bed bioreactor (LSCFB) with anoxic and aerobic beds and lava rock as a biofilm carrier media was used. As shown in Fig. 2.9, the system was consisted of two columns, the riser column which was operated as anoxic reactor and the downer column which was operated as aerobic reactor. This study was conducted at three different COD: N ratios

(Phase I COD: N = 10:1, Phase II COD: N = 6:1, Phase III COD: N = 4:1) at room temperature (22-28°C). The influent acetate rich synthetic wastewater during Phase I was characterized by COD, total Kjeldahl nitrogen (TKN), and TP concentrations of 244±21, 27±3.1, and 4.25±0.43 mg/L but there was a spike in ammonium during Phases II and III to achieve the required COD: N ratios. Total estimated SRT in this system was 36-45 d and HRTs in the anoxic and aerobic tanks were 0.39 h, and 1.65 h, respectively. Results showed that more than 90% of the influent organic matter was removed throughout the study with 58% removal in the anoxic column in Phase III. Total nitrogen removal efficiencies in Phases I, II, and III were 91%, 82% and 71%, respectively, and simultaneous nitrification-denitrification occurred in the aerobic downer.

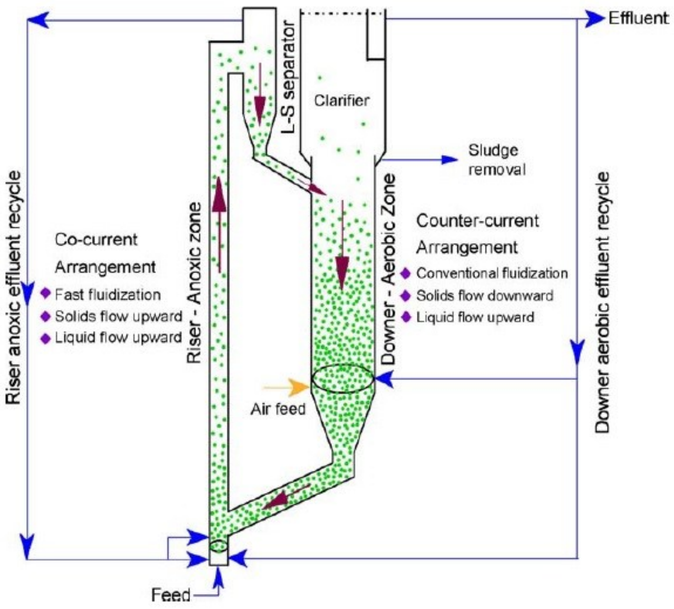


Fig. 2.9. Schematic diagram of the LSCFB system (Islam et al., 2009).

The performance of a new reactor configuration consisting of a fixed bed reactor was evaluated by Moura et al. (2012). This system was operated under continuous feeding and intermittent aeration for nitrogen removal. Polyurethane foam cylinders were vertically installed inside the reaction zone to support the biomass on the fixed bed. The experimental setup consisted of a continuous up-flow reactor with an internal diameter of 14.5 cm, a height of 80 cm. The composition of the influent synthetic wastewater to feed the system contained COD, TKN, and TN concentrations equal to 364, 25, and 29.7 mg/L, respectively. The reactor was operated as a continuous flow system under intermittent aeration (2 h aeration and 1 h without aeration) and a recirculation ratio (recycle from the effluent/influent) of 5. The system was operated under three different conditions (Phase I, Phase II, and Phase III) corresponding to HRT of 12 h, 8 h, and 10 h, respectively. In operation conditions of Phase I, the system achieved TN and COD removal efficiencies of 82% and 89%, respectively. At HRTs of 8 h and 10 h, TN removal was not stable, and the average resultant removal efficiencies were 49% and 45%, respectively. However, in Phases II and III, the removal efficiency of COD was high with mean values of 85% and 88%, respectively. To describe the TN removal in a single reactor, it can be attributed to the variations of DO concentration in the media. During aeration time, ammonium was oxidized and converted to nitrate, which was then consumed through denitrification during periods without aeration. By using a recirculation ratio of 5, it was possible to maintain constant effluent concentrations at the effluent of reactor, and no ammonium or nitrate peaks were observed. Efficient TN removal in a single reactor could also be attributed to the spatial distribution of microorganisms responsible for nitrification and denitrification and DO diffusion inside the support medium. These researchers concluded that maybe anoxic zone have been created inside the foam because the foam support had 3 cm diameter and that was enough to create anoxic zone. The internal anoxic zone was the favorite environment for denitrifying microorganisms due to a lack of DO. On the other hand, the oxygen availability was greater near the support medium surface, and thus nitrifying microorganisms were grown in its outer regions.

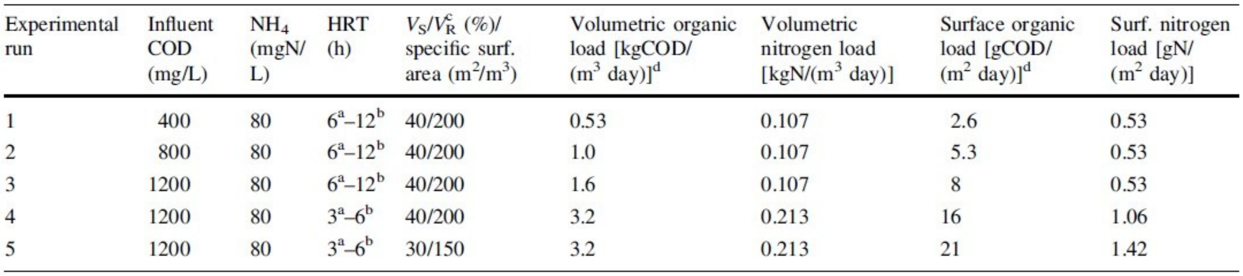
The primary objective of the study conducted by Hafez et al. (2010) was to investigate the feasibility of operating MLE as a biological nutrient removal (BNR) processes at a reduced HRT. A MLE pilot plant process with operating anoxic, aerobic, and secondary clarifier was set-up to treat the primary effluent of the Greenway Pollution Control Plant, London, ON. The internal mixed liquor recirculation rate from the aeration tank to the anoxic tank was $3.75Q_{in}$ while the return activated sludge flow was set at $2Q_{in}$ due to the relatively low return activated sludge (RAS) suspended solids concentrations. The real wastewater contained TCOD= 235 ± 28 mg/L; TKN 25.9 ± 3.3 mg/L; TP 4.7 ± 0.6 mg/L; COD:N:P ratio of 100:11:2. The results from the MLE pilot plant clearly indicate that despite operating at temperatures as low as 12°C, the MLE pilot plant achieved full nitrification and 75-80% nitrogen removal from this low-strength MWW at anoxic and aerobic HRTs of 1.5 h and 4.6 h, respectively. The SRT of 7 d in the aerobic tank was sufficient for complete nitrification even at low temperatures and the main reactions that occurred in the aerobic reactor were nitrification, oxidation of accumulated polyhydroxyalkonates and organic matter. On the other hand, denitrification was the main reaction in the anoxic reactor and approximately 5.1 g COD were consumed to denitrify 1.0 g NO_3^--N but phosphorus release were observed in the anoxic reactor as well. This study confirmed through both pilot testing and modeling that BNR systems treating low-strength municipal wastewater can be designed for short HRTs in cold climates.

The feasibility and process performance of a newly designed MLE combined with a post-denitrification reactor (PDMLE) using electroflotation (EF) as a secondary clarifier was investigated by Chung et al. (2012). Three different types of system were used in this study. Two systems maintained a combination of PDMLE with EF clarifier (EF-PDMLE) and anaerobic/anoxic/aerobic with EF clarifier (EF-A^2O) while the other one was maintained by combining A^2O and gravity settling clarifier (GS-A^2O). Throughout the experimental phases, HRT of each tank was equal to 1, 2.5, and 2.5 h, respectively. The RAS was maintained at $0.5Q_{in}$, while the internal recycling from the aerobic tank to the previous anoxic tank was set at the ratio of $2Q_{in}$. The DO in the aerobic tank was 3-4 mg/L, and SRT of EF-PDMLE, EF-A^2O, and GS-A^2O were kept at around 60, 60, and 30 days, respectively. This study was conducted to evaluate the nitrogen removal with two different C/N ratios. Phase I with synthetic wastewater of C:N ratio 10:1 (COD 300 mg/L, NH_4^+-N 30 mg/L) and the phase II with synthetic wastewater of C:N ratio 5:1 (COD 150 mg/L, NH_4^+-N 30 mg/L). COD removal efficiencies of all bioreactors exceeded 95% for the whole phases in this study. On the other hand, the EF-PDMLE system showed better performance on nitrogen removal compared with the EF-A^2O and the GS-A^2O regardless of the influent concentration. At phase I, stable TN removal efficiencies were 91, 75, and 74% for the EF-PDMLE, EF-A^2O, and GS-A^2O, respectively. At the beginning of phase II, high fluctuations of the TN removal efficiency were observed for all the reactors because of the C:N ratio changes in the influent from 10 to 5. However, from day 94 to the end of the phase II, the TN removal efficiencies were 59, 49 and 46% for EFPDMLE, EF-A^2O and GS-A^2O, respectively. Generally, the nitrogen removal efficiencies for all bioreactors in phase II were significantly lower than in phase I. This can be described by this fact that the influent C:N ratio is the major parameter for TN removal efficiency. Nevertheless, the EF-PDMLE performed better than the others with respect to nitrogen removal in the phase II as well. The unique configuration of EF-PDMLE implied that denitrification can occur in the pre-anoxic and post-anoxic tank and these tanks contributed to the nitrogen removal of 78% and 22%, respectively. In the EF-PDMLE process, 86% of the COD was removed in the pre-anoxic tank, and the removal of the remaining COD occurred in the aerobic tank. Therefore, these researchers concluded that the influent organic carbon was consumed by denitrification in the pre-anoxic tank. On the other hand, the major parts of COD were consumed during anaerobic phase in the EF-A^{2}O and GS-A^{2}O. During the organic matter removal in the anaerobic stage, microorganisms would store the organic carbon as PHB for later use in the aerobic stage. In the pre-anoxic tank of the EF-PDMLE system and because of the presence of nitrate, denitrification could occur using the influent organic matters before being utilized by polyphosphate accumulating organisms (PAOs). The presence of the pre-anoxic tank caused proper nitrification in the aerobic tank of the EF-PDMLE system, by using 86% of the influent COD and it could also reduce the inhibitory effects of organic loads on nitrification. The results confirmed that in the EF-PDMLE system, nitrate was further removed during the post-anoxic stage with removal efficiency of 20%, leading to an excellent nitrogen removal. Since the post-anoxic tank showed a small COD consumption without the addition of an external carbon, possibly endogenous denitrification occurred during denitrification in the post-anoxic tank. The reason for the high denitrification rate in this study could be explained by high mixed liquor suspended solids (MLSS) concentration in the bioreactor (5,350±352 mg/L). The high MLSS concentration could provide high endogenous substrate and an increase in denitrification efficiency without addition of external carbon source. The EF-PDMLE system could remove 97% of total nitrogen during the phase I, but this removal efficiency decreased to 59% in phase II.

Ghosh and LaPara (2004) investigated the performance of two modified MLE membrane-coupled bioreactors for the purpose of removing both nitrogenous and carbonaceous pollutants from a synthetic wastewater. Two different reactor configurations similar to the MLE systems tested in this work and the reactors were used to examine the effects of the different HRTs and the recirculation rate from the nitrification reactor (s) to the denitrification reactor on total nitrogen removal efficiency. The operational conditions for first MBR was HRT= 12 h; recirculation ratio (recycle from the nitrification tank to the denitrification tank/influent)= (8-14)Q_{in}; COD= 250 mg/L; and TN= 70 mg/L. Second MBR was operated with HRT= 12-24 h; recirculation ratio= (4-11)Q_{in}; COD= 250-500 mg/L; TN= 70 mg/L. During the first MBR operation, removal efficiencies were high (more than 90%) for COD and ammonium, but total nitrogenous removal efficiency was poor (around 25%). During the initial run of the second MBR system, COD and ammonium removal efficiencies were similar to the first operation of MBR until the COD of the influent wastewater was increased to provide additional electron donors to support denitrification. Finally, TN removal efficiencies achieved more than 90%, with an HRT of 24 h and a recirculation ratio of 8Q_{in}. However, the ammonium removal efficiency was adversely affected by decreasing the HRT to 12 h. A subsequent increase in the HRT to 18 h improved the performance of system and removal efficiencies increased and achieved more than 85% and 70% for ammonium and TN, respectively.

A moving-bed biofilm reactor (MBBR) system can be used for organic carbon and nitrogen content removal (Barwal and Chaudhary, 2017). A two-stage anoxic/aerobic MBBR system was studied by Lima et al. (2016) for the long-term assessment of the COD and nitrogen conversions under increasing organic loading rates. DO in the aerobic stage was adjusted below 2 mg/L in order to simulate a low dissolved oxygen environment for nitrification. The first anoxic reactor (MBBR1) was kept under anoxic conditions by sparging with nitrogen gas but the subsequent aerobic reactor (MBBR2) had DO concentration mostly between 1 and 2 mg/L throughout the whole experiment by combining appropriate amounts of compressed air and nitrogen gas in the aerobic tank. Both reactors were filled with Kaldnes K1 media and the recirculation ratio from MBBR2 to MBBR1 was set at 4Q_{in}. The experiments were conducted using synthetic wastewater at room temperature (23±2°C), and the biofilm system has undergone a gradual increase in the volumetric organic load, which was obtained by either increasing the influent COD or reducing the HRT. The influent ammonium concentrations were kept constant but COD:N ratio was increased from 5 to 15 by adding more COD. Table 2.1 depicts the operating conditions of the two-stage MBBR system in this study. The results showed that the two-stage MBBR system used in this study was very effective in removing COD and nitrogen under increasing organic loading rates up to 21 g COD/(m^2.d). The average COD and total nitrogen removal in this research were around 95 and 85 %, respectively. It was found that the denitrification was the removal pathway for most of the incoming organic matter at the low surface organic loadings (3 g COD/(m^2.d)). However, when the surface organic load reached 5.3 g COD/(m^2.d) or higher during runs 2-5, a lower percentage of the total organic matter was removed anoxically. The aerobic reactor played an important role in the COD removal, and the higher surface organic loadings in the system resulted in limited supply of the nitrite/nitrate from the aerobic tank and limited anoxic removal of the COD. This was especially determined during runs 3-5, the period when the influent COD:N ratio was around 15. Under these operating conditions, $MBBR_2$ was responsible for at least 50% of the incoming COD removed in the system. The DO concentration directly influenced the nitrite or nitrate production, particularly for the influent COD loads ranging from 2.6 to 8 g COD/(m^2.d). However, increasing the organic loading rates to 16 and 21 g COD/(m^2.d) led to incomplete ammonium removal (Lima et al., 2016).

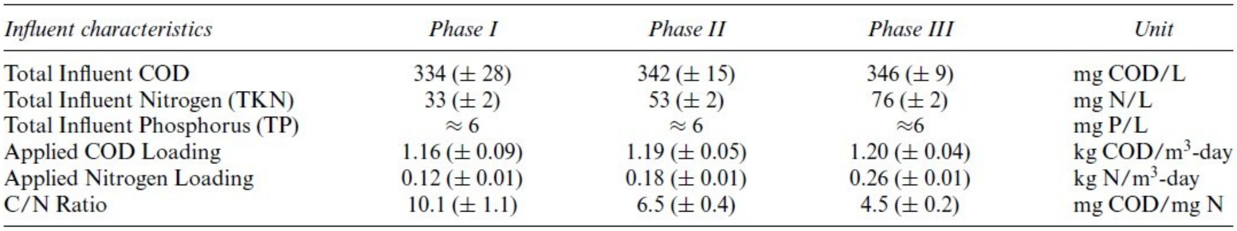
Table 2.1. Operating conditions of the two-stage MBBR system by Lima et al. (2016).



Experimental run	Influent COD (mg/L)	NH_4 (mgN/L)	HRT (h)	V_S/V_R^c (%)/ specific surf. area (m^2/m^3)	Volumetric organic load [kgCOD/(m^3 day)]^d	Volumetric nitrogen load [kgN/(m^3 day)]	Surface organic load [gCOD/(m^2 day)]^d	Surf. nitrogen load [gN/(m^2 day)]
1	400	80	6^a–12^b	40/200	0.53	0.107	2.6	0.53
2	800	80	6^a–12^b	40/200	1.0	0.107	5.3	0.53
3	1200	80	6^a–12^b	40/200	1.6	0.107	8	0.53
4	1200	80	3^a–6^b	40/200	3.2	0.213	16	1.06
5	1200	80	3^a–6^b	30/150	3.2	0.213	21	1.42

Two conventional MLE configuration pilot-scale biological nitrogen removal systems were operated in parallel by Sriwiriyarat et al. (2008) under different nitrogen loadings and DO concentrations to determine the effects of DO concentrations on the biological nitrogen removal. A Bioweb media was installed inside of the aerobic tank of one of the MLE system, designated as integrated fixed film activated sludge (IFAS), whereas another MLE was used as a control system. The carbonaceous and nitrogen removals from synthetic wastewater were investigated at different COD/Nitrogen (C/N) ratios of 4, 6, and 10, and DO concentrations of 2, 4, and 6 mg/L. Each system was consisted of one pre-anoxic reactor (HRT=1.7 h) and one aerobic reactor (HRT=5.1 h) in series. Systems were operated at 28.4°C with SRT=5.5±0.5 d, recycle ratio=1.5Q_{in}, and RAS=Q_{in}. Synthetic wastewater characteristics at different C/N ratios have been presented in Table 2.2. The results showed that the COD removal was nearly completed because glucose was used as a primary carbon source in the wastewater; therefore, there is no effect of DO concentration on the COD removal in both systems. However, the lower DO concentration maintained in the aerobic reactor resulted in more COD removal by denitrification. Optimum DO concentration for nitrification was determined 2 mg O_2/L when system was running with underloading nitrogen conditions at C/N ratio of 10. There was not advantage in installing media in the IFAS system at this C/N ratio. However, when the systems were operated at the C/N ratios of 4 and 6 (phases II and III according to the Table 2.2) which contained more nitrogen, the IFAS system was superior to the MLE system in terms of nitrification capacity as a result of supplemented biomass in the system. Based on the different operational conditions, optimal DO concentrations were determined to achieve the maximum nitrification rates. More nitrification rates resulted in more denitrification in the anoxic zones and finally more nitrogen removals in the system. The optimal DO concentration for the C/N ratios of 4 and 6 was found 6 mg O_2/L.

Table 2.2. Wastewater characteristics at different C/N ratios at different phases (Sriwiriyarat et al., 2008).



Influent characteristics	*Phase I*	*Phase II*	*Phase III*	*Unit*
Total Influent COD	334 (± 28)	342 (± 15)	346 (± 9)	mg COD/L
Total Influent Nitrogen (TKN)	33 (± 2)	53 (± 2)	76 (± 2)	mg N/L
Total Influent Phosphorus (TP)	≈ 6	≈ 6	≈6	mg P/L
Applied COD Loading	1.16 (± 0.09)	1.19 (± 0.05)	1.20 (± 0.04)	kg COD/m^3-day
Applied Nitrogen Loading	0.12 (± 0.01)	0.18 (± 0.01)	0.26 (± 0.01)	kg N/m^3-day
C/N Ratio	10.1 (± 1.1)	6.5 (± 0.4)	4.5 (± 0.2)	mg COD/mg N

Rodriguez et al. (2011) conducted a study in eight different stages of SBR operational conditions in which both the organic loading rate (OLR) and ammonium loading rate (ALR) was varied in order to monitor the performance of nitrification and denitrification processes. To achieve the various OLR and ALR in the influent, two types of meat processing wastewater were mixed in different proportions. SRT was kept constant at 30 days to promote the slow growth of nitrifying and denitrifying bacteria. The SBR cycles were always 8 h, with 6h intermittent aeration (8 min aeration) and anoxic (15 min mixing) and 2 h for the sedimentation, performing the filling in an average time of 35 min in the mixing and aeration cycle. OLR and ALR values varied between 0.71-4.55 g COD/(L.d) and 0.33-1.37 g NH_4^+-N/(L.d), respectively. The highest removal efficiencies occurred during phase IV, with COD, BOD, and ammonium removal percentages around 99, 98, and 71%, respectively, with corresponded OLR and ALR of 2.49 g COD/(L.d) and 1.02 g NH_4^+-N/(L.d). Nitrification-denitrification was the main process for organic carbon and nitrogen removal, and this study showed that the maximum density of nitrifying and denitrifying bacteria was observed at these loading rates.

Carrera et al. (2004) carried out a research with the goal to quantify the influence of the influent COD/N ratio on a BNR process of an industrial high-strength ammonium wastewater (N-wastewater) produced in a winery industry. In addition, the company generates a wastewater without nitrogen, mainly containing of organic matter (C-wastewater). The main objective was to optimize the proportion between both types of wastewaters in order to achieve maximum nitrogen removal. A pilot-scale MLE system was used and fed with industrial wastewater from the site. Operational conditions were as follows: total HRTs= 2.2-4 days; SRT=25 days; nitrogen loading rate= 0.035-0.14 g NH_4^+ /(g VSS.d); and COD loading rate= 0.10-0.15 g COD/(g VSS.d). Six different runs were performed throughout the study with different nitrogen loading rates (NLR) and COD/N ratios. Main mechanisms of nitrogen and organic carbon removals were determined as nitrification-denitrification and 7.1 g COD/g N was required to achieve the total denitrification. Because of the alkalinity requirement during nitrification, alkalinity was added manually by dissolving sodium bicarbonate in the N-wastewater until the amount of alkalinity stoichiometrically required was reached. As the influent ammonium concentration increased, higher amounts of alkalinity were required by this system which was not a simple task. Ammonium accumulation occurred if the required alkalinity was not available in this system. The lack of alkalinity can cause serious problems, since proper nitrification process needs alkalinity. With operational conditions at 25°C and pH of 8.0, inhibition of microorganism that oxidise ammonium to nitrite was initiated at concentrations of 150 mg/L NH_4^+-N. This study described that nitrification capacity was influenced by the relationship between organic carbon and ammonium in the influent, and nitrification was highly influenced by competition established between heterotrophic and autotrophic microorganisms.

Hu et al. (2013) fulfilled a study by using laboratory bench-scale anoxic/oxic sequencing batch reactors (A/O SBRs), in which duration of each cycle was 8 h and consisted of 10 min feeding, 2 h anoxic phase, 4 h oxic phase, 40 min settling, 10 min decanting, and 1 h idling. The goal of this study was to examine the characteristics of N_2O emissions from five parallel A/O SBR systems with different operational conditions. During this study, ammonium concentrations in all influents were approximately 60 mg/L N, but different COD concentrations were used ranging from 88.4 to 872.2 mg/L in order to achieve different C/N ratios. Different C/N ratios (1.5, 4.0, 7.5, 10.5, and 14.5) and SRTs (infinite, 60, 30, 20, and 10 days) were applied for R1, R2, R3, R4, and R5, respectively. These researchers defined that infinite SRT means that there was no sludge wastage in R1 during the whole experiments. The performance under different operational conditions showed that when the C/N ratios (not COD/N) were higher than 4.0, the ammonium removal efficiencies were similar, all above 90.0%. The main pollutants removal pathway was nitrification-denitrification, and during anoxic phase, denitrification occurred and when appropriate carbon source was supplied. However, NO_X accumulation happened in the anoxic phase of R1 because of the reduction of denitrification caused by insufficient organic carbon supplement. In addition, COD removal efficiencies were relatively low in R1 and R2, but in R3, R4, and R5 increased to 92, 90, and 93%, respectively.

Generally, in conventional pre-anoxic systems such as MLE, A^2O, University of Cape Town (UCT), etc., nitrogen is removed through nitrification-denitrification which is accompanied by organic carbon removal, along with the bacterial assimilation. During this process, 6.6 g COD will be removed per each g NO_3^- as elector donor (Metcalf and Eddy, 2014). The removal pathways with these systems are the same for treating conventional municipal wastewater and high-strength wastewater.

COD and ammonium can be removed by conventional aerobic processes, but these treatment methods are more common for treating wastewaters with low concentrations of organic matters. Various biological aerobic treatment processes have been tried in the past (Grady et al., 2011), but high energy consumption and sludge production in aerobic processes are serious drawbacks of these systems for treating high-strength wastewaters (Wang et al., 2016). In these conventional aerobic systems, some of the COD will be removed during denitrification, and the remaining COD will be consumed in the aerobic reactor by aerobic heterotrophic bacteria, while ammonium will be removed through the conventional nitrification-denitrification process, which needs more oxygen. Nitrifiers are slow-growing bacteria, and they need a longer SRT (around 15 days) for complete nitrification. These conventional aerobic systems, efficiently and cost-effectively remove organic carbon and nitrogen via nitrification-denitrification process from low to medium-strength wastewaters.

Anaerobic digestion is the best alternative to aerobic processes for the treatment of highly polluted streams, and is being used for treating various suitable industrial wastewaters. However there are some disadvantages in these systems, such as the fact that the biological removal of nutrients is not possible and so further treatments with aerobic treatment processes are needed to meet the effluent discharge standards, and that there are difficulties in achieving stable reactor performance as a result of sensitivity to fluctuations in temperature and operating conditions (Metcalf and Eddy, 2014). Anaerobic digestion needs longer HRT and SRT as well as a heating system and corrosion-resistant equipment, and so on, which makes the operation of these systems more intensive.

However, treatment of highly polluted streams such as slaughterhouse, dairy, and food processing wastewaters, and landfill leachates with high concentrations of organic carbon and nutrients needs special consideration, and anaerobic or aerobic treatment methods alone cannot be successful. For example, the anaerobic treatment alone does not produce effluents that comply with the effluent discharge limits, and despite the high efficiency of these processes, complete stabilization of the organic matter and removal of nutrients is not possible. The final effluent produced by the anaerobic treatment process contains solubilized organic matter and nutrients which is suitable for subsequent aerobic treatment processes to meet the required effluent discharge (Chan et al., 2009). Therefore, combined systems which have the drawbacks of being complicated, expensive, and more intensive with respect to operation have been applied as a way to meet discharge standards.

In combined systems (combination of at least two single-processes in series), the leading anaerobic digestion unit removes organic carbon but produces ammonia via ammonification (ammonia production) process. Then, the following aerobic system removes the remaining COD, and ammonia through conventional nitrification-denitrification. However, as a result of carbon source scarcity in the subsequent aerobic system, external organic carbon such as methanol is needed for denitrification. Furthermore, external alkalinity should be added to the anaerobic digestion unit in order to maintain the pH in the appropriate level.

El-Kamah et al. (2010) investigated a combined system consisting of two-stage up-flow anaerobic sponge reactors (UASRs) followed by activated sludge (AS) as a post-treatment system for the treatment of fruit juice industry wastewater, and the system achieved more than

97.5% COD removal. Bustillo-Lecompte and Mehrvar (2017) investigated the feasibility of using a combined system for the treatment of slaughterhouse wastewater. This combined treatment method was composed of an anaerobic baffled reactor (ABR) process followed by an AS, and the system achieved maximum removals of 85.03% and 72.10% for organic carbon and nitrogen, respectively. Hassan et al. (2020) monitored the performance of a combined MBBR-conventional activated sludge process for organic carbon and nutrients removal, and they concluded that the results were satisfying. However, combining two single-processes is not a simple task, and operation of these system are difficult. For example, if the heading single-process does not work properly; therefore, there will not be an appropriate feed for the subsequent single-process, and the final effluent of the system will be ruined.

According to the literature review, it was not identified a study to apply a single-process system to remove organic carbon and nitrogen content from the highly polluted streams. Therefore, the current thesis research is targeting to try the possibility of an effective application of a modified single-process system for treating highly polluted streams. The possible modified system is assumed to be a single-process which will have simple operation in comparison to the combined systems. In addition, the efficiency of the modified single-process system in this thesis research may be similar to the combined systems.

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3. Simultaneous anaerobic oxidation/partial nitrification-denitrification for cost-effective and efficient removal of organic carbon and nitrogen from highly polluted streams

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Abstract

Laboratory bench-scale anoxic/aerobic reactors with complete mix and continuous flow conditions were operated with high-strength synthetic wastewater to achieve simultaneous COD and nitrogen removal. High concentrations of organic carbon and nitrogen can be found in slaughterhouse, dairy, and food processing wastewaters, and also in some landfill leachates. Therefore, the goal of this study is to find a simple, efficient, reliable, cost-effective, and general solution for organic carbon and ammonia removal from streams with high influent concentrations of more than 5000 mg/L COD and 250 mg/L NH_3–N. The highest COD (97%) and NH_3–N (91%) removal efficiencies were obtained with initial COD and ammonia concentrations of 5211 mg/L and 262.8 mg/L NH_3–N with volumetric loading rates of 11.26 kg COD/m^3 d and 0.57 kg NH_3–N/m^3 d for COD and ammonia, respectively. Anaerobic oxidation is the main COD removal pathway in a simultaneous anaerobic oxidation/partial nitrification–denitrification (SAO/PND) system, and nitrogen removal significantly occurs via bacterial assimilation and partial nitrification–denitrification pathways. There are several advantages for this proposed SAO/PND system from a practical point of view, such as feasibility of

simultaneous COD and nitrogen removal in a single reactor; simple operation; flexibility and practicality of this system as a general solution and cost effectiveness.

Keywords: Highly polluted; SAO/PND; Simultaneous removal; COD; Nitrogen

3.1. Introduction

The activated sludge process is one of the most widely used biological treatments for wastewaters that contain carbon and nitrogen pollutants (Li et al., 2014; Hamoda and Bin-Fahad, 2012). This treatment should include processes for the oxidation of biodegradable carbon and nitrification-denitrification of nitrogen. If not removed to below standard levels, organic carbon and ammonia nitrogen cause oxygen depletion and toxicity in receiving water bodies (Rodgers et al., 2006). There are efficient and reliable treatment methods for treating conventional wastewaters like municipal wastewater, but most of these methods remove organic carbon through biological aerobic methods, and remove nitrogen through bacteria assimilation and nitrification-denitrification. Fig. 3.1 depicts two modes of nitrogen removal that occur in conventional biological processes: bacteria assimilation, and nitrification-denitrification reduction pathway (Metcalf and Eddy, 2003).

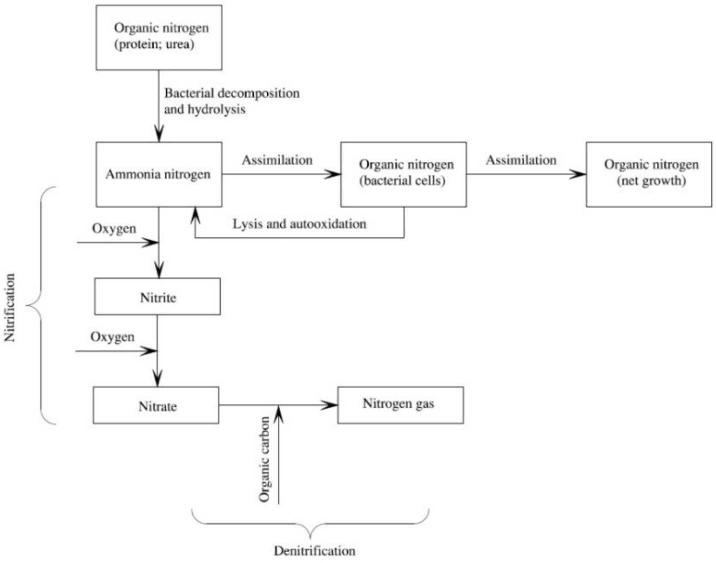

Fig. 3.1. Nitrogen transformations in biological treatment processes (Metcalf and Eddy, 2003).

Biological nitrification coupled with denitrification is commonly used in wastewater treatment plants to simultaneously remove carbon and nitrogen. Nitrification is achieved through the aerobic oxidation of ammonium (NH_4^+) or ammonia (NH_3) into nitrite (NO_2^-) by ammonia oxidizing bacteria (AOB), often *Nitrosomonas spp.*, followed by the oxidation of the nitrite into nitrate (NO_3^-) by nitrite-oxidizing bacteria (NOB), often *Nitrobacter spp*. The former is termed nitritation and the latter nitratation. Denitrification process consists of consecutive reaction steps in which nitrate is reduced to nitrogen gas by denitrifying bacteria using the organic matter of wastewater under anoxic conditions: the reduction of nitrate via nitrite and nitric oxide to nitrous oxide or nitrogen gas (Xu et al., 2014; Chen et al., 2013; Guo et al., 2013; Liu et al., 2013; Chung et al., 2012; Wunderlin et al., 2012; Andalib et al., 2011; Kim et al., 2011; Paetkau et al., 2011; Virdis et al., 2010; Adav et al., 2009; Kampschreur et al., 2009; Chiu et al., 2007; Al-Ghusain et al., 2002). However, in the nitrification process, it has been reported that ammonia concentrations higher than 150 mg/L NH_3-N will significantly inhibit the activities of both AOB and NOB (An et al., 2007).

Treatment of highly polluted streams such as slaughterhouse, dairy, and food processing wastewaters, and landfill leachates with high concentrations of organic carbon and nutrients needs special consideration. Most of the treatment processes used for such wastewaters are costly, complicated with respect to operations and maintenance, sensitive to changes, and need long HRTs. Also, the removals happen in different reactors, and none of these treatment methods can remove organic carbon and nitrogen simultaneously in a single reactor. Typical ranges of organic carbon and nitrogen for highly polluted streams reported in the literature include: slaughterhouse wastewater (COD=876-9,300 mg/L and ammonia=84-584.2 mg/L NH_3-N) (Pan et al., 2014; Fongsatitkul et al., 2011; Lemaire et al., 2009; Li et al., 2008; Moreira et al., 2008; Meknassi et al., 2005; Meknassi et al., 2004), dairy wastewater (COD=1,150-9,200 mg/L and ammonia=14-288 mg/L NH_3-N) (Chokshi et al., 2016; Lu et al., 2015; Mutamim et al., 2012; Sarkar et al., 2006; Demirel et al., 2005), food processing wastewater (COD=1,000-18,000 mg/L and ammonia=53-1,000 mg/L NH_3-N) (Galib et al., 2016; Giustinianovich et al., 2014; Rodríguez et al., 2011; He et al., 2005), and many landfill leachates in advanced countries with appropriate solid waste management systems, such as in most Western countries (COD=1,740-13,800 mg/L and ammonia=72-800 mg/L NH_3-N) (Wang et al., 2014; Wei et al., 2012; Eldyasti et al., 2010; Renou et al., 2008; Laitinen et al., 2006; Kennedy et al., 2000).

There are different kinds of treatment methods for nitrogen removal, but generally, most of the treatment methods for high concentrations of organic carbon are anaerobic methods. Anaerobic processes have been successfully applied to streams with high concentrations of soluble organic carbon. These processes are also feasible for the treatment of low-strength wastewaters, such as domestic wastewater, particularly under tropical conditions in which the supply of heat is not required. However, post treatment of the anaerobic effluent is necessary to further polish the remaining organic pollutants and nutrients (Mendes et al., 2016). Operational stability in anaerobic systems is one of the major concerns as these systems are highly sensitive to temperature and operational changes that can cause adverse effects. In addition, anaerobic treatment needs a following aerobic treatment process to meet discharge requirements (Metcalf and eddy, 2003).

Anaerobic digestion (AD) processes are usually classified as mesophilic AD (30-35°C) or thermophilic AD (55°C), but there is a relatively new trend which involves operating the AD process at a higher temperature range (65-80°C), which is referred to as hyper-thermophilic AD (Alqaralleh et al., 2016). AD processes are advantageous because of the lower biomass yields (0.05-0.1 g VSS/g COD) and energy demand, as methane can be recovered from the biological conversion of organic substrates. Typical volumetric organic loading rates for anaerobic suspended growth processes at 30°C with HRTs of 15-30 d are 1-5 kg COD/(m^3.d). On the other hand, typical volumetric organic loading rates for complete mix and suspended growth aerobic processes at HRTs of 3-5 hrs are 0.3-1.6 kg BOD/(m^3.d), and the yield of these systems is 0.3-0.5 g VSS/g biodegradable COD (Metcalf and eddy, 2003).

When treating high strength organic wastewaters, aerobic or anaerobic treatment alone do not produce effluents that comply with effluent discharge limit (Chan et al., 2009). Therefore, conventional biological treatment methods for highly polluted streams consist of combined anaerobic-aerobic treatment processes. The final effluent produced by anaerobic treatment contains solubilized organic matter, and this is a suitable feed for a following aerobic treatment to meet the effluent discharge standards (Chan et al., 2009). In general, in these combined systems, the majority of COD will be removed by AD in anaerobic units, with accompanying ammonia production by ammonification (ammonia production). Then, the following aerobic system will remove the remaining COD aerobically, and ammonia through nitrification-denitrification. This means that conventional combined anaerobic-aerobic treatment processes are expensive and more complicated with respect to operations and maintenance.

Combined anaerobic-aerobic treatment processes are conventional biological treatment methods for high strength organic wastewaters, and there have been no reports on biological treatment processes capable of simultaneously removing high concentrations of ammonia and COD in a single reactor. Therefore, the main objective of this study is to develop a novel, low intensity operation, and cost effective solution for simultaneous organic carbon and nitrogen removal from highly polluted streams. Based on COD and ammonia ranges presented earlier, the target influent COD and ammonia concentration values considered in this research are those higher than 5,000 mg/L and 250 mg/L NH_3-N, respectively.

3.2. Material and methods

3.2.1. Laboratory Reactor Setup

Following the main objectives, in this research a laboratory bench-scale system with complete mixed and continuous flow conditions was set up in the environmental engineering laboratory of the Civil Engineering department at the University of Ottawa. A Simultaneous Anaerobic Oxidation/ Partial Nitrification-Denitrification (SAO/PND) system with anoxic/aerobic reactors followed by a clarifier was used to simultaneously remove carbon and nitrogen from highly polluted streams (Fig. 3.2). The system was originally seeded by using waste activated sludge (WAS) from the Robert O. Pickard Environmental Centre (ROPEC) wastewater treatment plant in Ottawa, Canada to promote the growth of the microbial population. This plant was designed with a solids retention time (SRT) of 5 days for biological BOD and phosphorus removals without nitrogen removal. However, during warm seasons, like the sampling period for this research, limited nitrification occurs without denitrification.

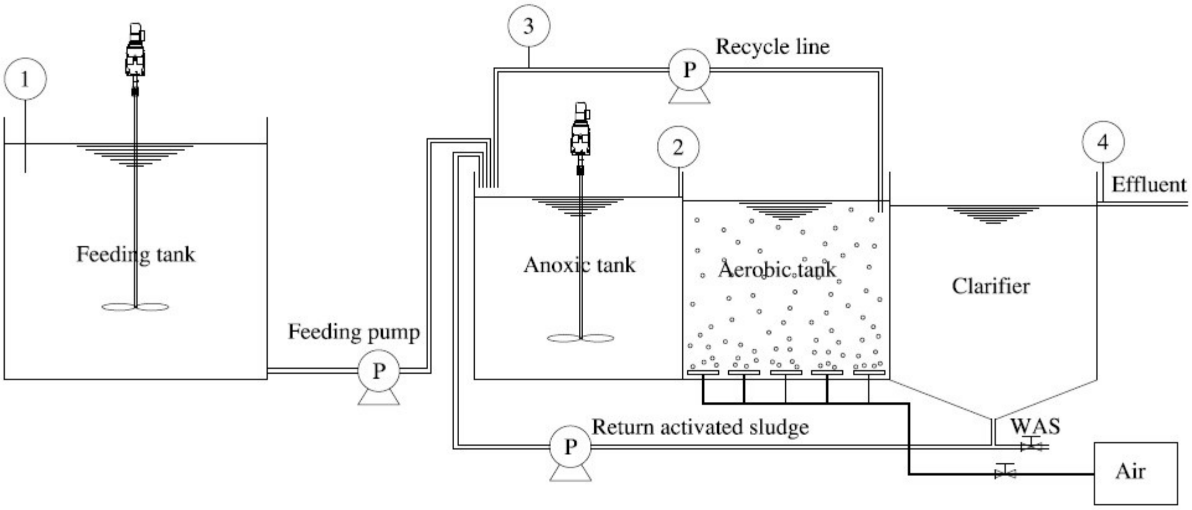

Fig. 3.2. Schematic flow diagram of bench-scale SAO/PND system with sampling points 1, 2, 3, and 4 (Not to Scale).

The effluent of the anoxic tank was fed into the aerobic tank, and there was a recycle line from the effluent of the aerobic tank to the beginning of the anoxic tank. The purpose of this line was to bring the produced nitrite/nitrate from the end of aerobic tank back to the anoxic tank. The effluent of the aerobic tank was connected to a clarifier, and there was another line to bring the return activated sludge (RAS) back to the beginning of the anoxic tank. The flowrate of this line was adjusted to maintain the required mixed liquor suspended solids (MLSS) in the biological reactors. Adjustable speed peristaltic pumps (Cole Parmer Instrument Company) were used to control the flowrates of the influent, recycle, and return lines.

The separation of solids in the activated sludge process is a very important function in order to provide well-clarified effluent and concentrated solids that are returned to the biological treatment system or are wasted to the solids processing facilities (Metcalf and Eddy, 2003). The excess produced sludge in the system was wasted manually on a daily basis to adjust the MLSS in the reactors and SRT of the system. The MLSS and SRT adjustments were done by RAS line and daily wasted sludge.

In order to run the system at constant temperature (25±1°C) during the experiments, temperature was maintained in both reactors by using an aquarium heater (Tetra HT30, 100w) attached to the inside wall of the aerobic reactor. Regular aquarium air stones from a pet store were purchased and used to aerate the aerobic reactor. Also, the air flow line was supplied from the laboratory air pipeline and the amount of applied air flowrate was enough for complete mixing in this reactor. A variable-speed overhead stirrer (Talboys Engineering Corp.; Model 134-2) set to 150 rpm was used for mixing purposes in the anoxic tank.

To monitor the performance of the overall system and each reactor, there were four different sampling points in the system: in the influent, anoxic tank effluent, recycle line and effluent (as depicted by sampling point numbers 1, 2, 3, and 4 respectively in Fig. 3.2).

3.2.1.1. Preliminary Experiments

Preliminary experiments were conducted before running the main phase of the experiments in order to optimize the operating conditions of the system to achieve stable steady-state conditions. During the preliminary experiments of this research, different design and operational scenarios were tried, and after 6 trials which resulted in system failure, the optimum conditions were determined. As stated previously, the main goal of this study is simultaneous removal of organic carbon and nitrogen, and failure was defined as at least one of these components (COD or nitrogen) not being removed. During these experiments, different HRTs, Q_r/Q_{in} ratios, MLSS in the reactors, Q_{RAS}/Q_{in} ratios, DO in the aerobic reactor, and SRTs were tried to find the optimum conditions in order to apply during the main phase of the research. In order to see the effects of each item during the preliminary experiments, only one parameter at a time was changed and the other parameters remained unchanged.

3.2.1.2. Main phase of SAO/PND Operation with Synthetic Wastewater

The SAO/PND consisted of three Plexiglas reactors with effective volumes of 7.8 L, 10.8 L, and 5.5 L for the anoxic, aerobic, and clarifier reactors, respectively. Influent flowrate to the system was 33 L/d between days 1 to 108, and then reduced to 17 L/d from day 109 till the end. The reduction in flowrate was done due to an observed decline in COD and nitrogen removal efficiency and in order to maintain the same removal efficiency in the system. The HRTs were 5.7 hrs and 7.85 hrs for the anoxic and aerobic tanks respectively from days 1 to 108, but were increased to 11 hrs and 15.2 hrs from days 109 to 172 as a result of reduction in flowrate.

During this phase, only COD and ammonia concentrations were measured in the influent. Nitrite and nitrate concentrations were confirmed to be negligible in the feeding tank. For the remaining sampling points, dissolved COD, ammonia, nitrite, and nitrate were measured from other sampling points on a weekly basis. In this research, total inorganic nitrogen (TIN) is defined as the sum of ammonia, nitrite, and nitrate.

The effluent of the anoxic tank was fed into the aerobic tank, and there was a recycle line from the effluent part of aerobic tank to the beginning part of the anoxic tank, and flowrate of this line was twice of influent ($Q_r=2Q_{in}$). Also, the return sludge flowrate was optimized in order to maintain the required MLSS in the biological reactors. The optimized return flow was equal to the influent flowrate ($Q_{RAS}=Q_{in}$).

As mentioned before, a fraction of the produced sludge in the system was wasted manually on a daily basis to keep the MLSS in the anoxic/aerobic reactors around 3,500±500 mg/L. The required MLSS in the system was adjusted by the RAS line and the daily wasted sludge. Under these conditions, the SRT in the system was around 4±1 days.

After finishing the preliminary and main phase of the experiments, a set of complementary experiments was fulfilled in order to clarify the COD and nitrogen removal pathways of the SAO/PND system. All descriptions of the removal pathways in the complementary experiments have been presented based on mass balance calculations in steady-state conditions and mean values of triplicates with related standard deviations.

3.2.2. Synthetic Wastewater

A stock solution was prepared and stored weekly or biweekly in the fridge at +4°C, and this stock solution was used to prepare synthetic wastewater with different concentrations. All stock solutions were prepared by diluting chemicals with distilled water in a 1000 mL volumetric beaker, and a magnetic stirrer helped dissolve the chemical compounds in distilled water. In addition, three different 1000 mL volumetric flasks were used to store the stock solutions: one flask for carbon source, one flask for the macronutrients and another one for micronutrients. All chemical compounds were purchased from the Fisher Scientific Company.

A common recipe for synthetic wastewater with small modifications was used to prepare synthetic wastewater. The stock solution for carbon source was composed of: 75g $C_6H_{12}O_6$, 75g Peptone, and 40g NaAc. The stock solution for macronutrients contained: 0.225g $FeCl_3.6H_2O$, 40g NH_4Cl, 22.05g $KH_2PO_4.2H_2O$, 1.5g EDTA, and 75g $NaHCO_3$, and the stock solution for micronutrients consisted of: 10g $MgSO_4.7H_2O$, 5.3g $CaCl_2$, 0.0225g H_3BO_3, 0.005g $CuSO_4.5H_2O$, 0.027g KI, 0.018g $MnCl_2.4H_2O$, 0.018g $ZnSO_4.7H_2O$, 0.05g $Na_2MoO_4.2H_2O$, and 0.005g $CoCl_2.6H_2O$ (Black et al., 2014; Zhao et al., 2008). To start up the system with low concentrations, 80 mL of each prepared stock solution was added and diluted with distilled water in a 40 L container to achieve around 390 mg/L COD and 22 mg/L NH_3-N concentrations in the influent. The influent COD and ammonia concentrations were increased stepwise by diluting more stock solutions in the 40 L container to increase the influent concentrations. After almost two months of operation, due to high consumption of the stock solutions, more concentrated stock solutions were prepared and used to achieve the desired influent COD and ammonia values.

3.2.3. Analytical Methods

Soluble COD, ammonia, nitrite, nitrate, alkalinity, total suspended solids (TSS), pH, and temperature were monitored during operation in order to investigate the performance of the SAO/PND system. In addition, total phosphate (TP), and total nitrogen (TN) were measured during the complementary experiments. HACH vials TNT 822, TNT 832, TNT 840, TNT 835, TNT 870, TNT 845, and TNT 827 were used to measure soluble COD, ammonia, nitrite, nitrate, alkalinity, TP, and TN concentrations, respectively, by a HACH DR 6000^{TM} UV VIS. Concentrations of soluble COD, ammonia, nitrite, nitrate, alkalinity, TP, and TN measured by the spectrophotometer were mg/L COD, mg/L NH_3-N, mg/L NO_2-N, mg/L NO_3-N, mg/L $CaCO_3$, mg/L PO_4-P, and mg/L N-N, respectively.

TSS was measured based on the analytical procedure in the standard methods (APHA, 1998). Dissolved oxygen (DO), pH, and temperature were directly measured during this experiment by a HQ40d Portable Meter with LDO101 optical dissolved oxygen and PHC101 pH probes (HACH Company, USA).

3.3. Results and Discussion

3.3.1. COD Removal and Loading Rates

COD samples were collected and analyzed from the influent, anoxic tank effluent, recycle line, and effluent of the system (sampling points shown in Fig. 3.2) for more than five months during the main phase of this study. The results are presented in Fig. 3.3. The effluent COD of the system remained constant and below 150 mg/L until day 152 (97% COD removal) and started to increase to 480 mg/L by increasing the influent COD until the system was stopped on day 172 (92% COD removal).

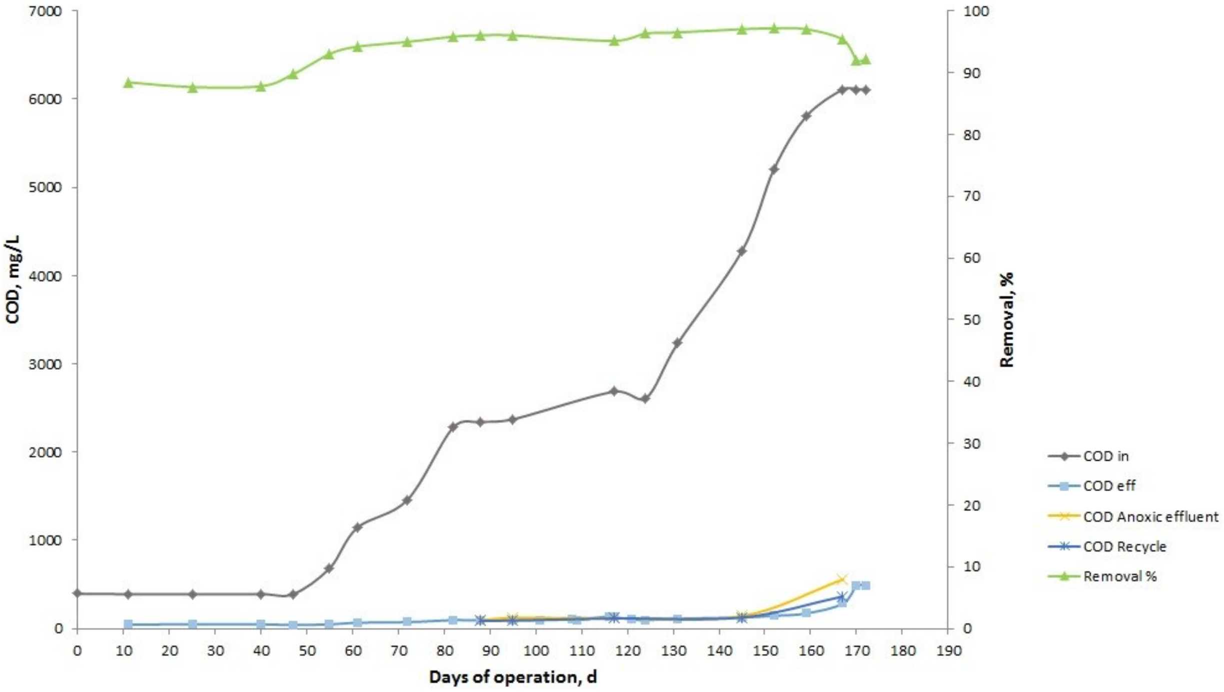

Fig. 3.3. COD changes in the SAO/PND system and removal percentages during operational days.

After the start-up of the system, the influent COD concentrations were gradually increased stepwise to reach the concentrations representing high strength wastewaters from industries such as slaughterhouses, dairy farms, and food processing, as well as landfill leachates. After each increase, it took 7 days to reach steady-state conditions. The results presented in Fig. 3.3 show that a maximum 97% COD removal was achieved from the start until day 152 before the system started to show signs of stress and failure. The removal pathway is discussed later in the complementary experiments section. The overall COD removal percentages for the SAO/PND system presented in Figs. 3.3 and 3.4 were calculated using Equation (3.1):

$$\text{COD removal}, 100\% = \frac{COD_{in} - COD_{eff}}{COD_{in}} \times 100\%, \qquad (3.1)$$

where COD_{in} is the influent COD to the system from sampling point 1 as shown in Fig. 3.2, and COD_{eff} is the effluent COD of the SAO/PND system from sampling point 4.

Volumetric COD loading rates are presented only for the anoxic tank because almost all COD removal happened in this reactor as discussed later. Fig. 3.4 depicts the volumetric loading rates and removal percentages for COD in the anoxic tank. The highest volumetric COD loading rate of the anoxic tank with highest removal efficiency was 11.26 kg COD/(m^3.d) after 152 days of operation. In addition, the equivalent highest COD mass loading rate on day 152 was 5.10 kg COD/(kg VSS.d). As mentioned before, the influent flowrate to the system was 33 L/d between days 1 to 108, and then reduced to 17 L/d from day 109 till the end. As a result of this flowrate change, a volumetric COD loading drop occurred in the anoxic tank which can be seen in Fig. 3.4.

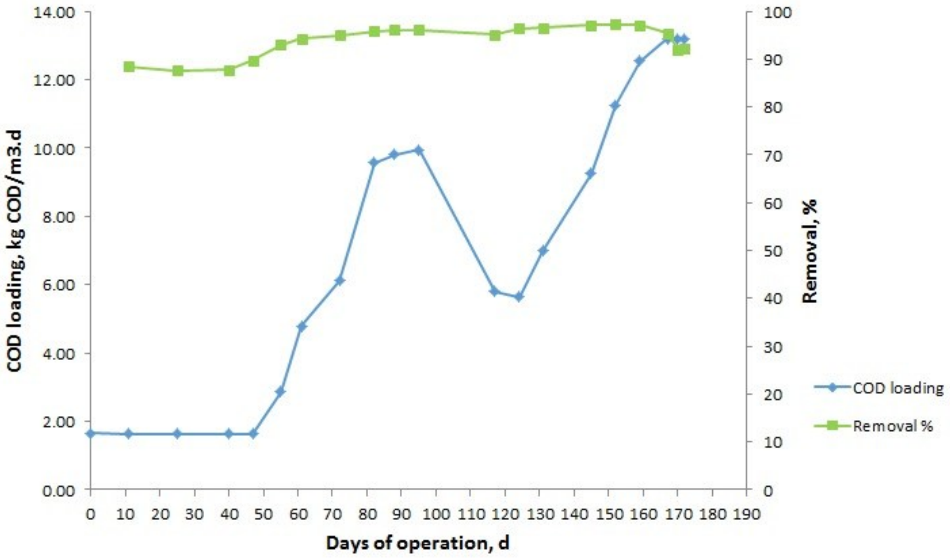

Fig. 3.4. COD Volumetric loading rates and removal percentages in the anoxic tank during operational days.

Hamoda and Bin-Fahad (2012) conducted a pilot-scale study to investigate the effect of hydraulic loading on nitrogen removal in-parallel testing of aerated submerged fixed-film (ASFF) and anoxic/aerobic submerged fixed-film (A/ASFF). They used a four-compartment reactor that was packed with Biolace media and operated at loadings of 0.03 to 0.3 g BOD/g BVS. d, 0.01 to 0.11 g NH_3/g BVS. d, HRTs 0.7 to 8 h, C/N of 6, and 28±2 °C. Biomass volatile solids (BVS) is the summation of mixed liquor volatile suspended solids (MLVSS) and attached volatile solids (AVS). All compartments of the ASFF reactors were aerated, but in the A/ASFF, only the second to fourth compartments were aerated. The results showed that the system effectively treated municipal wastewater with COD, BOD and ammonia removals of up to 75%, 98%, and 97%, respectively. Also, increasing of loading rates up to 10-fold did not have negative effects on the overall performance of operation modes. The results from Hamoda and Bin-Fahad (2012) depicted that COD and nitrogen removal percentages were higher than those removal percentages achieved by the SAO/PND system. On the other hand, the applied mass loading rates by Hamoda and Bin-Fahad (2012) were lower than those achieved by the SAO/PND system.

3.3.2. Nitrogen Removal and Loading Rates

Fig. 3.5 presents the change in TIN at different sampling points during the main phase of this study. The nitrogen removal pathway is discussed later. After 108 days of operation, the efficiency of the nitrogen removal declined, and; therefore the influent flowrate was decreased to 17 L/d on day 109, and HRT in the anoxic and aerobic reactors increased to 11 hrs and 15.2 hrs, respectively. Longer HRTs effectively increased the nitrogen removal efficiency and 91% ammonia removal was achieved on average by this system, but the COD removal improvement was not significant. The nitrogen removal percentages in Figs. 3.5 and 3.6 are the overall TIN removal percentages achieved by the SAO/PND system and were calculated using Equation (3.2):

$$\text{TIN removal}, 100\% = \frac{TIN_{in} - TIN_{eff}}{TIN_{in}} \times 100\%, \qquad (3.2)$$

where TIN_{in} is the influent TIN to the system from sampling point 1 in Fig. 3.2, and TIN_{eff} is the effluent TIN of the SAO/PND system from sampling point 4.

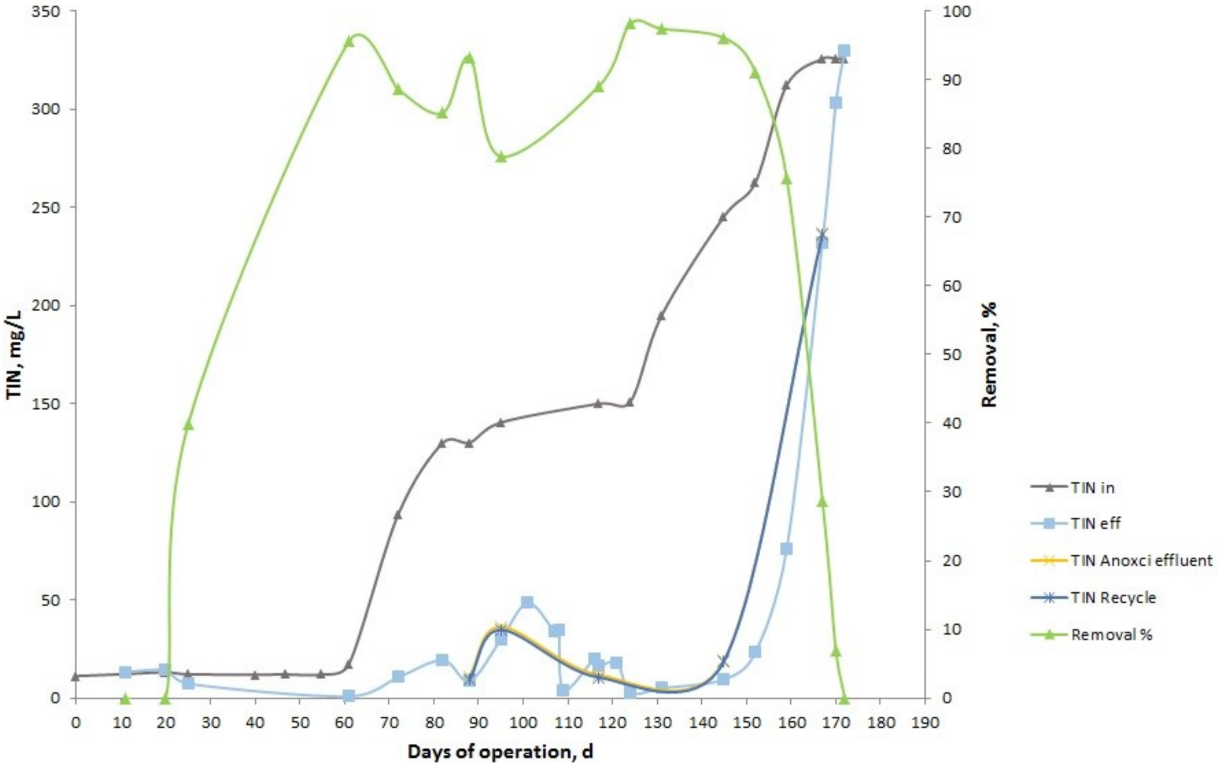

Fig. 3.5. TIN changes in the SAO/PND system and removal percentages during operational days.

The TIN volumetric loading rates have been presented for the anoxic tank only, because most of the TIN removal took place in this reactor. Fig. 3.6 shows the volumetric loading rates and overall removal percentages of TIN in the anoxic tank. The highest volumetric nitrogen loading in the anoxic tank with high removal efficiencies was 0.57 kg TIN/(m^3.d) after 152 days of operation. In addition, the highest equivalent TIN mass loading rate on day 152 was 0.26 kg TIN/(kg VSS.d). As mentioned before, the nitrogen removal percentages achieved by the SAO/PND system are lower than those achieved by Hamoda and Bin-Fahad (2012), but the nitrogen mass loadings were higher.

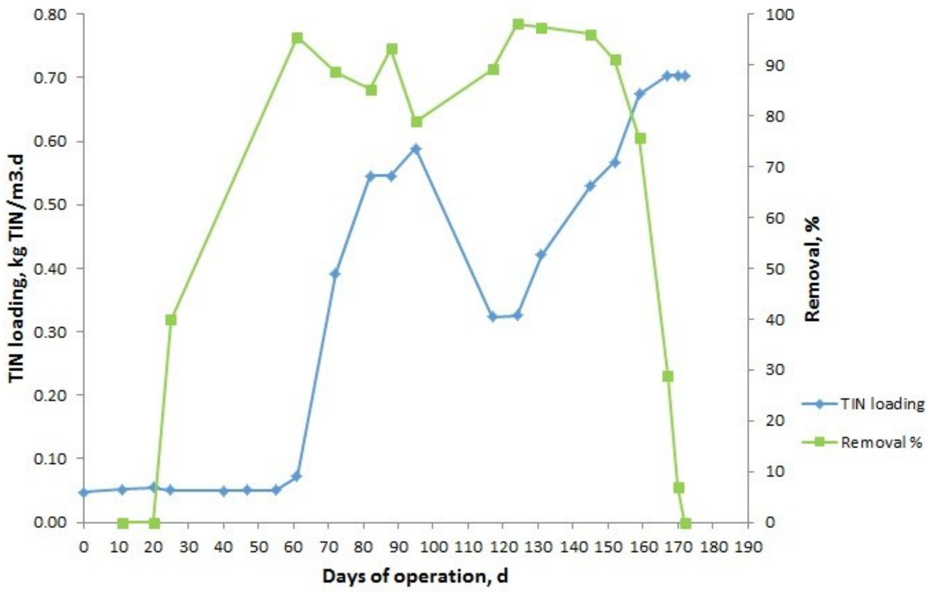

Fig. 3.6. TIN Volumetric loading rates and removal percentages in the anoxic tank during operational days.

Hamoda and Bin-Fahad (2012) reported that both the ASFF and A/ASFF systems achieved high specific nitrification rates of up to 0.096 g N/g BVS. D. In addition, their research showed the presence of active nitrifying microorganisms mainly in the second and third compartments at all loading rates and presence of nitrates in the aerobic compartments confirmed the activity of the nitrifying bacteria. Nitrogen transformations happened during nitrification process, and ammonia was oxidized in the presence of oxygen as an electron acceptor (Hamoda and Bin-Fahad, 2012). However, only 17% of nitrogen removal pathway of the SAO/PND system was nitrification-denitrification which was similar to the research conducted by Hamoda and Bin-Fahad (2012) as will be explained later in the complementary experiments section.

Hamoda and Bin-Fahad (2012) observed that the A/ASFF was more stable and efficient at higher loadings, with 60% COD removal and 90% BOD removal in the anoxic stage. On the other hand, 99% of COD removal occurred in the anoxic reactor in the SAO/PND system, as will be discussed later in the complementary experiments section. In addition, Hamoda and Bin-

Fahad (2012) showed that denitrification with organic carbon removal occurred in the A/ASFF system, which eliminated 6.6 g COD (or 3.35 g BOD) per each gram of denitrified NO_3-N. As a general rule, it has been estimated that for denitrification processes, 4 g of BOD is needed per 1 g of NO_3-N reduced to provide sufficient amount of electron donors for nitrate removal (Metcalf and eddy, 2003). Hamoda and Bin-Fahad (2012) clarified that COD and BOD were consumed in the anoxic reactor as carbon source by denitrifying bacteria that was the great advantage of implementing the anoxic process for organic carbon removal. On the other hand, only 5% of organic carbon was removed by denitrification in the SAO/PND system as will be discussed later in the complementary experiments section.

After 152 days of SAO/PND operation, the highest influent COD and ammonia concentrations were 5,211 mg/L and 262.8 mg/L NH_3-N respectively, with 97% and 91% removal efficiencies respectively. These were the maximum COD and ammonia concentrations that the SAO/PND system could remove with high efficiency, and higher concentrations of ammonia proved to be toxic to the system, as it failed with the higher concentrations.

When the SAO/PND system failed, two different and distinct types of sludge were observed in the clarifier: a discrete floating sludge layer at the top, and a denser settled sludge layer at the bottom, with a clear layer between the two layers. After system failure, daily WAS was taken only from the floating sludge, and after several days of repeating the same procedure, almost all of the floating sludge layer had disappeared. The results on day 159 showed that the COD removal efficiency was still high (around 97%), but the nitrogen removal had declined to 76% and continued to decrease to 7% on day 170. The COD removal efficiency on day 170 was still high, showing 92% removal. Finally, on day 172, the COD removal remained at 92%, but nitrogen production began occurring in the system instead of nitrogen removal.

A possible scenario for this reactor upset and system response could be that the COD and nitrogen removal bacteria were from different species and they were cooperating effectively with each other to achieve simultaneous COD and nitrogen removal in the SAO/PND system. The bacteria responsible for nitrogen removal were more sensitive to ammonia toxicity, while the bacteria responsible for carbon removal could tolerate the toxic ammonia conditions and continued to work efficiently.

It should be mentioned that the main phase of this study was repeated again and similar trends were observed, which showed failure happening again at maximum COD and ammonia concentrations of 5,412 mg/L and 288.6 mg/L NH_3-N respectively, with 97% and 92% removal efficiencies respectively.

3.3.3. pH Changes

Fig. 3.7 presents the changes in pH during operations, and pH was between 7 and 8 in both the anoxic and aerobic reactors. Total alkalinity was not measured routinely, but was measured occasionally to make sure there was enough alkalinity in the system (at least 80 mg/L $CaCO_3$ is required for appropriate nitrification). In this study, total alkalinity in the anoxic and aerobic tanks was always enough for nitrifiers. However, alkalinity in the anoxic tank was more than in the aerobic tank, which was because of alkalinity consumption by nitrifiers for nitrification purposes in the aerobic reactor.

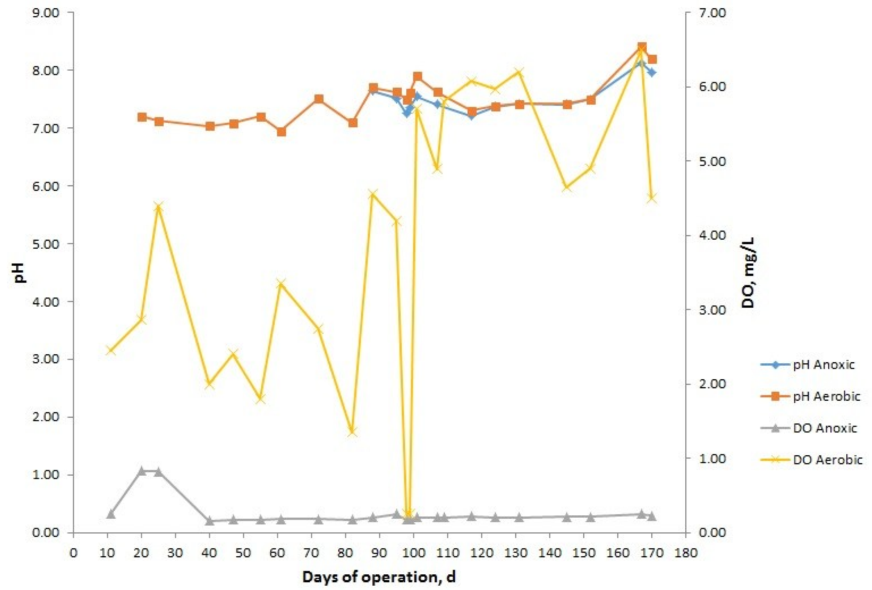

Fig. 3.7. pH and DO changes in the anoxic and aerobic reactors during operational days.

3.3.4. DO Changes

DO concentrations in the aerobic tank were adjusted to higher than 3 mg/L, because in conventional nitrification systems, DO of lower than 3 mg/L is not favorable for nitrification (Metcalf and eddy, 2003). DO adjustments were conducted by changing the influent air flowrate to the reactor and measuring DO inside this reactor with a DO meter. As depicted in Fig. 3.7, DO in the aerobic tank was around 4-5 mg/L and DO was not a limiting factor for nitrification in this research. On the other hand, recommended DO concentrations in the anoxic tank should be lower than 0.25 mg/L for conventional denitrification purposes, and DO in the anoxic tank was generally less than 0.25 mg/L (Fig. 3.7).

During the preliminary experiments, different DO concentrations in the aerobic tank was tried, and there was not a big difference in the efficiency of overall nitrogen removal in the SAO/PND system because nitrification-denitrification is not the main nitrogen removal pathway, as will be explored in more detail in the nitrogen removal pathway discussion. However, the aerobic tank played a key role in this system and if this tank was removed from the set-up, the system would run under unfavorable anaerobic conditions, which would completely ruin the performance of the SAO/PND system.

As mentioned before, temperature was kept constant during operations by a simple aquarium-type heater at around 25±1°C, which is a favorable temperature for the active bacteria in this system.

3.3.5. Complementary Experiments

After finishing the main phase of the experiments and in order to clarify the COD and nitrogen removal pathways of the SAO/PND system, a set of complementary experiments were conducted. This phase was operated for a month under steady-state conditions with constant influent concentrations, Q_{in}=17 L/d, Q_r=2Q_{in}, and Q_{RAS}=Q_{in}. As already discussed, the highest influent concentrations treated by SAO/PND in main phase of this study with high removal efficiencies were COD=5,211 mg/L and ammonia=262.8 mg/L NH_3-N. Therefore, it was decided to run complementary experiments with safer concentrations, and 80% of influent concentrations were selected as feed in order to run the system without it failing. The average constant influent COD and ammonia concentrations in the system during complementary experiments were 4,116 mg/L and 210.9 mg/L NH_3-N, as depicted in Fig. 3.8.

Soluble COD, ammonia, nitrite, nitrate, alkalinity, TP, and TN were monitored from all four sampling points in the complementary experiments, as shown in Fig. 3.8. In addition, unfiltered TN and TP from the influent and WAS were monitored to determine the amount of bacterial assimilation.

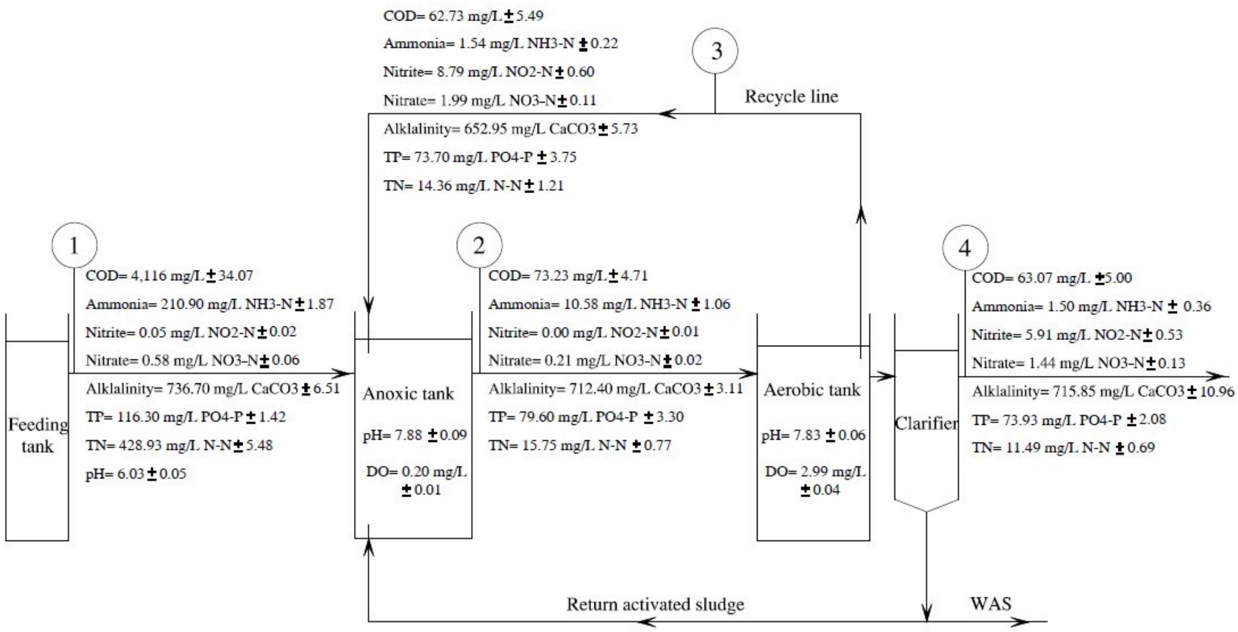

Fig. 3.8. Performance of the SAO/PND system during complementary experiments.

The removal pathways of the SAO/PND system are based on mass balance calculations in steady-state conditions, and the mean values of triplicates with standard deviations have been presented in Fig. 3.8. Different COD and nitrogen mass balance calculations were made around each reactor separately as well as the overall system in order to clarify the COD and nitrogen removal pathways. In addition, individual mass balances were made for ammonia, nitrite, nitrate, and TIN separately around the anoxic and aerobic reactors and the overall system to monitor the nitrogen conversions in the SAO/PND process.

3.3.5.1. COD Removal Pathway

Fig. 3.8 depicts that 99% COD removal occurs in the anoxic reactor, and that the subsequent aerobic reactor removes the remaining 1%. The main mechanisms of COD removal by the SAO/PND process include: i) 73% anaerobic oxidation; ii) 21% cell synthesis and biomass production; iii) 5% denitrification; and iv) 1% by aerobic heterotrophs in the aerobic reactor.

There is some evidence confirming that the anoxic tank behaves like an anaerobic reactor, as explained below:

(1) The biomass yield (Y) for aerobic activated sludge systems is 0.64 g VSS/g BOD but this value is 0.13 g VSS/g BOD for anaerobic systems (Metcalf and eddy, 2003). The determined biomass yield for the SAO/PND system is 0.15 g VSS/g BOD, and this value is close to $Y_{anaerobic}$.

(2) The typical volumetric organic loading rates for anaerobic suspended growth processes at 30°C with an HRT of 15-30 d are 1-5 kg COD/(m^3.d) (Metcalf and Eddy, 2003). The highest volumetric COD loading rate by the SAO/PND system in the anoxic tank is 11.26 kg COD/(m^3.d), and this value is similar to anaerobic processes.

(3) In addition, Xie et al. (2012) reported that anaerobic conditions prevail at COD/NO_x-N >53. This ratio in the anoxic tank of SAO/PND system is 350/1, which proves the anaerobic conditions in this reactor.

The above reasons confirm that the main mechanism of organic carbon removal in the SAO/PND system is anaerobic oxidation in the anoxic reactor. However, bacterial growth and denitrification processes consume some portions of the available organic carbon as well. The active denitrifiers in the anoxic reactor are heterotrophic bacteria that consume COD as an electron donor during nitrite and nitrate reduction.

3.3.5.2. Nitrogen Removal Pathway

The detection of new organisms is making the nitrogen cycle increasingly complicated, to the point that traditional descriptions like nitrification-denitrification are rather simplistic and insufficient for the explanation of nitrogen pathways in real life. The nitrogen removal pathways in SAO/PND include: i) 46% assimilation and synthesis into organism compositions; ii) 17%

partial nitrification-denitrification; iii) 26% experimental errors; and iv) 11% still lacking and unknown.

Mass balance calculations of unfiltered TN around the overall system revealed that 46% of nitrogen is assimilated and exits the system as sludge. In addition, individual mass balance calculations of filtered ammonia, nitrite, nitrate, and TIN around the anoxic and aerobic reactors proved the occurrence of 17% ammonia conversion in the aerobic tank. Mass balance calculations revealed that 17% of the total influent ammonia is converted to mostly nitrite and nitrate via partial nitrification in the aerobic reactor, and the NO_2^-/NO_3^- ratio production is 4.9/1. These oxidized nitrogen species are recycled back to the anoxic reactor for consumption by heterotrophic denitrifiers as electron acceptors, and is accompanied by 5% of organic carbon removal.

In general, the complementary experiments in this research show that only 17% of the nitrogen and 5% of the organic carbon removal pathways in SAO/PND are similar to common pre-anoxic nitrification-denitrification systems. In addition, almost all the COD and nitrogen removal occurs in the anoxic reactor, and the subsequent aerobic reactor plays a polishing role. However, the aerobic tank is part of the SAO/PND design which plays a critical role in the successful operation of this treatment method. As part of the design, this reactor helps to provide a suitable environment for all the adapted bacteria to cooperate properly with each other. Finally, it should be mentioned that the performance of SAO/PND process will be ruined if the aerobic tank is excluded.

3.3.6. Advantages and Cost Effectiveness of SAO/PND

There are many advantages of this new system, such as: i) feasibility of efficient and simultaneous COD and nitrogen removal from highly polluted streams in a single reactor; ii) simple operation; iii) flexibility and practicality of this system as a general solution for different kinds of streams ranging from conventional municipal wastewaters to highly polluted streams like industrial wastewaters with high organic carbon and nitrogen; and iv) very cost-effective in comparison with other treatment methods. The cost-effectiveness of SAO/PND is elaborated further below:

(1) Very low oxygen consumption. Aerobic treatment processes use oxygen to remove organic carbon and nitrogen from streams, and as such, higher influent concentrations of organic carbon and nitrogen need higher oxygen consumption. Also, dissolving the high amounts of required oxygen is not simple, and will need special consideration in some cases. However, the SAO/PND system could reduce huge amount of oxygen requirements in comparison with aerobic treatment methods and related costs.

(2) Very low alkalinity consumption. In order to remove high concentrations of nitrogen in conventional complete nitrification-denitrification systems, large amounts of alkalinity should be added. Also, it should be mentioned that dissolving high amounts of alkalinity in wastewaters is a big issue, and is not a simple and easy task. Since nitrification-denitrification is not the main nitrogen removal pathway in the proposed SAO/PND system, there is less alkalinity requirement. In addition, most wastewater streams contain enough alkalinity, and thus the SAO/PND system does not need alkalinity addition.

(3) Low sludge production. In comparison with the aerobic treatment methods, management of small quantities of the sludge produced by the SAO/PND system will be simpler and less costly.

(4) Low recycle (Q_r/Q_{in}) ratio. Conventional nitrification-denitrification systems need higher recycle ratios, which results in bigger recycling pumps with larger diameters of recycle line piping. However, the recycle ratio in the SAO/PND system is low, and handling the line will be simpler and more cost-effective.

(5) Short HRT. Conventional anaerobic processes need longer HRTs in order to remove high concentrations of organic carbon, but the SAO/PND system needs shorter HRTs which results in savings.

(6) Simple operation. Anaerobic treatment processes need higher-skilled operators in order to be able to run the system in stabilized conditions with high efficiency without system failure. However, operation of the SAO/PND system is simple and can be kept stabilized without higher-skilled staff, and this will save on operational costs.

(7) Low maintenance and equipment costs. In order to run anaerobic treatment processes in stable conditions, special considerations with higher costs are needed, such as proper heating systems, sealed and airtight reactors, proper mixing, etc. However, the SAO/PND system does not need these consideration and will save on costs related to maintenance and equipment.

(8) Very low energy consumption. As already stated, aerobic processes have huge oxygen requirements that need energy, and anaerobic processes use energy for heating and other equipment, but the SAO/PND system results in savings due to lower energy costs.

3.4. Conclusions

The present study is an attempt to investigate the treatability of some strong wastewaters by using the SAO/PND system. Simultaneous organic carbon and nitrogen removal were observed mostly in the anoxic tank, but the subsequent aerobic reactor played a polishing role. However, the aerobic reactor plays a key role in the SAO/PND system, and exclusion of this reactor will destroy the system's overall performance. This study showed 97% COD and 91% nitrogen removal by the SAO/PND system, with influent COD and ammonia concentrations of 5,211 mg/L COD, and 262.8 mg/L NH_3-N, respectively. In addition, maximum volumetric organic loading rates of 11.26 kg COD/(m^3.d), and 0.57 kg TIN/(m^3.d) were reported on day 152, with equivalent mass loading rates of 5.10 kg COD/(kg VSS.d) and 0.26 kg TIN/(kg VSS.d).

The results of the complementary experiments revealed that the COD removal pathway is: i) 73% anaerobic oxidation; ii) 21% cell synthesis and biomass production; iii) 5% denitrification; and iv) 1% by aerobic heterotrophs in aerobic reactor. On the other hand, the nitrogen removal pathway is composed of: i) 46% assimilation and synthesis into organism compositions; ii) 17% partial nitrification-denitrification; iii) 26% experimental errors; and iv) 11% still lacking and unknown.

There are several advantages for this proposed SAO/PND system from the practical point of view, such as: feasibility of simultaneous COD and nitrogen removal in a single reactor; simple operation; flexibility and practicality of this system as a general solution for different kinds of streams ranging from conventional municipal wastewaters to highly polluted streams like industrial wastewaters with high organic carbon and nitrogen; and very cost-effective in comparison with other treatment methods.

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4. Determination of design loading rates for simultaneous anaerobic oxidation/partial nitrification–denitrification process and application in treating dairy industry effluent

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Abstract

Organic carbon and nitrogen removal from highly polluted streams by using a simultaneous anaerobic oxidation/partial nitrification–denitrification (SAO/PND) system are investigated. Design loading rates of the chemical oxygen demand (COD) and total inorganic nitrogen (TIN) in this new developed process are recommended as 11.80 kg COD/(m^3.d) and 0.63 kg TIN/(m^3.d). The results showed 97% COD and 92% TIN removal efficiencies. High performance of the system was achieved in treating real dairy wastewater as well, and the effluent complied with international discharge standards into inland surface waters. Organic carbon was removed through anaerobic oxidation, assimilation, P–uptake by polyphosphate accumulating organisms (PAOs) and denitrifying PAOs (DPAOs), aerobic heterotrophs, and denitrification by DPAOs and denitrifying ordinary heterotrophic organisms (OHOs). Nitrogen removal mechanisms were assimilation, partial nitrification–denitrification, anaerobic ammonium oxidation (anammox), and small portion uncharacterized processes. The SAO/PND system is found as an efficient, innovative, cost-effective, energy efficient, and small footprint process with less intensive operation and maintenance requirements. This system has been verified as a robust treatment method, and will yield new insights into the design of treating highly polluted streams in the future.

Keywords: Design loading rate; Organic carbon; Total inorganic nitrogen; Dairy wastewater; Cost-effective.

4.1. Introduction

The dairy industry is growing around the world, and significantly contributes to the economy of many countries. Population growth in the world is accompanied with a high amount of milk product consumption. Water consumption in the dairy industry is high, and the production of dairy products generates high quantities of wastewater that originate from processing practices, milking, cleaning of process lines and equipment, sanitization, and floor washing (Chokshi et al., 2016). This wastewater is highly polluted, and includes complex and concentrated organic matters and other pollutants (Shams et al., 2018). Various factors related to the production and cleaning practices can affect the quantity and quality of dairy wastewater (Sarkar et al., 2006). Hence, the chemical oxygen demand (COD) and nitrogen content concentrations of dairy effluents vary significantly, with COD concentrations conventionally being between 1150 and 9200 mg/L and ammonium nitrogen being between 14 and 288 mg/L NH_4^+–N (Hosseinlou et al., 2019).

Due to the large quantity of generated wastewater with high organic concentrations, dairy wastewaters require efficient treatment prior to the release of this highly concentrated wastewater which can destroy receiving aquatic environment (Weerden et al., 2016). Various treatment methods are available but generally, physico–chemical and biological methods are more common. Biological processes are more acceptable because of lower costs and higher organic carbon removal efficiencies (Demirel et al., 2005). Chemical mechanisms need high reagents costs, and the COD removal efficiencies are low. Nanofiltration, adsorption, membrane bioreactors, reverse osmosis, electrochemical treatment, and coagulation–flocculation treatment methods have been tried as well (Davarnejad et al., 2016), but biological processes are still preferred.

Biological treatment methods such as ponds, aerobic lagoons, constructed wetlands, up–flow anaerobic sludge blanket (UASB), anaerobic contact reactors, and anaerobic filters require long retention times (Aziz et al., 2019). Anaerobic digestion is a common treatment process for highly polluted streams (Alqaralleh et al., 2019), and is often the preferred treatment process in comparison with aerobic methods (Andalib et al., 2011). However, the effluent organic carbon and nitrogen concentrations of anaerobic digestion are elevated relative to discharge requirements, and further treatment processes are needed to decrease the effluent pollutants (Lu et al., 2015). In addition, anaerobic digestion requires longer hydraulic retention time (HRT) and solids retention time (SRT). Anaerobic digestion require heating systems and corrosion–resistant systems, which makes the operation of these systems more intensive.

Alternatively, COD and nitrogen can be removed by conventional aerobic processes as well (Fulazzaky et al., 2014), but these treatment methods are more common for treating wastewaters with lower influent concentrations of organic matters compared to anaerobic systems. Various biological aerobic treatment processes and operations have been tried in the past to effectively treat high-strength wastewaters, but high energy consumption and sludge production in aerobic processes are serious drawbacks of these systems under these conditions (Wang et al., 2016). In general, if the influent COD concentrations are higher than 1300–1500 mg/L, these systems are not recommended, and they should be switched to anaerobic digestion.

Therefore, biological treatment of highly contaminated effluents like industrial wastewaters have been shown to require combined anaerobic and aerobic treatment methods to effectively reach required effluent discharge concentrations (Chan et al., 2009). However, the combined systems have the drawbacks of being complicated, expensive, and more intensive with respect to the operation.

In order to overcome the difficulties in combined processes, it is paramount to develop a single process to provide cost-effective, energy–effective, and small footprint solution capable of efficient treatment method with simple operation. The simultaneous anaerobic oxidation/partial nitrification–denitrification (SAO/PND) system is a proposed two–basin, single biological treatment method, which demonstrated the simultaneous ability to remove COD and nitrogen from synthetic wastewater (Hosseinlou et al., 2019).

Although the SAO/PND system has demonstrated the potential for sustainable, innovative, cost-effective, energy efficient, small footprint, and robust on–site technologies with less intense operation and maintenance treatment of highly concentrated influent COD and ammonium wastewaters at elevated loading rates, there remains a gap of knowledge with respect to the design and operation of these systems. As such, this research aims to determine the effective design COD and total inorganic nitrogen (TIN) loading rates through the use of controlled experiments, and hence synthetic wastewater feed. Secondly, the research will investigate the potential of this system to treat real dairy wastewaters and present an appropriate solution to on–site treatment of dairy industry effluents. Finally, this study will move beyond the demonstration of effluent concentrations, removal rates and efficiencies, and will endeavour to identify the COD and TIN removal mechanisms occurring within the SAO/PND treatment process.

4.2. Materials and methods

4.2.1. Experimental setup and operational conditions

A lab–scale SAO/PND system was used to carry out all experiments in this study, and a flow diagram of this system with four sampling points is shown in Fig. 4.1. The volume of the pre–anoxic reactor was 7.8 L, and the aerobic reactors had a 10.8 L capacity. The system was fed by using a peristaltic pump with an influent flowrate (Q_{in}) of 33 L/d during operational days 1 to 38. During this period of time, the HRTs for the pre–anoxic and aerobic reactors were around 5.7 h and 7.9 h, respectively. The Q_{in} was reduced to 17 L/d for the rest of the experiments (days 39 to 139) in order to maintain high organic matter and nitrogen content removal efficiency in the system, where the HRTs of the pre–anoxic and aerobic reactors were increased to around 11 h and 15.2 h, respectively. All pumps in this study were variable speed peristaltic pumps and purchased from Cole Parmer Instrument Company.

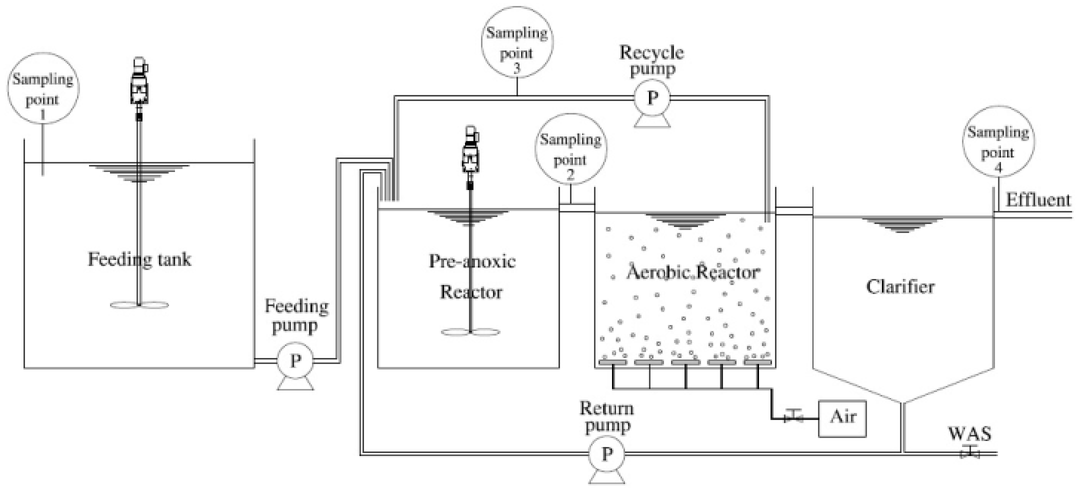

Fig. 4.1. Experimental setup of the lab–scale SAO/PND system, with sampling points identified (not to scale).

The effluent of the aerobic reactor was recycled back to the pre–anoxic reactor, and this line was adjusted at a recycle ratio (Q_r/Q_{in}) of 2, and the return activated sludge line from the bottom of the clarifier to the pre–anoxic tank had a return ratio (Q_{RAS}/Q_{in}) equal to 1. Daily excess sludge was removed from the clarifier to maintain the SRT at 4±1 d and mixed liquor suspended solids (MLSS) at 3000 to 4000 mg/L. The temperature was maintained at approximately 25°C by an aquarium heater (Model Tetra HT30, 100W) installed inside the aerobic tank.

Synthetic wastewater was used between days 1 to 122 in order to find the maximum influent COD and ammonium concentrations with high removal efficiencies by the optimized SAO/PND system. The system was subsequently fed with real dairy wastewater between days 123 to 139. After successfully completing these stages, the system was continued to operate being fed again with synthetic wastewater under steady-state (SS) conditions to gain further knowledge into the dominant COD and TIN removal mechanisms.

4.2.2. Wastewater composition

4.2.2.1 Synthetic solution

The synthetic solution was composed of distilled water, carbon sources, macronutrients, and micronutrients (Hosseinlou et al., 2019) as follows: 150 mg/L $C_6H_{12}O_6$, 80 mg/L NaAc, 150 mg/L Peptone, 0.45 mg/L $FeCl_3.6H_2O$, 80 mg/L NH_4Cl, 44.10 mg/L $KH_2PO_4.2H_2O$, 3 mg/L EDTA, 150 mg/L $NaHCO_3$, 20 mg/L $MgSO_4.7H_2O$, 10.60 mg/L $CaCl_2$, 0.05 mg/L H_3BO_3, 0.01 mg/L $CuSO_4.5H_2O$, 0.05 mg/L KI, 0.04 mg/L $MnCl_2.4H_2O$, 0.04 mg/L $ZnSO_4.7H_2O$, 0.10 mg/L $Na_2MoO_4.2H_2O$, and 0.01 mg/L $CoCl_2.6H_2O$. This resulted in a wastewater which had a COD of 390 mg/L and ammonium of 22 mg/L NH_4^+–N. More chemicals were added in order to elevate influent loading rates and concentrations to simulate high-strength wastewaters. The chemicals used in this study were all obtained from the Fisher Scientific Company, USA.

4.2.2.2. Real dairy wastewater

Untreated dairy wastewater was taken from a dairy farm located 30 km southwest of Ottawa, Canada. The wastewater was mainly a mixture from dairy farming, milking, and equipment and floor–washing. The dairy wastewater was filtered using a No. 80 sieve (nominal opening size 0.177 mm), and the filtered wastewater was stored in the laboratory fridge at 4°C before to the start of the experiments. The characteristics of the filtered dairy wastewater were: 4,105 mg/L COD, 64.01 mg/L NH_4^+–N, 0.68 mg/L NO_2^-–N, 3.24 mg/L NO_3^-–N, and pH of 6.01. After running the system with the raw dairy wastewater, a spike of ammonium was supplied between days 133 to 139 to investigate the system's performance with high influent nitrogen content as well.

4.2.3. Sampling method and analysis

Samples were taken from the four sampling locations which are shown in Fig. 4.1 to evaluate the effectiveness of the SAO/PND treatment method. Soluble COD, NH_4^+–N, NO_2^-–N, NO_3^-–N, alkalinity, total phosphate (TP), total nitrogen (TN), pH, temperature, total suspended solids (TSS), and volatile suspended solids (VSS) were monitored. TNT testing vials (HACH Co., Loveland, CO, USA) were used, as follows: TNT 822, 832, 840, 835, 870, 845, and 827 in order to measure the soluble COD, NH_4^+–N, NO_2^-–N, NO_3^-–N, alkalinity, total phosphate, and total nitrogen concentrations, respectively. A spectrophotometer from HACH Co., Loveland, CO, USA (DR 6000^{TM} UV VIS) was used to read the absorbance of the various testing vials. The TSS and VSS were tested according to standard methods (APHA, 1998). Dissolved oxygen (DO) was measured by a LDO101 optical DO probe of HQ40d Portable Meter (HACH Co., Loveland, CO, USA), while the pH and temperature were measured by a PHC101 pH probe with the same device.

4.3. Results and discussion

4.3.1. Determination of design loading rates

As already mentioned, first goal of this study was determined to find the design loading rates of COD and nitrogen by the SAO/PND process, and the results have been presented separately in the following subsections.

4.3.1.1. Organic carbon removal

The COD removal rates and removal percentages were evaluated at various COD loading rates during the operational days (Fig. 4.2). The COD was significantly removed in the pre–anoxic reactor, and the volumetric loading rates of the COD are presented only for this reactor.

The COD removal rates at various COD loading rates were monitored by stepwise increasing of applied loading rates as shown in Fig. 4.2. The incoming organic carbon to the system was gradually increased as much as possible to simulate highly polluted streams like industrial wastewaters. As can be seen in Fig. 4.2, there was a drop in the loading rates after day 38, which was because of the Q_{in} reduction from 33 L/d to 17 L/d to maintain high removal efficiency of both organic carbon and nitrogen in the SAO/PND treatment process.

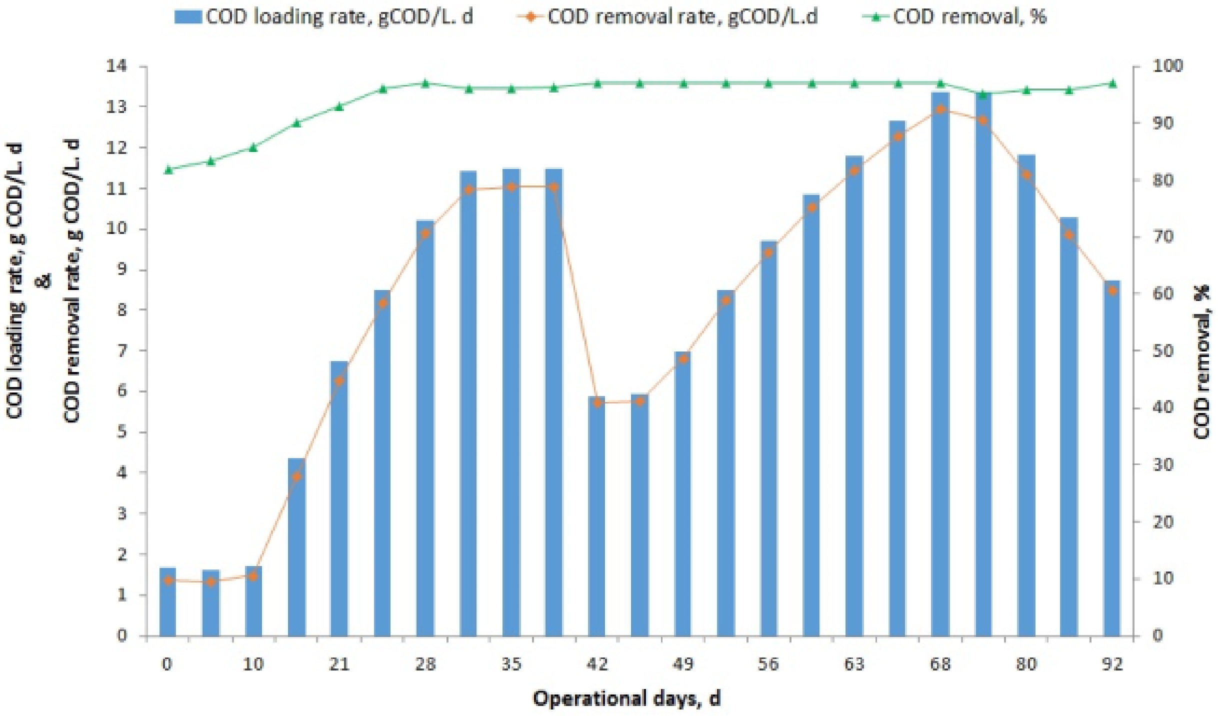

Fig. 4.2. COD removal rates and removal percentages at various COD loading rates during operational days.

The COD loading rate on day 63 was found to be the maximum applicable loading rate with the highest performance of the system without failing. On this day, the highest volumetric and mass organic carbon loading rates of the pre–anoxic unit were reported as 11.80 kg COD/(m^3.d) and 5.32 g COD/(g VSS.d), respectively, with 97% COD degradation (Fig. 4.2). This day was selected as day with maximum loading rate because the system was efficiently removing both organic carbon (97%) and nitrogen content (92%) at the same time. On some other days such as day 73, the COD loading rates were higher than on day 63, but the system was not working very well with respect to the nitrogen removal. Therefore, the design criteria of the COD loading rate in the SAO/PND system was recommended as 11.80 kg COD/(m^3.d) which was found on day 63.

The equivalent maximum incoming organic carbon concentration was 5412 mg/L as COD along with the highest organic carbon and nitrogen removal efficiencies which was achieved on day 63. During the first phase of this study which was conducted between days 0 to 92, the leaving COD concentrations at the effluent of the system were always less than 150 mg/L, and organic carbon degradation was remained around 97 %.

Fig. 4.2 depicts that the highest applied COD loading rate was 13.36 kg COD/(m^3.d) on day 73, with 95% COD removal, but the system was failing regarding to the nitrogen removal efficiency which was declined to around 51%. In an effort to prevent the failure and be able to recover the system, it was decided to decrease the influent COD and ammonium loading rates to around 75% of the maximum influent loading rates on day 63.

4.3.1.2. TIN Removal

The TIN is consisted of NH_4^+–N, NO_2^-–N, and NO_3^-–N, and the TIN removal rates and removal percentages were evaluated at various TIN loading rates during the operational days (Fig. 4.3). As a result of the poor performance in removing nitrogen content on day 38, the incoming flowrate to the system was reduced to 17 L/d on day 39 for performance improvement. The flowrate reduction caused an increase in the HRT values in the pre–anoxic unit (11 h) and aerobic unit (15.2 h). The HRT increases recovered the process and successfully improved the removal of the TIN up to 92% with influent ammonium concentrations of 288.6 mg/L NH_4^+–N till day 63, when failure signs were again evident (Fig. 4.3), but the organic carbon degradation remained almost unchanged (Fig. 4.2).

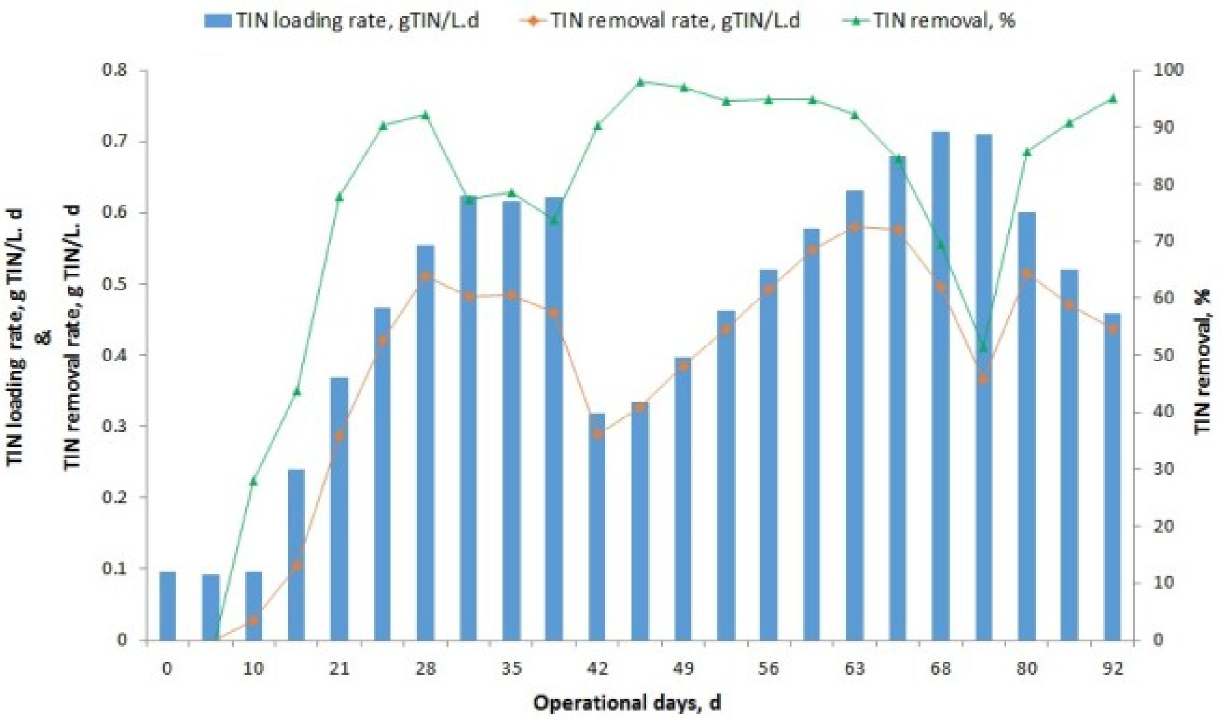

Fig. 4.3. TIN removal rates and removal percentages at various TIN loading rates during operational days.

Volumetric and mass loading rates for the TIN are shown in Fig. 4.3 only for the pre–anoxic unit because almost the entire TIN was removed in this unit, as will be explained later. On day 63, the maximum achieved volumetric and mass TIN loading rates in the pre–anoxic unit for the synthetic wastewater feed were reported, with 0.63 kg TIN/(m^3.d) and 0.28 g TIN kg VSS (m^3.d), respectively. Day 63 was selected as a day for maximum loading rates because the system was working very well with respect to the both COD and nitrogen removal at the same time. Therefore, the recommended design TIN loading rate in the SAO/PND system was reported as 0.63 kg TIN/(m^3.d) which was found on day 63.

Due to overloading the SAO/PND system regarding to the organic matter and nitrogen, the system showed signs of stress and failure on day 73, and nitrogen removal efficiency declined to around 51%. Similar failure signs as a result of overloading were observed in the study which was carried out by Hosseinlou et al. (2019). After a temporary failure in this research, the system was recovered, as can be seen in Figs. 4.2 and 4.3, and conducting the rest of the experiments became possible and continued. It was concluded that the maximum possible influent ammonium for the system was below 290 mg/L NH_4^+–N.

This study obtained high COD (97%) and nitrogen (92%) removal efficiencies with influent COD and nitrogen content of 5412 mg/L and 288.6 mg/L NH_4^+–N respectively, with loadings of 11.80 kg COD/(m^3.d) and 0.63 kg TIN/(m^3.d), respectively. The applied influent concentrations in this study are greater than the ones in the study by Hosseinlou et al. (2019), which contained COD of 5,211 mg/L and ammonium of 262.8 mg/L NH_4^+–N. The COD removal efficiency in the current study is in agreement with the findings of Hosseinlou et al. (2019) which used the SAO/PND system for treating highly polluted streams. In spite of the higher applied influent concentrations in the current study, the nitrogen removal showed a 1% improvement in comparison with the results of Hosseinlou et al. (2019).

In a study, Zwain et al. (2013) used a modified anaerobic baffled reactor (MABR) which was consisted of five chambers for COD removal purposes from recycled paper mill wastewater which was highly biodegradable. They separated each chamber by a modified vertical baffle, and applied 5 days of HRT and COD loading rate of 0.2 kg COD/(m^3.d). In spite of the lower loading rates compared to the current study, they achieved lower COD removal efficiency (71%). A sequencing batch reactor (SBR) system with intermittent aerobic and anoxic phases was applied by Rodriguez et al. (2011) in a research for treating meat industry effluent at different loading rates. They found that the highest removal efficiencies happened during loading rates of 2.48 kg COD/(m3.d) and 1.10 kg NH_4^+–N/(m^3.d), which were 99% COD and 71% ammonium removal. The COD removal efficiency is comparable with the current study, but they observed lower nitrogen removal.

Lima et al. (2016) carried out a study by suing a two–stage anoxic/aerobic moving bed biofilm reactor (MBBR) under increasing organic loading rates. They found 95% COD and 85% nitrogen removal, with loading rates equal to 3.20 kg COD/(m^3.d) and 0.21 kg TN/(m^3.d). Despite the underloading conditions, they determined lower organic carbon and nitrogen removals compared to the current study. Abdullah et al. (2011) applied aerobic granules (AG) process with SBR configuration for treating palm oil mill effluent with COD loading rate of 2.5 kg COD/(m^3.d). They could achieve 91.1% COD removal, which is lower than the COD removal efficiency of the current study. It should be mentioned that the operation of the AG system is not a simple task. Major challenges of the AG systems are attributed to the operation and maintenance, and granule formation and washout which can lead to unstable operational conditions and system failure. On the other hand, simple operation of the SAO/PND system is one of its advantages.

The results of the current study can be compared with the results obtained by El–Kamah et al. (2010), who used a combined system composed of a two–stage up–flow anaerobic sponge reactors (UASRs) followed by an activated sludge (AS) process. They achieved 97.5% COD removal, in which sludge age was 76 d. In addition, the results of the current study show higher organic carbon and nitrogen content removal in comparison with the study carried out by

Bustillo–Lecompte et al. (2017). These researchers used a combined system consisted of an anaerobic baffled reactor (ABR) process followed by an AS system for treating slaughterhouse wastewater, and reported 85.03% organic carbon and 72.10% nitrogen removal.

The results confirm that new developed SAO/PND system is an efficient and innovative treatment method with less intensive operation and maintenance requirements.

4.3.2. Performance of the SAO/PND system for dairy wastewater treatment

The SAO/PND process was underloaded, and was running in SS conditions between days 93 to 122 (Figs. 4.4 and 4.5), with influent COD concentrations around 4100 mg/L and ammonium concentrations of 215 mg/L NH_4^+–N, in order to recover and stabilize the system before switching to feed the system with real dairy wastewater. The system was recovered, and the COD and TIN removal increased and was maintained at around 98% COD and 96% TIN removal.

On day 123, the SAO/PND system was fed with real dairy wastewater which had loading rates below the recommended design loading rates, as shown in Figs. 4.4 and 4.5. The system was fed with real dairy wastewater step by step, with mixing conditions as follows: day 123 (25% real dairy ww + 75% synthetic ww), day 124 (50% real dairy ww + 50% synthetic ww), day 125 (75% real dairy ww + 25% synthetic ww), and from day 126 till the end (100% real dairy ww). During mixing on days 123, 124, and 125, the COD removal sharply dropped to 93% but then increased to 95% from day 126 onwards.

After feeding the system with real dairy wastewater between days 123 to 139, the effluent COD concentrations increased sharply, to around 230 mg/L, and COD removal decreased to 95%. Glucose, peptone, and sodium acetate were used to simulate the organic carbon in synthetic wastewater, and these compounds are readily biodegradable substances which can be easily consumed by heterotrophic organisms; however, the real dairy wastewater contained some complex and non–biodegradable organic carbon compounds. After feeding the system with complex organic carbon substrates, the COD removal declined to 95%.

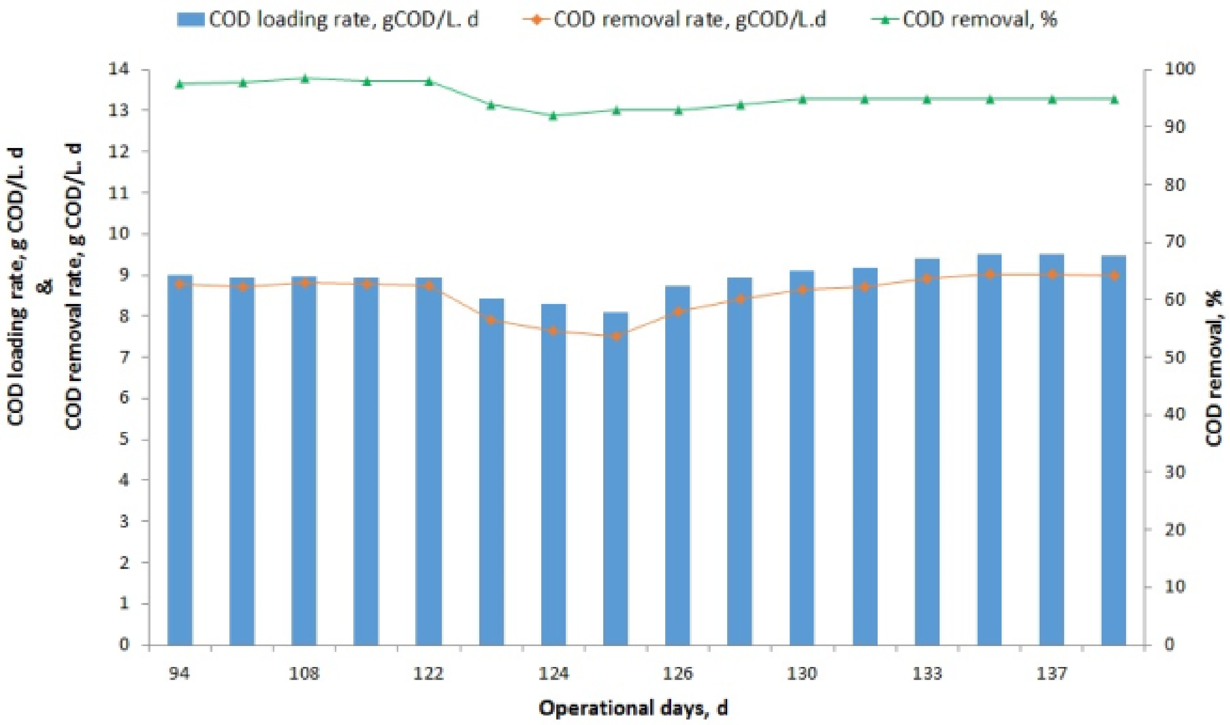

Fig. 4.4. COD removal rates and removal percentages at various COD loading rates during operational days.

As already mentioned, the feed for the system was switched from synthetic wastewater to real dairy wastewater step by step over 3 days. TIN removal was increased and maintained to around 99% till the end of the experiments. As a result of the low TIN concentrations in the collected real dairy wastewater sample in this study, a spike of ammonium was supplied to simulate high-strength (with respect to ammonium) dairy wastewater. Between days 133 and 139, the influent real dairy wastewater was also spiked stepwise by adding more ammonium to increase the TIN loading rates and concentrations.

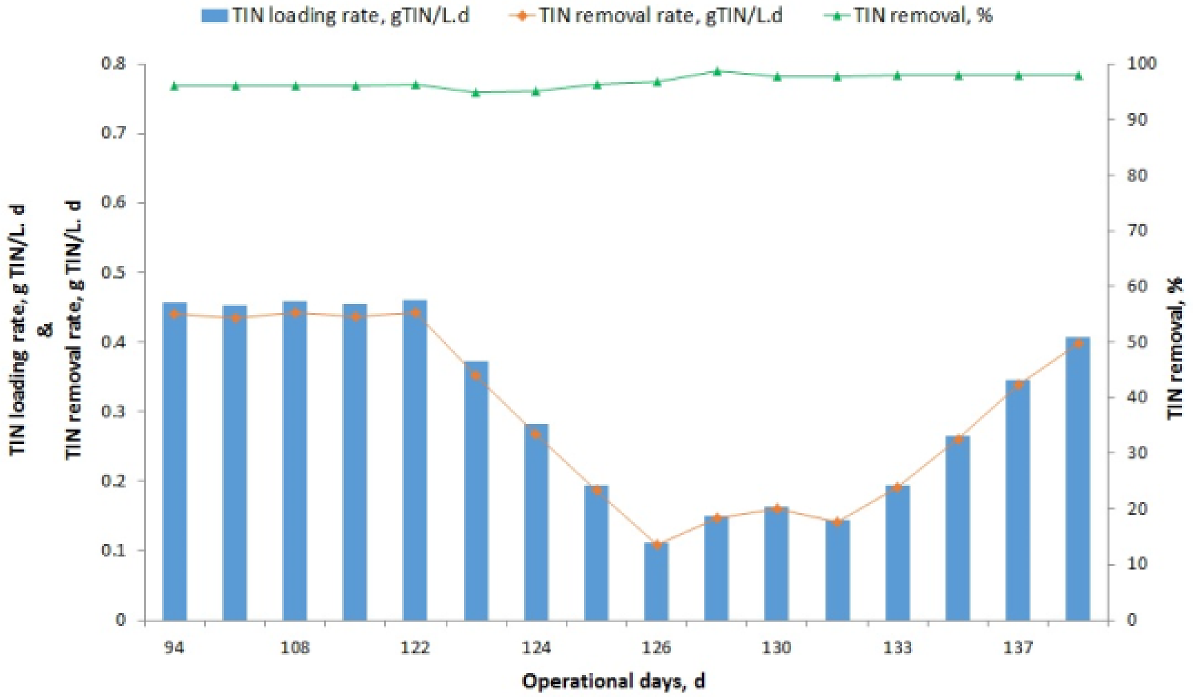

Fig. 4.5. TIN removal rates and removal percentages at various TIN loading rates during operational days.

This phase of the study finished on day 139 with COD (95%) and nitrogen (99%) removal efficiencies with applied COD and TIN loading rates at 9.47 kg COD/(m^3.d) and 0.41 kg TIN/(m^3.d) (Figs. 4.4 and 4.5), with equivalent incoming COD and TIN concentrations of 4347 mg/L and 186.90 mg/L TIN–N. The SAO/PND system showed a high potential to remove higher concentrations of influent feed nitrogen content, but this phase of the study finished on day 139 without a determination of the highest possible influent nitrogen concentrations.

Ji et al. (2020) conducted a research on dairy wastewater treatment by an integrated system consisting of an ABR and a following UASB. They achieved 98% COD removal which is greater than the removal efficiency of the current study. However, the operation and maintenance of an integrated system such as ABR–UASB is more intensive in comparison with the SAO/PND process. Also, McAteer et al. (2020) used a UASB system in treating dairy wastewater under different operational conditions. The optimum operational conditions of their study showed 92% COD removal efficiency in loading rates of 7.5–9 kg COD/(m^3.d), which is lower than the achieved results by the SAO/PND system in the current study. A SBR MBBR system with anaerobic, anoxic, and aerobic sequence of operation stage configuration was used by Ozturk et al. (2019) for treating dairy wastewater, in which the system could achieve 81.8% COD and 85.1% ammonium removal. In comparison, the SAO/PND system in the current study showed a higher performance in treating dairy wastewater.

The results of this study prove the high performance of the SAO/PND system for treating highly polluted streams, and demonstrate the successful operation of this process, which produces treated dairy industry effluent complying with international discharge standards into inland surface waters. The COD discharge limit for industrial effluents into inland surface waters according to international discharge standards is less than 250 mg/L (Gupta et al., 2008).

4.3.3. pH and DO fluctuations

Fig. 4.6 shows consistent pH ranges of around 7 to 8 inside the pre–anoxic and aerobic units during the operational period of this study, which is in a good agreement with the optimum pH found by Hosseinlou et al. (2019). Between days 31 and 38 and 66 and 80, the pH increased to above 8, and this high pH in the system was a clue regarding system failure. A pH above 8 was toxic to the SAO/PND process, and the system started to fail at this point. Hosseinlou et al. (2019) observed the similar failure signs in this pH range. A pH above 8 results in more free ammonia (NH_3), which is more toxic to bacterial communities than ionized ammonium (NH_4^+) because it diffuses more rapidly through the cell membrane, and causes bacterial death and system failure.

Alkalinity was consumed during partial nitrification in the aerobic tank, and the alkalinity concentrations in this tank were always lower than in the pre–anoxic tank, which is in agreement with the findings of Hosseinlou et al. (2019).

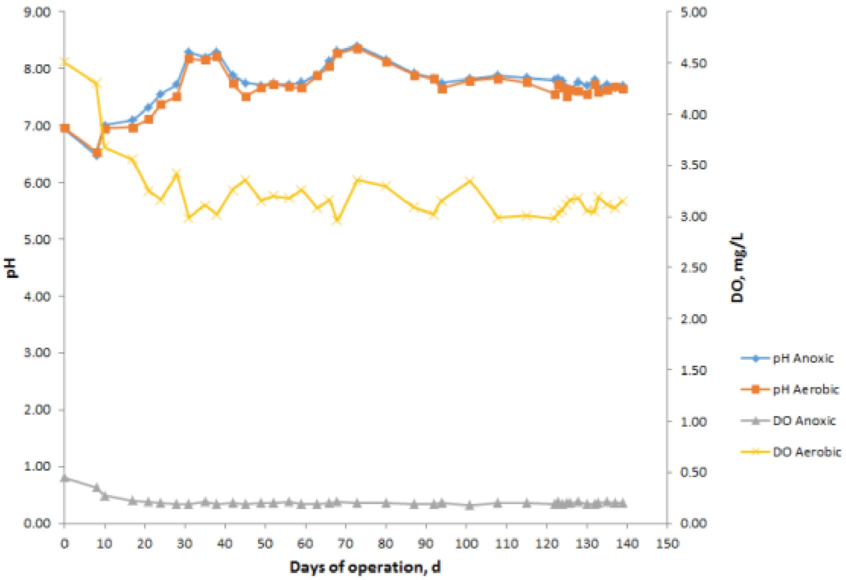

Fig. 4.6. pH and DO fluctuations of the pre–anoxic unit and aerobic unit.

Factors such as pH, alkalinity, oxygen, and organic carbon can influence the nitrification as well as the denitrification process, and DO concentrations below 3.0 mg/L are inappropriate for nitrifiers (Hameed et al., 2020; Metcalf and Eddy, 2003). DO concentrations in the aerobic unit process were maintained between 3.0 and 3.5 mg/L to make sure that DO concentrations were not limiting factor for nitrifiers in this study (Fig. 4.6). Oxygen levels in the pre–anoxic unit were consistently kept at around 0.20 mg/L to favor a possible denitrification process (Fig. 4.6). DO concentrations in the aerobic and pre–anoxic units by Hosseinlou et al. (2019) were around 4.0–5.0, and 0.25 mg/L, respectively. The current study found that because there was only 17.1% nitrogen consumption by the partial nitrification–denitrification mechanism in the SAO/PND process, various DO concentrations in the aerobic reactor did not significantly affect the overall nitrogen removal efficiency.

4.3.4. Removal mechanisms in the SAO/PND process

To investigate the mechanisms involved in the SAO/PND treatment method regarding the organic matter and nitrogen content removal, as well as to be able to write the mass balance (MB) calculations and stoichiometric equations, a set of experiments with the same operational conditions was conducted under SS conditions. The system was fed with synthetic wastewater with influent COD, ammonium, nitrite, nitrate, alkalinity, TP, and TN of around 69972 mg/d COD, 3587 mg/d NH_4^+–N, 0.9 mg/d NO_2^-–N, 9.9 mg/d NO_3^-–N, 12524 mg/d $CaCO_3$, 1972 mg/d PO_4^{3-}–P, and 7293 mg/d N–N, respectively.

4.3.4.1. Mechanisms involved in organic carbon degradation

The results showed that 68911 mg/d (98%) of the influent COD (69972 mg/d) were removed in the SAO/PND system. The MB calculations of COD around each individual reactor and the entire system revealed that the COD was significantly degraded inside the pre–anoxic unit (68197 mg/d), and that the rest of the COD (714 mg/d) was consumed in the aerobic unit process. The MB calculations showed 67% of influent COD removal by anaerobic oxidation. The very limited oxygen and NO_x as electron acceptors in the pre–anoxic unit, low observed biomass yield (0.15 mg VSS/mg BOD), high organic carbon loading rate (11.26 kg COD/(m^3.d), and high ratio of COD over NO_x (349/1) confirms the occurrence of anaerobic oxidation in the first reactor of the SAO/PND system. Daily solids production (excess sludge) in the SAO/PND system was around 10404 mg VSS/d, and by using 1.42 (Metcalf and Eddy, 2003) as the conversion factor for the VSS to COD, it is clear that 21.4% of the influent COD was assimilated by various active bacterial communities in this system, which is in good agreement with the study conducted by Hosseinlou et al. (2019).

Thirty–six percent (728 mg/d PO_4^{3-}–P) of the influent phosphorus (1972 mg/d PO_4^{3-}–P) was removed in the SAO/PND process, and the MB calculations of the dissolved TP around each reactor showed 45% (327 mg/d PO_4^{3-}–P) phosphorus removal in the pre–anoxic reactor, and 55% (401 mg/d PO_4^{3-}–P) phosphorus removal in the aerobic process unit. These phosphorus removal levels are attributed to the denitrifying polyphosphate accumulating organisms (DPAOs) in the pre–anoxic unit and the polyphosphate accumulating organisms (PAOs) in the following aerobic unit. From the MB data based on the unfiltered total phosphorus in the entire system, and considering the solids production, it was revealed that 100% of the influent phosphorus was recovered in the system as excess sludge and clarified effluent (around 2000 mg/d TP–P), and all the removed phosphorus was stored in the body of bacteria. During the phosphorus removal, the COD was stored internally as poly–b–hydroxyalkanoates (PHA) and poly–b–hydroxybutyrate (PHB) in the body of PAOs and DPAOs in the anoxic reactor. The average COD consumption for the phosphorus uptake was around 10 mg COD/mg PO_4^{3-}–P, and the MB calculations showed 5.8% and 4.7% COD removal of the influent COD (69972 mg/d) by the PAOs and DPAOs, respectively.

As will be discussed in Section 3.4.2, incomplete nitrification process inside the aerobic unit led to NO_x production (NO_3^-–N and mainly NO_2^-–N) in this reactor, which was recycled back and consumed in the pre–anoxic reactor. The anaerobic ammonium oxidation (anammox) bacteria consumed NO_2^-–N and produced NO_3^-–N via the anammox process inside the pre–anoxic unit. The recycled NO_3^-–N from the aerobic reactor and the NO_3^-–N produced through the anammox process, were consumed by denitrifying ordinary heterotrophic organisms (OHOs) and DPAOs via the denitrification and P–uptake processes, which was accompanied by influent organic carbon consumption during the exogenous denitrification and P–uptake processes. Phosphorus removal was carried out via phosphorus uptake by the PAOs and DPAOs together.

Anammox bacteria were competing with the DPAOs over the nitrite, and were more successful in using nitrite in the presence of ammonium, and then produce nitrate which was consumed by the DPAOs and other denitrifying OHOs as an electron acceptor. The DPAOs and denitrifying OHOs used COD as their carbon sources in the denitrification process. The DPAOs also converted a portion of the COD to PHA and PHB, and stored in their bodies for consumption during the P–uptake. It was discovered that the DPAOs and PAOs co–existed to degrade the organic carbon and phosphorus. The DPAOs were able to consume either NO_3^- or O_2, but the PAOs could only use O_2 as an electron acceptor.

The system was originally seeded with activated sludge obtained from a lab–scale SAO/PND process, with stabilized organic carbon and nitrogen content removal in laboratory operational conditions. The mentioned system was already enriched with PAOs, DPAOs, denitrifying OHOs, anammox bacteria, nitrifiers, and anaerobic and aerobic heterotrophs. The P–uptake took place by the DPAOs in the anoxic phase, in which their stored PHA or PHB sources were first consumed.

During the aerobic phosphorus uptake inside the aerobic unit, some PAOs did not consume nitrate, but accumulated organic carbon in the case of oxygen availability.

Inside the pre–anoxic unit, a portion of the influent COD was consumed via denitrification, and partly stored as intracellular carbon by the DPAOs and PAOs. The extended pre–anoxic stage helped the intracellular carbon accumulation for the phosphorus uptake, and this made available enough organic carbon for the denitrification process. Organic carbon in the pre–anoxic reactor was consumed during the exogenous denitrification of the nitrate, and was internally accumulated by the bacteria (DPAOs and PAOs) as well.

The filtered TP and NO_3^-–N MB calculations around the anoxic reactor revealed that there was enough NO_3^-–N in this reactor for the DPAOs during the anoxic P–uptake. The nitrate entering the anoxic reactor from the aerobic tank through the recycle line, as well as the nitrate produced by anammox was denitrified by the DPAOs and denitrifying OHOs inside the anoxic reactor. The DPAOs consumed 93% of the available NO_3^-–N in this reactor, and the remaining nitrate (7%) was removed by the denitrifying OHOs. The active denitrifying OHOs in the anoxic reactor were heterotrophic bacteria which consumed 0.1% of the influent COD as electron donors during the nitrate reduction, with 6.6 mg of COD consumption via denitrification for each mg of NO_3^-–N.

The aerobic reactor was only responsible for removing 1% (714 mg/d) of the influent COD which consumed by ordinary aerobic heterotrophs when O_2 was available as electron acceptor.

4.3.4.2. Mechanisms involved in nitrogen degradation

Because of the new–found microorganisms and processes, the nitrogen cycle has been more complicated, and therefore nitrogen removal via conventional nitrification–denitrification processes is not enough to describe the nitrogen removal pathway. A new cost–effective (less energy consumption and sludge production) and efficient biological treatment process like the SAO/PND process has been recently developed to explain this removal pathway in more detail.

A significant portion of the nitrogen content removal in this new treatment process was attributed to the nitrogen assimilation, and the MB data for the unfiltered total nitrogen in the entire system showed that 3360 mg/d TN–N (45.9%) of the influent TN (7293 mg/d TN–N) was consumed for bacterial cell growth and solids production, which exited the system as daily waste activated sludge (WAS).

The biological complete nitrification through the two phases of ammonium oxidation (Liu et al., 2020) is described in Eqs. (1) and (2) (Fulazzaky et al., 2015):

$$\mathrm{NH_4^+} + 1.5O_2 \rightarrow \mathrm{NO_2^-} + 2H^+ + \mathrm{H_2O} \quad (1)$$

$$\mathrm{NO_2^-} + 0.5O_2 \rightarrow \mathrm{NO_3^-} \quad (2)$$

Nitrifiers are slow growth bacteria and short SRT in the SAO/PND system, favored the retention of ammonia oxidizing bacteria (AOB), and meanwhile suppressed nitrite oxidizing bacteria (NOB), which resulted in only 17.1% incomplete nitrification inside the aerobic unit.

The influent ammonium to the system was 3587 mg/d NH_4^+–N, and the anoxic reactor removed about 83% of the influent ammonium and left about 17.1% of ammonium in the effluent (615 mg/d NH_4^+–N), which entered the aerobic reactor for partial nitrification. The percentages for the nitrogen assimilation and partial nitrification in this research are in good agreement with the research by Hosseinlou et al. (2019).

Inside the aerobic unit, NH_4^+–N was declined but NO_2^-–N and NO_3^-–N were produced, and all influent ammonium to the reactor (615 mg/d NH_4^+–N) was converted and oxidized in the presence of oxygen. During the partial nitrification process in this system, ammonium was transformed mainly to nitrite and some to nitrate with a molar ratio of NO_2^-–N over NO_3^-–N of around 5/1. Because of the small portion of organic nitrogen conversion to ammonium inside the aerobic reactor, the NO_x at the effluent of this reactor was slightly more than the ammonium consumption inside this reactor. Controlled oxygen concentrations along with the short SRT of the aeration unit reduced the microbial activity of the conventional nitrifiers which led to incomplete nitrification. This incomplete nitrified stream was recycled from the aerobic unit back to the pre–anoxic tank for removal.

Nitrification uses alkalinity via the production of hydrogen ions, and the theoretical stoichiometric balance yields a consumption of 7.14 mg CaCO3/mg NH_4^+–N during the complete nitrification (Metcalf and Eddy, 2003). However, the measured alkalinity consumption during the partial nitrification in the SAO/PND system was slightly lower than the theoretical value, and 6.6 mg of CaCO3 was consumed for each mg of NH_4^+–N. This difference was because of the fact that the theoretical value based on Eqs. (1) and (2) did not consider the conversion of some of the ammonium to cellular nitrogen.

As a result of the incomplete nitrification in the aerobic unit, nitrite was produced and conveyed to the pre–anoxic unit through the recycle line, which was one of the substrates for the anammox process in the pre–anoxic unit. For a long time, anammox bacteria were considered to be slow growth (Strous et al., 1999) with a minimum of eleven days as a requirement for doubling (Strous et al., 1998), but surprisingly a high maximum specific growth rate has been newly observed. It has been suggested that the maximum specific growth rate was not an intrinsic property and could be increased by optimizing the growth operational conditions (Lotti et al., 2015).

Anammox is a biological mechanism to remove nitrogen which needs low energy, and generally the anammox reaction can be specified in Eq. (3) (Wang et al., 2010):

$$NH_4^+ + 0.13H^+ + 0.066HCO_3^- + 1.32NO_2^- \rightarrow 1.02N_2 + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O + 0.26NO_3^- \quad (3)$$

Interactions between anammox bacteria and heterotrophs are very complicated, and Du et al. (2017) showed the co–existence of denitrifying and anammox bacteria in the denitrifying ammonium oxidation (DEAMOX) process.

The combination of nitritation and anammox processes is a more cost-effective method than the common biological nitrogen content removal process via denitrification. In the new system applied in this research, NO_2^-–N and NO_3^-–N were conveyed to the pre–anoxic unit via a recycle line and were then consumed by anammox, DPAOs, and denitrifying OHOs. The anoxic reactor was a complete mixed reactor, and concentrations inside this unit were equal to the effluent. COD concentrations at the effluent of the anoxic reactor were always below 80 mg/L, which was equal to the concentrations inside of the reactor. Low concentrations of COD inside the anoxic reactor with the presence of nitrite favored the proliferation of anammox bacteria.

The SAO/PND process integrated incomplete nitrification, anammox, and denitrification processes to remove nitrogen. Autotrophic nitrification bacteria were involved in the aerobic reactor, and NH_4^+–N was oxidized to NO_2^-–N and NO_3^-–N through incomplete nitrification in this unit. Inside the anoxic reactor, NO_2^-–N was consumed with NH_4^+–N through anammox, and NO_3^-–N was used via denitrification by the DPAOs and denitrifying OHOs.

According to the stoichiometric equations of anammox (Eq. 3) and MB calculations around the anoxic reactor, 303 mg/d NH_4^+–N with 400 mg/d NO_2^-–N was consumed during the anammox process in this reactor, which produced 79 mg/d of NO_3^-–N. Afterwards, all the NO_3^-–N produced via anammox, influent and recycled NO_3^-–N (in total 167 mg/d NO_3^-–N) were consumed during the denitrification process by the DPAOs (93%) and denitrifying OHOs (7%) in the anoxic reactor.

Anammox removed 303 mg/d NH_4^+–N (8.5%) of the influent ammonium (3587 mg/d NH_4^+–N), and according to the anammox process in the SAO/PND system, NH_4^+–N and NO_2^-–N were consumed and NO_3^-–N was produced under stoichiometric molar ratios of 1.00/1.32/0.26 for ammonium and nitrite consumption, and nitrate production, respectively.

The bacterial living conditions facilitated competitive advantages for the anammox bacteria over the DPAOs and denitrifying OHOs for nitrite consumption in the anoxic reactor. The nitrite accumulation during the incomplete nitrification in the aerobic unit which was conveyed to the pre–anoxic unit through the recycle line provided a substrate for the anammox reaction. All the nitrite produced during the incomplete nitrification inside the aerobic unit was consumed in coincidence with ammonium by the anammox bacteria to produce nitrate for subsequent use by the DPAOs and denitrifying OHOs in the pre–anoxic unit. Generation of NO_3^-–N during the anammox process was a critical factor for the desirable performance of the SAO/PND process, and was subsequently consumed by the denitrifiers.

For nitrite consumption, there is a competition between denitrifying and anammox bacteria (Mozumder et al., 2014), but when both NO_2^-–N and NO_3^-–N are available inside the pre–anoxic unit, the denitrifiers prefer to reduce nitrate, which other studies have also shown (Du et al., 2017). Recycled nitrate from the aerobic reactor to the pre–anoxic unit and the nitrate produced via the anammox process were consumed by the DPAOs and denitrifying OHOs as electron acceptors during the denitrification process, and was accompanied by organic carbon removal. Both ammonium and nitrate concentrations decreased in the anoxic reactor, suggesting the possible occurrence of denitrification–anammox reactions, which is in agreement with other reports. COD, nitrogen, and phosphorus removal mechanisms in the anoxic reactor confirm a favorable environment for the co–existence of anaerobic bacteria, DPAOs, anammox, and denitrifying OHOs in the anoxic reactor. The operational conditions exerted selective pressures on the SAO/PND process, favoring the growth and cooperation of all the mentioned bacterial communities.

Organic matter and nitrogen were significantly removed inside the pre–anoxic unit, but the aerobic unit was crucial in the successful operation of the overall system. The pre–anoxic unit at the head of the system, by degrading most of the influent organic matter helped the occurrence of incomplete nitrification inside the aerobic unit. The aerobic reactor worked under aerobic conditions, where incomplete nitrification and aerobic P–uptake took place together with very limited aerobic organic carbon removal. This tank was vital for successful operation and exclusion of this unit from the system configuration undermined the effectiveness of the entire system, and finally caused failure.

Based on the MBs performed on nitrogen for each unit process and the entire treatment system, 28.5% of the influent nitrogen was missing in this treatment process, which attributed to the possible laboratory errors and uncharacterized processes.

Appropriate bacterial living conditions provided a favorable environment inside the pre–anoxic reactor to promote the growth and co–existence of anaerobic heterotrophs, DPAOs, denitrifying OHOs, and anammox bacteria. The aerobic unit could successfully enrich the AOB and suppress NOB, which resulted in partial nitrification in this reactor.

4.4. Conclusions

This study recommended COD and TIN loading rates of 11.80 kg COD/(m^3.d) and 0.63 kg TIN/(m^3.d) for designing the SAO/PND treatment process with high removal efficiencies. The SAO/PND system was successfully applied for the treatment of high-strength synthetic wastewater and real dairy industry effluent. High performance for real dairy wastewater treatment was achieved while providing treated effluent complied with the international discharge standards into inland surface waters. Mass balance calculations for phosphorus, organic carbon, and nitrogen content showed that P was removed by the DPAOs and PAOs, while the COD was removed by anaerobic oxidation, bacterial growth, DPAOs, denitrifying OHOs, PAOs, and aerobic heterotrophs. Bacterial assimilation and partial nitrification–denitrification, and anammox processes were responsible for the TIN removal. The results prove that the SAO/PND system is a sustainable, efficient, and cost–effective solution with simple operation treatment method which can be applied in the future for treating highly polluted streams like dairy wastewater.

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5. Application of an efficient, cost-effective and newly developed single-process SAO/PND technology for treating brewery industry effluent

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Abstract

Simultaneous anaerobic oxidation/partial nitrification-denitrification (SAO/PND) technology, as a newly developed biological treatment method, removes organic carbon and nitrogen content from various streams both efficiently and cost-effectively. This study uses the SAO/PND system as a single-process for the treatment of real brewery wastewater with the aim of developing a robust alternative to conventional technologies. A laboratory bench–scale setup of this system is used to achieve this goal. In addition, the system is fed with a synthetic solution during a set of complementary experiments to reveal more details about the removal mechanisms. The highest COD (92%) and nitrogen (99%) removal efficiencies are obtained for treating real brewery wastewater with influent concentrations of 9036 mg/L COD and 187.52 mg/L TIN–N. The organic carbon volumetric and mass loading rates of the pre-anoxic reactor with high removal efficiency are determined as 19.95 kg COD/(m^3.d) and 8.75 kg COD/(kg VSS.d), respectively. The results revealed the high potential of the SAO/PND system for nitrogen removal, but the highest possible influent nitrogen concentrations with this system are not determined. The single-process SAO/PND treatment method showed a promising alternative

to conventional technologies, as it combines efficiency and cost-effectiveness with simple operation and maintenance.

Keywords: SAO/PND; brewery wastewater; simultaneous removal; COD; nitrogen

5.1. Introduction

Beer is the fifth most consumed beverage in the world behind tea, carbonated beverages, milk and coffee, and brewery is a growing industry which plays an important economic role in many countries (Verhuelsdonk et al., 2021; Sawadogo et al., 2018; Hultberg and Bodin, 2017). Brewing is an industry which use large quantities of water and generates large volumes of highly polluted wastewater (Manyuchi et al., 2018; Simate et al., 2012; Mata et al., 2012). It is estimated that the brewery industry typically generates 3–10 L of wastewater per liter of beer production, depending on the product and specific water usage (Papadopoulos et al., 2020; Chen et al., 2016; Seluy and Isla 2014; Olajire, 2012; Kanagachandran and Jayerantene, 2006; Fillaudeau et al., 2006).

Brewery wastewater is highly polluted, and typically contains very high levels of organic carbon and other pollutants which is characterized by large variations in these parameters (Raposo et al., 2010; Rao et al., 2007). Nitrogen and phosphorus concentrations are variable, and are mainly dependent on the handling of the raw materials and the amount of available yeast (Simate et al. 2011). In this industry, high amounts of water are consumed for: i) the main ingredient of the beer as a final product; and ii) the parts of the brewing process for steam raising, cooling, packaging, cleaning of the brew house, and floor washing. The quality of brewery wastewaters depends on the type of products, equipment, production method, system management, and washing mechanism. Therefore, the concentrations of pollutants in brewery wastewater varies significantly, and have been reported as COD=920–14,880 mg/L and nitrogen=16.4–280 mg/L TN–N (Oljira et al., 2018; Chen et al., 2016; Zheng et al., 2015; Simate et al., 2011; Feng et al., 2008; Connaughton et al., 2006; Tam et al., 2005; Herrmann and Janke, 2001; Ahn et al., 2001).

Because of the scarcity of fresh water around the world and competing demands for water resources, the discharge of industrial effluents into aquatic environments has become an important issue, and the abundance of organic substances in brewery wastewater causes serious pollution in receiving water bodies (Lyu et al., 2021; Asaithambi et al., 2020; Jaiyeola et al., 2016; Weirich et al., 2011). Therefore, in order to maintain the water quality, organic matter in brewery wastewater has to be removed before discharge to the environment (Gong et al., 2019; Bakare et al., 2017: Zheng et al., 2015). There are some physical, chemical, and biological treatment methods for this, but generally, biological treatment processes are more viable options than physico–chemical methods (Jaiyeola et al., 2016; Simate et al., 2011).

Different biological aerobic treatment processes have been tried in the past, but the high energy consumption and sludge production from these processes significantly raise the cost of system operations for treating high-strength wastewaters (Wang et al., 2016; Zheng et al., 2015; Grady et al., 2011). On the other hand, anaerobic processes play important roles for treating highly polluted streams, and are effective treatment methods for various industrial wastewaters (Tan et al., 2021; Tawfik et al., 2021; Gao et al., 2021; Tallou et al., 2020; Alqaralleh et al., 2020: Hultberg et al., 2017). However, one of the biggest problems with these systems is attributed to their high sensitivity to fluctuations of temperature and operating conditions, which can result in low system performance. Furthermore, these anaerobic processes need subsequent aerobic treatment systems to achieve high removal efficiencies and meet discharge requirements (Osmani et al., 2021; Arantes et al., 2017; Metcalf and Eddy, 2014).

In high-strength wastewater treatment, neither aerobic nor anaerobic treatment methods as single processes can produce treated effluents that comply with discharge standards. Therefore, combinations of separate treatment processes in series have been used to achieve acceptable performance for treating highly polluted wastewaters. Most conventional combined treatment methods are a combination of anaerobic systems followed by subsequent aerobic treatment processes (Arantes et al., 2017; Chan et al. 2009).

In combined anaerobic systems–aerobic systems in series, the anaerobic treatment processes remove the majority of the organic carbon, and produce a favorable feed for the polishing subsequent aerobic systems. Then, the organic carbon contained in the effluent from the anaerobic processes will be further polished by the aerobic processes for potential compliance with discharge standards (Chan et al., 2009). In the mentioned combined systems in series, organic carbon will mainly be consumed under anaerobic digestion (AD) conditions in anaerobic units; meanwhile, ammonia will be produced by an ammonification process. Afterwards, subsequent aerobic treatment methods such as aerobic activated sludge will remove the rest of the COD and produced ammonia (Akizuki et al., 2021). This means that combined systems are more expensive and complicated, and synchronizing the two separate processes in series in combined systems is more intensive regarding the operation and maintenance as well. These are some drawbacks with these complex treatment methods, but these should be solved by the development of new systems.

Hosseinlou et al. (2019) developed a new single-process wastewater treatment method known as simultaneous anaerobic oxidation/partial nitrification-denitrification (SAO/PND) in order to overcome the issues related to combined systems. This system simultaneously, efficiently, and cost-effectively removed organic carbon and nitrogen content from a synthetic solution. In addition, Hosseinlou (2021) determined the design loading rates for this process by using a synthetic solution, and confirmed this new system as a robust on-site process for treating real dairy industry effluent.

The SAO/PND system has been claimed as a general solution for treating various high-strength wastewaters, and the current study aims to evaluate the accuracy of this claim by feeding the SAO/PND system with real brewery industry effluent. Regarding the potential of the SAO/PND system for brewery wastewater treatment, and to the best of the knowledge of the author, no technical studies have yet been reported on this subject. Therefore, the main objectives of this research are: to evaluate the performance of the SAO/PND system as a single-process method for treating real brewery wastewater, and to confirm the removal mechanisms by using a synthetic solution.

5.2. Materials and methods

5.2.1. Experimental setup and operational conditions

As shown in Fig. 5.1, a newly developed single-process SAO/PND system which consisted of two continuous stirred tank reactors (CSTR) and one settler was used in this study. A laboratory-scale setup of the system was used, which also ensured homogeneous mixing conditions in both the pre-anoxic and aerobic reactors. The optimum operational conditions for the single-process SAO/PND treatment method used in this study were determined by Hosseinlou et al. (2019).

The sequential anoxic-aerobic-clarifier treatment process was continuously operated to simultaneously, efficiently, and economically treat a synthetic solution and real brewery wastewater containing organic carbon and nitrogen. Inoculation sludge was taken from a bench–scale SAO/PND system, which had achieved a stable performance of biological organic carbon and nitrogen removal for more than five months. According to the findings of Hosseinlou (2021), the parent system contained polyphosphate accumulating organisms (PAOs), denitrifying PAOs (DPAOs), denitrifying ordinary heterotrophic organisms (OHOs), anaerobic ammonium oxidation (anammox) bacteria, nitrifiers, and anaerobic and aerobic heterotrophs.

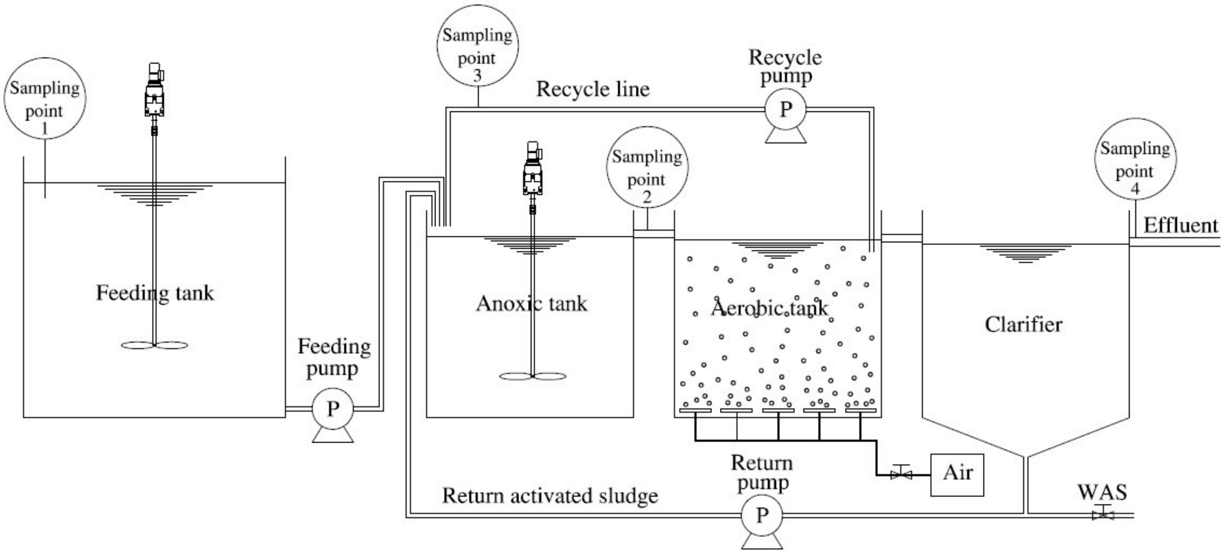

Fig. 5.1. Schematic flow diagram of the single-process SAO/PND system (NTS).

The reactors were made of Plexiglas with effective volumes of 7.8 liters, 10.8 liters, and 5.5 liters for the pre-anoxic tank, aerobic tank, and settler unit, respectively, with four sampling ports (Fig. 5.1). The effective size (L*W*D) of the pre-anoxic reactor was 30 cm * 15 cm * 17.3 cm, and the effective size of the aerobic reactor was 30 cm * 15 cm * 24 cm. When partial nitrification occurred in the aerobic tank, the nitrified effluent from this tank was recycled back to the pre-anoxic tank with twice the influent rate ($Q_r=2Q_{in}$). The separation of solids in the clarifier provided a well clarified effluent and concentrated solids, of which a portion was returned to the anoxic reactor through the return activated sludge (RAS) line with an equal rate of influent ($Q_{RAS}=Q_{in}$). In addition, excess sludge was withdrawn from the clarifier on a daily basis as waste activated sludge (WAS). The sludge retention time (SRT) and mixed liquor suspended solids (MLSS) were controlled by RAS and WAS lines to make sure the SRT and MLSS were adjusted at around 4±1 days and 3500±500 mg/L.

All experiments were performed under a controlled temperature of 25±1 ^{O}C by using an aquarium heater which was installed in the aerobic reactor. To ensure complete mix conditions in the pre-anoxic tank, a mechanical mixer set to 150 rpm was placed in this tank. Air from the laboratory air pipeline was continuously pumped to the aerobic reactor to maintain DO concentrations at 3-3.5 mg/L by using regular aquarium air stones. As a result of enough air flowrate in the aerobic reactor, appropriate complete mix conditions were achieved and the contents of this reactor were homogenous.

The influent flow to the system was 17 L/d, which was adjusted by using peristaltic pumps. Hydraulic retention time (HRT) for the pre-anoxic unit was 11 h, and this time was 15.2 h for the aerobic tank. To start up the single-process SAO/PND system, it was constantly fed with the synthetic solution over days 0-12. Then, during days 13 to 32, the system was operated with real brewery wastewater in order to evaluate the performance of the system for treating real high-strength wastewater.

After finishing the aforementioned stages, a set of complementary experiments was performed under steady-state conditions with the aim of explaining the removal mechanisms for the COD and nitrogen content in more detail. These experiments lasted for a month with the synthetic solution as the feed. More tests were conducted on samples collected from all four sampling points in order to better clarify the removal pathways. Mass balance calculations were carried out to describe and confirm all the removal mechanisms involved in the newly developed single-process method. During this operating period and under stable conditions, different parameters were tested from all four sampling points to monitor the overall performance and removal pathways. In order to conduct the mass balance calculations, various boundary limits were applied around the pre-anoxic and aerobic reactors separately as well as the entire system. The mass balance calculations are presented as mass according to Eq. (1)

$$\mathrm{M} = Q \times \mathrm{C} \qquad (1)$$

In the above equation, *M, Q, and C* represent the mass (mg/d), flowrate (L/d), and concentration (mg/L), respectively.

5.2.2. Composition of solutions

5.2.2.1. Synthetic wastewater

The synthetic wastewater's chemical composition comprised carbon sources, macronutrients, micronutrients, and distilled water, and was the same recipe as the one used in the study by Hosseinlou et al. (2019). To prepare different concentrations of the synthetic wastewater, three different stock solutions of carbon sources, macronutrients, and micronutrients (Table 5.1) were prepared weekly or biweekly and stored at 4 ^{O}C in the fridge. Three 1000 mL volumetric beakers were used in order to dilute the chemical compounds with distilled water for each stock solution. When preparing the stock solutions, magnetic stirrers were used inside of each beaker to dissolve the chemicals in distilled water. Afterwards, the prepared stock solutions were stored in the fridge for the required concentrations for the experiments.

Table 5.1. Synthetic wastewater composition.

Compound	Concentrations in stock solution (g/L)
Carbon sources:	
$C_6H_{12}O_6$	75
Peptone	75
NaAc	40
Macronutrients:	
$FeCl_3.6H_2O$	0.225
NH_4Cl	40
$KH_2PO_4.2H_2O$	22.05
EDTA	1.5
$NaHCO_3$	75
Micronutrients:	
$MgSO_4.7H_2O$	10
$CaCl_2$	5.3
H_3BO_3	0.0225
$CuSO_4.5H_2O$	0.005
KI	0.027
$MnCl_2.4H_2O$	0.018
$ZnSO_4.7H_2O$	0.018
$Na_2MoO_4.2H_2O$	0.05
$CoCl_2.6H_2O$	0.005

The daily usage solutions were prepared by diluting the three stock solutions in a container. Dissolving 80 mL of stock solutions in 40 L of distilled water produced a solution which contained 380 mg/L COD and 22 mg/L ammonia/ammonium. By diluting higher amount of stock solutions in the same 40 L container, higher concentrations were achieved which represented high-strength wastewaters.

5.2.2.2. Brewery wastewater

Raw brewery wastewater without any pretreatment was taken from a local Beau's brewery factory located 100 km east of Ottawa, Canada. This solution came mainly from the processing line and from the washing of equipment and floors. A No. 80 sieve was used for filtration in order to remove bigger particles from the collected brewery solution. The filtered solution was kept at 4 ^{O}C in the fridge to minimize substrate decomposition prior to the experiments. The chemical composition of the filtered brewery solution was as follows: 9140 mg/L COD, 15.1 mg/L ammonia/ammonium, 0.21 mg/L nitrite as NO_2^-–N, 4.66 mg/L nitrate as NO_3^-–N, and 9.01 pH. Ammonium augmentation was also applied between days 23-32 to evaluate the efficiency of the system for nitrogen content removal at higher concentrations.

5.2.3. Analytical methods

To evaluate the performance of the SAO/PND system, different samples were collected from the four sampling ports (Fig. 5.1), and the COD removal percentages were calculated according to Eq. (2):

$$\text{Removing COD as percent} = \frac{COD_{in} - COD_{eff}}{COD_{in}} \times 100\%, \qquad (2)$$

In the above equation, COD_{in} and COD_{eff} represent the COD concentrations in the influent and effluent of the single-process SAO/PND technology from sampling ports 1 and 4, respectively (Fig. 5.1).

The total inorganic nitrogen (TIN) is the summation of the ammonium plus nitrite and nitrate, and the removal percentages were obtained by Eq. (3):

$$\text{Removing TIN as percent} = \frac{TIN_{in} - TIN_{eff}}{TIN_{in}} \times 100\%, \qquad (3)$$

In the above equation, TIN_{in} and TIN_{eff} represent the TIN concentrations in the influent and effluent of the single-process SAO/PND technology from sampling ports 1 and 4, respectively (Fig. 5.1).

The COD, ammonia/ammonium, NO_2, NO_3, alkalinity, total phosphorus, and total nitrogen were measured using different HACH TNT vials (HACH Co., Loveland, CO, USA). As well, a HACH spectrophotometer (DR 6000^{TM} UV VIS) was used to read the absorbance of the various testing vials. A HACH HQ40d portable meter with different probes (LDO101 and PHC101) was used for measuring and collecting the data for DO, pH and temperature. The total suspended solids (TSS) and volatile suspended solids (VSS) were also measured according to the Standard Methods (APHA, 1998).

5.3. Results and discussion

5.3.1. Organic carbon

This study is mainly focused on organic carbon and nitrogen content removal, and this section evaluates the effectiveness of the single-process SAO/PND technology regarding these components, as well as explaining the attributed removal mechanisms. To investigate the efficiency of the system regarding the COD, samples were taken from four different sampling ports and then tested (Fig. 5.1). The COD concentrations in the system were tracked in order to evaluate the performance of the single-process SAO/PND technology, and the results are shown in Fig. 5.2. During the initial operational days 0-12, the system was run under steady-state conditions and constantly fed with a synthetic solution which represented high-strength wastewater (4100 mg/L COD and 210 mg/L ammonia/ammonium). There was no evidence of failure in the system, and the effluent concentrations were consistently maintained below 60 mg/L and COD removal remained high at around 99%. In the meantime, 96% nitrogen removal was achieved as well.

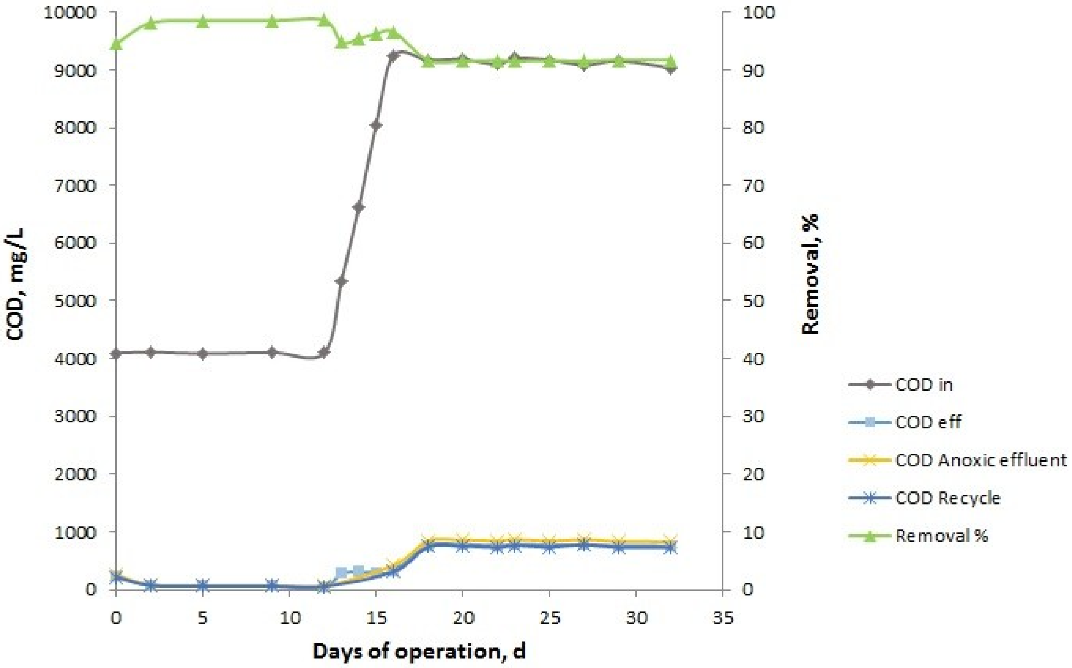

Fig. 5.2. COD removal with the single-process SAO/PND technology over time.

After day 12, the feed composition was changed stepwise over three days from the synthetic solution to real brewery wastewater with different mixing ratios. The mixing ratios on a volume basis (real brewery wastewater/synthetic solution) on days 13, 14, and 15 were 1/3, 1/1, and 3/1, respectively. From day 16 onwards, the system was run with real brewery wastewater. After switching the feed to the real brewery wastewater, the results showed a sudden increase in effluent COD concentrations of up to 780 mg/L, and COD removal sharply dropped and remained constant at 92% till the end of the experiments. This sharp decrease in the removal efficiency of the system was because of the fact that the accessible food was more difficult for the heterotrophs to consume. Previously, the chemicals for carbon sources in the synthetic solution recipe (glucose+peptone+sodium acetate) could be simply degraded and consumed by these organisms, whereas after changing to the real brewery wastewater, the more complex organic ingredients involved were not as easily degradable by the bacterial community. Figs. 5.2 and 5.3 depict the overall COD removal percentages.

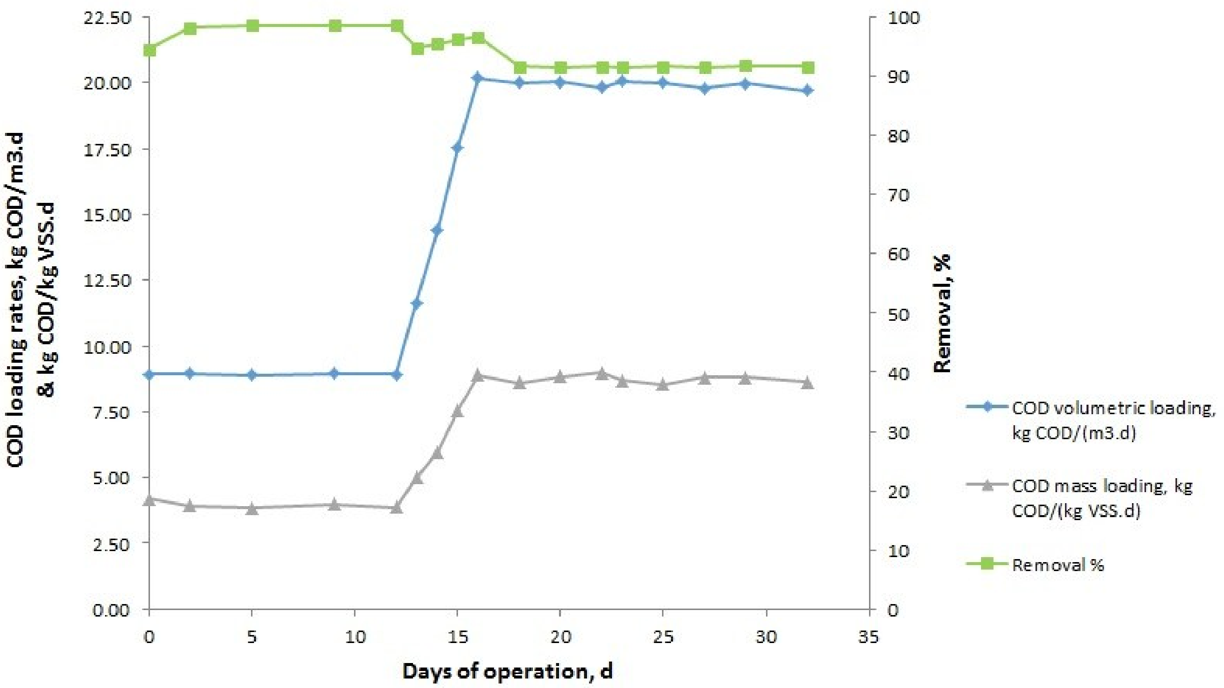

Fig. 5.3. COD loading rates in the pre-anoxic tank along with removals as percent.

The complementary experiments showed that the majority of COD removal occurred in the pre-anoxic tank. Fig. 5.3 depicts the loading rates (volumetric and mass) of this component for the pre-anoxic tank, along with the removal percentages. Between days 0 and 12, the organic carbon loading rates, which were accompanied with high removal efficiencies, were reported as 8.93 kg COD/(m^3.d) and 3.97 kg COD/(kg VSS.d), respectively, with a 99% COD removal efficiency (Fig. 5.3). Hosseinlou et al. (2019) showed that these values are not the maximum possible loading rates, and that the single-process SAO/PND technology can handle higher loads. The current study showed that after switching the feed to the real brewery wastewater between days 13 and 32, higher organic carbon loading rates were achieved with high removal efficiencies which were reported as 19.95 kg COD/(m^3.d) and 8.75 kg COD/(kg VSS.d), with a 92% COD removal efficiency (Fig. 3). These results prove the excellent efficiency of the single-process SAO/PND technology in removing organic carbon from real brewery wastewater.

Hosseinlou et al. (2019) reported 97% COD removal under 11.26 kg COD/(m^3.d) loading rates by the single-process SAO/PND technology with a synthetic solution as the feed. The current study shows a lower COD removal efficiency (92%), which can be attributed to the complexity of the real brewery wastewater used in the current study compared to the synthetic solution as a simple compound for bacterial consumption in the previous research. However, the COD loading rate in this study (19.95 kg COD/(m^3.d)) was significantly higher than the loading rate in the research by Hosseinlou et al. (2019).

Hosseinlou (2021) fed the SAO/PND system with a synthetic solution and determined 11.80 kg COD/(m^3.d) as a design loading rate with 97% removal efficiency. However, this researcher achieved 95% COD removal when treating real dairy wastewater under a 9.47 kg COD/(m^3.d) loading rate. The current study shows comparable COD removal efficiency (92%) under higher loading rates (19.95 kg COD/(m^3.d) for treating real brewery wastewater.

5.3.2. Nitrogen content

Fig. 5.4 depicts the fluctuations of TIN, which are attributed to the performance of the overall system. Between days 0 and 12, the system was steadily fed by ammonia/ammonium at around 210 mg/L, and the results showed a 96% nitrogen content removal without any signs of stress or failure (Fig. 5.4). The influent feed solution to the system was changed stepwise from synthetic to real brewery wastewater during days 13, 14, and 15, and nitrogen content removal declined slightly to 93% during these switching days. However, after completing the switch to real brewery wastewater on day 16, the overall nitrogen content removal performance improved, achieving 99% until the end.

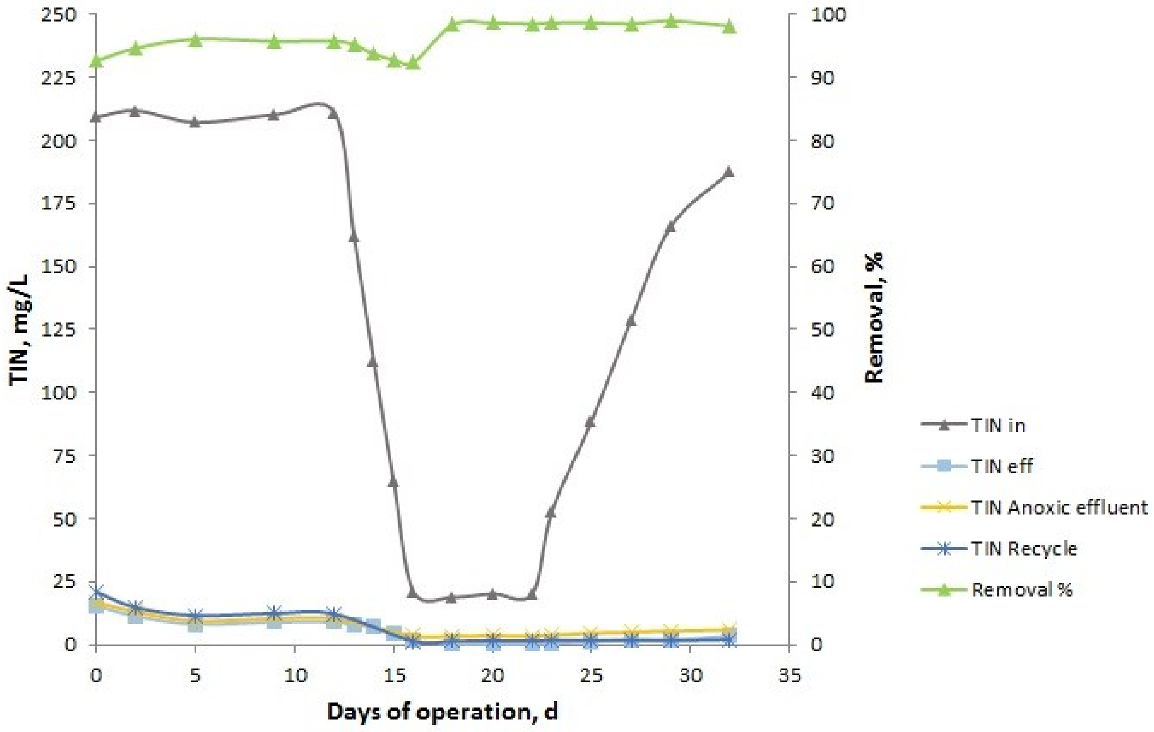

Fig. 5.4. TIN removal with the single-process SAO/PND technology during operational days.

Due to the lower amount of nitrogen content in the real brewery solution, it was decided to supply ammonium augmentation to the solution in an endeavour to raise the concentration of this component in order to simulate streams with strong nitrogen content. The ammonium augmentation process was undertaken step by step during days 23-32, and resulted in raised nitrogen concentrations in the feed to the system. The current study achieved 92% and 99% of organic carbon and nitrogen content removal, respectively, by feeding the system with 9036 mg/L COD and 187.52 mg/L TIN–N. This research showed that the single-process SAO/PND technology could be overloaded regarding TIN; however, this stage of the experiments was terminated on day 32, and did not find the maximum possible incoming concentrations for this component. Figs. 5.4 and 5.5 depict the overall TIN removal percentages.

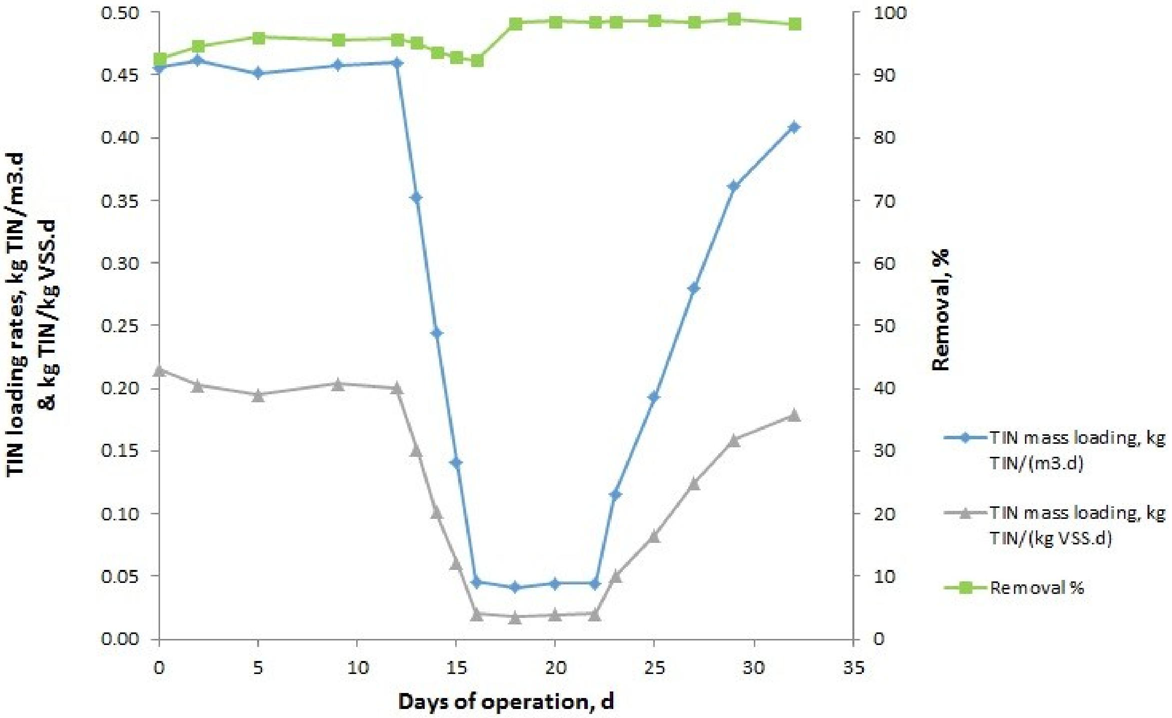

Fig. 5.5. Nitrogen content loading rates in the pre-anoxic tank along with removals as percent.

Complementary experiments showed that the majority of TIN removal happened in the pre-anoxic tank, and Fig. 5 depicts the loading rates (volumetric and mass) of this component for the pre-anoxic tank along with the removal percentages. Between days 0 and 12, the nitrogen content loading rates that were accompanied with high performance were reported as 0.46 kg TIN/(m^3.d) and 0.20 kg TIN/(kg VSS.d), respectively, with a 96% TIN removal efficiency (Fig. 5.5).

During the five months of running the single-process SAO/PND technology with synthetic solution conducted by Hosseinlou et al. (2019), they achieved a 91% nitrogen removal efficiency with a volumetric loading rate of 0.57 kg TIN/(m^3.d). On the other hand, the current study achieved a higher TIN removal efficiency of 96%, and this proves the high effectiveness of the single-process SAO/PND technology in removing nitrogen content from real brewery wastewater.

By feeding the SAO/PND system with a synthetic solution, Hosseinlou (2021) determined 0.63 kg TIN/(m^3.d) as a TIN loading rate, with a 92% removal efficiency. In addition, Hosseinlou (2021) fed the system with real dairy wastewater and found a 99% TIN removal with a 0.41 kg TIN/(m^3.d) as loading rate. The current study shows comparable results, with 96% TIN removal with a 0.46 kg TIN/(m^3.d) loading rate, and proves the high efficiency of the single-process SAO/PND system for nitrogen removal from real brewery wastewater.

Biase et al. (2020) conducted a study by using anaerobically pretreated brewery wastewater as a feed for three aerobic granule sludge (AGS) tanks under different loading rates. Various organic loading rates of 0.80-4.10 kg COD/(m^3.d) and total ammonia nitrogen (TAN) loading rates ranging from 0.04-0.41 kg TAN/(m^3.d) were applied, and approximately 80% COD and 60% nitrogen removal were achieved. In comparison, the current study used the single-process

SAO/PND system with higher loading rates, and achieved higher efficiencies for COD and nitrogen removal from brewery wastewater without any pretreatment.

The degradation of organic matter in AD systems does not need aeration, and this is an advantage of these processes. However, they are often not able to remove nutrients, and need the use of subsequent processes. Upflow anaerobic sludge blanket (UASB) is the most common anaerobic process for treating brewery wastewaters (Arantes et al., 2017). A combination of UASB and microbial fuel cell (MFC) were used for treating brewery wastewater by Liu et al. (2020). Their combined system (UASB-MFC) was able to remove 90% of the COD and maintain ammonium concentrations below 15 mg/L; however, the pairing of these two separate processes is more intensive regarding the operation and maintenance. On the other hand, the single-process SAO/PND system provided better performance with simpler operation and maintenance.

Papadopoulos et al. (2020) used a combined system composed of an electrocoagulation (EC) system and cyanobacteria-based cultivation process for treating brewery wastewater. Under the optimum operational conditions, they could achieve 91.6% COD and 89.4% total Kjeldahl nitrogen (TKN) removal. It should be mentioned that synchronizing and operating combined systems which are composed of two separate systems is not a simple task, and that in contrast, it can be stated that the efficiency of the SAO/PND technology is higher, with easier operation and maintenance as a single-process treatment method.

The results of the current research prove the high efficiency of the single-process SAO/PND technology for the treatment of real brewery industry wastewater, and this system as a single-process method has a big advantage over the combined systems. Therefore, the single-process

SAO/PND system can be used as a cost-effective and robust on-site technology which is easy to operate for treating real brewery industry effluents in the future.

5.3.3. DO and pH fluctuations

The ideal pH range for successful operation of the single-process SAO/PND technology was determined to be between 7 and 8 (Hosseinlou, 2021; Hosseinlou et al., 2019), and the current study was run under stable conditions with pH fluctuating in this optimum range (Fig. 5.6). In addition, it was reported that pH values higher than 8 would create toxicity for the single-process SAO/PND technology which would end up producing signs of stress and failure (Hosseinlou, 2021; Hosseinlou et al., 2019). Total alkalinity was not monitored regularly, but in order to provide enough alkalinity for partial nitrification, this parameter was measured occasionally and there was always 80 mg/L $CaCO_3$ in the system as a minimum amount for nitrification. In addition, as a result of the alkalinity consumption during the limited nitrification process which was occurring in the aerobic unit, the measured alkalinity values in the pre-anoxic tank were slightly higher than in the aerobic tank. It is worth mentioning that in combined systems in which AD is chosen for the pretreatment ahead of the subsequent processes, pH stabilization could be a challenging issue and would result in intensive operation and maintenance requirements.

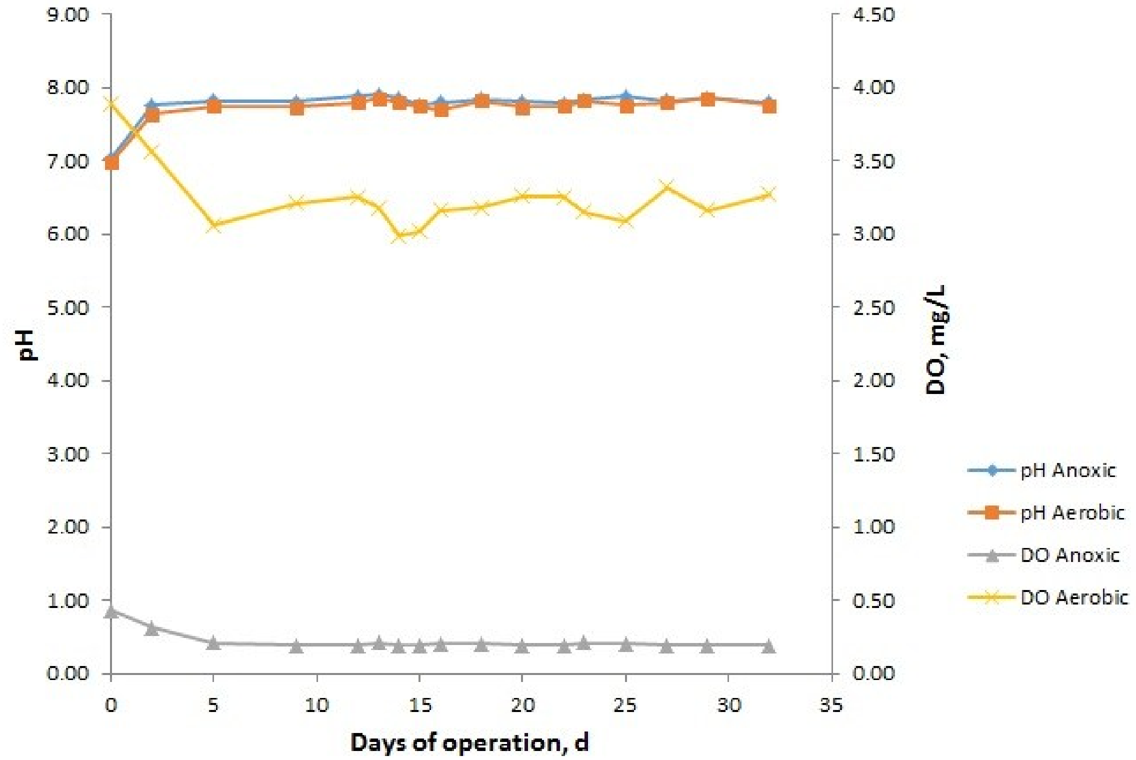

Fig. 5.6. DO and pH fluctuations in reactors over time.

Metcalf and Eddy (2014) recommended DO values above 3.0 mg/L to make sure that this component does not negatively impact the nitrification process; therefore, the DO values in the aerobic tank in the current research were kept between 3 and 3.5 mg/L in order to provide an appropriate environment for possible incomplete nitrification (Fig. 5.6). However, DO concentrations below 0.25 mg/L are recommended to create favorable conditions for conventional denitrification. To provide a favorable environment for the occurrence of denitrification in the pre-anoxic tank, the DO values in this unit were adjusted to below 0.25 mg/L (Fig. 5.6). As mentioned before, the temperature was always maintained at 25±1 ^{O}C by using a simple aquarium type heater in the aerobic unit, and this temperature was found to be the optimum for the bacterial community in the single-process SAO/PND treatment method.

5.3.4. Removal pathways involved in the single-process SAO/PND system

Complementary experiments were completed to determine the organic carbon and nitrogen removal mechanisms which occurred in the single-process SAO/PND system. These experiments were run at steady-state operational conditions for a month, and the performance of the system is presented in Fig. 5.7. Various mass balances for different components were calculated for each individual tank and the entire system as well, and stoichiometric ratios were derived. The synthetic influent solution used as a constant feed contained 70312 mg/d COD, 3606 mg/d ammonia/ammonium, 1.0 mg/d NO_2^-–N, 10.4 mg/d NO_3^-–N, 1998 mg/d PO_4^{3-}–P, 7363 mg/d N–N, and 12600 mg/d alkalinity as $CaCO_3$.

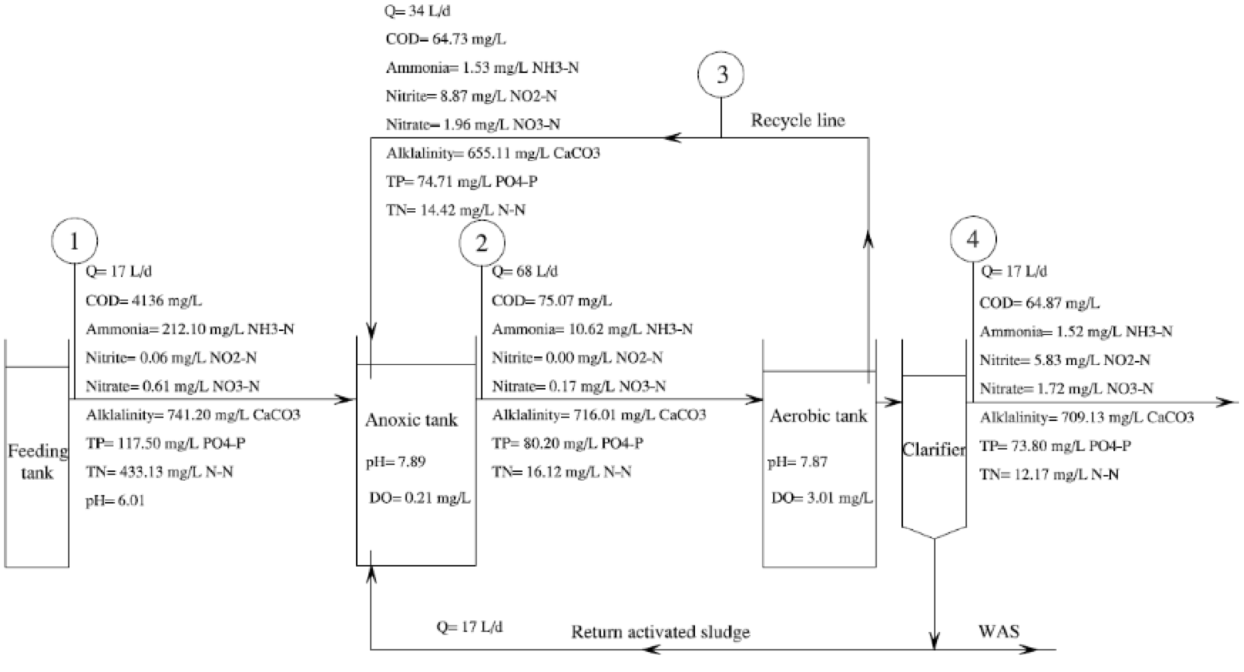

Fig. 5.7. Performance of the system under steady-state conditions during complementary experiments.

5.3.4.1. Organic carbon removal

The results revealed that 97.4% (68511 mg/d) of the organic carbon was removed in the pre-anoxic reactor, and the rest of this component was degraded in the following aerobic unit 1%

(703 mg/d), which resulted in an overall 98.4% (69214 mg/d) COD removal in the entire system. Anaerobic oxidation was determined to be the main COD removal mechanism, with mass balance calculations showing that a 67.9% COD removal was completed by this process, and the rest by other processes, as will be explained. The high organic loading rates and limited amount of electron acceptors such as oxygen and NO_x in the pre-anoxic unit, along with the lower Y_{obs} (0.15 mg VSS/mg BOD) and higher COD/NO_x ratio (442/1), created a favourable anaerobic oxidation environment in this reactor. Sludge production of 10103 mg VSS/d on a daily basis showed that 20.7% of the incoming COD was consumed for cell growth by different active microorganisms in the single-process SAO/PND system. A conversion rate of VSS to COD equal to 1.42 (Metcalf and Eddy, 2014) was applied to determine the organic carbon consumption for biological assimilation.

Incoming phosphorus to the system was 1998 mg/d PO_4^{3-}–P, and in total, 37.2% was removed. Dissolved total phosphorus mass balance calculations around each individual reactor tank revealed that 47.6% of phosphorus was removed by DPAOs inside the pre-anoxic tank, and 52.4% was removed by PAOs inside the subsequent aerobic tank. Applying an unfiltered TP mass balance around the overall system, and taking into account the solids generation, showed that phosphorus was stored in the bacterial communities as excess sludge. The internal storage of organic carbon as PHA and PHB during the phosphorus removal occurred by DPAOs and PAOs. During the P-uptake process, 10 g COD was consumed per each g PO_4^{3-}–P, and mass balance calculations showed that 4.9% of the incoming COD was degraded by DPAOs and 5.4% by PAOs.

Nitrification, which partially occurred inside the aerobic unit, resulted in mainly nitrite and limited nitrate. These NO_x products were conveyed to the pre-anoxic unit for degradation, and details about the nitrogen conversions and removals will be explained later in subsection 5.3.4.2. Nitrite from the recycle line was consumed due to the anammox process in the pre-anoxic tank, and in the meantime, nitrate was generated. Then, nitrate from the aerobic tank plus anammox activity inside the pre-anoxic tank was consumed in denitrification as electron acceptor by denitrifying OHOs and DPAOs, which led to organic carbon removal as well.

By applying separate mass balance computations for dissolved total phosphorus and nitrate around the pre-anoxic tank, it was found that DPAOs had access to enough nitrate for anoxic phosphorus uptake in this reactor. 92.8% of the available nitrate (recycled plus that generated by anammox) was removed by DPAOs, and the remaining 7.2% of this component was consumed by denitrifying OHOs. The latter denitrifiers, as ordinary heterotrophs, used 6.6 g COD per each g NO_3^-–N through the denitrification process, which was equal to a removal of 0.12% of COD from the system's incoming COD. Finally, in the presence of oxygen as an electron acceptor in the aerobic reactor, active ordinary aerobic heterotrophic bacteria inside this tank could remove 1% of the incoming COD, equal to 703 mg/d.

5.3.4.2. Nitrogen content removal

As a result of the innovative processes and new active biological communities in these systems, nitrogen transformations and cycles have become more complex, and a result, simple conventional processes like nitrification followed by denitrification cannot properly explain all the nitrogen removal mechanisms. The single-process SAO/PND method in this research, as a newly developed system, aims to reveal details about nitrogen conversion and removal pathways. Biological assimilation played an important role in removing the nitrogen content in this system. The mass balance for unfiltered TN was computed around the entire process, and it was found that 46.3% of the nitrogen content removal occurred through the assimilation mechanism in the system, ending up in excess sludge. Based on the calculations, 7402 mg/d TN–N entered the system, and the unfiltered total nitrogen which exited the system as part of the daily waste sludge was 3425 mg/d TN–N, thus proving that 46.3% of the nitrogen content was assimilated and wasted from the entire system.

In total, 99.3% (3580 mg/d) ammonia/ammonium was removed, and a significant portion of the incoming ammonia/ammonium to the system was degraded (82.1% or 2961 mg/d) in the pre-anoxic tank, with the rest of ammonia/ammonium (17.2% or 619 mg/d) being conveyed to the subsequent aerobic tank and oxidized to nitrite and nitrate in the presence of O_2 via partial nitrification, and finally sent to the head of the system for degradation. Conventional complete nitrification is a slow mechanism which needs a longer SRT to support this process (Metcalf and Eddy, 2014). On the other hand, the short SRT values accompanied by controlled O_2 concentrations in the single-process SAO/PND system caused a suppression of complete nitrification, with partial nitrification with a nitrite/nitrate molar ratio of 4.96/1 occurring in the aerobic tank.

In theory, stoichiometric alkalinity consumption for conventional complete nitrification is 7.14 g CaCO3 per each g NH_4^+–N (Metcalf & Eddy, 2014). However, this study found that 6.7 g CaCO3 was used per each g NH_4^+–N, and that the lower conversion value can be attributed to the transformation of a small portion of ammonium into cellular nitrogen under laboratory conditions.

In the nitrogen removal processes, nitritation and anammox together can save on costs compared to conventional denitrification processes. In this research, nitrite was the main NO_x product of the partial nitrification mechanism in the aerobic unit. After the production of nitrate in the aerobic tank, it was sent to the head of the process and consumed as one of the chemical compounds in the anammox activity. The pre-anoxic tank as a CSTR reactor always had COD concentrations less than 80 mg/L, with nitrite available in this reactor, and these operational conditions prepared an appropriate environment for the growth of an anammox bacterial community. Anammox as an effective biological process for removing nitrogen with less energy requirements is presented in Eq. (4) (Mojiri et al., 2021; Xie et al., 2020):

$$\mathrm{NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 2.03H_2O + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15}} \quad (4)$$

Based on the pre-anoxic mass balance computations, 304.3 mg/d NH_4^+–N and 401.7 mg/d NO_2^-–N were consumed by anammox activity, which ended up as 79.1 mg/d NO_3^-–N production. The total amount of available nitrate in the pre-anoxic tank was 173.8 mg/d NO_3^-–N, which was the summation of the nitrate from the recycled partial nitrification plus the anammox process. Nitrate was removed at 92.8% by DPAOs and 7.2% by denitrifying OHOs during denitrification occurring in the pre-anoxic tank. During the anammox process, 8.4% (304.3 mg/d NH_4^+–N) was removed, which was accompanied by NO_2^-–N reduction and NO_3^-–N generation with a stoichiometry of 1/1.32/0.26 for NH_4^+–N and NO_2^-–N consumption, and NO_3^-–N generation.

According to the nitrogen mass balance computations around each tank and for the entire process, 28.1% of the incoming nitrogen content was still unidentified, which can be related to experimental errors under laboratory conditions and some unknown processes. The explained removal pathways in the current study showed strong agreement with the results obtained by Hosseinlou (2021). Biological studies have been fulfilled as well, and they completely confirm all the explained removal mechanisms. However, the results of the biological studies are not a part of the current research, and will be presented as a separate article.

5.4. Conclusions

The results showed 92% COD and 99% nitrogen content removal from real brewery wastewater by using the single-process SAO/PND technology, with 9036 mg/L COD and 187.52 mg/L TIN–N influent concentrations. For treating real brewery wastewater, the maximum COD loading rates for the pre-anoxic unit which was accompanied by the highest removal efficiencies were reported as 19.95 kg COD/(m^3.d) and 8.75 kg COD/(kg VSS.d). It should be mentioned that the single-process SAO/PND technology showed a strong potential for nitrogen removal with more influent nitrogen concentrations as feed, but this study did not determine the highest possible influent nitrogen concentrations. The COD and nitrogen removal mechanisms were determined during a month of complementary experiments, in which the system was running under steady-state conditions with a synthetic solution as a feed. It was determined that the COD removal mechanisms with the single-process SAO/PND technology consisted of anaerobic oxidation, cell synthesis and biomass growth, denitrification by DPAOs and denitrifying OHOs, PAOs, and a negligible amount by aerobic heterotrophic bacteria. Furthermore, the nitrogen removal pathways were as follows: bacterial consumption for growth, incomplete nitrification followed by denitrification, and anammox. The results of the current study support the high potential of the single-process SAO/PND technology for the treatment of strong wastewaters, and that this system can be used as a robust, cost-effective, and efficient on-site process for treating brewery industry effluents in the future.

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6. Significant cost savings using a newly developed single-process technology compared to conventional processes for treating high-strength effluents

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Abstract

This study investigates the performance of a newly developed technology, known as simultaneous anaerobic oxidation/partial nitrification-denitrification (SAO/PND), for treating strong wastewater under laboratory conditions. Excellent results are achieved, with maximum COD and nitrogen removal of 98% and 96%, respectively, when treating synthetic wastewater with influent 4120 mg/L COD and 210 mg/L NH_4^+–N. Appropriate living environment in this single-process technology favored the co-existence of various bacterial communities, and removal mechanisms by these organisms are identified and confirmed. The results showed that the single-process SAO/PND technology required 95% and 90% less oxygen compared to conventional aerobic processes and combined systems, respectively. Furthermore, this newly developed technology produced 60% and 44% less sludge respectively in comparison with mentioned conventional systems. This study shows that the single-process SAO/PND technology is a promising sustainable alternative to conventional systems, as it combines high efficiency and cost-effectiveness with simple operation and maintenance requirements.

Keywords: COD; Cost comparison; Nitrogen; SAO/PND; Simultaneous; Strong stream

6.1. Introduction

Because of the scarcity of fresh water around the world, preserving water bodies is vital, and releasing untreated strong wastewaters can destroy aquatic environments, as the abundance of organic substances in these wastewaters can cause serious water pollution in receiving waters (Simate et al., 2011; Zheng et al., 2015; Jaiyeola et al., 2016). Therefore, organic matter and nitrogen content from strong wastewaters have to be removed before discharging to the environment in order to conserve the water quality (Zheng et al., 2015). The treatment of strong wastewaters such as from meat processing, landfill leachates, dairy products, brewery, and food industry effluents, which are rich in organic matter and nutrients, requires specific considerations. There is a wide range in the quality and quantity of strong wastewaters, but some of these effluents have been found to contain as much as 1000-13800 mg/L COD and 14-800 mg/L NH_3–N (Hosseinlou et al., 2019). For treating strong wastewaters, there are some physical, chemical, and biological treatment methods, but in general, biological treatment processes are preferred (Simate et al., 2011; Jaiyeola et al., 2016).

It is possible for organic matter and nutrients to be removed by conventional aerobic treatment processes such as the modified Ludzack Ettinger (MLE) as an activated sludge system, and various types of these systems have been applied in different studies. However, there are serious drawbacks when using these aerobic processes for treating strong wastewaters, as they consume huge amounts of oxygen for COD and nitrogen removal, along with a significant amount of sludge production (Zheng et al., 2015; Wang et al., 2016). In conventional aerobic systems such as MLE, which consist of anoxic/aerobic reactors, complete nitrification occurs under aerobic conditions and results in nitrite/nitrate generation. These nitrite/nitrates will be recycled back to the pre-anoxic reactor for denitrification. Then the COD will be removed during denitrification in this pre-anoxic reactor, and the rest of the COD aerobically in the aerobic tank by aerobic heterotrophic bacteria. Nitrogen removal through complete nitrification-denitrification process needs more oxygen. It is worth mentioning that during the nitrification process in aerobic processes, ammonia concentrations above 150 mg/L would create a toxic environment for nitrifiers, resulting in significant nitrification inhibition (An et al., 2007).

Anaerobic digestion (AD) is the best alternative to aerobic processes for the treatment of strong wastewaters (Ulgudur & Demirer, 2019; Xin et al., 2020; Gao et al., 2020; Garg et al., 2020), but there are some disadvantages, such as: i) biological nitrogen and phosphorus removal is not possible; ii) requirement for further treatment with a subsequent aerobic treatment process in series to meet discharge requirements; and iii) difficulties in achieving stable reactor performance as a result of sensitivity to fluctuations in temperature and operating conditions (Andalib et al., 2011; Metcalf & Eddy, 2014; Ambaye et al., 2021).

AD treatment method has been identified as an efficient system for removing organic carbon from strong wastewaters, but this system as a single-process cannot produce treated effluent that meets discharge standards; therefore, existing conventional treatment technologies are combined systems. These conventional combined systems involve at least two different systems which work together in series. Most of these combined methods apply the AD as a head of the system, which is followed by a polishing aerobic process such as conventional activated sludge for carbon and nitrogen removal. The AD head system removes organic carbon but produces ammonia via ammonification (ammonia production) process, and so external alkalinity should be added to the AD in order to maintain the pH at the appropriate level (Arantes et al., 2017; Osmani et al., 2021).

AD systems used as the head in combined systems can be processes such as anaerobic ponds, upflow anaerobic sludge blankets (UASBs), anaerobic contact reactors, anaerobic sequencing batch reactors (SBRs), anaerobic filters, anaerobic expanded granular sludge beds, and anaerobic fixed film reactors. Subsequent aerobic systems in these combined technologies can involve such methods as activated sludge processes, constructed wetlands, rotating biological contactors, trickling filters, aerobic ponds, aerobic lagoons, moving bed biofilm reactors (MBBRs), and intermittent sequencing batch reactors (Aziz et al., 2019).

The effluent from AD is suitable to feed to a subsequent polishing aerobic system for further removal to meet the effluent discharge standards (Chan et al., 2009); however, as a result of carbon scarcity in subsequent aerobic systems such as MLE, an external organic carbon source such as methanol is needed for denitrification. The polishing aerobic process as the second treatment step removes organic carbon via denitrification and aerobic degradation, as well as nitrogen via conventional complete nitrification and then denitrification (Akizuki et al., 2021). This means that synchronizing the two different processes in series in a combined system is expensive and complicated, and requires highly skilled operators to successfully run the system. Hence, in order to overcome these concerns, it is paramount to develop an efficient and less expensive single-process technology with relatively simple operation. With this in mind, a single-process simultaneous anaerobic oxidation/partial nitrification-denitrification (SAO/PND) technology, as a sustainable, robust, and cost-effective system, was developed by Hosseinlou et al. (2019) and removed both organic carbon and nitrogen from synthetic wastewaters. Further, Hosseinlou (2021) determined the appropriate design loading rates of this newly developed technology and successfully applied it for treating real dairy wastewater. The high performance of the system has also been confirmed for the treatment of real brewery industry effluent (Hosseinlou 2022).

Although the single-process SAO/PND technology has been identified as a system with strong capabilities; however, there is still a knowledge gap regarding the cost savings from this system compared to conventional technologies. Therefore, the main goal of the current research is to perform cost comparisons of the single-process SAO/PND technology with two conventional systems, which are: i) a conventional aerobic process such as MLE composed of anoxic/aerobic reactors; and ii) a conventional combined system which is composed of an AD and a subsequent aerobic system such as MLE. To achieve this goal, the performance of the single-process SAO/PND technology is monitored under steady-state conditions with a synthetic solution as a feed. The organic carbon and nitrogen removal mechanisms in this technology are further confirmed through complementary experiments.

6.2. Materials and methods

6.2.1. Experimental setup and operational conditions

In the current study, a laboratory-scale single-process SAO/PND technology (Fig. 6.1) is designed and employed based on the optimum operational conditions found by Hosseinlou et al. (2019). Those researchers tried different operational conditions in laboratory conditions applying various design criteria such as solids retention time (SRT), hydraulic retention time (HRT), mixed liquor suspended solids (MLSS), dissolved oxygen (DO), recycle flowrate (Q_r), and return activated sludge flowrate (Q_{RAS}). After several incidents of system failure, they were able to determine the optimum operational conditions and design criteria for this newly developed technology (Hosseinlou et al., 2019).

In the current study, the system was run under steady-state conditions for a period of one month with a synthetic solution simulating strong wastewater. Herein, strong wastewater is a term used to define wastewaters with high concentrations of organic carbon and nitrogen content. For inoculation purposes in order to start the system, sludge was collected from a stabilized single-process SAO/PND technology which had been running for five months under laboratory conditions. Based on Hosseinlou (2021), the mentioned parent setup was rich in anaerobic and aerobic heterotrophic bacteria, polyphosphate accumulating organisms (PAOs), denitrifying ordinary heterotrophic organisms (OHOs), denitrifying PAOs (DPAOs), nitrifiers, and anaerobic ammonium oxidation (anammox) bacterial community.

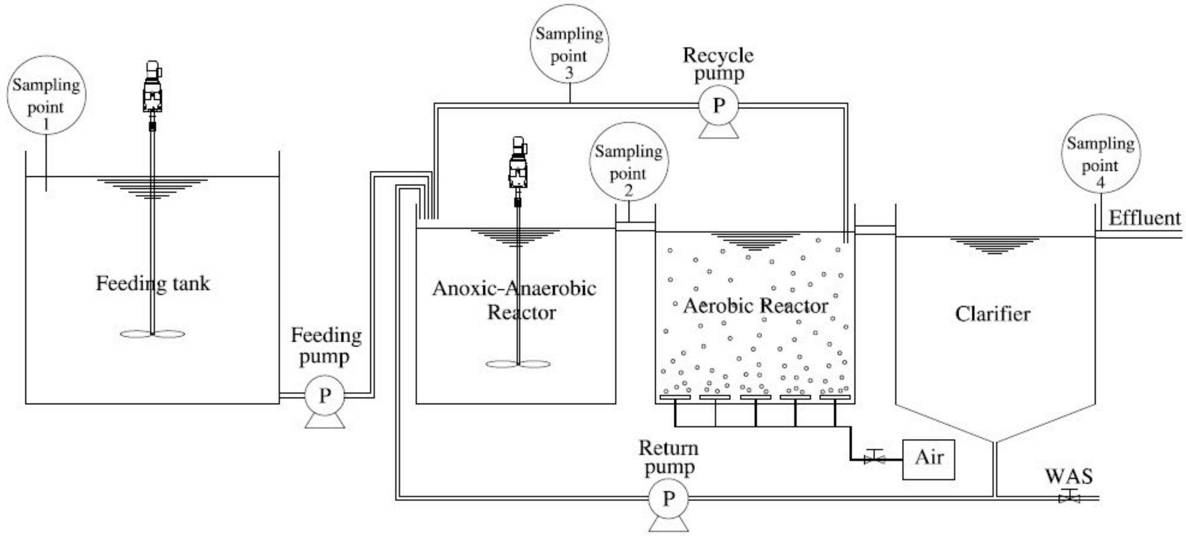


Fig. 6.1. Laboratory scale single-process SAO/PND technology with four sampling ports (NTS).

After inoculation and successfully running the system under stable laboratory conditions, excess sludge was withdrawn on a daily basis from the settler tank as waste activated sludge. The MLSS and SRT were controlled by return activated sludge (RAS) and waste activated sludge

(WAS) lines in order to maintain the SRT and MLSS around the required optimum operational conditions as presented in Table 6.1.

Table 6.1. Operational conditions in this study.

Paratmeters		**Units**	**Values**
Overall System:			
	influent flowrate (Qin)	L/d	17
	recycle ratio (Q_r/Q_{in})	-	2
	return ratio (Q_{RAS}/Q_{in})	-	1
	MLSS in reactors	mg/L	3500±500
	SRT	d	4±1
	temperature	°C	25±1
Pre-anoxic reactor:			
	volume of reactor	L	7.8
	HRT	hr	11
	DO	mg/L	0.2
	pH	-	7.9
	mixing speed in the pre-anoxic reactor	rpm	150
Aerobic reactor:			
	volume of reactor	L	10.8
	HRT	hr	15.2
	DO	mg/L	3.0-3.5
	pH	-	7.8

6.2.2. Synthetic wastewater composition

The main ingredients in the synthetic solution used in this study consisted of carbon sources, macronutrients, micronutrients, and distilled water. This recipe is a slightly modified version of the synthetic solution applied by Zhao et al. (2008). Three different stock solutions of these ingredients were prepared and stored at 4°C in a refrigerator. Volumetric beakers with a nominal capacity of 1000 mL were used as containers for diluting the chemical compounds in distilled water to prepare the stock solutions, and magnetic stirrers were used inside of each beaker to dissolve the chemicals in the distilled water. After preparing each stock solution, they were stored in separate 1000 mL volumetric flasks in the refrigerator to provide the required concentrations for the experiments. The components of each stock solution are presented in Table 6.2.

Table 6.2. Chemical composition of the synthetic wastewater.

Compound		Concentrations in stock solution (g/L)
Carbon sources:		
	$C_6H_{12}O_6$	75
	Peptone	75
	NaAc	40
Macronutrients:		
	$FeCl_3.6H_2O$	0.225
	NH_4Cl	40
	$KH_2PO_4.2H_2O$	22.05
	EDTA	1.5
	$NaHCO_3$	75
Micronutrients:		
	$MgSO_4.7H_2O$	10
	$CaCl_2$	5.3
	H_3BO_3	0.0225
	$CuSO_4.5H_2O$	0.005
	KI	0.027
	$MnCl_2.4H_2O$	0.018
	$ZnSO_4.7H_2O$	0.018
	$Na_2MoO_4.2H_2O$	0.05
	$CoCl_2.6H_2O$	0.005

The system was continuously fed by preparing synthetic solution in a 40 L container as a storage feeding tank. After adding 800 mL of the stock solutions to this storage tank, distilled water was used for dilution, with synthetic solution produced containing concentrations of around 4120 mg/L COD and 210 mg/L NH_3–N, to be consumed as influent.

6.2.3. Sampling and analysis

To evaluate the performance of the single-process SAO/PND technology, samples were taken from the four sampling ports (Fig. 6.1). Different TNT vials from the HACH Company (HACH Co., Loveland, CO, USA) were used to measure the COD, ammonia/ammonium, nitrite, nitrate, alkalinity, total phosphorus, and total nitrogen. To monitor the DO, temperature, and pH in the system, a portable probe meter from the HACH Company (HQ40d) was used, and this device had different probes (LDO101 and PHC101) for measuring the mentioned items. In addition, the TSS and VSS were measured and analyzed using standard methods (APHA, 1998).

The results of the SAO/PND technology in this research were obtained from the experiments under laboratory conditions; however, for cost comparisons of the SAO/PND technology with two conventional systems, theoretical calculations of the conventional systems were carried out based on Metcalf & Eddy (2003, 2014). Table 6.3 presents the design criteria, sludge production, and oxygen consumption for the mentioned conventional systems: i) conventional aerobic processes; and ii) conventional combined systems composed of an AD and subsequent aerobic system.

Table 6.3. Design criteria and theoretical calculations of two conventional systems according to Metcalf & Eddy (2003, 2014).

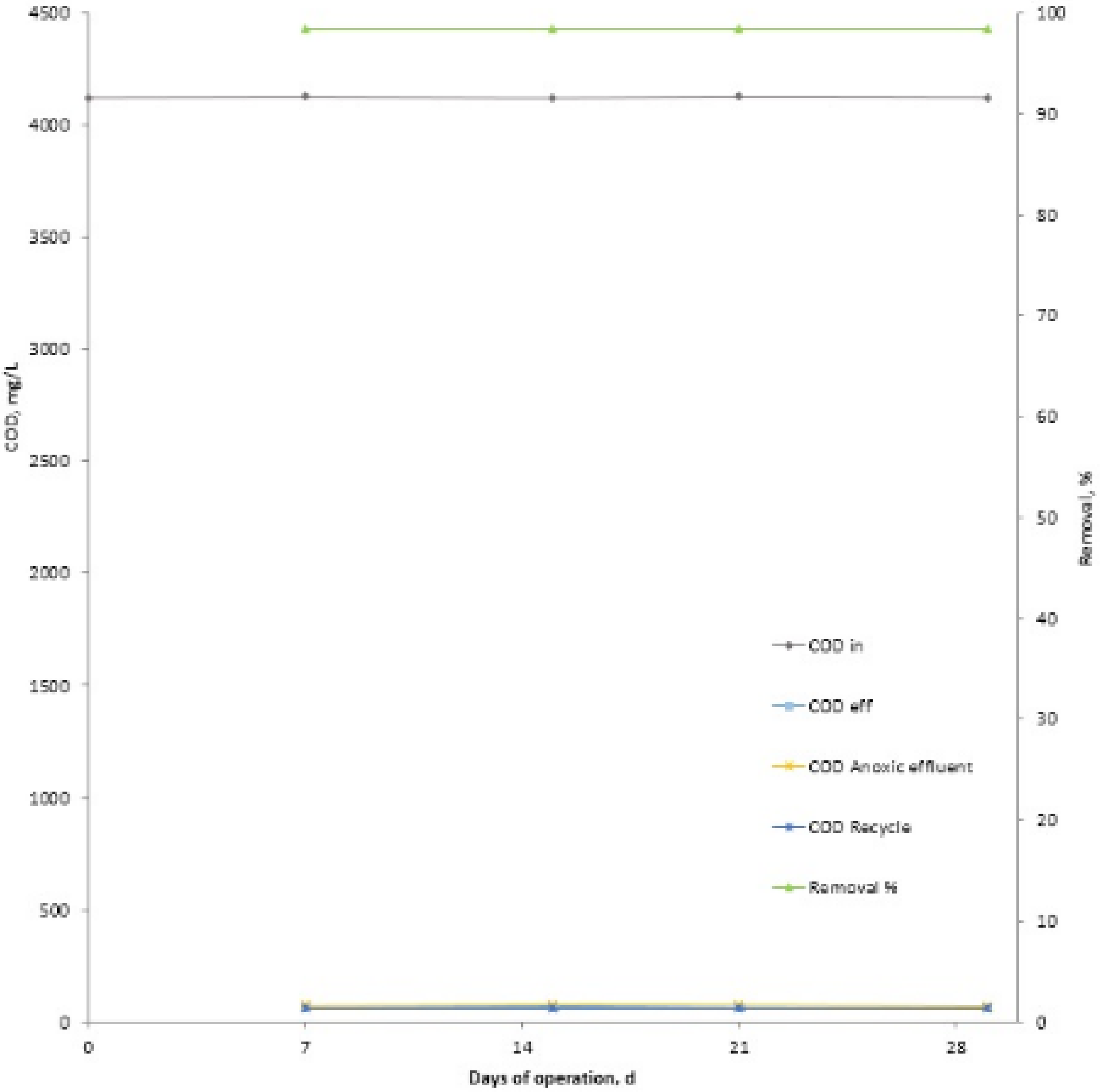
Conventional aerobic processes		Conventional combined systems	
		Head AD	**Subsequent aerobic processes**
T	25°C	25°C	25°C
μ_m	8.42 g VSS/g VSS.d	0.2 g VSS/g VSS.d	8.42 g VSS/g VSS.d
$\mu_{n,m}$	1.052 g VSS/g VSS.d		1.052 g VSS/g VSS.d
K_n	0.96 g NH_4–N/m^3		0.96 g NH_4–N/m^3
K_d	0.146 g VSS/g biomass.d	0.03 g VSS/g biomass.d	0.146 g VSS/g biomass.d
K_{dn}	0.097 g VSS/g biomass.d		0.097 g VSS/g biomass.d
Y_n	0.12 gVSS/g NH_4–N	Y= 0.08 gVSS/g COD	0.12 gVSS/g NH_4–N
f_d	0.15 g VSS/g VSS	0.15 g VSS/g VSS	0.15 g VSS/g VSS
SRT	12.5 d	45 d	12.5 d
Theoretical sludge production	25.81 g VSS/d	18.62 g VSS/d	
Theoretical oxygen requirements	46.88 g O_2/d	26.14 g O_2/d	

6.3. Results and discussion

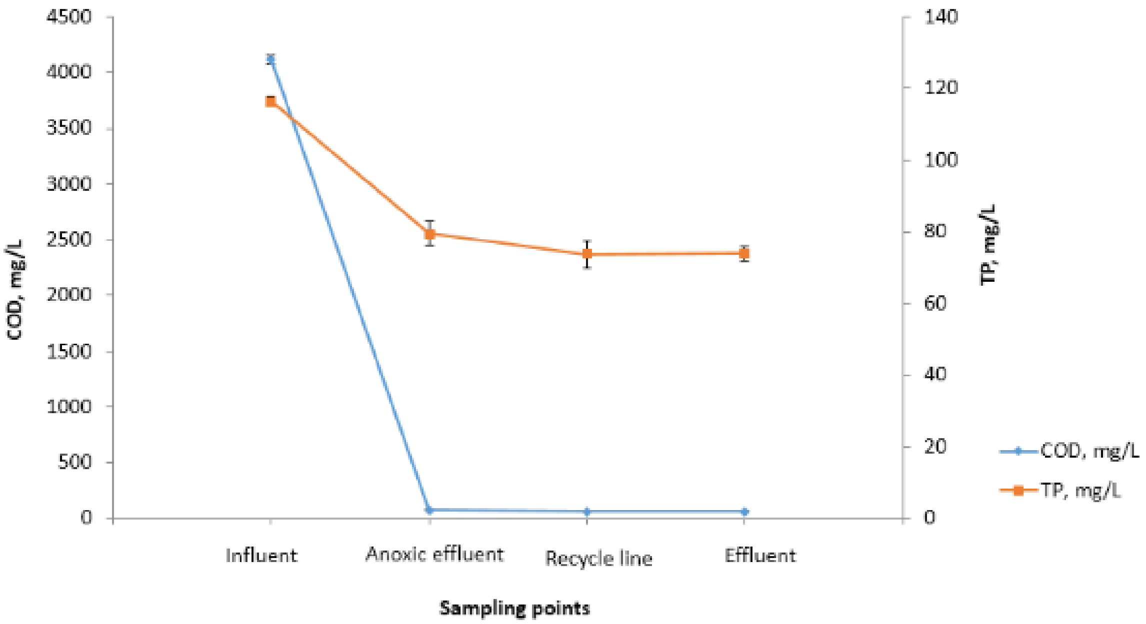
The time series of the reactor performance regarding the organic carbon and nitrogen content removal under steady-state operational conditions are presented. For a better understanding of the removal mechanisms in this system, the components (COD, ammonia/ammonium, nitrite, nitrate, TP, TN, and Alkalinity) were also monitored from the four sampling ports over a single day (day 15), and similar performance was consistently found during the entire period of the experiments. As the system was running under steady-state conditions, any single day could have been chosen, but day 15 was selected as a day representing half the period of the treatment process. Efficiency and cost savings of this new single-process SAO/PND technology were compared with those of the two conventional systems. During the complementary experiments, the organic carbon and nitrogen removal mechanisms were also confirmed and explained in detail.

6.3.1. Organic carbon removal

To monitor the COD degradation in the system, COD samples were taken from the four different sampling ports as shown in Fig. 6.1. The single-process SAO/PND technology was fed continuously by incoming COD concentrations of around 4120 mg/L under steady-state conditions for a month, and the results are presented in Fig. 6.2a. In order to show the overall performance of the system, all the influent and effluent COD concentrations are presented in a single graph (Fig. 6.2a), and this resulted in the graphed effluent lines being very close to each other. The average concentrations of triplicates with error bars representing standard deviations for the COD and TP are depicted in Fig. 6.2b for a single day (day 15). It should be mentioned that at some points, the error bars are negligible and cannot be recognized in this graph due to similar readings for the triplicates. This technology is found to be a robust system for removing COD, with effluent COD concentrations always staying below 65 mg/L and COD removal remaining high at around 98%. Organic carbon was mainly removed in the pre-anoxic unit, and the removal mechanisms are explained in Subsection 6.3.3. It is worth noting that to confirm the high efficiency of the system, more studies are required for treating real strong wastewaters in the future.



a)

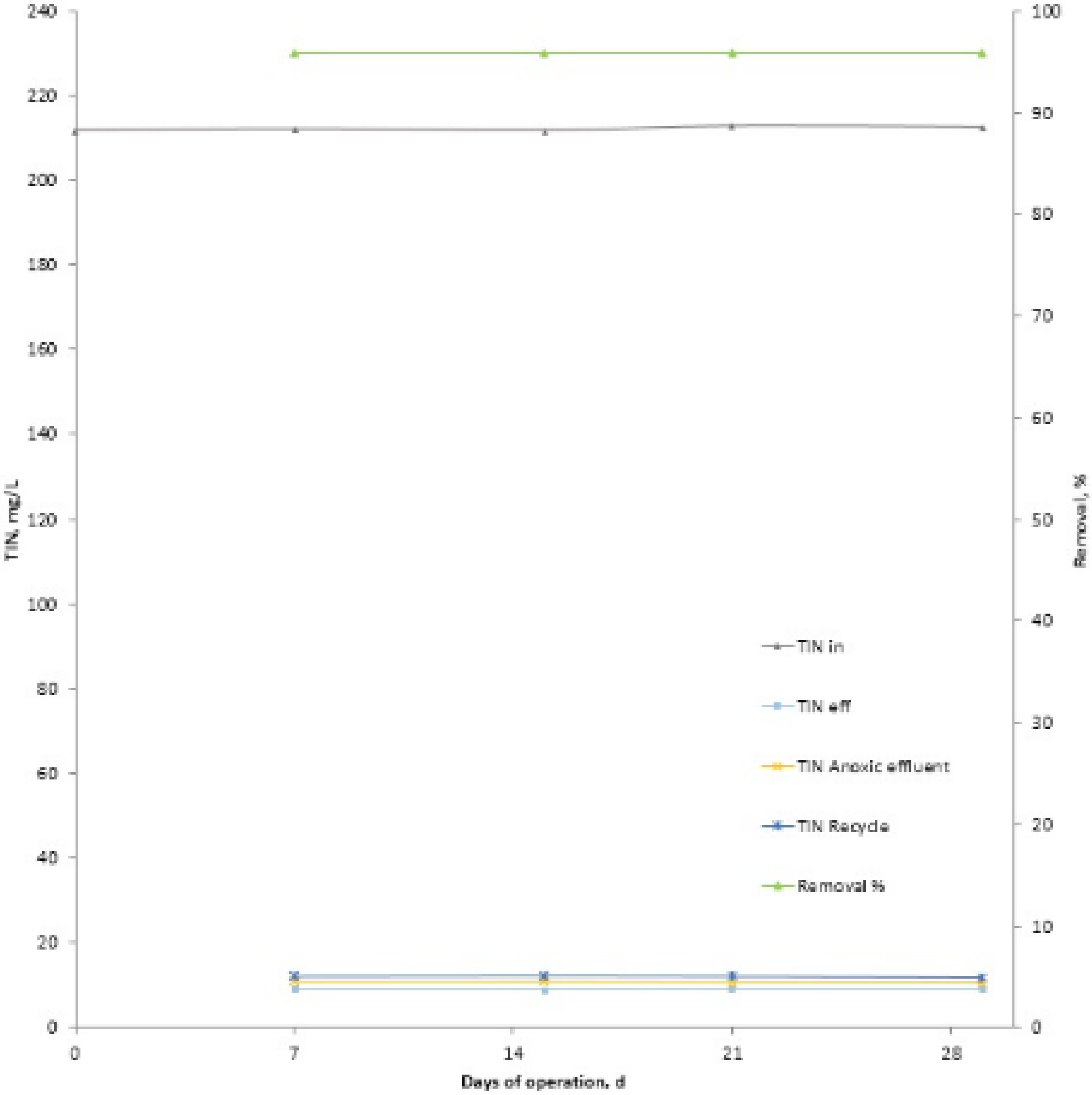


b)

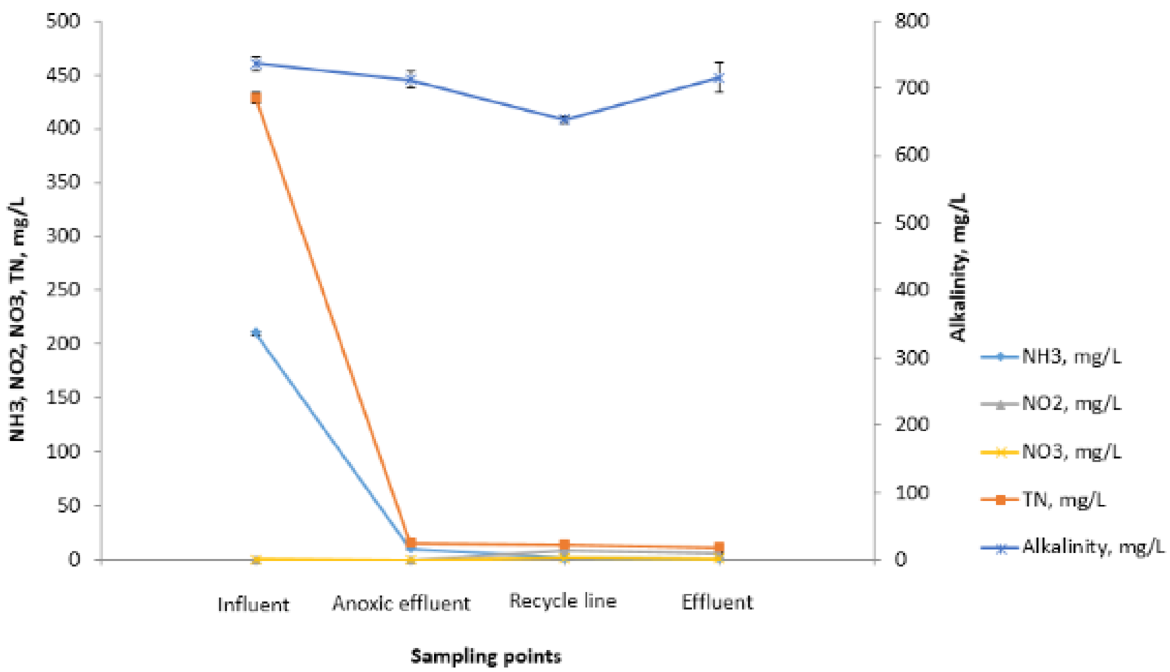
Fig. 6.2. a) Time series of COD removal performance of the single-process SAO/PND technology; b) COD and TP fluctuations over a single day (day 15).

6.3.2. Nitrogen content removal

Fig. 6.3a depicts the time series performance of the single-process SAO/PND technology for nitrogen content removal for a period of one month under steady-state operational conditions. The system was continuously fed by incoming ammonia/ammonium concentrations of around 210 mg/L. All the influent and effluent concentrations of total inorganic nitrogen (TIN) are presented in a single graph (Fig. 6.3a) to show the overall performance of the system regarding the nitrogen content removal, and this resulted in the effluent lines being very close to each other in this graph. The average concentrations of triplicates with error bars representing standard deviations for ammonia/ammonium, nitrite, nitrate, total nitrogen, and alkalinity as $CaCO_3$ are shown in Fig. 6.3b for a single day (day 15). Due to the excellent performance of this single-process technology, a 96% nitrogen content removal was achieved. The pre-anoxic tank was mainly responsible for removing the nitrogen content, with only a small portion of the ammonia/ammonium being conveyed to the partial nitrification-denitrification process. All the removal mechanisms are presented in Subsection 6.3.3.



a)



b)

Fig. 6.3. a) Time series of TIN removal performance of the single-process SAO/PND technology; b) ammonia/ammonium, nitrite, nitrate, total nitrogen, and alkalinity fluctuations over a single day (day 15).

Ebrahimi et al. (2018) employed a combined technology which was composed of an anaerobic rotating biological contactor (AnRBC) process in conjunction with an aerobic MBBR process for treating high-strength organic wastewater. Under optimum operational conditions (HRT of 5 d, organic loading rate of 2 kg COD/m^3.d, and disk rotational speed of 7 rotations per minute) with the influent COD concentrations of 10000 mg/L, by connecting the two separate processes together in a series, they could remove 98% of the influent COD. Pairing and synchronizing of these two separate processes to work together require intensive operation and maintenance; in comparison, the SAO/PND technology achieved the same removal efficiency as a single-process with less complexity.

In a study on treating slaughterhouse wastewater, Bustillo-Lecompte and Mehrvar (2017) applied a 50 L combined anaerobic baffled reactor-aerobic activated sludge (ABR-AS) system which was composed of a 36 L ABR with five equal volume chambers with a 45 slanted edge baffle within each chamber. The ABR was followed by a 14 L aerobic AS reactor with DO concentrations over 2.0 mg/L. By successfully operating the combined system with influent concentrations of 87-2080 mg/L COD and 161-255 mg/L TN, the authors removed 85% of the organic carbon and 72% of the nitrogen. In another research on the treatment of brewery wastewater with 1420 mg/L COD and 84 mg/L total Kjeldahl nitrogen (TKN), Papadopoulos et al. (2020) applied a hybrid system which was composed of an electrocoagulation (EC) system and a cyanobacteria-based cultivation process. The EC was carried out in magnetically stirred batch reactors with a 0.5 L volume, with each reactor containing two parallel, flat, rectangular aluminum or iron electrodes immersed in the solution (total effective area of 12 cm^2), spaced 3 mm apart. Three different electric current densities of 10, 30 and 40 mA/cm^2 were applied, and the temperature was kept constant at 25±1°C during the experiments. Following the EC treatment, the brewery wastewater was left to settle for 30 min for sludge precipitation/separation, and then the supernatant was collected for use in 1 L batch photobioreactors for the cyanobacteria-based treatment. Under the optimum operational conditions, these researchers achieves 92% COD and 89% TKN removal. It is worth mentioning that the operation and maintenance of these complex systems can be more intensive and require special consideration. In comparison, the SAO/PND technology, as a single-process with simple operation, could achieve higher efficiency.

Ozay et al. (2018) carried out a study combining electrochemical technology and an AD process in order to efficiently remove COD from raw pistachio processing wastewater which was rich in organic carbon (22000±530 mg/L COD). The EC experiments were performed in a glass reactor with a working volume of 800 mL, and the process was maintained at room temperature

(25±1°C). Aluminium and iron electrodes were used, with a 54 cm^2 immersed active surface area for each electrode, and various operational conditions (50-100 A/m^2 for current density, 2-6 cm for electrode distance, and 60-300 min for reaction time) were tested. A glass serum bottle with a working volume of 400 mL was used for batch anaerobic digestion under mesophilic conditions (35°C). The researchers determined the best possible operational environment and ran this hybrid system under optimum conditions, and reported 86.4% COD degradation. In another study, for the treatment of slaughterhouse industry effluent, Vidal et al. (2019) used a combined UASB process as an AD unit which was followed in series by a solar photoelectron-Fenton (SPEF) system. The UASB reactor was used at two different organic load rates of 3.94 and 8.15 kg $COD/m^3.d$, and the reactor temperature was maintained at around 37°C. The SPEF was carried out in a photoelectrochemical reactor, and four current densities of 2, 5, 10, and 25 mA/cm^2 were applied. Running this hybrid system under the best operational scenario resulted in 91% COD removal. In addition, Mortezaei et al. (2018) reported that by using an optimized combined system consisting of an anaerobic expanded granular sludge bed (EGSB) with a following fixed bed (FB) process in series (HRT of 27 hr and temperature of 35±1°C), 90% COD removal was achieved from yogurt industry wastewater with an influent COD of 11200 mg/L. All of these studies showed lower efficiency compared to the single-process SAO/PND technology, which has simple operation and less maintenance requirements.

The results of the current study proved the successful application and high performance of the SAO/PND technology in removing organic matter and nitrogen content from strong streams, as discharge standards were met by the treated effluent before release to surface water bodies. According to international discharge standards, the organic carbon limitation for industrial wastewater discharges to the surface water bodies is below 250 mg/L (Gupta et al., 2008). For further consideration, the effluent discharge standards regarding organic carbon and nitrogen content from the European Union (EU), Central Pollution Control Board (CPCB) of India, and People's Republic of China Ministry of Environmental Protection are 125, 250, and 100-300 mg/L COD, and 10-15, N/A, and 15-20 mg/L TN, respectively (Aziz et al., 2019).

The currently studied system, as a single-process technology, involves simple operation and maintenance, which is a big advantage over conventional combined systems. Therefore, the single-process SAO/PND technology can be considered as a robust, sustainable, on-site technology which is easy to operate for treating real strong wastewaters such as various industrial effluents.

6.3.3. Removal pathways in the SAO/PND technology

Complementary experiments with more tests were performed to determine and confirm the organic carbon and nitrogen removal mechanisms in the single-process SAO/PND technology. These experiments were run under steady-state operational conditions. Various mass balances of different components were calculated for each tank and the overall process, and stoichiometric ratios were determined as well. The influent synthetic solution used as a constant feed contained 70091 mg/d COD, 3599 mg/d NH_4^+–N, 0.85 mg/d NO_2^-–N, 10.20 mg/d NO_3^-–N, 1991 mg/d PO_4^{3-}–P, 7363 mg/d N–N, and 12560 mg/d alkalinity as $CaCO_3$.

6.3.3.1. Organic carbon removal pathway

In total, 98.5% (68999 mg/d) of COD was removed through the entire system. The pre-anoxic tank degraded 97.5% (68393 mg/d) of the organic carbon, and the subsequent aerobic tank removed 1% (606 mg/d). According to the mass balance calculations, anaerobic oxidation removed 66.5% of the incoming COD. The pre-anoxic tank had appropriate conditions for anaerobic oxidation as a result of the high organic loading rates, limited electron acceptors (O_2 and NO_x), low Y_{obs} (0.15 mg VSS/mg BOD), and high COD/NO_x ratio (364/1). Sludge production in the system (10402 mg VSS/d) determined the amount of organic carbon used for biological growth, and this was equal to 21.4% of the influent COD consumption. In order to convert the measured VSS in the sludge to the equivalent COD values, a conversion rate of 1.42 (Metcalf & Eddy, 2014) was used.

Mass balance calculations of the dissolved phosphorus showed that 1991 mg/d PO_4^{3-}–P entered the system via the influent line, and 36.3% was removed through the system (41.8% removal by DPAOs inside the pre-anoxic unit and 58.2% by PAOs inside the subsequent aerobic unit). Also, the mass balance calculations of the unfiltered TP around the entire system revealed that the phosphorus removed in the system was stored inside the bacterial communities and wasted as sludge. In the meantime, the organic carbon was stored internally as poly–b–hydroxyalkanoates (PHA) and poly–b–hydroxybutyrate (PHB) by the DPAOs and PAOs. The organic carbon consumption was 10 g COD/g PO_4^{3-}–P, and this resulted in a 4.6% COD removal by DPAOs and 6.4% by PAOs.

Partial nitrification in the aerobic tank led to NO_x production (mainly nitrite and limited amounts of nitrate), and these components were recycled to the pre-anoxic tank at the head of the system for removal. As will be described later in Subsection 6.3.3.2, the nitrite was consumed via the anammox process in the pre-anoxic unit, along with nitrate production. Afterwards, both the recycled and anammox related nitrates were used as electron acceptors by the DPAOs and denitrifying OHOs during the denitrification process inside the pre-anoxic unit, which was accompanied by organic carbon removal. There was enough nitrate for anoxic phosphorus uptake by the DPAOs in the pre-anoxic unit, and they consumed 95.5% of the available nitrate.

The rest of the nitrate (4.5%) was degraded by the denitrifying OHOs, and 0.1% COD removal happened in this process (6.6 g COD/g NO_3^-–N) as well.

In the aerobic unit, a small amount of influent organic carbon (1% or 606 mg/d COD) was consumed by ordinary aerobic heterotrophs.

6.3.3.2. Nitrogen content removal pathway

The unfiltered TN mass balance calculations around the overall system showed that assimilation was responsible for removing 46.3% of the nitrogen content, and this nitrogen was stored inside the body of bacterial community and exited the system as daily excess sludge. As a result of the partial nitrification in the aerobic tank, 16.7% (602 mg/d) of the influent ammonia/ammonium was converted to nitrite and nitrate (with a molar ratio of nitrite/nitrate equal to 5.48/1) and then recycled to the pre-anoxic tank at the head of the system for removal. Also, 82.5% (2969 mg/d) of the influent ammonia/ammonium was degraded in the pre-anoxic tank. Therefore, the total amount of influent ammonia/ammonium removal was 99.2% (3570 mg/d).

Equations (1) and (2) show the complete nitrification process in conventional systems, which needs a long SRT (Metcalf & Eddy, 2014):

$$\mathrm{NH_4^+} + \frac{3}{2}O_2 \rightarrow \mathrm{NO_2^-} + \frac{4}{2}H^+ + \mathrm{H_2O} \qquad (1)$$

$$\mathrm{NO_2^-} + \frac{1}{2}O_2 \rightarrow \mathrm{NO_3^-} \qquad (2)$$

However, complete nitrification was suppressed in the SAO/PND technology as a result of the short SRT values and controlled oxygen concentrations inside the aerobic unit, which led to partial nitrification.

For theoretical complete nitrification in conventional systems, 7.14 g of $CaCO_3$/g NH_4^+–N is needed (Metcalf & Eddy, 2014), but the SAO/PND technology consumed 6.33 g of the $CaCO_3$/g NH_4^+–N. This lower alkalinity consumption in the SAO/PND system was possibly because of the laboratory conditions in this research and the small amount of ammonia/ammonium transformation to cellular nitrogen.

Partial nitrification in the aerobic tank led to nitritation (nitrite production); then, all the nitrite was recycled back to the pre-anoxic tank and consumed through the anammox process. The low COD concentrations (below 80 mg/L) and sufficient accessible nitrite inside the pre-anoxic unit made favorable conditions for the anammox bacteria. Therefore, the combination of nitritation and anammox mechanisms in the single-process SAO/PND technology could save on nitrogen removal costs in comparison with conventional systems.

Anammox is well known as a low energy consuming and eco-friendly biological treatment method for nitrogen removal according to Equation (3) (Xie et al., 2020):

$$\mathrm{NH_4^+} + 1.32\mathrm{NO_2^-} + 0.066\mathrm{HCO_3^-} + 0.13\mathrm{H^+} \rightarrow 1.02\mathrm{N_2} + 2.03\mathrm{H_2O} + 0.26\mathrm{NO_3^-} + 0.066\mathrm{CH_2O_{0.5}N_{0.15}} \qquad (3)$$

As a result of the anammox inside the pre-anoxic tank, 8.3% (299.8 mg/d) ammonia/ammonium removal occurred which was accompanied by nitrite removal (395.8 mg/d) and nitrate production (77.9 mg/d) with a stoichiometry of 1/1.32/0.26. There was 159.1 mg/d NO_3^-–N in the pre-anoxic tank from two sources: i) recycled nitrate from the aerobic tank as a portion of partial nitrification product; and ii) nitrate generated during the anammox process inside the pre-anoxic tank. DPAOs and denitrifying OHOs were responsible for the denitrification inside the pre-anoxic reactor, with 95.5% and 4.5% nitrate removal, respectively.

It should be mentioned that 28.7% of the nitrogen removal pathway is not identified, which can be attributed to possible errors because of the laboratory experimental conditions as well as unknown processes.

In summary, the COD degradation mechanisms are determined as follows: i) 66.5% anaerobic oxidation; ii) 21.4% cell growth; iii) 6.4% PAOs and 4.6% DPAOs through P-uptake; iv) 0.1% ordinary denitrifying heterotrophs; and v) 1% aerobic heterotrophic bacteria. In addition, nitrogen content removal occurred via: i) 46.3% bacterial assimilation; ii) 16.7% partial nitrification and then denitrification; iii) 8.3% anammox; and iv) 28.7% experimental errors and unknown mechanisms. The presented removal mechanisms are in a strong agreement with the findings of Hosseinlou (2021).

6.3.4. Cost comparisons

There are no technical or economic justifications for aerobically removing organic matter and nitrogen from strong wastewater, because this treatment strategy needs a significant amount of oxygen and produces huge amounts of sludge. The higher oxygen requirements mean higher capital and operational costs related to energy and equipment. More sludge production increases the sludge management and disposal costs as well, which comprise a big portion of costs related to wastewater treatment plants.

Complete nitrification-denitrification accompanied with organic carbon removal mechanisms, which happens in conventional aerobic processes such as MLE composed of anoxic/aerobic tanks, requires more oxygen and increases the costs. In these systems, complete nitrification happens in the aerobic reactor, and the produced nitrite/nitrate will be recycled and removed in the anoxic tank via denitrification. In contrast, in the single-process SAO/PND technology, only 16.7% of the incoming nitrogen is removed through the partial nitrification-denitrification process, and 1% of the organic carbon via aerobic degradation. This leads to very low oxygen consumption during nitrogen and organic carbon removal in the single-process SAO/PND technology.

Furthermore, sludge production in the single-process SAO/PND technology is low compared to conventional systems. Table 6.4 presents the measured sludge characteristics and oxygen requirement in this newly developed treatment method.

Table 6.4. Measured sludge characteristics, oxygen and alkalinity requirements of the single-process SAO/PND technology in this study.

Parameters	Units	Values
Y_{obs}	g VSS/g COD	0.15
VSS/TSS ratio	–	0.71
$WAS_{daily\ TSS}$	g TSS/d	14.65
$WAS_{daily\ VSS}$	g VSS/d	10.40
F_{TSS}	kg TSS/m^3.d	0.79
F_{VSS}	kg VSS/m^3.d	0.56
R_{O2}	g O_2/d	2.54
alkalinity consumption	g $CaCO_3$/g NH_4^+–N	6.33
external alkalinity	g $CaCO_3$/d	Not needed
external carbon source	g COD/d	Not needed

There are several cost saving factors in the single-process SAO/PND technology in comparison with the two conventional systems. The latter systems involve the following: i) conventional aerobic processes which aerobically remove nitrogen along with COD; and ii) conventional combined systems which are composed of an AD followed in series by an aerobic process. Table 6.5 compares these systems, and summarizes the economic advantages. In this table, values inside the parentheses represent cost savings by the single-process SAO/PND technology in comparison with each of the mentioned conventional systems. As can be seen, there are extraordinary cost savings with the single-process SAO/PND technology compared to conventional aerobic processes for nitrogen and COD removal, which are attributed to the oxygen and energy requirements, sludge production, and equipment costs. Furthermore, significant cost reductions in the single-process SAO/PND technology in comparison with conventional combined systems (AD followed by a subsequent aerobic nitrogen and COD removal) are found which are related to these items: oxygen and energy consumption, sludge production, methanol supply, alkalinity addition, operational and maintenance needs, and structural and equipment costs.

Table 6.5. Cost comparisons of the single-process SAO/PND technology with: i) conventional aerobic nitrogen and COD removal system; and ii) conventional combined AD followed by aerobic process.

Costs		Conventional aerobic nitrogen and COD removal system	Conventional combined AD followed by aerobic system
Capital:			
	aeration equipment	(95%)	(90%)
	structural work	comparable	(78%)
	sludge disposal equipment	(60%)	(44%)
Operational:			
	oxygen consumption	(95%)	(90%)
	energy consumption	(95%)	(90%)
	sludge production	(60%)	(44%)
	methanol addition	–	(USD 1.04/m^3)
	alkalinity addition	–	(USD 0.40/m^3)
	operation & maintenance	Comparable	More intensive
	staff & operators	Comparable	More intensive

Table 6.5 depicts 95% and 90% less oxygen consumption in the single-process SAO/PND technology compared to conventional aerobic processes and combined AD/aerobic systems, respectively. This high amount of oxygen reduction in this newly developed technology means significant cost savings on aeration equipment and energy consumption.

In wastewater treatment plants, up to 60% of the total costs are dedicated to sludge management and disposal (Nghiem et al., 2014; Maragkaki et al., 2018). Therefore, as presented in Table 6.5, the 60% and 44% sludge reduction in the single-process SAO/PND technology as compared to conventional aerobic processes and conventional combined AD/aerobic systems, respectively, shows very significant cost reductions.

The results not only prove the high efficiency of the single-process SAO/PND technology in treating strong wastewater, but also its accompanying significant cost savings. Therefore, this technology can be considered as a sustainable, economic, robust, and efficient alternative to conventional systems for treating highly polluted streams such as from various industrial wastewaters.

6.4. Conclusions

The removal of 98% of COD and 96% of nitrogen was achieved from synthetic wastewater with incoming average concentrations of 4120 mg/L COD, and 210 mg/L NH_4^+–N. A high performance for treating strong wastewater was achieved, and the treated effluent of this technology met the standards for release to surface water bodies. According to the mass balance calculations and by running the system under steady-state conditions, the removal mechanisms are recognized. The COD degradation mechanisms are determined as follows: i) anaerobic oxidation; ii) cell growth; iii) P-uptake (performed by PAOs and DPAOs); iv) denitrification (completed by DPAOs and ordinary heterotrophs); and v) insignificant amounts by aerobic heterotrophic bacteria. In addition, the nitrogen content removal mechanisms comprised: i) bacterial assimilation; ii) partial nitrification and then denitrification; and iii) anammox. Significant cost reductions were achieved in comparison with two other conventional treatment methods, with the results showing 95% and 90% less oxygen necessity and 60% and 44% sludge reduction with the single-process SAO/PND technology as compared to conventional aerobic processes and conventional AD followed by aerobic processes, respectively. The high efficiency of the single-process SAO/PND technology along with its economic advantages and sustainability define this process as a robust on-site technology for treating strong wastewaters and as a serious alternative to conventional treatment methods.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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7. General summary of results

An effective single-process was configured in treating highly polluted streams focusing on organic carbon and nitrogen content removal. The key contribution of this single-process was recognizing the interaction between reactors and optimizing HRT and SRT. Under laboratory conditions, it was demonstrated that there are experimental results theoretically better on organic carbon and nitrogen removal compared to the conventional systems. Using synthetic solution as a feed in the same environment, less oxygen requirement and sludge production was found, which resulted in significant cost reductions related to these two key costly components (sludge and O_2). In addition, high performance of the system was confirmed by feeding the system with real dairy and brewery wastewater.

There were some assumptions and limitations in this thesis. As biological studies have not been carried out; therefore, the explained organic carbon and nitrogen removal pathways have been hypothesized based on the mass balance calculations. As a portion of the nitrogen removal has not been identified, it can be assumed that a portion of ammonia has been volatilized. Also, there is a limitation about comparing the laboratory results in this thesis with those theoretically designed as conventional systems. In addition, generalizing high performance of the system based on the only synthetic solution and two real wastewaters is not enough, and more investigation should be fulfilled. Specially, performance of the system should be evaluated in treating landfill leachate which can be toxic for the bacterial community in this system, along with organic carbon which are not readily biodegradable. Finally, it should be noted that, due to time constraints and COVID-19 season, lab access was limited; therefore, it is better to do more experiments and repeat the performance of the system with more real samples.

The application of a modified single-process system (MLE reactor configuration) for treating highly polluted streams was investigated, and a laboratory scale system was used to carry out experiments in this thesis. These are the similarities between the modified single-process systems in this thesis and the MLE treatment method as follows: i) reactors configuration; and ii) recycle and return lines. However, wastewaters with influent ammonia concentrations more than 150 mg/L can cause system failure in the MLE system, and high amounts of oxygen and alkalinity requirements in treating strong wastewaters can result in operational difficulties, as well as significant amounts of sludge will be produced.

During the preliminary experiments of this research, synthetic solution was used as a feed, and different operational scenarios were tried, and after six trials which resulted in system failure, the optimum conditions were determined. Operational conditions of the optimized SAO/PND system is presented in Table 6.1. The main goal of this study was identified as organic carbon and nitrogen content removals together in a single-process system, and failure was defined as at least one of these components (COD or nitrogen content) not being removed. During the preliminary experiments, optimum operational conditions were determined, and in order to see the effects of each item during the preliminary experiments, only one parameter at a time was changed and the other parameters remained unchanged.

After determining the optimized modified single-process system, main phases of this research were started by using the optimized system. During more than 5 months of operation of the modified system by synthetic wastewater, 97% and 91% organic carbon and nitrogen content removals were obtained with influent concentrations of 5,211 mg /L COD, and 262.8 mg/L NH_3-N. Then, during another run of experiments and feeding the system with synthetic solution, volumetric design loading rates were determined as 11.80 kg COD/(m^3.d) and 0.63 kg TIN/(m^3.d) with high efficiency.

In order to investigate the removal mechanisms involved in the modified single-process system regarding the organic matter and nitrogen content, as well as to be able to write the MB calculations and stoichiometric equations, a set of experiments was conducted under SS conditions. More research is needed to confirm (see Chapter 8 – Conclusions and future work recommendations), but organic carbon and nitrogen removal pathways are explained as a hypothesis in this thesis. Organic carbon degradation mechanisms were hypothesized as follows: i) anaerobic oxidation; ii) cell growth; iii) P-uptake that performed by PAOs and DPAOs; iv) denitrification process which completed by DPAOs and ordinary heterotrophs; and v) negligible amount by aerobic heterotrophic bacteria. In addition, nitrogen content removal mechanisms were hypothesized to happen via: i) bacterial assimilation; ii) incomplete nitrification and then denitrification; iii) anammox; and iv) small portion uncharacterized processes and experimental errors.

The modified single-process system in this research has been aimed as a general solution for treatment of various highly polluted streams; therefore, real dairy and brewery industry wastewaters were used as a feed to the system in separate sets of experiments. The results showed 95% COD and 99% nitrogen content removal efficiencies for treating real dairy industry wastewater with applied COD and TIN loading rates at 9.47 kg COD/(m^3.d) and 0.41 kg TIN/(m^3.d), with equivalent incoming COD and TIN concentrations of 4,347 mg/L and 186.9 mg/L TIN–N. Also, the highest COD (92%) and nitrogen content (99%) removal efficiencies were obtained for treating real brewery wastewater with influent concentrations of 9,036 mg/L

COD and 187.5 mg/L TIN–N. Applied volumetric and mass loading rates (organic carbon) with high removal efficiencies were 19.95 kg COD/(m^3.d) and 8.75 kg COD/(kg VSS.d).

Finally, cost-effectiveness of the modified single-process system was compared with two conventional technologies as follows: i) conventional aerobic processes such as MLE; and ii) conventional combined processes. The results revealed 95% and 90% less oxygen consumption in the modified single-process system compared to the conventional aerobic processes such as MLE and conventional combined systems, respectively. This high amount of oxygen reduction means significant cost savings in aeration equipment and energy consumption. As in the wastewater treatment plants, up to 60% of total costs are dedicated to sludge management and disposal; therefore, reducing the sludge production by new systems can save costs in treatment plants. The results of this study showed that the modified single-process system produced 60% and 44% less sludge compared to the mentioned conventional systems, respectively, which lead to significant cost savings.

8. Conclusions and future work recommendations

Performance of a modified single-process system was evaluated in this thesis. In the treatment of a high organics, high ammonia strength wastewater at 25°C through operating condition optimization the MLE process configuration performs like an entirely different process. That is: a) a very large portion of the incoming COD is oxidized and assimilated in the lead pre-anoxic reactor; b) a great deal of the influent ammonia is transformed in the pre-anoxic tank; c) the recycled nitrate/nitrate is transformed in the pre-anoxic tank without the addition of a supplemental carbon source. This process has been named SAO/PND.

Despite the fact that much information and significant outcomes were obtained in this thesis, there are still a number of other aspects that can be considered for future studies in order to improve the SAO/PND system. The following recommendations are presented:

- Given that the results are explained using many different bacterial mechanisms, it is critical that the bacterial populations in the wastewaters in both reactors be identified by microbiological techniques in order to ascertain if the hypothesized mechanisms are correct.
- Given that the reactor configuration of the SAO/PND system is the same as that of the MLE process, the same reactor configuration should be studied with different strength wastewaters to identify at what organic feed concentrations the bacterial processes switch from those of MLE to those of the SAO/PND.
- Possibility of nitrogen losses through nitrogen gas can be monitored to quantify the ammonia emissions from the reactors.

- As the SAO/PND system has been considered as a general solution for treating high-strength streams, it is necessary to investigate the performance of this system with various real strong wastewaters as a feed.
- Landfill leachates are known as one of the challenging strong streams for treatment, and it is worth assessing the efficiency of the modified single-process system in removing pollutants from landfill leachates. Landfill leachates contain toxic compounds and organic carbon which are not readily biodegradable; therefore, there is not a guarantee that the SAO/PND system can successfully treat landfill leachates.
- Performance of the modified single-process system should be evaluated under running in different temperatures, especially the application of this system is needed to be assessed for using in cold climate regions.
- Application of various C/N/P ratios in feed are recommended to be tested in this system for better understanding, and finding optimum ratios of these components for operation.
- There is a possibility in achieving higher performance of the system by adding plastic carriers and switching the conditions from suspended growth to attached growth; therefore, it is recommended this possibility to be assessed.
- Pre-anoxic and aerobic reactors can be compartmentalized, and the effect of these changes can be monitored for a possible better performance.
- Pilot studies are strongly recommended before using this system as a full-scale treatment method for industrial effluents.
- More experiments by other researchers under a wide variety of conditions are needed to fully understand and validate.

Appendix A: Notes on Chapter 3

In chapter 3, the article is presented identical to what is published. Significant feedback was received after the paper was accepted for publication. Detailed notes are included here to comment the publication to acknowledge some weaknesses or limitations and add some clarifications in the context of the thesis.

Presented in first published article: Title: "Simultaneous anaerobic oxidation/partial nitrification-denitrification for cost-effective and efficient removal of organic carbon and nitrogen from highly polluted streams"

Clarification: Anaerobic oxidation process is happening in the pre-anoxic tank, and the name of this tank is chosen anoxic because there are electron acceptors such as nitrite and nitrate. The mentioned "anaerobic oxidation" is not strict anaerobic process because there are some oxygen as well. It can be stated that the pre-anoxic tank works as an anoxic and anaerobic reactors together.

Published: The last sentence of the abstract: "There are several advantages for this proposed SAO/PND system from a practical point of view, such as feasibility of simultaneous COD and nitrogen removal in a single reactor; simple operation; flexibility and practicality of this system as a general solution and cost effectiveness."

Suggested improvement: It is better to use "single-process" instead of "single reactor".

Published: The last part of the introduction: "Combined anaerobic-aerobic treatment processes are conventional biological treatment methods for high strength organic wastewaters, and there have been no reports on biological treatment processes capable of simultaneously removing high concentrations of ammonia and COD in a single reactor. Therefore, the main objective of this study is to develop a novel, low intensity operation, and cost effective solution for simultaneous organic carbon and nitrogen removal from highly polluted streams."

Suggested improvement: It is better to use "single-process" instead of "single reactor".

Published: Fig. 3.2.

Clarification: Schematic flow diagram (Fig. 3.2) which has been presented in the "Material and methods" section looks like MLE system regarding the reactor configuration, but the performance of the system is different. In summary, MLE has greater SRT and shorter HRT which significantly affect the performance of the system.

Published: Third paragraph of section 3.2.1.2: "The effluent of the anoxic tank was fed into the aerobic tank, and there was a recycle line from the effluent part of aerobic tank to the beginning part of the anoxic tank, and flowrate of this line was twice of influent ($Q_r=2Q_{in}$). Also, the return sludge flowrate was optimized in order to maintain the required MLSS in the biological reactors. The optimized return flow was equal to the influent flowrate ($Q_{RAS}=Q_{in}$)."

Clarification: Recycle and return lines in the SAO/PND and MLE systems are similar to each other. In conventional aerobic systems such as MLE, increasing flowrate of the recycle line can improve the nitrogen removal performance of the system.

Published: End of 3.2.1.2: "mean values of triplicates with related standard deviations"

Clarification: Triplicate measurement of experiments.

Published: Section 3.3.1: “After each increase, it took 7 days to reach steady-state conditions.”

Clarification and suggested improvement: As rule of thumb to reach steady-state biological systems need to be operated for 2-3 times the SRTs. Therefore, it’s better to remove the published sentence. However, there are studies which they choose 1 time SRT for achieving steady-state conditions.

Published: Section 3.3.1: “The results presented in Fig. 3.3 show that a maximum 97% COD removal was achieved from the start until day 152 before the system started to show signs of stress and failure.”

Clarification: In this thesis, failure means one of the key components (organic carbon or nitrogen content) was not being removed. And in most cases of failure, nitrogen content was a responsible component.

Published: Section 3.3.1: “Volumetric COD loading rates”

Clarification: Volumetric COD loading rates are calculated based on the applied influent COD concentrations and volume of anoxic reactor ($\frac{Q\times C}{V}$).

Published: Section 3.3.2: “Hamoda and Bin-Fahad (2012)”

Clarification and suggested improvement: This study has been chosen for comparing the results, but it would be more informative to present other studies as well.

Published: Section 3.3.2: “TIN Volumetric loading rates”

Clarification: TIN Volumetric loading rates are calculated based on the applied influent TIN concentrations and volume of anoxic reactor ($\frac{Q \times C}{V}$).

Published: Section 3.3.2: "However, only 17% of nitrogen removal pathway of the SAO/PND system was nitrification-denitrification which was similar to the research conducted by Hamoda and Bin-Fahad (2012) as will be explained later in the complementary experiments section"

Clarification and suggested improvement: 17% of nitrification-denitrification was found in the SAO/PND system not in the study by Hamoda and Bin-Fahad (2012); therefore, "Hamoda and Bin-Fahad (2012)" should be removed from the sentence. The similarity of these two studies was the nitrification-denitrification process, not 17% nitrogen removal. Also, the modified sentence should be moved to the section 3.3.5.

Published: Section 3.3.2: "On the other hand, only 5% of organic carbon was removed by denitrification in the SAO/PND system as will be discussed later in the complementary experiments section."

Clarification and suggested improvement: For denitrification in the pre-anoxic tank, organic carbon was needed, and mass balance calculations showed 5% of COD removal during this process. Also, this sentence should be moved to the section 3.3.5.

Published: Section 3.3.5: "Different COD and nitrogen mass balance calculations were made around each reactor separately as well as the overall system in order to clarify the COD and nitrogen removal pathways. In addition, individual mass balances were made for ammonia, nitrite, nitrate, and TIN separately around the anoxic and aerobic reactors and the overall system to monitor the nitrogen conversions in the SAO/PND process."

Clarification: The following general term has been used for mass balance calculations:

$$\begin{aligned}&(Accumulation\ of\ mass\ in\ the\ system)\\&\qquad = (mass\ into\ the\ system) - (mass\ out\ of\ the\ system) + (generation)\\&\qquad - (consumption)\end{aligned}$$

Published: Section 3.3.5.1: "The main mechanisms of COD removal by the SAO/PND process include: i) 73% anaerobic oxidation; ii) 21% cell synthesis and biomass production; iii) 5% denitrification; and iv) 1% by aerobic heterotrophs in the aerobic reactor."

Clarification and suggested improvement: All these mechanisms are based on mass balance calculations, and is better to be written as a hypothesis.

Published: Section 3.3.5.1: "The above reasons confirm"

Suggested improvement: The above reasons suggest

Published: Section 3.3.5.2: "The detection of new organisms is making the nitrogen cycle increasingly complicated, to the point that traditional descriptions like nitrification-denitrification are rather simplistic and insufficient for the explanation of nitrogen pathways in real life."

Suggested improvement: As a result of newly identified processes in nitrogen cycle, common processes of nitrification-denitrification are rather simplistic which are not sufficient to explain nitrogen removal pathways (Andalib et al., 2011).

Andalib, M., Nakhla, G., McIntee, E., Zhu, J., 2011. Simultaneous denitrification and methanogenesis (SDM): Review of two decades of research. *Desalination,* 1(3), 1–14.

Published: Section 3.3.5.2: “The nitrogen removal pathways in SAO/PND include: i) 46% assimilation and synthesis into organism compositions; ii) 17% partial nitrification-denitrification; iii) 26% experimental errors; and iv) 11% still lacking and unknown.”

Clarification and suggested improvement: All these mechanisms are derived from the mass balance calculations. In addition, it is better to report 37% as experimental errors and unidentified processes. Also, this sentence should be moved before the last paragraph of section 3.3.5.2.

Published: Section 3.3.6: “The cost-effectiveness of SAO/PND is elaborated further below:”

Suggested improvement: As the specific values are not given to the various parameters mentioned in points 3, 4, and 5; therefore, it is better to remove these points. However, an article about cost savings has been published as fourth paper in this thesis which has presented the required information.

Published: Section 3.4: “The results of the complementary experiments revealed that the COD removal pathway is: i) 73% anaerobic oxidation; ii) 21% cell synthesis and biomass production; iii) 5% denitrification; and iv) 1% by aerobic heterotrophs in aerobic reactor.”

Clarification and suggested improvement: As the biological studies are not presented in this thesis; then, writing tone should be soften like this: The results of the complementary experiments hypothesized that the COD removal pathway is: i) 73% anaerobic oxidation; ii) 21% cell synthesis and biomass production; iii) 5% denitrification; and iv) 1% by aerobic heterotrophs in aerobic reactor.

Published: The experiments in this research have been conducted under 25^{O}C as room temperature.

Clarification: It is likely that operating in colder weather or cold regions in the world increase operational costs.

Appendix B: Notes on Chapter 4

In chapter 4, the article is presented identical to what is published. Significant feedback was received after the paper was accepted for publication. Detailed notes are included here to comment the publication to acknowledge some weaknesses or limitations and add some clarifications in the context of the thesis.

Presented in second published article: Last paragraph of section 4.1:"Although the SAO/PND system has demonstrated the potential for sustainable, innovative, cost-effective, energy efficient, small footprint, and robust on–site technologies with less intense operation and maintenance treatment of highly concentrated influent COD and ammonium wastewaters at elevated loading rates, there remains a gap of knowledge with respect to the design and operation of these systems."

Clarification and suggested improvement: The tone needs to be softened, and a suggested statement can be like this: Although the SAO/PND system has demonstrated the potential for treating highly concentrated influent COD and ammonium wastewaters at elevated loading rates, there remains a gap of knowledge with respect to the design and operation of these systems.

Published: Last paragraph of section 4.1: "As such, this research aims to determine the effective design COD and total inorganic nitrogen (TIN) loading rates through the use of controlled experiments, and hence synthetic wastewater feed. Secondly, the research will investigate the potential of this system to treat real dairy wastewaters and present an appropriate solution to on–site treatment of dairy industry effluents. Finally, this study will move beyond the demonstration of effluent concentrations, removal rates and efficiencies, and will endeavour to identify the COD and TIN removal mechanisms occurring within the SAO/PND treatment process."

Clarification: This article use the same reactor setup as the first article but it consists of an entirely different run. The performance of the system showed a trend similar to the first article, but the system could be recovered without total collapse after appearance of initial failure signs, and design loading rates determined with higher concentrations. It should be mentioned that the explained removal pathways in the first article were correct, but they were not comprehensive; therefore, a comprehensive removal pathway is presented in the second article.

Published: Fig. 4.1

Clarification: In this figure, the first reactor is named as a "pre-anoxic" and this term is chosen to differentiate from similar "post-anoxic" reactor configurations. However, the "pre-anoxic" can be replaced by the "anoxic" term as well.

Published: Section 4.2.2.1: "More chemicals were added in order to elevate influent loading rates and concentrations to simulate high-strength wastewaters."

Clarification: For example, when all ingredients of the synthetic wastewater were doubled (2×150 mg/L $C_6H_{12}O_6$, 2×80 mg/L NaAc, 2×150 mg/L Peptone,…), this resulted in 2×390 mg/L COD and 2×22 mg/L NH_4^+–N. For higher concentrations of COD and nitrogen content, more chemicals were proportionally added.

Published: Section 4.2.2.1: "This resulted in a wastewater which had a COD of 390 mg/L and ammonium of 22 mg/L NH_4^+–N."

Clarification: All the COD in the synthetic solution was biodegradable.

Published: Section 4.3.1.2: “After a temporary failure in this research, the system was recovered, as can be seen in Figs. 4.2 and 4.3, and conducting the rest of the experiments became possible and continued.”

Clarification: In this thesis, system failure was defined as a condition that at least one of two key components (organic carbon and nitrogen content) were not being removed. Successful operations or recovery conditions refer to operational conditions with both organic carbon and nitrogen content removals at the same time.

Published: Section 4.3.1.2: “It was concluded that the maximum possible influent ammonium for the system was below 290 mg/L NH_4^+–N.”

Clarification and suggested improvement: This statement should be removed as supporting scientific reason is not provided.

Published: Section 4.3.1.2: “In a study, Zwain et al. (2013) used a modified anaerobic…”

Clarification and suggested improvement: In order to help the reader, having an orientation sentence will be helpful, and the paragraph can be started like this: Various studies have applied different treatment processes, and the results of some of them are presented in comparison with performance of the SAO/PND system. In a study, Zwain et al. (2013) used a modified anaerobic…

Published: Section 4.3.1.2: “Despite the underloading conditions”

Clarification: Underloading conditions mean Lima et al. (2016) applied organic loading rates less than those applied in the SAO/PND system.

Published: Section 4.3.2: “The system was fed with real dairy wastewater step by step, with mixing conditions as follows: day 123 (25% real dairy ww + 75% synthetic ww), day 124 (50% real dairy ww + 50% synthetic ww), day 125 (75% real dairy ww + 25% synthetic ww), and from day 126 till the end (100% real dairy ww).”

Clarification and suggested improvement: It should be clarified that the change took place over 5 days, not 3 days as stated in this article.

Published: Section 4.3.2: “After feeding the system with real dairy wastewater between days 123 to 139, the effluent COD concentrations increased sharply, to around 230 mg/L, and COD removal decreased to 95%. Glucose, peptone, and sodium acetate were used to simulate the organic carbon in synthetic wastewater, and these compounds are readily biodegradable substances which can be easily consumed by heterotrophic organisms; however, the real dairy wastewater contained some complex and non–biodegradable organic carbon compounds. After feeding the system with complex organic carbon substrates, the COD removal declined to 95%.”

Clarification and suggested improvement: As non–biodegradable organic carbon compounds were not measure; then, this can be improved like: … the real dairy wastewater contained some complex and non–biodegradable organic carbon compounds but they were not measured. …

Published: Section 4.3.2: “Between days 133 and 139, the influent real dairy wastewater was also spiked stepwise by adding more ammonium to increase the TIN loading rates and concentrations.”

Clarification and suggested improvement: Longer times are suggested to run the system in order to insure that steady-state conditions were achieved.

Published: Section 4.3.2: "However, the operation and maintenance of an integrated system such as ABR–UASB is more intensive in comparison with the SAO/PND process."

Clarification: Operating a hybrid system such as ABR–UASB which is composed of two different processes is not a simple task, and the successful operation of the following UASB depends on the successful performance of the head system (ABR). On the other hand, the SAO/PND system is a single-process which its performance is not dependent on other processes as it works alone.

Published: Section 4.3.2: "The COD discharge limit for industrial effluents into inland surface waters according to international discharge standards is less than 250 mg/L (Gupta et al., 2008)."

Clarification and suggested improvement: The COD discharge limit for industrial effluents into inland surface waters according to international discharge standards is less than 250 mg/L (Gupta et al., 2008), and the results of the current study showed COD effluents around 230 mg/L.

Published: Section 4.3.3: "Hosseinlou et al. (2019) observed the similar failure signs in this pH range."

Clarification: At the end of the experiments (first article) when system failed as shown in Fig. 3.7, pH was above 8 which is similar to the findings of the second article.

Published: Section 4.3.3: "Oxygen levels in the pre–anoxic unit were consistently kept at around 0.20 mg/L to favor a possible denitrification process (Fig. 4.6)."

Clarification and suggested improvement: The word "kept" implies that the author had direct control over the DO, but this is not correct; therefore, the word "kept" should be removed. Suggested improved sentence will be like this: Oxygen levels in the pre–anoxic unit were consistently at around 0.20 mg/L, and this favored a possible denitrification process (Fig. 4.6).

Published: Section 4.3.3: “The current study found that because there was only 17.1% nitrogen consumption by the partial nitrification–denitrification mechanism in the SAO/PND process, …”
Clarification: 17.1% is based on mass balance calculations, and section 4.3.4 presents more details.

Published: Section 4.3.4: “To investigate the mechanisms involved in the SAO/PND treatment method regarding the organic matter and nitrogen content removal, as well as to be able to write the mass balance (MB) calculations and stoichiometric equations, a set of experiments with the same operational conditions was conducted under SS conditions.”
Clarification: Different complementary experiments were run for different articles in this thesis. However, all these experiments carried out under very similar conditions, as they used synthetic solution, the same reactor setup, almost the same influent concentrations. In the first article, removal pathways were correct but they were not comprehensive. In the second article, comprehensive removal pathways were explained. As the SAO/PND system is claimed as a newly developed system, the removal pathways needed confirmations to insure that they are correct, and there was a necessity to determine the range of each removal pathway as percentages as well. In the third and fourth articles, the removal pathways were confirmed, and the range of each removal mechanism as percent were identified.

Published: Section 4.3.4.1: “The MB calculations showed 67% of influent COD removal by anaerobic oxidation.”

Suggested improvement: Based on the MB calculations, it is hypothesized 67% of influent COD removal by anaerobic oxidation.

Published: Section 4.3.4.1: “low observed biomass yield (0.15 mg VSS/mg BOD),…”

Clarification: The BOD values are not measured, but BOD and COD in synthetic solution are the same.

Published: Section 4.3.4.1: “Daily solids production (excess sludge) in the SAO/PND system was around 10404 mg VSS/d, and by using 1.42 (Metcalf and Eddy, 2003) as the conversion factor for the VSS to COD, it is clear that 21.4% of the influent COD was assimilated by various active bacterial communities in this system, which is in good agreement with the study conducted by Hosseinlou et al. (2019).”

Clarification and suggested improvement: As this value is an estimate based on assumed value of 1.42; then the sentence can be improved like this: Daily solids production (excess sludge) in the SAO/PND system was around 10404 mg VSS/d, and by using 1.42 (Metcalf and Eddy, 2003) as the conversion factor for the VSS to COD, it is hypothesized that 21.4% of the influent COD was assimilated by various active bacterial communities in this system, which is in good agreement with the study conducted by Hosseinlou et al. (2019).

Published: Section 4.3.4.1: “and all the removed phosphorus was stored in the body of bacteria.”

Clarification: MB of unfiltered TP around the entire system showed this.

Published: Section 4.3.4.1: “During the phosphorus removal, the COD was stored internally as poly–b–hydroxyalkanoates (PHA) and poly–b–hydroxybutyrate (PHB) in the body of PAOs and DPAOs in the anoxic reactor.”

Clarification: In this thesis, PHA and PHB were not measured, and this statement is based on the other studies. Measurement of these two components are recommended for future studies.

Published: Section 4.3.4.1: “The average COD consumption for the phosphorus uptake was around 10 mg COD/mg PO_4^{3-}–P, and the MB calculations showed 5.8% and 4.7% COD removal of the influent COD (69972 mg/d) by the PAOs and DPAOs, respectively.”

Clarification: Based on the MB calculations, the amount of denitrification by DPAOs were determined, and the equivalent COD removal by these bacteria determined during the P removal. The rest of P was removed by PAOs, and equivalent COD removal during this process determined.

Published: Section 4.3.4.1: “As will be discussed in Section 3.4.2, incomplete nitrification process inside the aerobic unit led to NO_x production (NO_3^-–N and mainly NO_2^-–N) in this reactor, which was recycled back and consumed in the pre–anoxic reactor.”

Clarification: As it was tried to discuss organic carbon and nitrogen content removal pathways separately, nitrogen removal pathway is referred to the next section. The author assumed that mixing both removal pathways together, will result in more confusion.

Published: Section 4.3.4.1: “The anaerobic ammonium oxidation (anammox) bacteria consumed NO_2^-–N and produced NO_3^-–N via the anammox process inside the pre–anoxic unit. The recycled NO_3^-–N from the aerobic reactor and the NO_3^-–N produced through the anammox process, were consumed by denitrifying ordinary heterotrophic organisms (OHOs) and DPAOs via the denitrification and P–uptake processes, which was accompanied by influent organic carbon consumption during the exogenous denitrification and P–uptake processes. Phosphorus removal was carried out via phosphorus uptake by the PAOs and DPAOs together.”

Clarification and suggested improvement: As biological results are not presented in this thesis; therefore, it is better to hypothesize all these removal pathways. The improved statement can be like this: It is hypothesized that the anaerobic ammonium oxidation (anammox) bacteria consumed NO_2^-–N and produced NO_3^-–N via the anammox process inside the pre–anoxic unit. As a hypothesis, the recycled NO_3^-–N from the aerobic reactor and the NO_3^-–N produced through the anammox process, were consumed by denitrifying ordinary heterotrophic organisms (OHOs) and DPAOs via the denitrification and P–uptake processes, which was accompanied by influent organic carbon consumption during the exogenous denitrification and P–uptake processes. Phosphorus removal was hypothesized to be carried out via phosphorus uptake by the PAOs and DPAOs together.

Published: Section 4.3.4.1: “Anammox bacteria were competing with the DPAOs over the nitrite, and were more successful in using nitrite in the presence of ammonium, and then produce nitrate which was consumed by the DPAOs and other denitrifying OHOs as an electron acceptor. The DPAOs and denitrifying OHOs used COD as their carbon sources in the denitrification process.

The DPAOs also converted a portion of the COD to PHA and PHB, and stored in their bodies for consumption during the P–uptake."

Clarification and suggested improvement: Again, because the biological results are not presented in this thesis; then, all these removal pathways should be hypothesized. The improved sentences should be like this: As a hypothesis, anammox bacteria were competing with the DPAOs over the nitrite, and were more successful in using nitrite in the presence of ammonium, and then produce nitrate which was consumed by the DPAOs and other denitrifying OHOs as an electron acceptor. It is hypothesized that the DPAOs and denitrifying OHOs used COD as their carbon sources in the denitrification process. The DPAOs also converted a portion of the COD to PHA and PHB, and stored in their bodies for consumption during the P–uptake as a hypothesis.

Published: Section 4.3.4.1: "The system was originally seeded with activated sludge obtained from a lab–scale SAO/PND process, with stabilized organic carbon and nitrogen content removal in laboratory operational conditions. The mentioned system was already enriched with PAOs, DPAOs, denitrifying OHOs, anammox bacteria, nitrifiers, and anaerobic and aerobic heterotrophs. The P–uptake took place by the DPAOs in the anoxic phase, in which their stored PHA or PHB sources were first consumed.

During the aerobic phosphorus uptake inside the aerobic unit, some PAOs did not consume nitrate, but accumulated organic carbon in the case of oxygen availability.

Inside the pre–anoxic unit, a portion of the influent COD was consumed via denitrification, and partly stored as intracellular carbon by the DPAOs and PAOs. The extended pre–anoxic stage helped the intracellular carbon accumulation for the phosphorus uptake, and this made available enough organic carbon for the denitrification process. Organic carbon in the pre–anoxic reactor was consumed during the exogenous denitrification of the nitrate, and was internally accumulated by the bacteria (DPAOs and PAOs) as well."

Clarification and suggested improvement: Again, biological studies are not presented in this thesis; hence, these sentences should be rewritten in a more hypothetical fashion. The improved statements could be like as follows: As a hypothesis, the system was originally seeded with activated sludge obtained from a lab–scale SAO/PND process, with stabilized organic carbon and nitrogen content removal in laboratory operational conditions. The mentioned system was already hypothesized to be enriched with PAOs, DPAOs, denitrifying OHOs, anammox bacteria, nitrifiers, and anaerobic and aerobic heterotrophs. The P–uptake took place by the DPAOs in the anoxic phase, in which their stored PHA or PHB sources were first consumed, and all these are a hypothesis.

Hypothetically during the aerobic phosphorus uptake inside the aerobic unit, some PAOs did not consume nitrate, but accumulated organic carbon in the case of oxygen availability.

As a hypothesis, inside the pre–anoxic unit, a portion of the influent COD was consumed via denitrification, and partly stored as intracellular carbon by the DPAOs and PAOs. It was hypothesized that the extended pre–anoxic stage helped the intracellular carbon accumulation for the phosphorus uptake, and this made available enough organic carbon for the denitrification process. Hypothetically organic carbon in the pre–anoxic reactor was consumed during the exogenous denitrification of the nitrate, and was internally accumulated by the bacteria (DPAOs and PAOs) as well, and all these were.

Published: Section 4.3.4.1: "The active denitrifying OHOs in the anoxic reactor were heterotrophic bacteria which consumed 0.1% of the influent COD as electron donors during the nitrate reduction, with 6.6 mg of COD consumption via denitrification for each mg of NO_3^-–N."

Clarification: These calculations are based on the nitrate MB around the pre-anoxic tank, and equivalent 6.6 mg of COD consumption during denitrification.

Published: Section 4.3.4.2: "A new cost–effective (less energy consumption and sludge production) and efficient biological treatment process like the SAO/PND process has been recently developed to explain this removal pathway in more detail."

Clarification and suggested improvement: This statement is unnecessary, and should be removed.

Published: Section 4.3.4.2: "Nitrifiers are slow growth bacteria and short SRT in the SAO/PND system,…"

Suggested improvement: "the" should be added before "short". Therefore, this sentence will be like this: Nitrifiers are slow growth bacteria and the short SRT in the SAO/PND system,…

Published: Section 4.3.4.2: "Inside the aerobic unit, NH_4^+–N was declined but NO_2^-–N and NO_3^-–N were produced, and all influent ammonium to the reactor (615 mg/d NH_4^+–N) was converted and oxidized in the presence of oxygen."

Suggested improvement: Inside the aerobic unit, NH_4^+–N declined but NO_2^-–N and NO_3^-–N were produced, and most of the influent ammonium to the reactor (615 mg/d NH_4^+–N) was converted and oxidized in the presence of oxygen.

Published: Section 4.3.4.2: “Because of the small portion of organic nitrogen conversion to ammonium inside the aerobic reactor, the NO_x at the effluent of this reactor was slightly more than the ammonium consumption inside this reactor.”

Suggested improvement: As a hypothesis because of the small portion of organic nitrogen conversion to ammonium inside the aerobic reactor, the NO_x at the effluent of this reactor was slightly more than the ammonium consumption inside this reactor.

Published: Section 4.3.4.2: “Nitrification uses alkalinity via the production of hydrogen ions, and the theoretical stoichiometric balance yields a consumption of 7.14 mg CaCO3/mg NH_4^+–N during the complete nitrification (Metcalf and Eddy, 2003).”

Suggested improvement: Standard nitrification uses alkalinity via the production of hydrogen ions, and the theoretical stoichiometric balance yields a consumption of 7.14 mg CaCO3/mg NH_4^+–N during the complete nitrification inside the aerobic tank (Metcalf and Eddy, 2003).

Published: Section 4.3.4.2: “As a result of the incomplete nitrification in the aerobic unit, nitrite was produced and conveyed to the pre–anoxic unit through the recycle line, which was one of the substrates for the anammox process in the pre–anoxic unit.”

Clarification and suggested improvement: As biological results are not presented to support this statement; hence, the improved sentence can be like this: Hypothetically as a result of the incomplete nitrification in the aerobic unit, nitrite was produced and conveyed to the pre–anoxic unit through the recycle line, which was one of the substrates for the anammox process in the pre–anoxic unit.

Published: Section 4.3.4.2: “In the new system applied in this research, NO_2^-–N and NO_3^-–N were conveyed to the pre–anoxic unit via a recycle line and were then consumed by anammox, DPAOs, and denitrifying OHOs.”

Clarification and suggested improvement: Again, biological studies are not presented to support. Then, the sentence should be improved like this: In the new system applied in this research, as a hypothesis, NO_2^-–N and NO_3^-–N were conveyed to the pre–anoxic unit via a recycle line and were then consumed by anammox, DPAOs, and denitrifying OHOs.

Published: Section 4.3.4.2: “According to the stoichiometric equations of anammox (Eq. 3) and MB calculations around the anoxic reactor, 303 mg/d NH_4^+–N with 400 mg/d NO_2^-–N was consumed… “

Suggested improvement: According to the stoichiometric equations of anammox (Eq. 3) and MB calculations around the anoxic reactor, 303 mg/d NH_4^+–N and 400 mg/d NO_2^-–N was consumed…

Published: Section 4.3.4.2: “Afterwards, all the NO_3^-–N produced via anammox, influent and recycled NO_3^-–N (in total 167 mg/d NO_3^-–N) were consumed during the denitrification process by the DPAOs (93%) and denitrifying OHOs (7%) in the anoxic reactor.”

Suggested improvement: As the anoxic reactor’s effluent contained no nitrate, then nitrate generated by anammox reaction must have been consumed by DPAOs (93%) and denitrifying OHOs (7%), and MB calculations support these percentages.

Published: Section 4.3.4.2: “Based on the MBs performed on nitrogen for each unit process and the entire treatment system, 28.5% of the influent nitrogen was missing in this treatment process, which attributed to the possible laboratory errors and uncharacterized processes.”

Clarification and suggested improvement: Nitrogen losses through nitrogen gas emissions from both reactors should be monitored to quantify ammonia emissions, and this is one of the recommendations for future works. In addition, “uncharacterized processes” should be changed to “unidentified processes”. Also, biological studies are not presented. Therefore, the improved sentence will be: As a hypothesis, and based on the MBs performed on nitrogen for each unit process and the entire treatment system, 28.5% of the influent nitrogen was missing in this treatment process, which attributed to the possible laboratory errors and unidentified processes.

Published: Section 4.4: “Mass balance calculations for phosphorus, organic carbon, and nitrogen content showed that P was removed by the DPAOs and PAOs, while the COD was removed by anaerobic oxidation, bacterial growth, DPAOs, denitrifying OHOs, PAOs, and aerobic heterotrophs. Bacterial assimilation and partial nitrification–denitrification, and anammox processes were responsible for the TIN removal.”

Clarification and suggested improvement: Again, the wording about explained removal mechanisms need to be softened and the identification of the microorganisms needs to be more presumptive. Therefore, the improved sentences should be like this: Mass balance calculations for phosphorus, organic carbon, and nitrogen content showed that hypothetically P was removed by the DPAOs and PAOs, while the COD was removed by anaerobic oxidation, bacterial growth, DPAOs, denitrifying OHOs, PAOs, and aerobic heterotrophs. As a hypothesis, bacterial assimilation and partial nitrification–denitrification, and anammox processes were responsible for the TIN removal.

Appendix C: Notes on Chapter 5

In chapter 5, the article is presented identical to what is published. Significant feedback was received after the paper was accepted for publication. Detailed notes are included here to comment the publication to acknowledge some weaknesses or limitations and add some clarifications in the context of the thesis.

Presented in third published article: Section 5.2.1: "In addition, excess sludge was withdrawn from the clarifier on a daily basis as waste activated sludge (WAS)."

Clarification: It should be mentioned that the SAO/PND is an activated sludge process as a part of sludge is remained from the clarifier and returned inside the system to keep the bacteria active in the system.

Presented: Section 5.2.1: "The sludge retention time (SRT) and mixed liquor suspended solids (MLSS) were controlled by RAS and WAS lines to make sure the SRT and MLSS were adjusted at around 4±1 days and 3500±500 mg/L."

Clarification: Flowrate of the RAS line was already determined during the optimization experiments, and was controlled to adjust required SRT and MLSS in the system. After finding the RAS flowrate, the amount of the WAS as daily wasted sludge from the clarifier was used to control the SRT and MLSS.

Presented: Section 5.3.1: "After switching the feed to the real brewery wastewater, the results showed a sudden increase in effluent COD concentrations of up to 780 mg/L, and COD removal sharply dropped and remained constant at 92% till the end of the experiments. This sharp decrease in the removal efficiency of the system was because of the fact that the accessible food was more difficult for the heterotrophs to consume. Previously, the chemicals for carbon sources in the synthetic solution recipe (glucose+peptone+sodium acetate) could be simply degraded and consumed by these organisms, whereas after changing to the real brewery wastewater, the more complex organic ingredients involved were not as easily degradable by the bacterial community. Figs. 5.2 and 5.3 depict the overall COD removal percentages."

Clarification: In this thesis, the non-biodegradable COD values were not measured. However, for the synthetic solution with simple organic carbon ingredients, bacterial communities can consume these organic carbon sources as biodegradable COD. On the other hand, the real brewery wastewater contained some complex organic carbon sources which bacteria could not consume and they were considered as non-biodegradable COD.

Presented: Section 5.3.1: "Hosseinlou et al. (2019) reported 97% COD removal under 11.26 kg COD/(m^3.d) loading rates by the single-process SAO/PND technology with a synthetic solution as the feed. The current study shows a lower COD removal efficiency (92%), which can be attributed to the complexity of the real brewery wastewater used in the current study compared to the synthetic solution as a simple compound for bacterial consumption in the previous research. However, the COD loading rate in this study (19.95 kg COD/(m^3.d)) was significantly higher than the loading rate in the research by Hosseinlou et al. (2019)."

Clarification: It is correct that the findings of this study shows high COD removal efficiency but is still lower than the previous study. Unfortunately, the maximum applicable ammonia concentrations were not identified and experiments stopped. That would be helpful to determine the point that system become stressed out and start showing the failure.

Presented: Section 5.3.2: “The current study shows comparable results, with 96% TIN removal with a 0.46 kg TIN/(m^3.d) loading rate, and proves the high efficiency of the single-process SAO/PND system for nitrogen removal from real brewery wastewater.”

Clarification: At the end of experiments, the TIN removal in the current study was less than the previous one, but the applied TIN loading rates were higher. However, in both set of experiments with real wastewaters, the maximum applicable ammonia concentrations were not identified and experiments stopped.

Presented: Section 5.3.4.1: “The results revealed that 97.4% (68511 mg/d) of the organic carbon was removed in the pre-anoxic reactor, and the rest of this component was degraded in the following aerobic unit 1% (703 mg/d), which resulted in an overall 98.4% (69214 mg/d) COD removal in the entire system. Anaerobic oxidation was determined to be the main COD removal mechanism, with mass balance calculations showing that a 67.9% COD removal was completed by this process,…”

Clarification and suggested improvement: Even there are some findings to support the anaerobic oxidation, but this statement should be written as a hypothesis, and the improved statement can be like this: The results revealed that 97.4% (68511 mg/d) of the organic carbon was removed in the pre-anoxic reactor, and the rest of this component was degraded in the following aerobic unit 1% (703 mg/d), which resulted in an overall 98.4% (69214 mg/d) COD removal in the entire system. Anaerobic oxidation was hypothesized to be the main COD removal mechanism, with mass balance calculations showing that a 67.9% COD removal was completed by this process,…

Presented: Section 5.3.4.1: “anoxic unit, along with the lower Y_{obs} (0.15 mg VSS/mg BOD) a…”

Clarification: In this thesis, the BOD values were not measured, but COD in synthetic solutions are equal to BOD values.

Clarification: The whole article. As the biological results are not presented; therefore, all the explained removal pathways should be improved by using hypothetical tone.

Clarification: Sample mass balance calculations under steady-state conditions, and daily VSS production are presented. The following general term has been used for mass balance calculations in this thesis:

$$(Accumulation\ of\ mass\ in\ the\ system) = (mass\ into\ the\ system) - (mass\ out\ of\ the\ system) + (generation) - (consumption)$$

COD mass balance around the anoxic reactor:

$$0 = \left(17\frac{L}{d} \times 4136\frac{mg}{L}\right) + \left(34\frac{L}{d} \times 64.73\frac{mg}{L}\right) + \left(17\frac{L}{d} \times 64.87\frac{mg}{L}\right) - \left(68\frac{L}{d} \times 75.07\frac{mg}{L}\right) + Generation - Consumption$$

$$Consumption = 68510.85\frac{mg}{d}$$

Ammonia mass balance around the anoxic reactor:

$$0 = \left(17\frac{L}{d} \times 212.1\frac{mg}{L}\right) + \left(34\frac{L}{d} \times 1.53\frac{mg}{L}\right) + \left(17\frac{L}{d} \times 1.52\frac{mg}{L}\right) - \left(68\frac{L}{d} \times 10.62\frac{mg}{L})\right) + Generation - Consumption$$

$$Consumption = 2961.4\frac{mg}{d}$$

Nitrite mass balance around the anoxic reactor:

$$0 = \left(17\frac{L}{d} \times 0.06\frac{mg}{L}\right) + \left(34\frac{L}{d} \times 8.87\frac{mg}{L}\right) + \left(17\frac{L}{d} \times 5.83\frac{mg}{L}\right) - \left(68\frac{L}{d} \times 0\frac{mg}{L})\right) + Generation - Consumption$$

$$Consumption = 401.71\frac{mg}{d}$$

Nitrate mass balance around the anoxic reactor:

$$0 = \left(17\frac{L}{d} \times 0.61\frac{mg}{L}\right) + \left(34\frac{L}{d} \times 1.96\frac{mg}{L}\right) + \left(17\frac{L}{d} \times 1.72\frac{mg}{L}\right) - \left(68\frac{L}{d} \times 0.17\frac{mg}{L})\right) + Generation - Consumption$$

$$Consumption = 94.69\frac{mg}{d}$$

TIN mass balance around the anoxic reactor:

$$0 = \left(17\frac{L}{d} \times 212.77\frac{mg}{L}\right) + \left(34\frac{L}{d} \times 12..36\frac{mg}{L}\right) + \left(17\frac{L}{d} \times 9.07\frac{mg}{L}\right) - \left(68\frac{L}{d} \times 10.79\frac{mg}{L})\right) + Generation - Consumption$$

$$Consumption = 3457.80\frac{mg}{d}$$

COD mass balance around the aerobic reactor:

$$0 = \left(68\frac{L}{d} \times 75.07\frac{mg}{L}\right) - \left(34\frac{L}{d} \times 64.73\frac{mg}{L}\right) - \left(34\frac{L}{d} \times 64.73\frac{mg}{L}\right) + Generation - Consumption$$

$$Consumption = 703.12\frac{mg}{d}$$

Ammonia mass balance around the aerobic reactor:

$$0 = \left(68\frac{L}{d} \times 10.62\frac{mg}{L}\right) - \left(34\frac{L}{d} \times 1.53\frac{mg}{L}\right) - \left(34\frac{L}{d} \times 1.53\frac{mg}{L}\right) + Generation - Consumption$$

$$Consumption = 618.12\frac{mg}{d}$$

Nitrite mass balance around the aerobic reactor:

$$0 = \left(68\frac{L}{d} \times 0\frac{mg}{L}\right) - \left(34\frac{L}{d} \times 8.87\frac{mg}{L}\right) - \left(34\frac{L}{d} \times 8.87\frac{mg}{L}\right) + Generation - Consumption$$

$$Generation = 603.16\frac{mg}{d}$$

Nitrate mass balance around the aerobic reactor:

$$0 = \left(68\frac{L}{d} \times 0.17\frac{mg}{L}\right) - \left(34\frac{L}{d} \times 1.96\frac{mg}{L}\right) - \left(34\frac{L}{d} \times 1.96\frac{mg}{L}\right) + Generation - Consumption$$

$$Generation = 121.72\frac{mg}{d}$$

TIN mass balance around the aerobic reactor:

$$0 = \left(68\frac{L}{d} \times 10.79\frac{mg}{L}\right) - \left(34\frac{L}{d} \times 12.36\frac{mg}{L}\right) - \left(34\frac{L}{d} \times 12.36\frac{mg}{L}\right) + Generation - Consumption$$

$$Generation = 106.76\frac{mg}{d}$$

$$Daily\ WAS\ measured\ in\ the\ lab = 14229\ mg\ TSS/d$$

$$\frac{VSS}{TSS} = 0.71$$

$$Daily\ VSS\ production = 0.71 \times 14229 = 10103\ mg\ VSS/d$$

Appendix D: Notes on Chapter 6

In chapter 6, the article is presented identical to what is published. Significant feedback was received after the paper was accepted for publication. Detailed notes are included here to comment the publication to acknowledge some weaknesses or limitations and add some clarifications in the context of the thesis.

Presented in fourth published article: Abstract: “mentioned conventional systems…”

Suggested improvement: The improved sentence should be like this: mentioned conventional systems (conventional aerobic processes and combined systems)…

Published: Section 6.1: ”releasing untreated strong wastewaters can destroy aquatic environments…”

Suggested improvement: The improved sentence can be like this: releasing untreated strong wastewaters can negatively impact the aquatic environments…

Published: Section 6.1: “consume huge amounts of oxygen…”

Suggested improvement: consume high amounts of oxygen…

Published: Section 6.1: “Then the COD will be removed during denitrification in this pre-anoxic reactor, and the rest of the COD aerobically in the aerobic tank by aerobic heterotrophic bacteria.”

Suggested improvement: Some of the COD will be removed during denitrification in this pre-anoxic reactor, and the rest of the COD aerobically in the aerobic tank by aerobic heterotrophic bacteria.

Published: Section 6.1: "Anaerobic digestion (AD) is the best alternative…."

Suggested improvement: The improved sentence should be like this: Anaerobic processes are better alternative….

Published: Section 6.1: "AD treatment method has been identified as an efficient system for removing organic carbon from strong wastewaters, but this system as a single-process cannot produce treated effluent that meets discharge standards; therefore, existing conventional treatment technologies are combined systems. These conventional combined systems involve at least two different systems which work together in series. Most of these combined methods apply the AD as a head of the system, which is followed by a polishing aerobic process such as conventional activated sludge for carbon and nitrogen removal. The AD head system removes organic carbon but produces ammonia via ammonification (ammonia production) process, and so external alkalinity should be added to the AD in order to maintain the pH at the appropriate level (Arantes et al., 2017; Osmani et al., 2021)."

Clarification and suggested improvement: As this idea is already presented, and in order to avoid the repetition, it is better to remove this paragraph.

Published: Section 6.1: "so external alkalinity should be added to the AD in order to maintain the pH at the appropriate level (Arantes et al., 2017; Osmani et al., 2021)."

Suggested improvement: so in some cases, external alkalinity should be added to the AD in order to maintain the pH at the appropriate level (Arantes et al., 2017; Osmani et al., 2021).

Published: Section 6.1: "The effluent from AD is suitable to feed to a subsequent polishing aerobic system for further removal to meet the effluent discharge standards (Chan et al., 2009); however,"

Clarification and suggested improvement: This is a repetition and is better to remove this paragraph.

Suggested improvement: "The polishing aerobic process as the second treatment step removes organic carbon via denitrification, as well as nitrogen via conventional complete nitrification and then denitrification (Akizuki et al., 2021)."

Published: Section 6.1: "With this in mind, a single-process simultaneous anaerobic oxidation/partial nitrification-denitrification (SAO/PND) technology, as a sustainable, robust, and cost-effective system, was developed by Hosseinlou et al. (2019) and removed both organic carbon and nitrogen from synthetic wastewaters."

Suggested improvement: With this in mind, a single-process simultaneous anaerobic oxidation/partial nitrification-denitrification (SAO/PND) technology, was developed by Hosseinlou et al. (2019) and removed both organic carbon and nitrogen from synthetic wastewaters.

Published: Section 6.1: "Therefore, the main goal of the current research is to perform cost comparisons of the single-process SAO/PND technology with two conventional systems, which are: i) a conventional aerobic process such as MLE composed of anoxic/aerobic reactors; and ii) a conventional combined system which is composed of an AD and a subsequent aerobic system such as MLE."

Clarification and suggested improvement: As a full cost comparison is not performed; therefore, the tone should be soften. In addition, the MLE has been considered as a representative for common aerobic systems; however, the MLE has an anoxic reactor but still is considered as an aerobic system in comparison with strict anaerobic systems. The improved sentence should be like this: Therefore, the main goal of the current research is to compare the cost key components of the single-process SAO/PND technology with those of two conventional systems, which are: i) a conventional aerobic process such as MLE composed of anoxic/aerobic reactors; and ii) a conventional combined system which is composed of an AD and a subsequent aerobic system such as MLE.

Published: Section 6.2.1: "Based on Hosseinlou (2021), the mentioned parent setup was rich in anaerobic and aerobic heterotrophic bacteria, polyphosphate accumulating organisms (PAOs), denitrifying ordinary heterotrophic organisms (OHOs), denitrifying PAOs (DPAOs), nitrifiers, and anaerobic ammonium oxidation (anammox) bacterial community.“

Clarification and suggested improvement: As biological results are not presented; therefore, the improved sentence will be like this: Based on Hosseinlou (2021), the mentioned parent setup was hypothesized to be rich in anaerobic and aerobic heterotrophic bacteria, polyphosphate accumulating organisms (PAOs), denitrifying ordinary heterotrophic organisms (OHOs), denitrifying PAOs (DPAOs), nitrifiers, and anaerobic ammonium oxidation (anammox) bacterial community.

Published: Section 6.2.2: “Volumetric beakers”

Clarification: “Volumetric beakers” and “beakers” are the same.

Published: Section 6.2.2: “were stored in separate 1000 mL volumetric flasks”

Clarification and suggested improvement: Volumetric flasks are expensive, so it is considered good lab practice to use volumetric flasks to prepare solutions and then transfer them to more sturdy (and cheaper) capped glass bottles. It is better to remove the part of storing the solutions in volumetric flasks.

Published: Section 6.2.2: Table 6.2. Chemical composition of the synthetic wastewater.

Clarification: In this thesis, the same recipe has been used for preparing synthetic wastewater, and Table 6.2 shows the same ingredients as other publications. In addition, this article use the same SAO/PND setup as other articles. However, each article has carried out its own runs and experiments which are entirely different from other runs and experiments in different articles.

Published: Section 6.2.3: “Table 6.3. Design criteria and theoretical calculations of two conventional systems according to Metcalf & Eddy (2003, 2014).”

Clarification and suggested improvement: The MLE is a representative of conventional aerobic system. In addition, oxygen requirements are calculated based on the COD and nitrogen content removals. All kinetic parameters should be defined first as follows:

μ_m	Maximum specific growth rate
$\mu_{n,m}$	Nitrification maximum specific growth rate

K_n	Nitrification half-velocity constant
K_d	Decay half-velocity constant
K_{dn}	Denitrification half-velocity constant
Y_n	Nitrification yield
f_d	Fraction of decay

Published: Fig. 6.2 in section 6.3.1: "; b) COD and TP fluctuations over a single day (day 15)."

Suggested improvement: b) COD and TP profile within the SAO/PND system during day 15.

Published: Section 6.3.2. "Nitrogen content removal"

Suggested improvement: Nitrogen removal

Published: Fig. 6.3 in section 6.3.2: "; b) ammonia/ammonium, nitrite, nitrate, total nitrogen, and alkalinity fluctuations over a single day (day 15)."

Suggested improvement: b) ammonia/ammonium, nitrite, nitrate, total nitrogen, and alkalinity profile within the SAO/PND system during day 15.

Published: Section 6.3.2: "Pairing and synchronizing of these two separate processes to work together require intensive operation and maintenance"

Clarification: As combined (hybrid) systems are composed of at least two different treatment technologies in a series, and they are not independent, and success of the overall system depends on successful operation of both systems. In cases that one of these treatment technologies do not work properly, it will ruin the successful performance of the entire system; therefore, the operation of hybrid systems are more intensive.

Published: Section 6.3.2: “In comparison, the SAO/PND technology, as a single-process with simple operation, could achieve higher efficiency.”

Clarification: It should be acknowledged that treating real wastewaters with higher non-biodegradable COD components can limit the COD removal by the SAO/PND system.

Published: Section 6.3.2: “and People's Republic of China Ministry of Environmental Protection…”

Suggested improvement: and the People's Republic of China Ministry of Environmental Protection…

Published: Section 6.3.2: “Therefore, the single-process SAO/PND technology can be considered as a robust, sustainable, on-site technology which is easy to operate for treating real strong wastewaters such as various industrial effluents.”

Clarification and suggested improvement: The tone should be soften; therefore, it is better to remove this statement.

Published: Section 6.3.3: “7363 mg/d N–N”

Clarification and suggested improvement: The N represents total nitrogen; therefore, it should be written like this: 7363 mg/d TN–N.

Published: Section 6.3.3.1: “In total, 98.5% (68999 mg/d) of COD was removed through the entire system. The pre-anoxic tank degraded 97.5% (68393 mg/d) of the organic carbon, and the subsequent aerobic tank removed 1% (606 mg/d).”

Clarification and suggested improvement: As the biological results are not presented; therefore, these sentences should be like this: Based on MB calculations, in total 98.5% (68999 mg/d) of COD was removed through the entire system. Hypothetically the pre-anoxic tank degraded 97.5% (68393 mg/d) of the organic carbon, and the subsequent aerobic tank removed 1% (606 mg/d).

Published: Section 6.3.3.1: “According to the mass balance calculations, anaerobic oxidation removed 66.5% of the incoming COD.”

Clarification and suggested improvement: Again, biological studies are not presented to support this statement; hence, this sentence should be like this: According to the mass balance calculations, and as a hypothesis, anaerobic oxidation removed 66.5% of the incoming COD.

The following general term has been used for mass balance calculations:

$$\begin{aligned}&(Accumulation\ of\ mass\ in\ the\ system)\\&\qquad = (mass\ into\ the\ system) - (mass\ out\ of\ the\ system) + (generation)\\&\qquad - (consumption)\end{aligned}$$

Published: Section 6.3.3.1: “low Y_{obs} (0.15 mg VSS/mg BOD),”

Clarification: In this thesis, the BOD values were not measured; however, the synthetic solution with equal COD and BOD values were used. Synthetic solution has BOD/COD ratio of 1/1.

Published: Section 6.3.3.1: “Mass balance calculations of the dissolved phosphorus showed that 1991 mg/d PO_4^{3-}–P entered the system via the influent line, and 36.3% was removed through the system (41.8% removal by DPAOs inside the pre-anoxic unit and 58.2% by PAOs inside the subsequent aerobic unit). Also, the mass balance calculations of the unfiltered TP around the entire system revealed that the phosphorus removed in the system was stored inside the bacterial communities and wasted as sludge. In the meantime, the organic carbon was stored internally as poly–b–hydroxyalkanoates (PHA) and poly–b–hydroxybutyrate (PHB) by the DPAOs and PAOs.”

Clarification and suggested improvement: As biological results are not presented; then, this paragraph should be like this: Mass balance calculations of the dissolved phosphorus showed that 1991 mg/d PO_4^{3-}–P entered the system via the influent line, and 36.3% was removed through the system (hypothetically 41.8% removal by DPAOs inside the pre-anoxic unit and 58.2% by PAOs inside the subsequent aerobic unit). Also, the mass balance calculations of the unfiltered TP around the entire system revealed that the phosphorus removed in the system was stored inside the bacterial communities and wasted as sludge. As a hypothesis, the organic carbon was stored internally as poly–b–hydroxyalkanoates (PHA) and poly–b–hydroxybutyrate (PHB) by the DPAOs and PAOs.

Published: Section 6.3.3.1: “The organic carbon consumption was 10 g COD/g PO_4^{3-}–P,…”

Suggested improvement: According to the literature review, the organic carbon consumption was 10 g COD/g PO_4^{3-}–P,…

Published: Section 6.3.3.1: “Partial nitrification in the aerobic tank led to NO_x production (mainly nitrite and limited amounts of nitrate), and these components were recycled to the pre-anoxic tank at the head of the system for removal.”

Suggested improvement: Partial nitrification in the aerobic tank led to NO_x production (mainly nitrite and limited amounts of nitrate production), and these components were recycled to the pre-anoxic tank at the head of the system for removal.

Published: Section 6.3.3.1: “(6.6 g COD/g NO_3^-–N) as well.”

Clarification: This value has been used according to the literature review.

Published: Section 6.3.3.2: “As a result of the partial nitrification in the aerobic tank, 16.7% (602 mg/d) of the influent ammonia/ammonium was converted to nitrite and nitrate (with a molar ratio of nitrite/nitrate equal to 5.48/1) and then recycled to the pre-anoxic tank at the head of the system for removal.”

Clarification: Both nitrite and nitrate were consumed in the pre-anoxic tank.

Published: Section 6.3.2: “a small portion of the ammonia/ammonium being conveyed to…”

Suggested improvement: a small portion of the ammonia/ammonium was conveyed to…

Published: Section 6.3.3.2: “As a result of the partial nitrification in the aerobic tank, 16.7% (602 mg/d) of the influent ammonia/ammonium was converted to nitrite and nitrate (with a molar ratio of nitrite/nitrate equal to 5.48/1) and then recycled to the pre-anoxic tank at the head of the system for removal. Also, 82.5% (2969 mg/d) of the influent ammonia/ammonium was degraded in the pre-anoxic tank. Therefore, the total amount of influent ammonia/ammonium removal was 99.2% (3570 mg/d)."

Clarification and suggested improvement: The SAO/PND system did not remove 100% of the influent ammonia/ammonium; therefore, this should be stated at the end of these sentences. The improved paragraph should be like this: As a result of the partial nitrification in the aerobic tank, 16.7% (602 mg/d) of the influent ammonia/ammonium was converted to nitrite and nitrate (with a molar ratio of nitrite/nitrate equal to 5.48/1) and then recycled to the pre-anoxic tank at the head of the system for removal. Also, 82.5% (2969 mg/d) of the influent ammonia/ammonium was degraded in the pre-anoxic tank. Therefore, the total amount of influent ammonia/ammonium removal was 99.2% (3570 mg/d); however, a small portion of this component was detected at the effluent.

Published: Section 6.3.3.2: "However, complete nitrification was suppressed in the SAO/PND technology as a result of the short SRT values and controlled oxygen concentrations inside the aerobic unit, which led to partial nitrification."

Clarification: According to the literature review, the Do levels above 3 mg/L are recommended for complete nitrification. However, there are other studies with lower DO values which reported complete nitrification.

Published: Section 6.3.3.2: "Partial nitrification in the aerobic tank led to nitritation (nitrite production); then, all the nitrite was recycled back to the pre-anoxic tank and consumed through the anammox process."

Suggested improvement: Partial nitrification in the aerobic tank led to nitritation (nitrite production); then, all the nitrite was recirculated to the pre-anoxic tank and hypothetically consumed through the anammox process.

Published: Section 6.3.3.2: “As a result of the anammox inside the pre-anoxic tank,…”

Clarification and suggested improvement: Again, as biological results are not presented; hence, this sentence should be like this: Presumably by the anammox inside the pre-anoxic tank,…

Published: Section 6.3.3.2: “In summary, the COD degradation mechanisms are determined as follows: i) 66.5% anaerobic oxidation; ii) 21.4% cell growth; iii) 6.4% PAOs and 4.6% DPAOs through P-uptake; iv) 0.1% ordinary denitrifying heterotrophs; and v) 1% aerobic heterotrophic bacteria. In addition, nitrogen content removal occurred via: i) 46.3% bacterial assimilation; ii) 16.7% partial nitrification and then denitrification; iii) 8.3% anammox; and iv) 28.7% experimental errors and unknown mechanisms.”

Clarification: These are based on mass balance calculations, and the following general term has been used for this purpose:

$$\begin{aligned}(Accumulation\ of\ mass\ in\ the\ system) \\ &= (mass\ into\ the\ system) - (mass\ out\ of\ the\ system) + (generation) \\ &- (consumption)\end{aligned}$$

Published: Section 6.3.4: “There are no technical or economic justifications for aerobically removing organic matter and nitrogen from strong wastewater, because this treatment strategy needs a significant amount of oxygen and produces huge amounts of sludge.”

Clarification and suggested improvement: This statement is confusing and should be clarified. The improved sentence can be like this: Aerobic organic matter and nitrogen removal from strong wastewater can result in high amounts of oxygen requirement and sludge production; therefore, aerobic treatment methods are not recommended for this purpose.

Published: Section 6.3.4: "Complete nitrification-denitrification accompanied with organic carbon removal mechanisms, which happens in conventional aerobic processes such as MLE composed of anoxic/aerobic tanks, requires more oxygen and increases the costs."

Clarification: Partial nitrification happens in the SAO/PND system, as well as only 17% of nitrogen are being removed via this process. These items result in cost savings in the SAO/PND system proportionally.

Published: Table 6.4 in section 6.3.4:

Clarification and suggested improvement: This paragraph should be added in the context after Table 6.4: "The presented values are measured under the laboratory conditions. Y_{obs} and VSS/TSS ratio pertain the process as a whole. $WAS_{daily\ TSS}$ and $WAS_{daily\ VSS}$ are the average values. F_{TSS}, F_{VSS}, and R_{O2} are fluxes of TSS, VSS, and oxygen requirements, respectively."

Published: Section 6.3.4: "As can be seen, there are extraordinary cost savings with the single-process SAO/PND technology compared to conventional aerobic processes for nitrogen and COD removal, which are attributed to the oxygen and energy requirements, sludge production, and equipment costs. Furthermore,"

Suggested improvement: As this statement is not too informative; hence, it is better to be removed.

Published: Table 6.5 in section 6.3.4:

Clarification and suggested improvement: Cost comparisons are based on the measure values in the SAO/PND system under laboratory conditions, and theoretical values in the conventional systems. In addition, this statement should be added to Table 6.5 as a legend: “Values in brackets represent cost savings by the SAO/PND technology compared to the conventional systems.”

Published: Section 6.3.3: Removal pathways in the SAO/PND technology

Clarification and suggested improvement: As the results of biological studies are not presented; therefore, all the removal pathways should be explained in a hypothetical language since this is a working hypothesis.

Published: Section 6.3.4: Cost comparisons

Clarification and suggested improvement: For a comprehensive cost comparisons and get actual cost estimates, computer program such as CapdetWorks should be used.