ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL

UNLOCKING SUSTAINABILITY IN WASTEWATER DENITRIFICATION THROUGH WASTE-DERIVED VOLATILE FATTY ACIDS

Ph.D. THESIS

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Department of Environmental Engineering

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AUGUST 2024

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

ATIKTAN KAYNAKLANAN UÇUCU YAĞ ASİTLERİ YOLUYLA ATIKSU DENİTRİFİKASYONUNDA SÜRDÜRÜLEBİLİRLİK KİLİDİNİN AÇILMASI

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Tuğba SAPMAZ WIKSTRÖM, a Ph.D. student of ITU Graduate School student ID 501172708, successfully defended the thesis/dissertation entitled "UNLOCKING SUSTAINABILITY IN WASTEWATER DENITRIFICATION THROUGH WASTE-DERIVED VOLATILE FATTY ACIDS", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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FOREWORD

My PhD journey felt quite long (perhaps because it involved not just one but two PhDs), so much so that I often wondered if it would ever reach its end. Imagine everything culminating in a single day–even things that we believe will never see an end, such as completing a PhD thesis. However, its real essence lies in how it appears when one arrives at that point. When you are smiling at the finish line, when you have had ample fun throughout the journey–even on the rainiest, coldest, and darkest days–and when you have gathered a diving trove of great memories with your companions and colleagues, that is when you know it has been a success. Throughout my PhD studies, I received great companionship and support from numerous individuals to whom I owe a great deal of appreciation. Now, I would like to take this opportunity to thank them all from the bottom of my heart.

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I am incredibly thankful to have a family that supports my every decision and always makes me feel loved and whole. I feel very fortunate and blessed to have my mom, Kadriye, and my dad, Ahmet. I would never have made it this far without their prayers and support. I consider myself the luckiest to have a little brother like Can and a sister like Büşra in my life. Thank you, Can, for consistently reminding me, sometimes through playful teasing, of how fortunate I am to be pursuing higher education, especially during times when I am traveling for a conference. Büşra, thank you for always being there for me regardless of the reason or time I need you. She listened attentively to me and cheered on me continuously. I greatly admire your energy. Thank you for those never-ending phone calls whenever I need to talk and vent, visiting us on holidays, and bringing Ahmet to cheer us up even more during the dark winter here in Sweden. It makes me appreciate every little time together. I would also like to thank my Swedish family (Jenny, Henry, Sara, Esmeralda, and Melvin) for providing me with a mental respite during the holiday gatherings, for all the wonderful meals and fikas we had together, for refreshing outdoor walks, for their unwavering support, along with curiosity and a positive and supportive attitude toward my narrow subject.

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With all my love and sincerity, I dedicate my PhD thesis to you Linus, whose unwavering presence has been the guiding light throughout my PhD pursuit.

January 2024

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(MSc Environmental Engineer)

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ABBREVIATIONS

AD : Anaerobic Digestion
BOD : Biochemical Oxygen Demand
COD : Chemical Oxygen Demand
HRT : Hydraulic Retention Time
MBBR : Moving Bed Biofilm Reactor
MBR : Membrane Bioreactor
NF : Nanofiltration
OLR : Organic Loading Rate
TN : Total Nitrogen
VFA : Volatile Fatty Acid
VS : Volatile Solids
WWTP : Wastewater Treatment Plant

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UNLOCKING SUSTAINABILITY IN WASTEWATER DENITRIFICATION THROUGH WASTE-DERIVED VOLATILE FATTY ACIDS

SUMMARY

Wastewater treatment is a crucial process, designed to remove contaminants and suspended solids from wastewater prior to its discharge into the environment. One of the primary nutrient contaminants found in municipal wastewater is nitrogen. Biological treatment process is the most common process employed in wastewater treatment plants (WWTPs) for nutrient removal. Traditionally, wastewater has undergone nitrogen removal through nitrification and denitrification processes, where organic carbon source has served as an electron donor in the denitrification process for heterotrophic denitrifiers. Therefore, carbon sources play an important role in wastewater treatment during biological nitrogen removal. The growing need for improved denitrification, driven by stricter nutrient discharge standards, is leading to an increased demand for external carbon sources. External carbon sources conventionally used in wastewater treatment such as methanol or ethanol are often acquired from fossil derived sources, raising environmental and economic concerns. To address these issues, this thesis explores a more effective solution for carbon source in denitrification: waste-derived volatile fatty acids (VFAs). VFAs are fatty acids with carbon chains ranging from 2 to 6 (such as acetic acid, propionic acid, butyric acid, etc.), generated and can be obtained during the anaerobic digestion (AD) of various organic waste materials as intermediate metabolites. The transformation of waste into VFAs also offers an additional means to reduce the ever-increasing amount of waste. Consequently, VFAs present a sustainable alternative that shows promise in optimizing denitrification processes while mitigating ecological impacts.

Long-term fermentation and filtration, affirming the viability of immersed anaerobic membrane bioreactors (MBRs) for sustainable VFA generation was constructed and rapid generation of VFAs at a concentration of up to 11.8 g/L could be successfully achieved. The effect of pH in the batch experiments on the production and yield of VFAs during acidogenic digestion was evaluated. Under the optimal conditions, a high yield of 0.65 g VFAs/g VS_{fed} was obtained for the organic loading rate (OLR) of 1 g VS/L/d using FPPL substrate and chicken manure as inoculum. The results show that the application of sequential multi-step bioconversion of potato starch industry residues has the potential to increase the variety of value-added products generated from a single organic residue while enhancing nutrient recovery capacity.

In the second experimental work, VFA effluent with high ammonium, chicken manure (CKM) derived VFA effluent, was subjected to ion exchange process to remove ammonium. Ammonium removal potential was explored by using a low-cost natural

ion exchanger, clinoptilolite. Experiments were conducted for both synthetic and actual VFA (AD-VFA) solutions, and the effects of different parameters were investigated with batch and continuous studies. It was observed that the Langmuir-type isotherm provided the best fit to the equilibrium data in the isotherm investigations carried out with the AD-VFA solution. The maximum adsorption capacity (q_m) was found as 15.7 mg NH_4^+/g clinoptilolite. The effect of some operational parameters on process performance such as pH, initial NH_4^+ loading and potassium ion (K^+) concentration were investigated. The pH had a negligible effect on ammonium removal for a pH range of 3-7, while the removal efficiency of ammonium decreased with the increase of initial NH_4^+ loading and K^+ concentration. At the optimum conditions determined in batch experiments, the ammonium removal from synthetic and AD-VFA solutions were compared and average ammonium removal efficiencies of 93 and 94% were found in 12 h equilibrium time for synthetic and AD-VFA solutions, respectively. Overall findings indicated that clinoptilolite has excellent potential for ion exchange when combined with biological processes such as acidogenic fermentation of VFAs to purify the solution from high-ammonium content.

Fossil-based materials such as methanol are frequently used in the denitrification process of advanced biological wastewater treatment as external carbon source. Volatile fatty acids (VFAs) produced by anaerobic digestion of food waste, are sustainable compounds with the potential to act as carbon sources for denitrification, reducing carbon footprint and material costs. The application of VFAs as external carbon source presents a novel possibility for sustainable denitrification in wastewater treatment. In the studies performed, the effectiveness of food waste-derived VFAs (AD-VFA) was investigated in the post-denitrification process in comparison with synthetic VFA and methanol as carbon sources. Acetic acid had the highest rate of disappearance among single tested VFAs with a denitrification rate of 0.44 g NO_x-N removed/m^2/day, indicating a preferential utilization pattern. While AD-VFA had a denitrification rate of 0.61 mg NO_x-N removed/m^2/day, sVFA had a rate of 0.57 mg NO_x-N removed/m^2/day, indicating that impurities in AD-VFA did not play substantial role in denitrification. AD-VFA proved to be promising carbon source alternative for denitrification in wastewater treatment plants. In the continuation study, although solely added AD-VFA_{PPL} as a carbon source had a slower denitrification capability (0.56 ± 0.13 mg NO_x-N removed/m^2/day) than methanol (1.04 ± 0.46 mg NO_x-N removed/m^2/day), up to 50% of the methanol can be replaced by waste-derived AD-VFA_{PPL} and achieve comparable performance (1.08 ± 0.07 mg NO_x-N removed/m^2/day) with the pure methanol. This proves that the co-addition of VFAs together with methanol can fully compete with pure methanol in performance. This integration emerges as a sustainable approach, attaining parallel denitrification performance while reducing reliance on fossil-derived sources. This proves that the co-addition of VFAs together with methanol can fully compete with pure methanol in performance, providing a promising opportunity for wastewater treatment plants to potentially reduce their carbon footprint and become more sustainable in practice while benefiting from recovered nutrients from waste.

In conclusion, this thesis reimagines WWTPs as places that recover valuable resources, going beyond only wastewater treatment. Bridging VFA generation with value-added usage holds the potential to strengthen biorefineries and promote environmentally friendly wastewater solutions.

ATIKTAN KAYNAKLANAN UÇUCU YAĞ ASİTLERİ YOLUYLA ATIKSU DENİTRİFİKASYONUNDA SÜRDÜRÜLEBİLİRLİK KİLİDİNİN AÇILMASI

ÖZET

Atık su arıtımı, atık suyun çevreye desarj edilmesi öncesi kirleticileri ve askıda katı maddeleri uzaklaştırmak için tasarlanmış çok önemli bir işlemdir. Evsel atık sularda bulunan başlıca besin kirleticilerinden biri nitrojendir. Biyolojik arıtma prosesi, atık su arıtma tesislerinde (AAT) besin giderimi için kullanılan en yaygın prosestir. Geleneksel olarak atık su, organik karbon kaynağının, heterotrofik denitrifikasyon maddeleri için denitrifikasyon prosesinde bir elektron vericisi olarak görev yaptığı nitrifikasyon ve denitrifikasyon prosesleri yoluyla nitrojenin uzaklaştırılmasına tabi tutulur. Bu nedenle biyolojik azot giderimi sırasında atıksu arıtımında karbon kaynakları önemli bir rol oynamaktadır. Dahada sıkılastırılan ya da öngörülen deşarj standartlarının etkisiyle artan denitrifikasyon ihtiyacı, harici karbon kaynaklarına olan talebin artmasına yol açıyor. Atık su arıtımında geleneksel olarak kullanılan metanol veya etanol gibi harici karbon kaynakları çoğunlukla fosil kökenli kaynaklari kullanarak elde ediliyor ve bu da çevresel ve ekonomik kaygıları artırıyor. Bu konuları ele almak için bu tez calışması, denitrifikasyonda karbon kaynağı için daha etkili bir çözümü araştırmaktadır: Atıklardan türetilen uçucu yağ asitleri (UYA'lar)'nin kullanımı. UYA'lar, karbon zincirleri 2 ila 6 arasında değişen (asetik asit, propiyonik asit, bütirik asit vb.) yağ asitleridir ve ara metabolitler olarak çeşitli organik atık malzemelerin anaerobik fermentasyonu (AF) sırasında elde edilebilir. Atıkların UYA'lara dönüştürülmesi, giderek artan atık miktarının azaltılmasına yönelik ek bir araç da sunuyor. Sonuç olarak UYA'lar, ekolojik etkileri azaltırken denitrifikasyon süreçlerini optimize etme konusunda umut vaat eden sürdürülebilir bir alternatif sunuyor.

Sürdürülebilir UYA üretimi için batık anaerobik membran biyoreaktörlerinin (MBR'ler) uygulanabilirliğini doğrulayan uzun vadeli fermantasyon ve filtrasyonu birleştiren sistem kurulmuştur ve 11,8 g/L'ye kadar konsantrasyonda hızlı UYA üretimi başarıyla gerçekleştirilmiştir. Optimum koşullar altında, fermente patates protein likörü (FPPL) substratı ve aşı olarak tavuk gübresi kullanılarak 1 g UKM/L/gün organik yükleme oranı (OLR) için 0,65 g UYA/g $UKM_{beslenen}$ gibi yüksek bir verim elde edildi.

İkinci deneysel çalışmada, yüksek amonyum içeren UYA çıkış suyu, tavuk gübresi (CKM) türevi UYA çıkış suyu, amonyumun uzaklaştırılması için iyon değiştirme işlemine tabi tutulmuştur. Amonyum giderme potansiyeli, düşük maliyetli bir doğal iyon değiştirici olan klinoptilolit kullanılarak araştırıldı. Maksimum adsorpsiyon

kapasitesi (q_m) 15,7 mg NH_4^+/g klinoptilolit olarak bulunmuştur. Belirlenen optimum koşullarda amonyum giderimleri karşılaştırılmış ve sentetik ve AF-UYA çözeltileri için 12 saatlik denge süresinde sırasıyla ortalama %93 ve %94'lük ortalama amonyum giderim verimleri bulunmuştur. Genel bulgular, çözeltiyi yüksek amonyum içeriğinden arındırmak için VFA'ların asidojenik fermantasyonu gibi biyolojik işlemlerle birleştirildiğinde klinoptilolitin mükemmel iyon değişimi potansiyeline sahip olduğunu gösterdi.

Metanol gibi fosil kökenli malzemeler, ileri biyolojik atıksu arıtımında denitrifikasyon prosesinde dış karbon kaynağı olarak sıklıkla kullanılmaktadır. Gıda atıklarının anaerobik parçalanması ile üretilen uçucu yağ asitleri (UYA'lar), denitrifikasyon için karbon kaynağı olarak hareket etme, karbon ayak izini ve malzeme maliyetlerini azaltma potansiyeline sahip sürdürülebilir bileşiklerdir. UYA'ların harici karbon kaynağı olarak uygulanması, atık su arıtımında sürdürülebilir denitrifikasyon için yeni bir olasılık sunmaktadır. Yapılan çalışmalarda, gıda atıklarından elde edilen UYA'ların (AF-UYA) denitrifikasyon sonrası süreçte etkinliği, karbon kaynağı olarak sentetik VFA ve metanol ile karşılaştırmalı olarak araştırılmıştır. Asetik asit, tek test edilen VFA'lar arasında en yüksek giderim verimine sahipti ve 0,44 g NO_x-N giderilen/m^2/gün denitrifikasyon oranıyla kıyaslanabilir bir verime işaret ediyordu. AF-UYA'da uzaklaştırılan NO_x-N/m^2/gün 0,61 mg denitrifikasyon oranına sahipken, sVFA'da uzaklaştırılan NO_x-N/m^2/gün oranı 0,57 mg idi, bu da AF-UYA'daki safsızlıkların denitrifikasyonda önemli bir rol oynamadığını göstermektedir . AF-UYA'nın atık su arıtma tesislerinde denitrifikasyon için umut verici bir karbon kaynağı alternatifi olduğu kanıtlandı. Devam çalışmasında, karbon kaynağı olarak yalnızca AF-UYA_{PPL} eklenmiş olmasına rağmen, metanolden (1,04 ± 0,46 mg NO_x-N uzaklaştırılmış/m^2/gün) daha yavaş bir denitrifikasyon kapasitesine (0,56 ± 0,13 mg NO_x-N uzaklaştırılmış/m^2/gün) sahip olmuştur. Metanolün %50'ye kadarı atıktan türetilmiş AF-UYA_{PPL} ile değiştirilebilir ve saf metanol ile karşılaştırılabilir performansa (1,08 ± 0,07 mg NO_x-N uzaklaştırılmış/m^2/gün) ulaşılabilir. Bu, VFA'ların metanolle birlikte eklenmesinin performans açısından saf metanol ile tamamen rekabet edebileceğini kanıtlanmaktadır. Bu entegrasyon, fosil kaynaklı kaynaklara olan bağımlılığı azaltırken paralel denitrifikasyon performansı sağlayan sürdürülebilir bir yaklaşım olarak ortaya çıkmıştır. Bu, UYA'ların metanolle birlikte eklenmesinin performans açısından saf metanol ile tamamen rekabet edebileceğini kanıtlamakta ve atık su arıtma tesislerine potansiyel olarak karbon ayak izlerini azaltmadir, aynı zamanda atıklardan geri kazanılan besinlerden faydalanırken pratikte daha sürdürülebilir hale gelme konusunda umut verici bir fırsat sunmaktadir.

Bu çalışmanın sonuçları, atık su arıtma tesislerinde sürdürülebilir denitrifikasyon için atıklardan türetilen UYA'larının kullanımının potansiyelini vurgulamaktadır. UYA'ların kullanımı, sadece çevresel etkileri azaltmakla kalmaz, aynı zamanda ekonomik açıdan da avantajlar sunar. Geleneksel fosil kökenli karbon kaynaklarına olan bağımlılığı azaltarak, atık su arıtma süreçlerinin daha sürdürülebilir ve maliyet etkin hale gelmesine katkıda bulunur. Ayrıca, atıkların değerli bir kaynağa dönüştürülmesi, döngüsel ekonomi prensiplerine uygun olarak atık yönetiminde yeni

bir paradigma oluşturur. Bu bağlamda, UYA'ların geniş çapta benimsenmesi, hem çevresel sürdürülebilirliği artıracak hem de atık su arıtma tesislerinin operasyonel verimliliğini yükseltecektir.

Sonuç olarak bu tez, atık su arıtma tesislerinin ötesinde, değerli kaynakların geri kazanıldığı yerler olarak AAT'leri yeniden tasavvur etmektedir. UYA üretimi ile katma değerli kullanım arasında köprü kurmak, biyorafinerileri güçlendirme ve çevre dostu atıksu çözümlerini teşvik etme potansiyelini barındırmaktadır.

1. INTRODUCTION

In an era characterized by an ever-escalating environmental crisis and an urgent need for sustainable solutions, wastewater management has emerged as a critical frontier. The widening discrepancy between the need of the industrialized world to ensure the availability of resources and nonrenewable feedstocks such as fossil fuels has had an alarmingly significant impact on global environmental problems. With growing awareness of the critical consequences of pollution on ecosystems and human health, there is a pressing global obligation to revolutionize our approach to wastewater treatment. However, rather than focusing solely on improving effluent wastewater quality, wastewater treatment plants (WWTPs), critical facilities for urbanization, are also coping with new challenges such as energy consumption, operating costs, and stringent effluent limits. Therefore, developing innovative strategies for next-generation WWTPs that consume less energy and are more sustainable is critical.

In conventional wastewater treatment, influent wastewater undergoes different treatment stages to remove organic matter and nutrients, such as nitrogen and phosphorus. Generally, biological nitrogen removal is accomplished by aerobic nitrification, i.e., the oxidation of ammonium (NH_4^+) to nitrate (NO_3^-), followed by anoxic denitrification, i.e., the reduction of NO_3^- to nitrogen gas (N_2). During the subsequent anoxic denitrification stage, denitrifiers produce N_2, released into the atmosphere by reducing the NO_3^- ions produced during nitrification (Kim et al., 2016).

The problem at the denitrification stage is that denitrifying bacteria, unlike nitrifying bacteria, requires organic carbon sources for growth and energy acquisition. In some wastewater treatment processes with stepwise denitrification following effective nitrification, or multistage nitrification and denitrification followed by a high-load activated sludge process with a limited area to expend further, most organic carbon sources are readily depleted/ exhausted for further processing. Therefore, influent wastewater in the post-denitrification stage requires the addition of a readily biodegradable external carbon source to achieve highly efficient biological nitrogen removal. Common external carbon sources, such as alcohols (e.g., methanol and

ethanol), acetate, and glycerol, are widely used for denitrification. However, the associated costs and environmental concerns, particularly with fossil-fuel-based options such as methanol, pose challenges. Issues include varying prices and availability, seasonal fluctuations, and the need for large on-site storage, thereby increasing process footprints. In conclusion, *ex situ* organic carbon source addition places external demands on the wastewater treatment facility, making it far from self-sustaining.

This thesis aims to contribute to reducing the dependence on fossil fuel sources by investigating alternative carbon sources for wastewater denitrification. To achieve this, denitrification potential of waste-derived volatile fatty acids (VFAs) as an alternative carbon source for the denitrification process and compared their performance with that of commonly used carbon sources were evalued in this thesis project.

1.1 Impact

The studies described in this thesis investigate the feasibility of using waste-derived VFAs, an acidogenic fermentation product, as a carbon source for denitrification in an attempt to provide answers to the following research questions:

- How can VFAs be produced, extracted, and improved for an end application in wastewater denitrification?
- What is the denitrification potential of waste-derived VFAs as an alternative carbon source in wastewater treatment, and how does it compare with that of commonly used carbon sources, e.g., methanol?
- How can sustainability be enhanced through wastewater treatment using waste-derived VFAs?

1.2 Purpose of Thesis

The main aim of this thesis was to evaluate the potential of waste-derived VFA-bearing anaerobic digestion (AD) effluents in the denitrification process during wastewater treatment and the replacement of fossil-based conventional carbon sources, e.g., methanol.

The specific objectives described below were to investigate:

1. A continuous VFA recovery technique from a waste stream using an immersed membrane bioreactor (Paper I);
2. Effects of high nitrogen concentrations of VFAs, and its possible risks related to the intended end use so evaluating the ion exchange process performance of waste-derived high-nitrogen VFA effluents (Paper II);
3. An evaluation of waste-derived VFAs as an alternative carbon source for denitrifying moving bed biofilm reactors (Paper III);
4. Effects of sequential and simultaneous supplementation of waste-derived VFAs and methanol as alternative carbon source blend for wastewater denitrification (Paper IV).

1.3 Thesis Structure

This thesis is divided into six chapters as follows:

Chapter 1 introduces the scope and the main objectives of the performed research;

Chapter 2 provides an information on the production of volatile fatty acids (VFAs) from the effluent of fungi biomass cultivation on low-grade residues from the potato starch industry with batch and semi-continuous membrane bioreactors (MBRs) containing the effluent of already fermented potato protein liquor (FPPL) inoculated with chicken and cow manure.

Chapter 3 describes the the upgrading process of waste-derived VFAs, the NH_4^+ removal strategy from N-rich VFA effluents using an ion exchange process. This study investigates the potential for removal of ammonium using a naturally occurring zeolite (clinoptilolite) from chicken manure (CKM) derived VFA effluent recovered from an anaerobic membrane bioreactor (MBR). Experiments were conducted for both synthetic and actual VFA (AD-VFA) solutions, and the effects of different parameters were investigated with batch and continuous studies.

Chapter 4 and **Chapter 5** discusses the direct use of VFA mixtures and the application of waste-derived VFAs as carbon sources for wastewater post-denitrification process. Volatile fatty acids (VFAs) produced by anaerobic digestion of food waste, chicken manure and potato protein liquor, are sustainable compounds with the potential to act as carbon sources for denitrification, potentially reducing

carbon footprint and material costs. In this study, the effectiveness of waste-derived VFAs were investigated in the post-denitrification process in comparison with synthetic VFA and methanol as carbon sources.

Chapter 6 presents the main conclusions of this research and offers insights for future research.

2. TOWARDS MAXİMUM VALUE CREATİON FROM POTATO PROTEİN LIQUOR: VOLATILE FATTY ACIDS PRODUCTION FROM FUNGAL CULTIVATION EFFLUENT[1]

2.1 Introduction

Potato can be grown under a wide range of conditions, making it a common crop in all EU countries and worldwide. The global annual potato production is now over 370 million tons, of which, in 2021, 826 400 tons was produced in Sweden, corresponding to an area under cultivation of 23 750 ha (Jordbruksverket, 2022). The yield per hectare for starch potato processing in Sweden is estimated at 41 950 kilos (Agriculture, 2022). However, the potato industry entails side streams that take up a significant part of the original biomass. Especially in southern Sweden, potato starch production generates a large amount of low-value residuals consisting of potato liquor and potato pulp (Kuktaite et al., 2015). The potato liquor is treated with steam to coagulate the protein fraction, which is separated using a decanter. The remaining potato liquor, which mainly consists of the soluble fraction of the waste from potato starch production, is concentrated to form potato protein liquor (PPL). Together with the processing costs and stricter EU requirements, the potato and starch industry is forced to use valuable resources responsibly and develop a more efficient approach that can produce more value-added products from the by-products (Ahokas et al., 2014). Increasing resource efficiency by maximizing the utilization of nutrients in a used resource is a step further than converting organic waste into bio-based resources and is a major contribution to sustainable production with higher circularity. Therefore, efficient utilization of protein-rich side streams is important for the starch industry.

[1]This chapter is based on: Sapmaz, T., Uwineza, C., Mahboubi, A., De Wever, H., Koseoglu-Imer, D. Y., & Taherzadeh, M. J. (2023). Towards maximum value creation from potato protein liquor: Volatile fatty acids production from fungal cultivation effluent. Biomass Conversion and Biorefinery, 1– 14.

In current practices, residual effluents from the potato starch industry usually end up as fertilizer (Souza Filho et al., 2017a).

This common scenario has been compared with other scenarios in different literature (Souza Filho et al., 2017a) and the use of PPL for the cultivation of fungal biomass and proteins (Schügerl & Rosen, 1997; Souza Filho et al., 2017b), yeast biomass (Lotz et al., 1991) and enzyme production (Klingspohn et al., 1993) were investigated. PPL has been valorized through conversion of a high fraction of its nutrients into food and feed grade fungal biomass in a previous study (Souza Filho et al., 2017b). This partial valorization approach generates another type of effluent stream that can be used to maximize value creation from PPL. In this regard, the well-known valorization method of anaerobic digestion can use the effluent from fungal cultivation that still contains high chemical oxygen demand (COD), unconverted organics and fungal metabolites.

A modified version of anaerobic digestion, acidogenic fermentation or acidogenic digestion which provides conditions for the production of volatile fatty acids (VFAs) rather than biogas (mainly methane) can help with further value creation from such effluents. VFAs including fatty acids with 2 to 6 carbon chains (acetic acid, propionic acid, butyric acid, etc.) are important platform chemicals produced in the anaerobic digestion process as an intermediate metabolites (Zhang et al., 2022). Traditionally, VFAs are synthesized commercially through petrochemical pathways. Due to the continuously rising environmental awareness on the excessive application consequences and resource scarcity of global oil sources, new alternative approaches such as VFA generation from low or negative value organic waste has attracted great attention. The generated VFAs through acidogenic fermentation or digestion have market demands in applications such as bioplastics and bioenergy production, external carbon sources for biological nutrient removal, and usage in pharmaceutical and food industries (Agnihotri et al., 2022; Sapmaz et al., 2022b; Wainaina et al., 2019b). Different sources of organic waste including food waste (Sapmaz et al., 2022b; Shewa et al., 2020; Wainaina et al., 2019a), lignocellulosic materials (Sun et al., 2021), waste activated sludge (Fang et al., 2020; Yuan et al., 2019), and various animal manures (Huang et al., 2016; Jomnonkhaow et al., 2021; Yin et al., 2021) have been used as feed stock for acidogenic digestion and VFAs production. The addition and type of inoculum are also essential for the effectivity of the anaerobic process because they provide the initial microbial population (Rajput & Sheikh, 2019). The main benefit of

employing inoculum from manure with AD process is related to have a faster starting time and well optimized digestion process (Paes et al., 2020).

This study focuses on a sequential biological treatment strategy for the production of different value-added products from potato starch industry residues. In this regard, the waste generated from the fungal cultivation of PPL was further valorized for the production of volatile fatty acids via acidogenic fermentation. The effect of pH adjustment through changes in the inoculum to substrate ratio, inoculum type (cow manure (CM) and chicken manure (CKM)) and methane production inhibition were initially evaluated in batch assays. In order to benefit from continuous VFA production from this rather low organic bearing effluent and in situ recovery of the particle- and microorganism-free VFA bearing stream, two immersed membrane bioreactors (iMBRs) operating with different inoculum type and at different organic loading rate were applied. Changes in the VFAs production and distribution, MBR performance (transmembrane pressure (TMP) and flux), medium characterization (viscosity, pH, extracellular polymeric substance (EPS), soluble microbial products (SMP), solid content), and inoculum activities were analyzed to determine the underlying mechanism for efficient VFA production by anaerobic digestion of FPPL. The introduced approach in this study targets maximum nutrient recovery and enhanced resource efficiency for industries generating organic residues through bioconversion approaches.

2.2 Materials and Methods

2.2.1 Substrate and inoculums

Potato protein liquor (PPL) was obtained from Lyckeby Starch AB (Kristianstad, Sweden) and used for fungal cultivation study in a pilot-scale reactor with 1200 L capacity (Process& Industriteknik AB, Kristianstad, Sweden) with results presented in another study by Sar et al. [24]. After completion of cultivation, the fermented broth was passed through a sieve in order to collect the fungal biomass that is a product of fungi fermentation process. The already fermented residues and a waste liquid (FPPL) from the pilot-scale reactor that were commonly drained to the sewage system were collected and stored at -18 °C to be used as substrate in this study. The composition of substrate used is shown in Table 2.1. The same batch of liquid was used for all experiments described in this article. For inoculum, cow manure (CM) was provided

by Hushållningssällskapet Sjuhärad (Länghem, Sweden) and chicken manure (CKM) was collected from Sjömarkens Höngårds (Sjömarken, Sweden). The as-received total solid (TS) of CKM and CM were 87.0 ± 0.47 and 45.2 ± 0.17 g/L), respectively, while volatile solids (VS) were 51.4 ± 0.77 and 32.3 ± 1.23 (g/L), respectively.

Table 2.1 : Composition of fermented potato protein liquor (FPPL).

Component	Value
pH	7.54
tCOD (g/L)	13.6 ± 0.55
sCOD (g/L),	13.3 ± 0.58
NH_4^+-N (mg/L)	381 ± 69.90
TKN (g/kg)	43.4 ± 0.15
Protein (%)	27.1 ± 0.09
TS (g/L)	18.1 ± 0.10
VS (g/L)	9.2 ± 0.08
TSS (g/L)	2.0 ± 0.05
VSS (g/L)	± 0.09

2.2.2 Batch digestion

CKM, CM and FPPL used in the anaerobic batch experiments were thermally treated at 80°C for 15 min (thermal shock) using a water bath to inhibit the activity and growth of methanogens (Mondylaksita et al., 2021). The batch assays were carried out under mesophilic conditions (37 ± 1°C) for 23 days, using 120 mL glass serum bottles as bioreactors. Batch acidogenic digestion experiments were performed in triplicate with a working volume of 80 mL and inoculum to substrate ratios of 1:1 and 1:4 (on VS basis) (Table 2.2). A total of 1 g/L VS including the substrate and inoculum mixture were loaded to each bioreactor. The inoculum controls contained same VS loading as in the mixture. Tap water was added to fill the serum bottles to the same working volume for each condition, and then the initial pH was measured. Based on the as-mixed pH of the inoculum and substrate, the pH of the controls (inoculum only) was defined. Prior to digestion, all bottles were tightly sealed and purged with nitrogen gas for 2 min to remove any residual oxygen and create anaerobic conditions. The reactors were placed in water bath shakers at 37 ± 1°C and 110 rpm for incubation. Liquid and gas samples were collected on day 1, 2, 3, 5, 7, 9, 11, 13, 15, 18 and 23. During

incubation, 250 µL of biogas was sampled using a gas-tight syringe (VICI, Precision Sampling Inc., USA) to analyze the volume and composition of the biogas. A 1 mL aliquot was taken from the digestion medium using a syringe to analyze the VFAs content and distribution.

Table 2.2 : Experimental conditions applied for batch assays.

FPPL with CKM as inoculum					FPPL with CM as inoculum				
Substrate and inoculum	**Organic loading rate (g VS/L)**	**Initial pH**	**Set pH**		**Substrate and inoculum**	**Organic loading rate (g VS/L)**	**Initial pH**	**Set pH**	
			pH: 7.90	**pH: 7.56**				**pH: 7.41**	**pH: 7.02**
CKM	0.5	8.68	◆		CM	0.5	7.79	◆	
CKM	0.2	8.68		◆	CM	0.2	7.79		◆
CKM and FPPL	0.5:0.5	7.90	as mixed		CM and FPPL	0.5:0.5	7.41	as mixed	
CKM and FPPL	0.2:0.8	7.56	as mixed		CM and FPPL	0.2:0.8	7.02	as mixed	

2.2.3 Anaerobic immersed membrane bioreactor (iMBR) setup and operation

Two identical iMBRs that house second generation Integrated Permeate Channel (IPC) membrane panels were used (Fig. 2.1). The MBRs were operated semi-continuously at 37 °C with a working volume of 3.5 liters. The IPC flat- sheet membranes were developed and supplied by the Flemish Institute for Technological Research (VITO NV, Mol, Belgium) (Doyen et al., 2010) . Flat sheet membrane panels were made of hydrophilic polyethersulfone (PES)/PVP membranes coated on both sides of a spacer fabric with a total effective area of 205.8 cm^2. Membranes had an average pore size of 0.3 µm and two different membranes were used in this regard in each MBR. Membrane specifications were the same, but the membrane age was different in the MBRs. MBR2 had a lower permeability from the beginning as they were not virgin membranes and have been used in different practices. Hence, the membranes were exposed to application and cleaning cycles which could not fully regenerate the capacity of the membrane. Each membrane panel had a total of 12 built-in air/gas diffusers on each side of the panel with a diameter of 0.5 mm. This allows effective membrane surface scouring during digestion and filtration and helps better mixing and mass transfer in the reactor using nitrogen gas. The iMBRs benefited from a gas flow meter (Dwyer Instruments, USA) to control the nitrogen flow. The nitrogen flow was set at 5 L/min during the filtration cycle and 3 L/min when filtration was not applied. During filtration, permeate flow was measured using Atrato 710-V11-D ultrasonic flowmeters

(Titan Enterprises Ltd, Dorset, England), while liquid pressure on the permeate line was measured using a PMC131 pressure sensors (Endress+Hauser AB, Solna, Sweden). Temperature and pH in the bioreactor were monitored real-time using a probe combined with a transmitter via fermenter PLC (Micro DCU-300, Germany). Peristaltic pumps (Watson Marlow 400) were used separately to withdraw permeate from the membrane modules in the reactors. The filtration process consisted of a 4-min filtration cycle followed by a 30-second backwash cycle. Data logging and control of the permeate pump, pressure sensors and flow meters were centrally controlled and automated with LabView-based MeFIAS® software (Doyen et al., 2003) kindly customized and developed for these specific lab-scale iMBRs by VITO NV. MBR1 was initially loaded with a total of 3.5 L of thermally treated FPPL (4 g VS/L) and chicken manure as inoculum (4 g VS/L). For MBR2 cow manure was used instead as inoculum with the same loading ratios. During the start-up phase (days 1-6), iMBRs were operated in batch mode. Then, the iMBRs were switched to semi-continuous mode by performing the filtration cycle before the daily feeding of the reactor. Separation of the VFA-bearing effluent was performed by filtering out 350 ml of permeate a specific initial flowrate of 0.3-0.4 L/h (gives the average flux of 20 L/m²/h (LMH)) before feeding the same volume of the thermally treated FPPL. To compare membrane filtration performance at a different organic loading and VFA production at different solids concentrations, feeding regime began with an organic loading rate (OLR) of 1 gVS /L/d on day 6^{th}, and later increased to 2 g VS/L/d from day 26^{th} (i.e., after approximately 2 hydraulic retention times (HRT) at which the VFA concentration remained stable or unchanged) until day 48^{th}. The initial pH of the MBRs were 8.15 and 8.28 for MBR1 and MBR2, respectively, and the pH during the digestion process was not controlled. Samples were collected every 24 hours from the MBRs' bulk medium for viscosity, COD, total solids (TS), total suspended solids (TSS), ammonium, extracellular polymeric substances (EPS) and soluble microbial product (SMP) analysis.

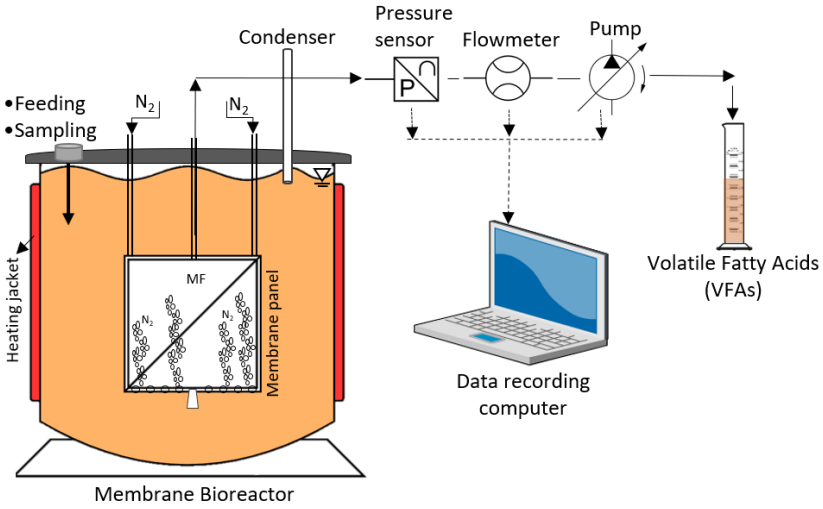

Figure 2.1 : Schematic diagram of the membrane bioreactor setup.

2.2.4 Analytical methods

Concentration and distribution of VFAs (acetate, propionate, iso-butyrate, butyrate, iso-valerate, valerate, and caproate) in the permeate and gas volume and compositions (H_2, CH_4, and CO_2) from the batch digestion were analyzed using Gas Chromatography (GC) (Clarus 550; Perkin-Elmer, Norwalk, CT, USA) as described by Jomnonkhaow et al. (2021). The volumetric gas measurements were transformed to standard conditions at 1.01 bar of pressure and 273.2 K of temperature and presented as NmL as in Fig. 2.3. Prior to analysis, the liquid samples were mixed with an acid mixture (25% (v/v) formic acid and 25% (v/v) ortho-phosphoric acid in a 1:3 ratio) to protonate the -COOH groups of the VFAs needed to facilitate extraction in the sample, and then centrifuged at 8000 rpm for 5 min and the supernatant was then filtered through 0.2 µm syringe filter to remove particulate matter before GC analysis. Butanol solution with a concentration of 1 g/L was used as an internal standard. The pH was determined using a pH meter (Mettler Toledo F20 FiveEasy, OH, USA). Viscosity measurements were carried out with a Vibro viscometer (SV -10, A&D Co., Ltd., Tokyo, Japan). TS, VS and TSS, volatile suspended solids (VSS) were measured using an oven and a muffle furnace at 105±1°C and 550±1°C, respectively, according to the American Public Health standard methods (Federation & Association, 2005). Total chemical oxygen demand (tCOD) and soluble chemical oxygen demand (sCOD) were measured using COD 15000 test kits (range of 1.0-15.0 g/L O_2) and ammonium nitrogen (NH_4^+-N) was analyzed using Ammonium 100 test kits (range of 0-80 mg/L) (Nanocolor, MACHEREY-NAGEL GmbH & Co. KG, Germany). sCOD samples were filtered through a 0.45 µm syringe filters before analysis. Concentrations of

tCOD, sCOD and NH_4^+-N were measured using Nanocolor 500D photometer (MACHEREY-NAGEL GmbH & Co. KG, Germany). For EPS and SMP analysis, protein content of EPS and SMP was measured according to the modified Lowry method (Frølund et al., 1996; Griebe & Nielsen, 1995), and carbohydrate content was analyzed according to Dubois et al. (1956) by using glucose (0-200 mg/L) as a polysaccharide standard and bovine serum albumin (0-200 mg/L) as a protein standard for calibration. SMP, loosely bound EPS (LB-EPS), and tightly bound EPS (TB-EPS) were extracted according to the method described by Li and Yang (2007).

For comparison of VFA yield data from batch experiments, statistical significance was determined using MINITAB® software package (version 17.1.0, Minitab Inc., State College, PA, USA) using one-way analysis of variance (ANOVA, $p<0.05$) with a 95% confidence interval. All experiments were performed in duplicates and the error bars indicated in the graphs represent two standard deviations.

2.3 Results and Discussion

In this study, the valorization of the effluent from fungal cultivation on PPL to VFAs using CKM and CM as inoculum was investigated using both batch assays and semi-continuous iMBRs.

2.3.1 Batch digestion of FPPL to VFAs

In order to investigate the potentials of VFAs generation from FPPL, batch tests were conducted considering thermal shocking, different types of inoculums (CKM and CM), and inoculum to substrate ratios. When exposed to extreme conditions such as high temperatures, acidogenic bacteria can generate spores, whereas methanogens cannot (Setlow, 2014; Valdez-Vazquez & Poggi-Varaldo, 2009; Wainaina et al., 2019a). The ability of acidogens to form spores has been exploited as a strategy to kill methanogens by applying 80°C for 15 min thermal shock pretreatment. The obtained maximum VFAs productions together with calculated yields are summarized in Table 2.3.

Table 2.3 : Summary of pH and produced VFAs from batch assays.

FPPL with CKM as inoculum						FPPL with CM as inoculum					
Substrate and inoculum	**Initial & set pH**	**Final pH**	**Maximum VFA $_{produced}$**			**Substrate and inoculum**	**Initial & set pH**	**Final pH**	**Maximum VFA $_{produced}$**		
			Day	**Concentration (g/L)**	**Yield ($gVFA/gVS_{added}$)**				**Day**	**Concentration (g/L)**	**Yield ($gVFA/gVS_{added}$)**
CKM (0.5 g VS)	7.90 ± 0.01	6.46 ± 0.01	18	2.64 ± 0.20	0.42 ± 0.01	CM (0.5 g VS)	7.41 ± 0.01	7.30 ± 0.10	23	1.76 ± 0.20	0.3 ± 0.01
CKM + FPPL (0.5 g VS:0.5 g VS)	7.90 ± 0.00	6.81 ± 0.10	18	5.56 ± 0.10	0.44 ± 0.01	CM + FPPL (0.5 g VS:0.5 g VS)	7.41 ± 0.01	6.88 ± 0.10	11	5.76 ± 0.20	0.5 ± 0.01
CKM (0.2 g VS)	7.56 ± 0.01	6.76 ± 0.10	18	1.88 ± 0.10	0.75 ± 0.01	CM (0.2 g VS)	7.02 ± 0.01	7.29 ± 0.10	13	1.04 ± 0.20	0.4 ± 0.01
CKM + FPPL (0.2 g VS:0.8 g VS)	7.56 ± 0.00	6.97 ± 0.10	18	5.89 ± 0.10	0.47 ± 0.01	CM + FPPL (0.2 g VS:0.8 g VS)	7.02 ± 0.01	7.04 ± 0.01	11	5.68 ± 0.40	0.4 ± 0.01

The results of VFA concentration and distribution during batch digestion under each of the planned conditions are shown in Fig. 2.2. The maximum VFA concentration of 5.89 ± 0.10 g/L and 5.76 ± 0.20 g/L was obtained from the conditions with CKM as inoculum with an I:S ratio of 1:4 (initial pH of 7.56) and CM as inoculum with an I:S ratio of 1:1 (initial pH of 7.41), respectively.

Normally, the higher the VFA production, the lower the final pH of the medium, however, this is not the case with CKM batch digestion. When comparing CKM + FPPL (1:4) and CKM + FPPL (1:1) results, the former happens to have a slightly higher final pH value. This can be explained by the difference in the starting I:S ratio and the buffering capacity of CKM in the I:S ratio of 1:4 during acidogenic digestion (Table 2.2). For both conditions, a total of 1 g VS was used in batch reactors and the yields obtained under these conditions were 0.47 g VFA/g VS_{added} and 0.46 g VFA/g VS_{added}, respectively. Both inoculums showed similar maximum yields for FPPL at similar pH. The yield for the CKM-only reactors was about 0.75 g VFA/g VS_{added}, while the VFA production was only 1.88 ± 0.10 g VFA/L produced only by 2 g VS of CKM at set pH of 7.56 as the inoculum itself was highly biodegradable compared to FPPL, resulting in higher bioconversion. The maximum VFA production yield for CM was 0.46 g VFA/g VS_{added} at initial pH 7.41. The reason for CKM-only batch fermentation produces a considerable amount of VFAs can be attributed to its substantial sCOD content.

The different digestion substrates/inoculums used affect the optimal pH in the VFA production system. In a batch digestion study (Yin et al., 2021) investigating the effects of initial pH (5.0, 6.0, 7.0, and 9.0) on VFA production from CKM, the highest yield was found to be 0.27 ± 0.01 at an initial pH of 6.0. In the same study, the highest net yield of VFAs was reported to be 0.53 g VFA/g VS (23.11 g/L) using heat shocked inoculum and substrate, and without pH control (Yin et al., 2021). The maximum yield found in the current study for the substrate + inoculum conditions, however, was comparatively lower at 0.47 ± 0.01 g VFA/g VS_{added} with an I:S ratio of 1:4 (initial pH of 7.56). The reason for that could be the initial microbial characteristics of the inoculums used. In another study on anaerobic co-digestion of sewage sludge and cattle manure, it was found that the initial pH of 9.0 significantly increased VFA production and provided a yield of 67.99 ± 1.44 g/kg TS (with a VS ratio of sewage sludge to cattle manure of 3:7) (Dai et al., 2016). Cow manure was also used as an

inoculum for acidogenic digestion of food waste, yielding a VFA production yield of 0.43 g VFAs/g VS where an initial pH of 9 dropped to 7 during VFA fermentation (Tampio et al., 2019). In the current study, the slighter lower yields achieved compared to the abovementioned studies could be due to the type of substrate and load of soluble organic matter and the non-optimal initial as-mixed pH values.

In addition to the efficiency in VFA production, the change in VFA composition due to use of 2 different inoculums (CKM and CM) was also investigated. As expected, acetic acid was the predominant VFA in most conditions, followed by propionic and butyric acids, except for days 2 and 3 in for the FPPL + CM assays. The results indicate that the VFA composition was not affected by the difference in inoculum. A similar conclusion was drawn by Atasoy et al. (2019), who worked with three different inoculum types (large and small granular, and slurry) and emphasized that inoculum type did not significantly affect the VFA composition. This shows that the microorganisms in inoculums are more acclimated to produce similar VFA patterns.

Fig. 2a, 2c, 2e and 2.2g show results acquired from batch reactors loaded only with inoculum (as control) along with the ones with a mixture of substrate and inoculum. The maximum total VFA (tVFA) concentrations of 5.56 ± 0.10 g/L and 5.89 ± 0.10 g/L were achieved for CKM + FPPL (1:1) and CKM + FPPL (1:4), respectively, on day 18. When CM was used as inoculum, 5.76 ± 0.20 g/L and 5.68 ± 0.40 g/L of VFAs were detected on day 11 for CM + FPPL (1:1) and CM + FPPL (1:4), respectively. As expected, the reactors operating with inoculum only had lower concentrations of VFAs than the reactors to which FPPL was added. According to the findings, substrate is crucial to the production of VFAs. The difference between the two shows the contribution of FPPL to the VFA generated.

The cumulative gas productions for different conditions in batch experiments are presented in Fig.2.3 presenting changes in H_2, CH_4 and CO_2 generation volumes. According to the obtained CH_4 production results for CKM (CKM + FPPL, pH 7.90 and CKM + FPPL, pH 7.56), it was found that methane production was almost completely inhibited in heat shocked assays as they showed almost no production and remained stable during digestion (Fig. 2.3b). However, for the inoculums (CKM, pH 7.90 and CKM, pH 7.56), signs of methane production were observed from day 13 and day 9, respectively. Moreover, in the case of the reactors inoculated by CM, thermal pretreatment was able to hinder methanogens during almost the entire digestion period

under all conditions, except for CM with pH 7.41, where 0.7 mL CH_4/g VS production started on the last day of digestion (day 23) (Fig. 2.3e). The effectiveness of heat shock treatment at different temperatures on methane inhibition has been confirmed in different literature, with some reporting methanogens recovering in long runs (Jomnonkhaow et al., 2021; Mei et al., 2016) which can back up what has been happening in this study. Carbon dioxide and hydrogen production commenced on day 1 in all batch fermenters. Carbon dioxide content appears to be higher for CM compared to CKM (Fig. 2.3c and Fig. 2.3f). The CO_2 yields for FPPL at initial pH of 7.90, FPPL at pH 7.56 together with CKM as inoculum and FPPL at pH 7.41, FPPL at pH 7.02 together with CM were similar under the tested conditions and ranged from 7.5-14 mL/g VS. Hydrogen gas that is produced and VFAs have some parallels as they are both mediators and precursors to biogas synthesis. As a result, hydrogen gas is typically created as a byproduct of bioprocesses that are advantageous for high-yielding VFA generation, and vice versa (Mondylaksita et al., 2021). CKM only at pH 7.9 showed the highest H_2 yield of 11.7 mL/g VS. Stabilization of H_2 production occurred shortly after for FPPL with CKM and CM batch reactors (Fig. 2.3a). Stabilization of the cumulative production data could be explained by gas production having come to a halt, interaction of other operating factors or the amount of gas produced being equal to the conversion of it in the complex anaerobic metabolism (Guo et al., 2010).

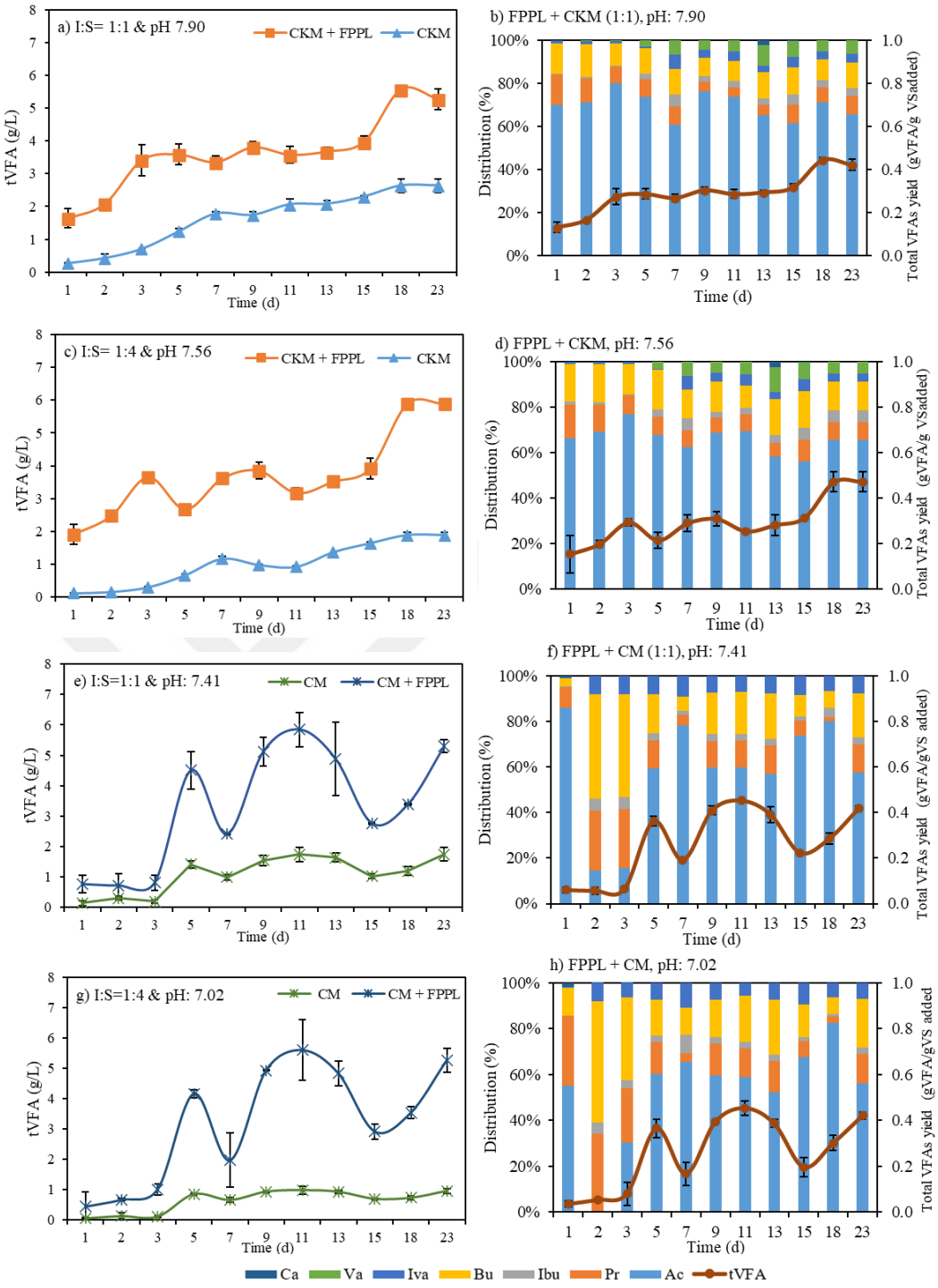

Figure 2.2 : VFAs concentration and distribution during batch experiments.

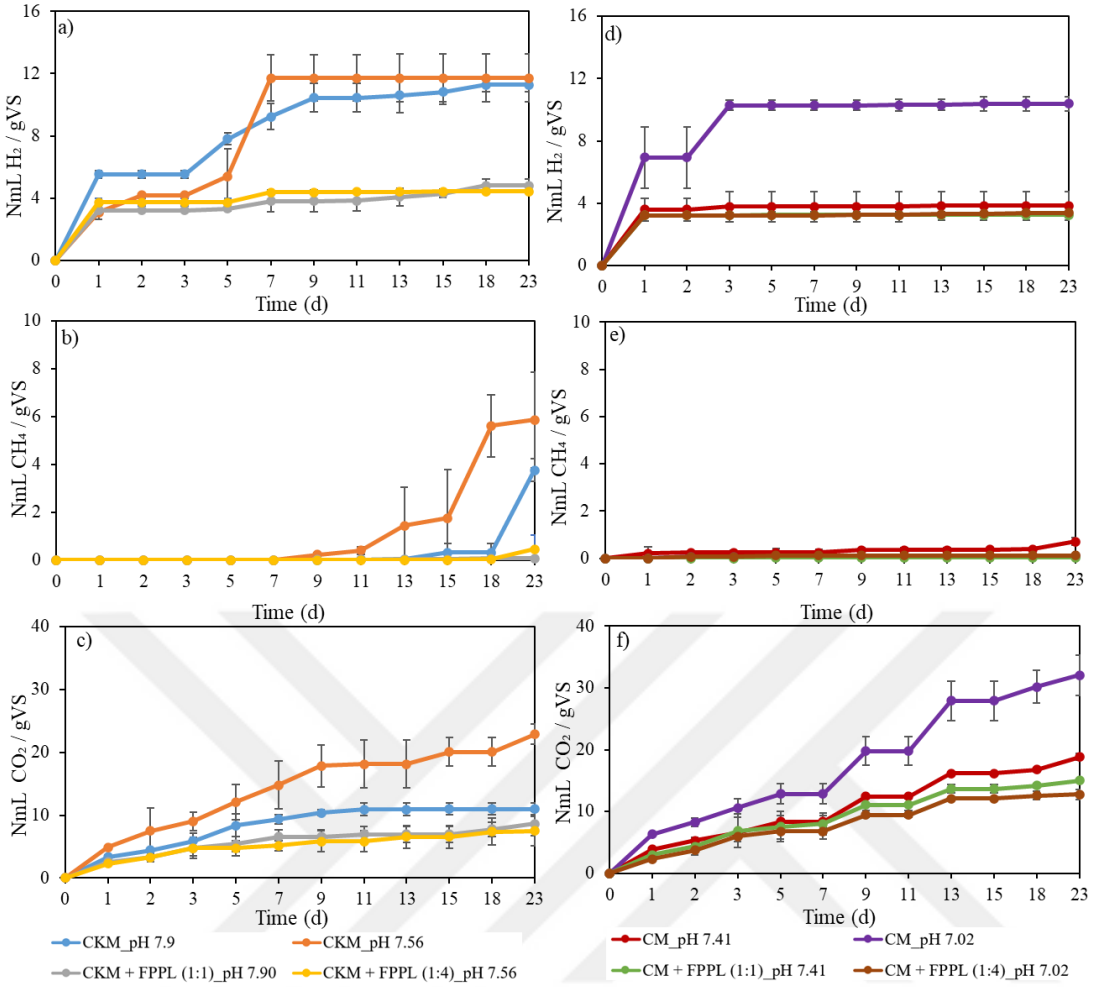

Figure 2.3 : Changes in the cumulative gas production yields (a) H_2, (b) CH_4, (c) CO_2 for (FPPL+CKM), and (d) H_2, (e) CH_4, (f) CO_2 for (FPPL+CM) during batch experiments.

2.3.2 VFAs production and recovery using iMBR

Semi-continuous anaerobic iMBRs were used for acidogenic fermentation and *in situ* recovery of VFA-bearing permeate using FPPL as feed and CKM (MBR1) and CM (MBR2) as inoculums. They were run for 48 days with 6 days of startup. The concentrations and compositions of VFAs in the iMBR during the anaerobic digestion process are shown in Fig. 2.4.

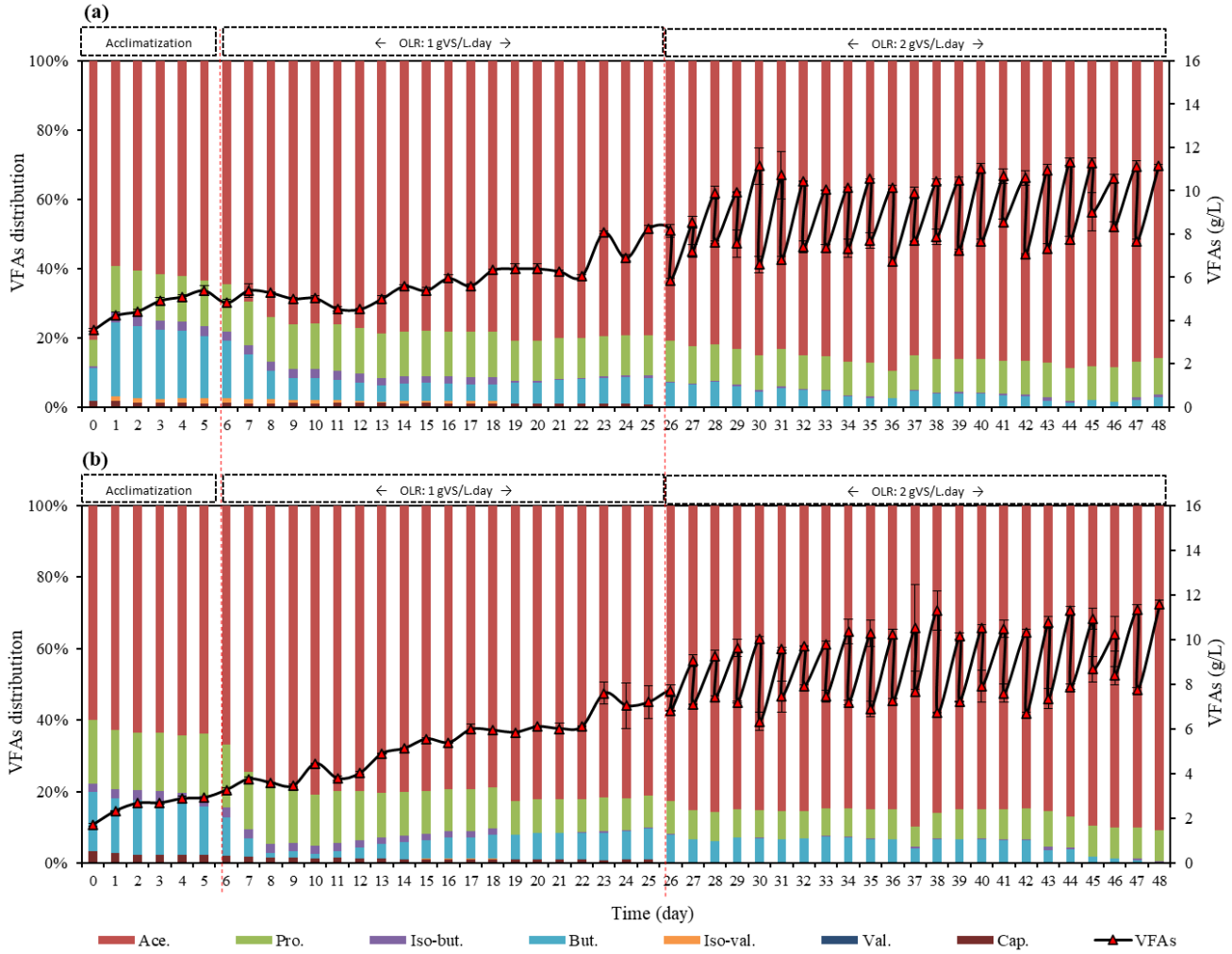

Figure 2.4 : Concentration and distribution of VFAs during digestion in the (a) MBR1 and (b) MBR2 at OLR 1 and 2 g VS/L/d.

Acetic acid is often the main component of the tVFAs during anaerobic digestion (Srisowmeya et al., 2020). This was also the case in the present study, where acetic acid comprised 59 – 97% of the tVFAs in both reactors and at both operating OLRs. The features of the substrate and inoculum, the operating conditions, and the initial pH and pH changes during digestion by the microbial community responsible for the specific acid type all have an impact on VFA distribution (Bedaso, 2019; Lee et al., 2014). Following acetic acid, butyric acid (1 – 21%) and propionic acid (6 – 18%) were the second and third largest VFA components in the digestion of FPPL (Fig. 2.4). Longer-chain fatty acids such as valeric and caproic acids were produced at very low concentrations. This is in line with the results from batch experiments (Fig.2.2).

The iMBRs were initiated in batch mode and accumulation of VFAs during batch mode reached 5.39 ± 0.23 g/L for MBR1 and 2.93 ± 0.02 g/L for MBR2 on day 5 and stabilized thereafter. Afterwards on day 6, batch mode was switched to semi-continuous mode with a OLR of 1 g VS/L/d. The VFA production in this OLR increased to a maximum of 8.25 g/L for MBR1 and 7.61 g/L for MBR2. The average of tVFAs on MBR1 and MBR2 were 5.84 ± 1.03 g/L and 5.26 ± 1.31 g/L, respectively, during the period of day 6 to day 26. In order to enhance production and yield of VFAs, OLR is one of the most important operational parameters to be focused on. Applied OLR also has a great impact on the microbial community structure (De Groof et al., 2021). The change in the microbial community can lead to changes in the metabolic pathways, which means a different distribution of the individual VFAs (Li et al., 2021). Therefore, the OLR was doubled to 2 g VS /L/d on day 26, which required feeding and filtration twice a day (one in the morning and one in the evening) due to the low VS content of the feedstock (9.2 ± 0.08 g/L). VFA samples were taken daily before the first and second filtration and feedings, and the results of both VFA profiles are shown in Fig. 2.2. When the OLR was doubled, the average total production of VFAs in both reactors increased to 10.40 ± 0.79 g/L for MBR1 and 10.22 ± 0.86 g/L for MBR2, which is an increase in VFA production of almost 44% and 48% for MBR1 and MBR2, respectively. The systems stabilized during day 40 - 48 in terms of VFA production. Further increases in the various OLRs could not be attempted due to the low VS of the feedstock. It was observed that doubling the OLRs resulted in a higher increase in MBR2, although the average concentrations for MBR2 were always lower compared to MBR1. Moreover, the maximum VFA yield was calculated as 0.65 g VFAs/g VS_{fed}

and 0.36 g VFAs/g VS_{fed} for OLR 1 and 2 g VS/L/d in MBR1, and 0.54 g VFAs/g VS_{fed} and 0.35 g VFAs/g VS_{fed} for OLR 1 and 2 g VS/L/d, respectively in MBR2. The reduction of the VFA yield by increasing substrate loading, and hence the presence of an optimum OLR value, has already been described in the literature (Jomnonkhaow et al., 2021). When this point is achieved, the first phase of acidogenic digestion becomes restricted. These studies attribute this point of inflection to the system's hydrolytic capacity with increased concentrations of different compounds such as K^+, Na^+, chlorophenols, and heavy metals (Cu>Zn>Cr>Cd>Ni>Pb) that are responsible for acidogenic inhibition might be an influencing parameter that leads to an inhibition of the acidogenic step (Magdalena et al., 2019). The pH of the reactors was not controlled or adjusted during the digestion which can be another reason of this reduction on VFA yields. Methanogens should be kept in an environment with controlled pH and temperature because these factors are essential for the anaerobic bacteria's ability to function. Methanogenesis requires pH levels of 6.5-7.5, while acidogenesis and acetogenesis need pH levels of 5-5.5 (Osman et al., 2021). The average pH during the whole semi-continuous process in present study was 8.10 ± 0.13 and 8.31 ± 0.15 at MBR1 and MBR2, respectively. In literature, the production of VFAs from potato related residues are limited to potato peel waste or waste potato (den Boer et al., 2016; Li et al., 2019; Lu et al., 2020; Ma et al., 2017). In one of these studies (Lu et al., 2020), the waste of peeled potatoes from a student cafeteria was used to produce VFAs and the highest VFA production was found to be 41.9 g COD /L and 632.2 mg COD /g VS_{fed} at a pH of 7.0. Li et al. (2019) also reported the importance of pH control and microbial diversity analysis for feasible and efficient acidogenic digestion in a study using potato waste from a vegetable processing center and an anaerobic sludge as inoculum for VFA production. The highest concentration and proportion of VFAs were observed as 7.8 g/L and 49.7% for butyric acid at pH 6.0 (Li et al., 2019).

2.3.3 iMBRs performance during anaerobic digestion and filtration

In the current research, two identical iMBRs were used to conduct in situ recovery of VFA-containing effluent during acidogenic digestion of FPPL. Semi-continuous production and recovery of VFAs was performed at an HRT of 10 days by filtering out 350 ml of the liquid medium as permeate before feeding on daily basis. Membrane filtration was performed over a 48-day period at a set initial permeate flux of 20 LMH. After approximately 2 HRTs of filtration and feeding at OLR of 1 g VS/L/d, the OLR

was increased to 2 g VS/L/d (day 26). During filtration, membrane filtration performance, and the changes in the membrane surface condition and the extent of fouling was tracked by observing changes in the filtration parameters (TMP and flux) and medium characteristics (SS, viscosity etc.).

As shown in Fig. 2.5a, the starting TMP for the MBR2 was slightly higher than that of MBR1 which is due to different ages (consecutive experiments that the membranes were exposed to previously) of the membranes in the MBRs. Another reason may be due to the characteristics of the inoculum (CKM and CM) that may have affected the initial TMP through initial adsorption of a layer of foulants to the membrane surface at first contact. TMP fluctuated between 0.011 - 0.103 bar throughout the digestion period for MBR1 (Fig. 2.5a). The trend for the increase in the concentrations of TSS in MBR1 indicated accumulation rates of 0.13 g TSS/L/d. The average TSS at OLR of 1 g VS/L/d was 4.71 ±1 g/L. Although, this value increased to 8.25 ± 2 g/L at OLR 2, an unlimited solid retention was considered where no drainage of the accumulated sludge was performed throughout the digestion. The average viscosity for MBR1 was 0.93 ± 0.12 mPa.s during OLR1 and 0.99 ± 1.10 mPa.s during OLR2. The difference in viscosity could not have been the cause of TMP difference as the viscosities are low and rather in the same range during the digestion period. TMP fluctuated between 0.064 - 0.219 bar throughout the digestion period for MBR2 (Fig 2.5a). The trend for the increase in the concentrations of TSS in MBR2 indicated accumulation rates of 0.17 g TSS/L/d. The average TSS at OLR of 1 g VS/L/d was 5.16 ± 1.5 g/L in MBR2. Similarly, as seen in MBR1, TSS value increased to 9.10 ± 2.3 g/L at OLR 2 for MBR2, an unlimited solid retention was considered where no drainage of the accumulated sludge was performed throughout the digestion. The low accumulation of TSS compared to similar studies (Barreto et al., 2017; Jomnonkhaow et al., 2021; Wainaina et al., 2020) is due to the low solid content (TS and VS) of the fed substrate in addition to the ease of breakdown and solubilization of organic suspended solids (Table 2.1).

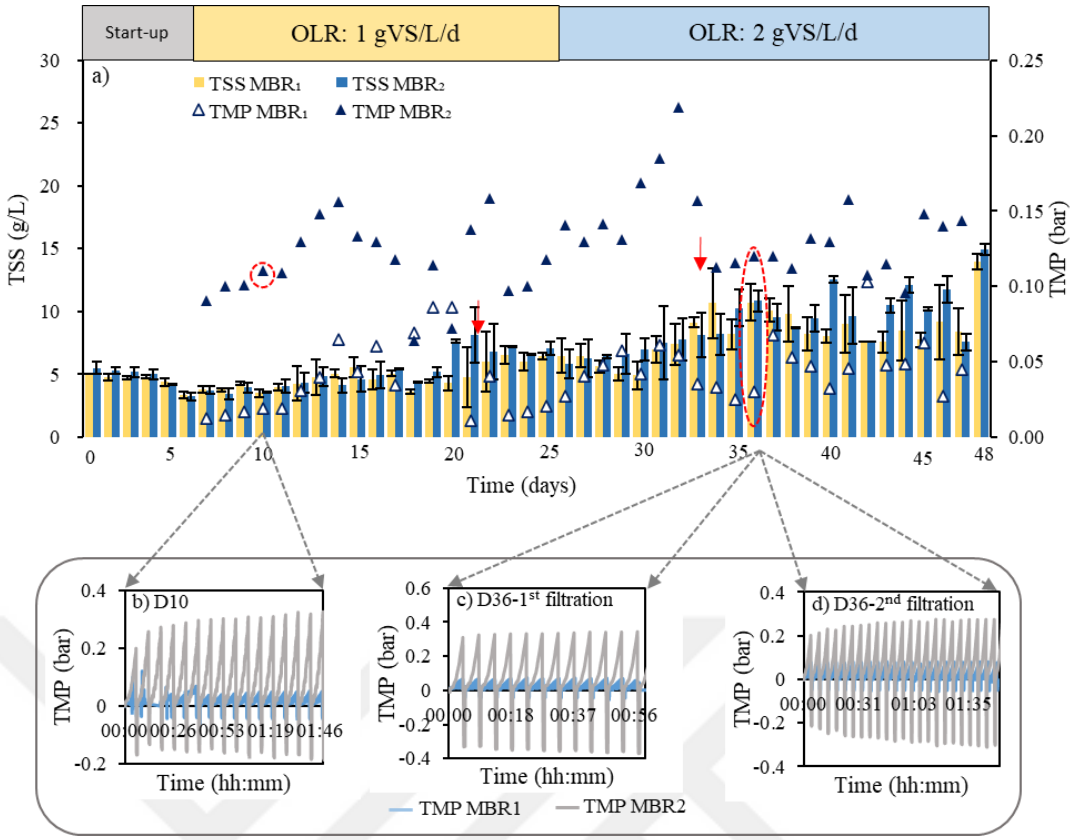

Figure 2.5 : Changes in TMP and TSS during digestion and filtration in the immersed membrane bioreactor at different OLRs (the red arrows represent membrane chemical cleaning incidents).

Considering the changes in the membrane surface condition and resistance to liquid permeation, the TMP of MBR1 and MBR2 maxed at 0.10 bar and 0.22 bar on day 42 and 32, indicating the highest membrane fouling throughout the period (Fig. 2.5a and 2.5b). At a relatively high flux of 20 LMH, a constant but gradual increase in TMP was observed even though TMP levels are very different in both MBRs. This increase can also be attributed to the changes in the membranes surface condition due to cake layer build up and its subsequent resistance to permeate flow (Judd, 2010). The increase in the TMP together with medium TSS content shows the gradual deposition of foulants on the membrane surface gradually adding cake layer resistance to membrane hydraulic resistance. As presented in Fig. 2.5b, 2.5c and 2.5d which illustrate TMP patterns at different intervals, MBR1 using CKM as inoculum was the least problematic when it comes to the changes in membrane hydraulic resistance. The first and second filtering cycles on day 36 appear to be perfect repeats in both MBRs, with no decrease in performance (Fig. 2.5c and Fig. 2.5d). As indicated in Fig. 2.5a with red arrows, membrane chemical cleaning was performed in two occasions when the TMP had values between 0.15-0.20 bar. Although chemical cleaning temporarily

reduced the TMP, fouling re-occurred progressively as the conventional physical fouling preventive method of gas scouring and backwashing proved rather ineffective. Although TSS has been quite stable, the development of a biofilm and the deposition of biopolymers (proteins, polysaccharides) that cannot be easily removed by backwashing could have contributed to hydraulic resistance (Meng et al., 2017; Sambusiti et al., 2020). For this reason, the concentration of carbohydrates, proteins and humic like substances in EPS and SMP were analyzed to determine their contribution to the rise in TMP observed during filtration.

2.4 Conclusions

The effluent from fungal cultivation on PPL was used to further maximize value creation from the potato starch industry through acidogenic digestion of VFAs. The effect of inoculum type and substrate to inoculum ratio on VFA yield on FPPL was investigated. iMBRs inoculated with either cow or chicken manure were used to generate a particle- and microorganism-free VFA-bearing effluent with a maximum yield of 0.65 g VFAs/g VS_{fed} recovered at a flux of 20 LMH in a long-term (48-day) digestion and filtration process. The findings prove that a multi-stage bioconversion of potato starch industry residues to value added products with the aim of nutrient reuse and resource circularity can be realized.

3. WASTE-DERIVED VOLATILE FATTY ACID PRODUCTION AND AMMONIUM REMOVAL FROM IT BY ION EXCHANGE PROCESS WITH NATURAL ZEOLITE[2]

3.1 Introduction

In order to meet the Sustainable Development Goals and prevent global warming, transmission from a linear to circular economy and conversion of waste back to resources should be implemented (Taherzadeh, 2019). One way to realize the abovementioned goals is to generate highly recovered and sought-after chemicals and materials from waste rather than fossil sources. Volatile fatty acids (VFAs) are sought-after platform chemicals such as acetic, propionic, and butyric acids with great market interest that have conventionally been synthesized from fossil resources. However, these acids are intermediates of microbial degradation of organic wastes, so they can be obtained from anaerobic digestion (AD) of organic wastes and thus contribute greatly to a sustainable circular economy. The acid-forming bacteria in AD processes, which include acetogenic bacteria and homoacetogenic bacteria, are the group of microbes that are primarily in charge of producing VFAs (Yuan et al., 2019). These VFAs represent a group of valuable carboxylic acids used for a variety of applications, including bioenergy production (Cesaro et al., 2020; Chen et al., 2017; Sydney et al., 2018), biodegradable plastics (Fradinho et al., 2014; Reddy et al., 2014), an additional carbon source for enhanced biological nitrogen and phosphorus removal from wastewater (Kim et al., 2016; Liu et al., 2018; Zhu et al., 2021). In addition, VFAs can also be used as food additives and animal feed supplements (Fujihara et al., 2002; Rigout et al., 2003).

[2]This chapter is based on: Sapmaz, T., Mahboubi, A., Taher, M. N., Beler-Baykal, B., Karagunduz, A., Taherzadeh, M. J., & Koseoglu-Imer, D. Y. (2022). Waste-derived volatile fatty acid production and ammonium removal from it by ion exchange process with natural zeolite. Bioengineered, 13(6), 14751–14769.

Anaerobic digestion, a common bioconversion approach used mainly for treating organic waste, is a promising option for producing VFAs from renewable and cheap resources in a sustainable manner. AD uses organic-rich waste streams as input material and traditionally converts them to the final product of biogas through a sequential microbial conversion process involving hydrolysis, acidogenesis, acetogenesis, and methanogenesis. VFAs are intermediate products of AD (generated during the acido- and acetogenesis stages) with a higher market value than biogas, which need to be efficiently produced**,** recovered, and purified from AD effluent in order to be commercialised. The purer VFA solution has a higher market price and a wider range of applications from production of pharmaceuticals to wastewater treatment (Atasoy et al., 2018). In-depth studies on traditional pre-treatment and co-fermentation methods have also been conducted in the literature with the aim of increasing VFA production and composition, as well as the effects of some key variables that influence VFA composition and production (Fang et al., 2020). However, the recovery process of VFAs still poses a great challenge because the AD effluent is a complex medium containing microorganisms, particulate matter, macromolecules, and dissolved nutrients such as nitrogen (N), potassium (K), and phosphorus (P) (Barłóg et al., 2020). Some separation techniques such as precipitation, distillation, adsorption, ion exchange, liquid-liquid extraction, and/or membrane processes can also be used to separate microorganisms and particles as well as macromolecules such as proteins and lipids from AD effluents (Aktij et al., 2020). Among the newly developed separation technologies for VFA recovery from anaerobic digestate, membrane-based processes are considered to be very attractive and competent methods (Aktij et al., 2020).

The high ammonia content in the AD effluents is one of the challenges in building a VFA platform. Considering the potential use of VFAs as an additional carbon source for wastewater denitrification process, or the production of polyhydroxyalkanoates (PHAs) as bioplastics, the NH_4^+ content of the effluent is an undeniable drawback. Although the presence of a certain amount of ammonium is beneficial for microbial growth in AD, inhibitory concentrations can be reached during the degradation of protein-rich substrates. Therefore, in processes where limited nitrogen concentrations are important, the excess amount of ammonium in the VFA-containing AD effluent should be removed with minimal loss of VFAs. Common technologies used for

ammonium removal include breakpoint chlorination, air stripping, nitrification-denitrification, and ion exchange (Metcalf et al., 1991). Among these methods, ion exchange has gained great research and industrial attention due to process simplicity and the application of low-cost abundant raw minerals as exchangers (Inglezakis et al., 2001a). For this purpose, natural zeolites such as clinoptilolite could be applied as the ion-exchanging material. The high selectivity of clinoptilolite for ammonium ion is an asset and gives it advantage over conventional and synthetic exchange resins such as purolite and amberlite (Jorgensen & Weatherley, 2006).

Ion exchange is a special type of adsorption based on electrostatic attraction. In this process, the pollutant (adsorbate) or compound to be removed is separated from the liquid phase and concentrated on the solid phase (adsorbent). Ion exchange can also be considered as a reversible chemical reaction driven by electrostatic attraction between the ions in solution (adsorbate) and the exchangeable ion on the ion exchanger (Weber Jr, 1972). There are many studies using the ion exchange process to recover nutrients from different types of waste streams (Beler-Baykal et al., 2011; Beler-Baykal et al., 1996; Ishii & Boyer, 2015; Kocatürk & Baykal, 2012). However, to the knowledge of the authors, the removal of ammonium from the organic waste derived AD-VFA effluent with clinoptilolite has not been studied. Therefore, it is of great importance to understand the ion exchange performance of clinoptilolite for the removal of ammonium from VFAs mixtures. This is an important application since production of VFAs and removing contaminants like ammonium may be key technology in the upcoming biorefineries. It is essential to carry out an experimental study to assess the different effects of the various processes on the functionality of VFA production and recovery to comprehend how this can be done and which operational elements influence the recovery of purer and higher concentrated VFAs from fermentation broths.

The objective of this study is to investigate a well-established process, ion exchange, for a new application, namely the removal of ammonium from bio-based VFAs mixtures. The purpose of this study is to research the removal of ammonium from synthetic and chicken manure derived AD-VFA solutions by ion exchange using natural zeolite (clinoptilolite). Following this, batch tests will be used to determine the effects of initial ammonium loading, pH, and the most common competing ion, K^+, on ammonium removal. The experimental steps of ion exchange process that were carried

out includes isotherm studies, batch-column experiments for ammonium removal, and continuous experiments for producing breakthrough curves.

3.2 Materials and Methods

3.2.1 Chemicals

Acetic acid (>99.7%), propionic acid (>99.5%), butyric acid (>99%), NH_4Cl and NaCl were purchased from Sigma-Aldrich (Burlington, MA, US) and KCl of reagent grade was provided from Merck (Darmstadt, Germany). The water used in the preparation of all synthetic solutions was ultrapure (Milli-Q at 25 °C).

3.2.2 The solid phase: Clinoptilolite

The natural zeolite, clinoptilolite, which is an alumina silicate was used as the ion exchanger. It was obtained from Rota Mining Corporation and originated from the mines of the Gordes region in Manisa, Turkey. Its mineralogical composition was determined by Rota Mining (Rota, 2021) as 90-95% clinoptilolite, 0-5% cristobalite, and 0-5% tridymite. The shared oxygen atoms between aluminium and silicon in clinoptilolite result in a negative charge that is balanced by the presence of exchangeable cations such as Ca^{2+}, K^+, and Na^+, depending on the composition. The chemical formula of clinoptilolite is $(Ca, K_2, Na_2, Mg)_4\ Al_8\ Si_{40}\ O_{96}.24H_2O$ with chemical composition shown in Table 3.1 (Rota, 2021). Its theoretical exchange capacity was 1.5-2.1 meq/g based on the mineralogical composition according to Methylene Blue Chloride Method as indicated in the product information sheet by Rota Mining Corporation (Rota, 2021). The apparent density of the clinoptilolite was found to be 0.86 g/cm^3 using the standard method. Clinoptilolite was firstly sieved to obtain a specific particle size, which in this study was 1-2 mm. The sieved clinoptilolite was then washed several times with distilled water to remove non-adherent impurities and small particles, followed by air-drying for 24 h to remove moisture. It was proven in the literature that the sodium form of clinoptilolite is the most recommended form to provide the highest capacity for the removal of ammonium, therefore, Na-form of clinoptilolite was used for the experiments (Yurtoğlu, 2007). Clinoptilolite has a monoclinic platy crystal structure with angular granules as its physical appearance *(Rota, 2021)*. The utilized particle size ranged from 1000 to 2000 μm. The conditioning of clinoptilolite aims to remove certain ions from the surface to replace them with

easily exchangeable ions prior to ion exchange application. Clinoptilolite in the sodium form is known to have a higher ammonium removal capacity as compared to its original form (Cincotti et al., 2001; Cooney et al., 1999; Inglezakis et al., 2001b; Yurtoğlu, 2007), therefore, using the sodium form is beneficial (Hedström, 2001). For this reason, prior to the ion exchange process, conditioning was carried out to increase the ammonium holding capacity of clinoptilolite. Using a 1 M NaCl solution according to the procedure recommended by Yurtoğlu (2007), at flow rate of 11.7 L/min for up to 48 h. Prior to use in experiments, the clinoptilolite was dried at 103-105 °C to remove moisture in an attempt to obtain constant weight. The same batch of conditioned clinoptilolite was used in all runs of the experimental work.

Table 3.1 : Characteristics of the clinoptilolite (from Rota (2021)).

Constituent	Properties/ Value (wt.%)
SiO_2	65 – 72
Al_2O_3	10 – 12
CaO	2.4 – 3.7
K_2O	2.5 – 3.8
Fe_2O_3	0.7 – 1.9
MgO	0.9 – 1.2
Na_2O	0 – 0.08
MnO	0 – 0.08
Cr_2O_3	0 – 0.01
P_2O_5	0.02 – 0.03

3.2.3 Liquid phase

Two different solutions were used: waste-derived anaerobic digestion VFA effluent (AD-VFA) and synthetic VFA solution (sVFA).

3.2.3.1 Anaerobic digestion VFA effluent (AD-VFA)

Chicken manure (CKM) was used as AD substrate and inoculum was collected from a laying hen farm Sjömarkens Hönsgård AB (Borås, Sweden) with total solid (TS) and volatile solid (VS) concentrations of about 320 and 220 g/L, respectively. To ensure consistency, as-received CKM was diluted with tap water to VS of about 120 g/L (~2 times dilution) and mixed with a high-performance blender (Waring® CB15, CT, USA) at high speed for about 30 s. The liquid phase was separated with a sieve (mesh size about 0.1 mm) and the characteristics were checked after thorough mixing.

Thermal pretreatment (thermal shock) was used to inhibit the activity of methanogens in CKM. Each feed solution was placed in a 100 mL of flask, then heated to 80 °C for 15 min in a water bath while being completely mixed at a speed of approximately 300 rpm to ensure a homogeneous temperature, and then cooled in an ice chamber. Some properties of the sieved thermally shocked CKM (the feed for AD) are pH of 7.9 ± 0.1, total VFA concentration of 4.5 ± 0.2 g/L, of which acetic acid content of 3.8 ± 0.2 g/L, and ammonium concentration of 4500 mg/L. The supernatant from the heat shocked CKM (mesh size approximately 0.1 mm) was diluted to approximately 10 g VS /L and used as inoculum for the production of VFAs effluent in an anaerobically digested membrane bioreactor (MBR). The operation of MBRs is described more detailed in Section 2.4.1. The VFA solution filtered from the reactor each day was collected and stored in the freezer before it was used further in experiments. AD-VFA solution that is mixed and stored had a pH of 6.8, ammonium of 1955 ± 135 mg/L and total VFA concentration of 7 ± 0.2 g/L (Table 3.2). AD-VFA solution was used in the isotherm studies and the batch mode fixed bed column operation during this study.

Table 3.2 : The properties of recovered AD-VFA.

Parameters	Unit	Range of the effluent during fermentation	VFA effluent used in this study
pH	/	6.02 – 8.18	6.8 ± 0.1
NH_4-N	mg/L	1381 – 2262	1955 ± 135
tCOD	g/L	12.0 – 68.7	13.8 ± 0.6
Acetic acid	g/L	5.0 – 11.9	5.37 ± 0.2
Propionic acid	g/L	0.8 – 3.1	0.81 ± 0.1
Butyric acid	g/L	0.6 – 2.4	0.75 ± 0.1
Caproic acid	g/L	0.05 – 0.15	0.07 ± 0.0
Total VFAs	g/L	6.50 – 18.0	7.0 ± 0.2
Na^+	mg/L	986.3 – 1045.7	1016 ± 29.7
K^+	mg/L	1535.6 – 1572.4	1554 ± 18.4
Ca^{2+}	mg/L	32 – 33	33 ± 0.0
Mg^{2+}	mg/L	59.4 – 63.6	61.5 ± 2.1
Fe^{2+}	mg/L	1.8 – 3.2	2.5 ± 0.7

3.2.3.2 Synthetic VFA solution (sVFA)

The synthetic VFAs solution was prepared by dissolving acetic acid, propionic acid, butyric acid and NH_4Cl in Milli-Q water to represent VFAs and ammonium content. The total concentration of VFAs was arranged as 7.1 g/L theoretically to simulate waste-derived VFA effluent (AD-VFA) content including acetic, propionic, butyric acids at the ratio of 4.5:2:0.6, respectively. pH of the solution was measured as 2.8 ± 0.2 and the amount of NH_4^+ was changed in the range of 500-2070 mg/L. In order to study the effect of K^+ ion on process performance, KCl solution was prepared as 0.5 and 2 M for required K^+ ion concentration. Synthetic VFA solution was used in batch experiments (the effect of initial loading, pH, and K^+ concentration), batch mode fixed bed column operation and breakthrough experiments to understand the system behaviour of processes investigated.

3.2.4 The experimental setup

3.2.4.1 Experimental set up and operation of MBRs for AD-VFA production

In order to generate the VFAs effluent required for this study, MBR using CKM as feed was used. The MBRs that were operated semi-continuously (Fig.2.1) used for the anaerobic digestion of CKM consisted of a reactor with a 2nd generation microfiltration integrated permeate channel membrane panel (PES, 0.3 µm average pore size; VITO NV, Belgium), with a filtration area of 205.8 cm^2 submerged in a reactor (Biotech GmbH, Germany) and with 3.5 L working volume. Permeate flow and membrane pressure were recorded automatically using a control recording system developed in-house. pH, the concentrations of VFAs, and ammonium concentrations from permeate in the reactor were recorded by daily. The 350 mL of permeate containing VFA was filtered off daily and kept in freezer before use in ion exchange experiment.

3.2.4.2 Isotherm experiments

Waste-derived AD-VFA was used in the isotherm experiments. In order to generate the isotherms for ammonium removal by clinoptilolite, the equilibrium time was determined first using AD-VFA solutions with concentrations of 1930, 1035, 801, 524 mg NH_4^+/L prepared by serial dilution of AD-VFA. After that, the isotherm experiments were carried out to determine the capacity of clinoptilolite for ammonium in AD-VFA mixture in duplicates (Eq. 3.1) (Balarak & McKay, 2021).

$$\text{Equilibrium capacity (mg } NH_4^+ \text{ /g clinoptilolite), } (q_e) = \frac{(C_0 - C_e) \times V}{m} \quad (3.1)$$

C_0 is the initial ammonium concentration (mg/L), C_e is the equilibrium concentration of ammonium in the solution (mg/L), V is the volume of the solution (L), m is the mass of the clinoptilolite used (g), q_e is the amount of adsorbate per mass of the adsorbent under equilibrium conditions (mg/g). During the isotherm experiments, 2.0, 4.0, 6.0, 10 and 20 g clinoptilolite was put in each flask including 50 mL of AD-VFA solution having 1955±135 mg/L of ammonium placed in a water bath-shaker (Grant OLS 200, Grant instrument ltd, UK) for 12 h which had been determined as the equilibrium time. The ammonium concentrations of the samples were measured at the beginning of experiment and then at the equilibrium time (12 h)**.** All experiments were performed at temperature of 22±2 °C. Isotherms were plotted as the amount of ammonium adsorbed onto the clinoptilolite, q_e, against the equilibrium concentration of ammonium in the solution, C_e. The experimental results were fitted to Langmuir Isotherm model (Eq. 3.2) and Freundlich model (Eq. 3.3) to determine the best isotherm model that represented the experimental data.

$$q_e = q_m K_L \frac{C_e}{1 + K_L C_e} \quad (3.2)$$

$$q_e = K_F C_e^{1/n} \quad (3.3)$$

In these non-linear expression of isotherm models, K_L is a Langmuir constant associated with adsorption energy or net enthalpy, K_F is the Freundlich constant and n is a constant with a function of adsorption intensity and q_m is the surface concentration obtained when all adsorption sites are exhausted (maximum capacity of adsorbent) (Chen, 2015).

3.2.4.3 The effects of pH, initial ammonium loading and competing cation (K^+) in batch experiments

Batch experiments were performed as duplicate to investigate the influence of pH (3, 5, 6, and 7), initial ammonium loading (2, 2.5, 5.0, and 10 mg NH_4^+/ g clinoptilolite), and competing ion-K^+ concentration (500, 1000, and 2000 mg/L) using a synthetically prepared VFA solution. The experiments were carried out in the batch mode using conical Erlenmeyer flasks of 250 mL (working volume of 200 mL) placed in a temperature-controlled water bath shaker at 110 rpm and with a temperature of 22±1 °C. The aforementioned synthetic VFAs mixture (Section 2.3.2) was used at this stage.

The effect of pH on the removal of ammonium by the ion exchange process with clinoptilolite has been studied in the literature (Karadag et al., 2008). In this study, since the removal of ammonium was studied in the presence of VFAs, and the tests were carried out at different pH values for observing the changing of VFA species with pH changes. The pKa values of the targeted VFAs are reported as 4.76 for acetic acid, 4.88 for propionic acid, and 4.82 for butyric acid. The pH values of AD-VFA solutions may change based on the substrate type and the operational parameters of the anaerobic digester. The pH values of the synthetic VFAs mixture and AD-VFA solution were measured as 2.8 ± 0.2 and AD-VFA and 6.8 ± 0.1, respectively. Thus, the effect of pH on overall ion exchange performance was investigated at 3.0, 5.0, 6.0 and 7.0. The ion selectivity of clinoptilolite was given by the manufacturer as $Cs^+ > NH_4^+ > Pb^{2+} > K^+ > Na^+ > Ca^{2+} > Mg^{2+} > Ba^{2+} > Cu^{2+} > Zn^{2+}$ (Rota, 2021). In the experiments of competing ion effect, different K^+ concentrations in the range of 500 to 2000 mg/L were tested due to high concentration of potassium ion (K^+: 1554 ± 18.4 mg/L) that could compete with the ammonium ion strongly in the exchange process (Table 3.2).

The effect of initial ammonium loading (mg NH_4^+ initially loaded per gram of clinoptilolite) was studied in the batch mode by changing clinoptilolite loading through the use of constant ammonium (760.50 ± 54.83 mg/L) and VFAs concentrations (7.46 ± 0.51 g/L) and variable amounts of clinoptilolite. All the experiments were carried out at the same stirring rate and in duplicates.

3.2.4.4 Batch fixed bed column experiments

In this part of experiments, the ion exchange experiments for the removal of ammonium from two different solutions, synthetic and AD-VFA solutions were carried out in fixed bed columns. The fixed bed columns were all made of Plexiglas and operated in duplicates. The columns were 3 cm in inner diameter and 35 cm in height (Fig. 1). The column experiments were carried out in batch and up-flow mode. Fixed bed column experiments were performed until equilibrium was attained, which had taken at most 30 hours. AD-VFA solution was fed to the column at a flow rate of 28 ± 1.6 mL/min using a peristaltic pump, Watson-Marlow 403 (Watson Marlow, United Kingdom). Bed volume was calculated by measuring the height of the bed and found as 0.23 L when filled with 200 g clinoptilolite for an initial loading 2.5 mg

NH_4^+/g clinoptilolite. The pH for synthetic VFA was adjusted to the same value as of AD-VFA (pH: 6.8). In this study, two columns were operated using two different solutions and samples were taken at certain time intervals, and the results were given with mean and standard deviation values.

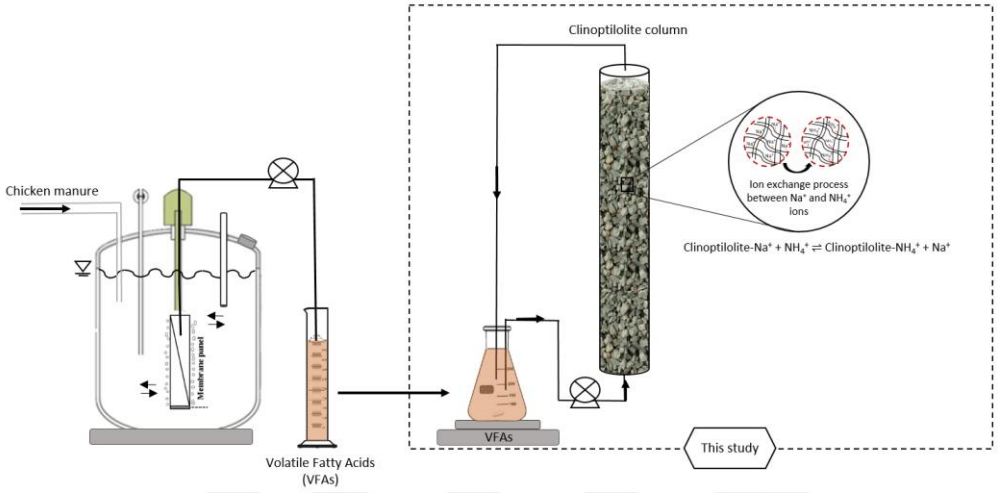

Figure 3.1 : Schematic overview of MBR setup for AD-VFA production and fixed bed batch column setup for ammonium removal.

3.2.4.5 Breakthrough experiments

In this step, breakthrough curves at three different ammonium concentrations were generated for ammonium in synthetically prepared VFA solution, to observe system behaviour. Breakthrough experiments were performed in up-flow fixed bed columns with continuous flow mode and at flowrates of 14 ± 1.5 ml/min. Synthetic solutions were prepared with different initial ammonium concentrations (570, 1135 and 2070 mg/L) and a constant VFA concentration of 7.1 ± 0.8 g/L. The height of the bed was kept constant, and the column was filled fully (bed height: 33 cm). Effluent from the column was sampled continuously and the samples were analyzed for ammonium concentrations. The experiments were conducted at 16.6 min empty bed contact time (EBCT) that was found with Eq. 3.4, where V_b is the bed volume and Q denotes the flowrate in the fixed bed column (Guida et al., 2021).

$$EBCT = V_b / Q \tag{3.4}$$

3.2.5 Analytical methods

For characterization of VFAs, total solid (TS) and total suspended solid (TSS) contents were measured using oven at 105 °C following the standard methods of the American

Public Health Association (APHA-AWWA-WEF, 2005). Total chemical oxygen demand (tCOD) was measured using the CSB 15000 test kits (range of 1.0–15.0 g/L O_2) (Nanocolor, MACHEREY-NAGEL GmbH & Co. KG, Germany) for characterization of AD-VFA. The concentration of tCOD was measured using the Nanocolor 500D photometer (MACHEREY-NAGEL GmbH & Co. KG, Germany). The analysis of trace elements (Fe, Mg, K, Na, and Ca) was performed using microwave plasma atomic emission spectrometry (MP-AES 4200, Agilent Technology, Santa Clara, USA). The pH was measured using a pH combination electrode from Orion Ross Model according to the manufacturer's recommendations. Ammonium concentration was measured using an Orion 720 ion meter and ammonia electrode. Prior to ammonium measurements and VFA analysis, samples were filtered through a 0.45 μm microporous membrane filter. VFA compositions (acetic, propionic, isobutyric, butyric, isovaleric, valeric acid and caproic acid) were analysed by means of Perkin-Elmer gas chromatograph (GC) (Clarus 590; Norwalk, CT, USA) equipped with a flame ionization detector (FID) and a capillary column (Elite-WAX ETR, 30 m × 0.32 mm × 1.00 μm, Perkin-Elmer, Shelton, CT, USA). The injection port and the detection temperature were maintained at 250°C and 300°C, respectively. Nitrogen was used as the carrier gas at a flow rate of 2 mL/min and a pressure of 20 psi. Prior to analysis, the liquid samples were mixed with an acid mixture (25% (v/v) formic acid and 25% (v/v) ortho-phosphoric acid in a 1:3 ratio) to pronate the -COOH groups of the VFAs needed to facilitate extraction in the sample, and then centrifuged at 10000×g for 5 min. The supernatant was then filtered through a 0.2 μm syringe filter to remove particulate matter. The range of VFA standards used were between 0.3125-10 g/L. Butanol solution with a concentration of 1 g/L was used as an internal standard.

3.3 Results and Discussion

Thermally pre-treated CKM was used as substrate in an immersed MBR for enhanced VFA production and in situ recovery of VFAs in a semi-continuous AD. It was aimed both to investigate the production of VFA and to remove NH_4^+ from this solution. Batch and continuous ion exchange studies were conducted to investigate ammonium removal from collected AD-VFA effluents by clinoptilolite. Batch tests were used to investigate the effects of some important parameters on ammonium removal, while column studies were used as preliminary studies for the practical application of

continuous VFA treatment. Within this context, the effects of initial ammonium loading, presence of K^+ ions and pH on ammonium removal were investigated in batch tests. Then, ammonium removal from synthetic and real VFA media (AD-VFA) was compared and batch column studies were conducted to investigate the effects of other constituents in AD-VFA on ammonium removal. In the final phase, breakthrough experiments were performed at constant empty bed contact time (EBCT) at different ammonium concentrations and a preliminary evaluation was made for future studies.

VFAs were produced from the fermentation of CKM at organic loading rates of 2 to 4 g VS/L/d. The effluent bearing VFAs that was to be use from ion exchange was obtained by in-situ recovery from anaerobic acid fermentation reactor using an immersed microfilter (unpublished work). The properties of the permeate effluent mixture is presented in Table 3.2. The organic composition of the substrate, as well as the source of the substrate influence the type of the VFAs produced (Zhang et al., 2020). It was also proposed that operational conditions may dictate which catabolic product would be dominant, allowing for more efficient growth in the mixed culture fermentation system at the same time (Jankowska et al., 2015). The VFA formed after fermentation is not pure, but rather contains a considerable mineral impurity in an aqueous solution (Reyhanitash et al., 2017). NH_4^+-N content in the permeate was in the range of 1381 – 2262 mg/L during the fermentation of CKM. The daily fermentation effluents from the reactor were homogeneously mixed, and the characteristics of the solution employed in this study were determined (Table 3.2). Ammonium content has been found to be 1955 ± 135 mg/L (Table 3.2). K^+ has the highest concentration (1554.0 ± 18.4) among other cations in the effluent solution (Table 3.2). The concentration of impurities may vary depending on the source of the wastewater/feedstock and the type and conditions of the fermentation (Reyhanitash et al., 2017). In the current study, in situ recovery of VFAs-rich filtrate by the MBR not only prevented the washing out of microorganisms, ensuring the continuous and stable operation of the anaerobic acid fermentation process (Jomnonkhaow et al., 2021; Wainaina et al., 2020), but also obtained a VFAs solution with low turbidity (Parchami et al., 2020), suitable for downstream processing such as ion exchange for ammonium removal.

3.3.1 Isotherm experiment results

In this study, isotherm experiments were performed in a real VFA solution (AD-VFA), and the isotherm constants and ammonium removal capacity were compared with the isotherm constants obtained in pure ammonium solution in distilled water without other constituents such as competing ions determined by using the same type of clinoptilolite [32]. The objective is to determine how the ammonium removal capacities change in AD-VFA solution. Such a comparison is critical for designing real applications. Isotherms were generated from equilibrium data using clinoptilolite and AD-VFA solution in an attempt to evaluate ammonium removal capacity. For this purpose, firstly, equilibrium time experiments were carried out at four different ammonium concentrations that may be expected to prevail in actual large-scale operation, for 30 hours as can be seen in Fig. 3.2, and 12 hours was chosen as the equilibrium time.

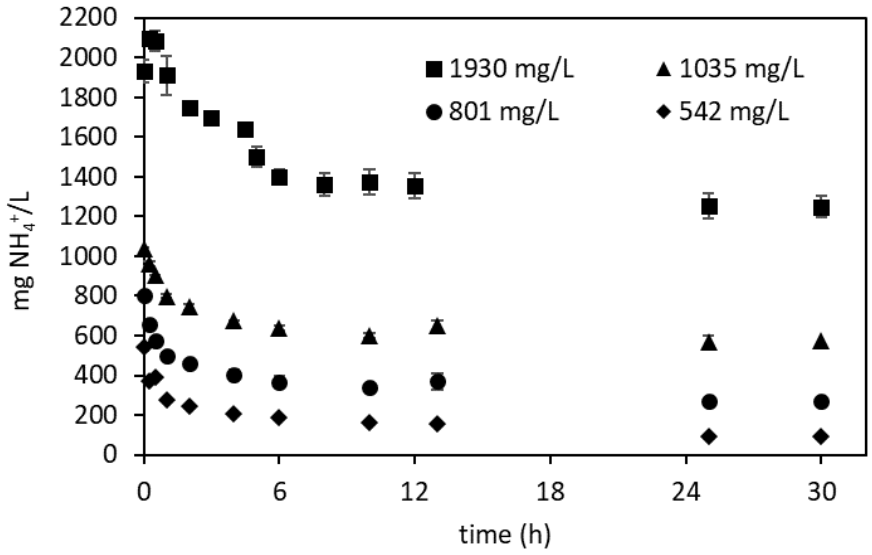

Figure 3.2 : Equilibrium time profile with different initial adsorbate concentrations.

Both the Freundlich and Langmuir models were used to evaluate the experimental results of ammonium removal from a real VFA solution. As can be seen from Table 3.3 and Fig. 3.3, Langmuir model had a slightly better fit compared to the Freundlich isotherm model, with a regression coefficient (R^2) value on Langmuir, which is 0.9998. A comparison of the regression coefficients in these two model solutions (pure ammonium and AD-VFA) reveals that the Langmuir model represent the system better than the Freundlich model. In the Langmuir model, an adsorbate is bound to the active adsorption site and all surface areas are assumed to have the same attractive force.

According to the Langmuir model, the maximum adsorption capacity (q_m) was found as 15.7 mg ammonium/g clinoptilolite (Table 3.3) while the equilibrium capacity (q_e) shows 11.3 mg/g for 1510 ± 85 mg NH_4^+/L equilibrium liquid phase concentration in Fig 3.3a.

The C_e values obtained in this study were placed in the Langmuir equation obtained by Allar (Allar, 2015), and while the q_m value was 27.4 mg/g, the q_e value for 1510 mg/L was found as 26.8 mg/g. The graph for pure solution and AD-VFA comparatively is shown in Fig. 3.4. The differences of equilibrium capacities between the pure solution and the AD-VFA solution is 15.5 mg NH_4^+/g. This difference shows that existence of ions other than ammonium in AD-VFA solution has reduced the equilibrium capacity for ammonium for this specific solution. The content and the concentration of competing cations was measured with MP-AES device and their concentrations are presented in Table 3.2. As shown in Table 3.2, the average potassium concentration in the real VFA environment was measured as 1554 ± 18.4 mg/L. In addition, the ammonium concentration was measured as 1955 ± 135 mg/L and was very similar to potassium concentration. K^+ competes with ammonium (Wang et al., 2007) and hence the ammonium removal by ion exchange of AD-VFA is lower than in pure solution. According to a study done by Rizzioli et al. (2021) on the purification and concentration of VFAs using various batch adsorption tests, Lewatit and Amberlyst ion exchange resins had respective adsorption yields of 40 and 27%. A real fermentate with an initial VFA concentration of roughly 18 g/L was subjected to the best adsorbent, Lewatit, and the best desorption conditions, resulting in a final VFA content that was three times higher than in the original solution (Rizzioli et al., 2021). Depending on the source and the fermentation process applied, Na^+, K^+, $H_2PO_4^-$ /HPO_4^{2-}, Cl^- and SO_4^{2-} ions are common in fermentation effluents (Reyhanitash et al., 2017). However, negatively charged ions are not expected to interfere with the cation exchange process and K^+ might be the most critical competing ion. In addition to these ions, AD-VFA contains other soluble organic compounds as well as VFA (Rizzioli et al., 2021).

Table 3.3 : The values of isotherm model equations.

	Langmuir model				Freundlich model			
	Equation	q_m	K_L	R^2	Equation	K_f	1/n	R^2
Pure solution (Allar (2015))	$q_e = \frac{0.9438Ce}{1+0.0345Ce}$	27.4	0.0345	0.9826	$q_e = 3.8966\ C_e^{0.2356}$	3.8966	0.2356	0.8723
AD-VFA (This study)	$q_e = \frac{0.02628Ce}{1+0.0017\ Ce}$	15.7	0.0017	0.9998	$q_e = 0.277 C_e^{0.5138}$	0.277	0.5138	0.9884

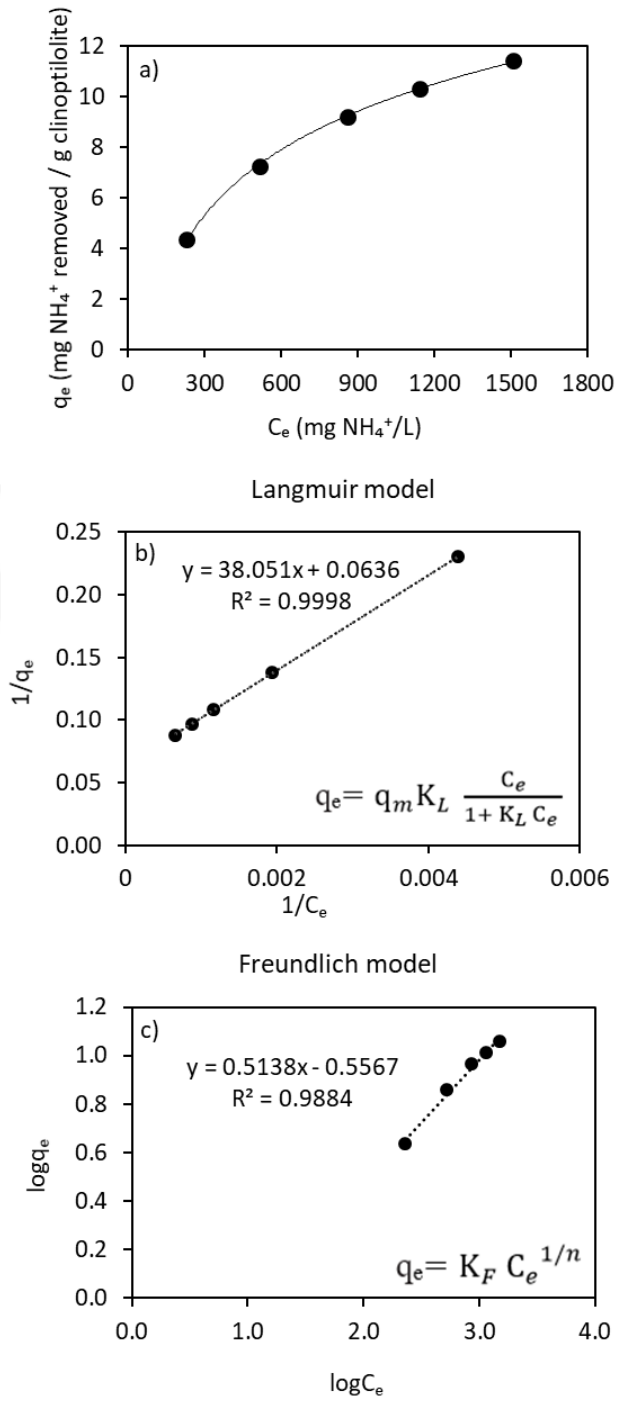

Figure 3.3 : (a) Isotherm curve and (b) Langmuir and (c) Freundlich isotherm models of ammonium with clinoptilolite for AD-VFA solution.

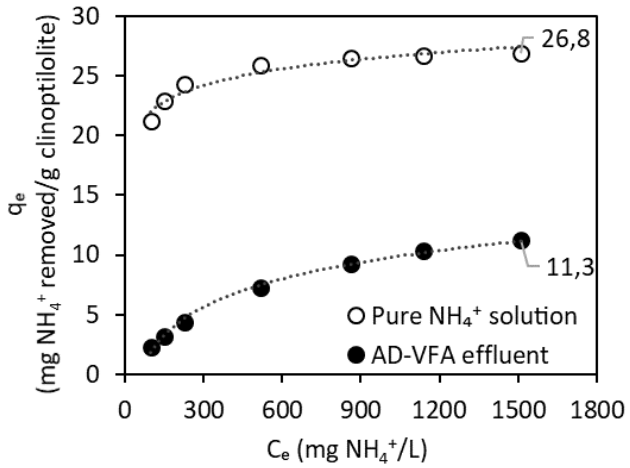

Figure 3.4 : Comparison between ion exchange isotherms of pure NH_4^+ solution, and AD-VFA with q_m values from Langmuir. The figure contains results from experiments that were done in a previous study done by Allar (2015).

3.3.2 The effect of initial NH_4^+ loading on ammonium removal

One of the most important design parameters in the ion exchange process is the initial ammonium loading which affects the removal efficiency of the system (Beler Baykal et al., 2009). The initial ammonium loadings (mass of NH_4^+/mass of clinoptilolite) were arranged as 2.0-2.5-5.0-10 mg NH_4^+/g clinoptilolite and the graph of ammonium removal is presented in Fig. 3.5a and Fig. 3.5b. The figures show that at the initial ammonium loading of 2.5 mg NH_4^+/g clinoptilolite, ammonium removal was found to be over 92%, and higher initial loadings, lead to relatively lower efficiencies which will not permit the product to be used effectively, as an external carbon supplement for denitrification for instance. It may be observed that ammonium removal increased with decreasing the initial loadings up to 2.5, whereas further reduction of initial loading down to 2 mg/g did not result in a significant improvement in ammonium removal. Increasing the amount of clinoptilolite leads to lower initial loadings per g of adsorbent within same initial ammonium concentration. The removal of ammonium is essentially unaffected up to the initial loading threshold of 10 mg NH_4^+/g clinoptilolite, and a removal efficiency of 94% was achieved for ammonium in a study on the transfer of plant nutrients from urine to clinoptilolite by examining the removal efficiency under various initial ammonium loadings (5-34 mg NH_4^+/g clinoptilolite) (Beler Baykal et al., 2009). Hence, in accordance with previous findings, increasing the amount of adsorbent to a certain extent resulted in increased removal of ammonium ions (Beler Baykal et al., 2009; Rengaraj et al., 2001). Therefore, initial ammonium loading, 2.5 mg NH_4^+/g clinoptilolite, was selected for the rest of the experiments.

There was no VFAs loss from the solution during the sets of initial loading tests (Fig. 3.5c).

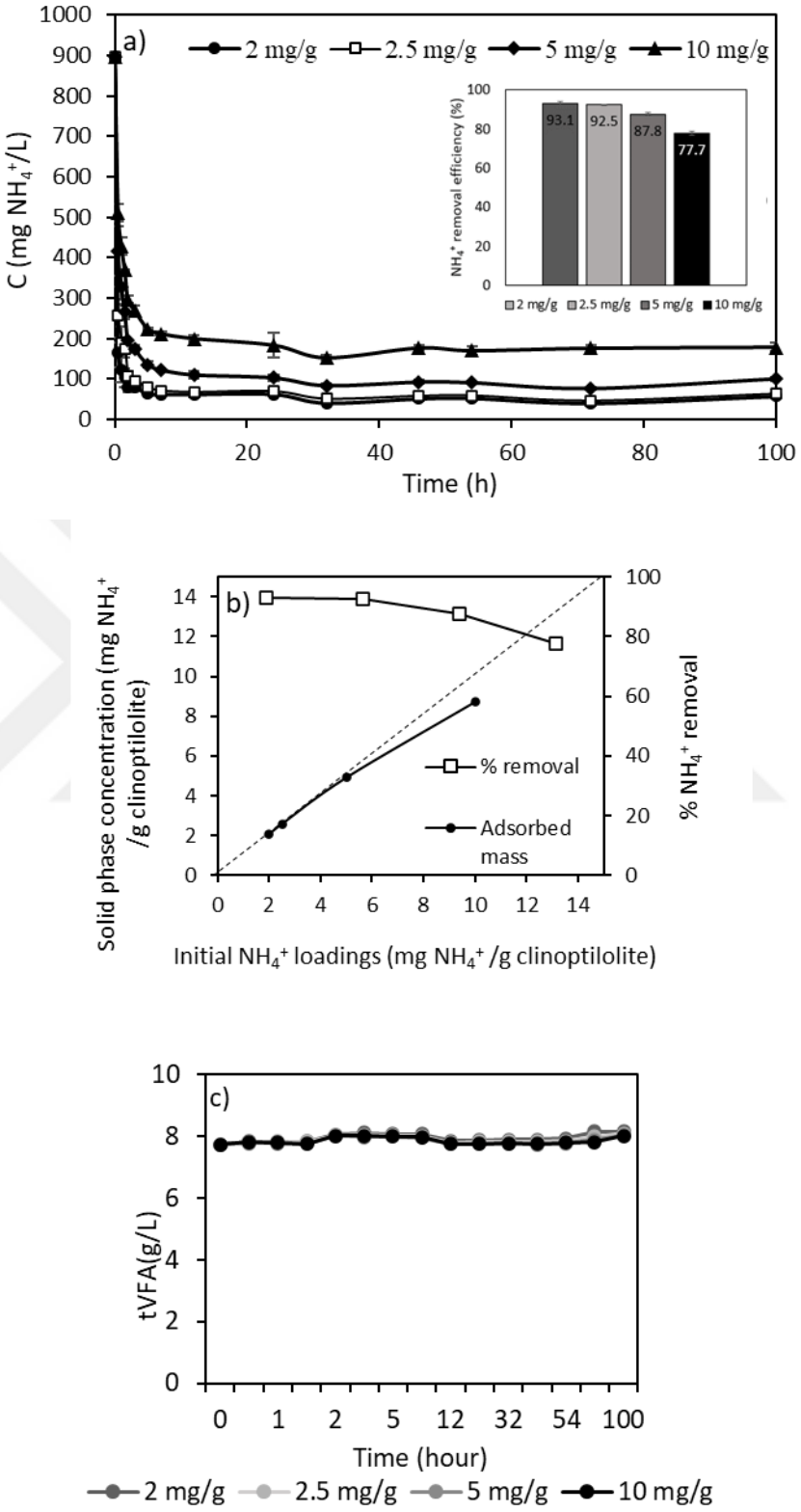

Figure 3.5 : Effect of initial loading on ammonium removal with ion exchange from sVFA solution.

3.3.3 The effect of pH

The pH of solutions was 2.8 ± 0.2 for synthetic VFA and 6.8 ± 0.1 for AD-VFA effluent. The pH is an important factor for ammonium/ammonia equilibrium in ion exchange processes. Although ammonium is the dominant form at low pH, its concentration starts to decreases at pH values above 8 (pK_a of ammonium is 9.25) as it transforms to the non-ionised form of ammonia (Dong & Sartaj, 2016) which cannot undergo ion exchange process. From the perspective of ammonium removal, pH values below 8 are not expected to be critical, and that is what the results show in this present work as well, as may be observed in Fig. 3.6a and Fig. 3.6b. Moreover, whether VFAs exist in dissociated or undissociated (free acid) form depends on pH as well (Ramos-Suarez et al., 2021), therefore, VFA concentrations were also measured during the ion exchange batch studies. This has led to conducting batch experiments at different pH values ranging from 3-7 to determine the effect of pH on the overall ion exchange process. In this range of pH, the efficiency of ammonium exchange was not directly affected by the change in pH. VFAs exist mainly as undissociated acids at pH values below the dissociation constant of acids (≤ 4.86 at 25 °C) (Wainaina et al., 2019a); however, the dissociated form of VFAs are dominant in higher pH values (Reyhanitash et al., 2017). As can be seen in Fig. 3.6a and Fig. 3.6b, pH had no significant effect on VFAs concentrations as well as ammonium removal. The maximum removal (about 96%) for ammonium was found at pH 3, as shown in Fig. 3.6a moreover, the removal efficiency of ammonium was still above 90% at all different pH values investigated in this work as expected. The results in this study show that pH values between 3 and 7 have no significant effect on the ammonium removal efficiency. It was evident that the presence of VFAs did not affect the removal of ammonium (with a 0.5-1% decrease only). There is nearly no VFAs loss from solution during ammonium adsorption (Fig. 3.6b). Similar results were obtained in a previous study using clinoptilolite for ammonium removal from leachate (Karadag et al., 2008). In that study, pH values of leachate were in the range of 4 to 10. It was shown that changes in the pH in the range between 6 and 8 had little effect on ammonium removal with the best removal performance was recorded at pH of 7 with 57.8% (Karadag et al., 2008). Specific anions in the solution could reduce ammonium adsorption due to hydrolyzation of ions and the associated pH change (Liu et al., 2010), therefore, the VFA concentrations were also controlled during the ion exchange batch

studies. As the synthetic solution used has both ammonium and VFAs it was of importance to see the effect of both cations and anions. There are also some studies on the removal of VFAs by anion exchange resins (Bhandari et al., 2000; Eregowda et al., 2020; Fufachev et al., 2020; Rebecchi et al., 2016; Takahashi et al., 2013); however, clinoptilolite is a cation exchanger. Hence, it does not affect the concentrations of deprotonated VFAs (carboxylate salts) in the effluent.

It can be concluded that addition of chemicals for pH adjustment would not be required when ion exchange system was adapted directly to the fermenter, which would mean that no additional cost would be required. This possible integration of processes eliminates the need to integrate an additional pH adjustment and control system, which indirectly reduces the cost.

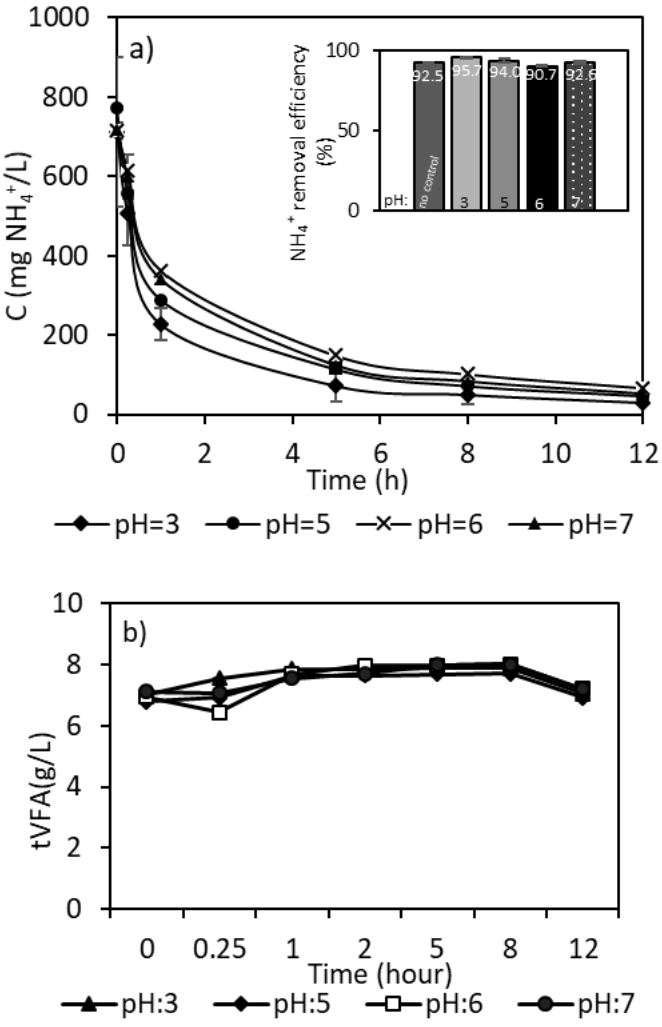

Figure 3.6 : Effect of pH on ammonium removal with ion exchange from sVFA solution.

3.3.4 The effect of K^+ concentrations on ammonium removal

In this part of the study, the effect of potassium ion on ammonium removal was studied since it is a constituent found in higher concentrations in real VFA effluent and has a competing effect on ammonium removal (Table 3.2). The cations in the solution and ammonium would compete with each other, resulting in a decrease in ammonium exchange by the ion exchanger (Lei et al., 2008). The selectivity order of clinoptilolite between cations at similar concentrations is $Cs^+ > NH_4^+ > Pb^{2+} > K^+ > Na^+ > Ca^{2+} > Mg^{2+} > Ba^{2+} > Cu^{2+}$, Zn^{2+} as it is given by manufacturer (Rota, 2021). Although the clinoptilolite used in this study is a highly ammonium-selective zeolite (Kithome et al., 1998), at comparable concentrations of the major competing cations, the increased concentration of K^+ and Ca^+ ions could alter the selectivity order of the clinoptilolite depending on the characterization of the fermentation liquid. As shown in Fig. 3.7a, with the addition of maximum 2 g/L K^+ compared to no addition of K^+, the removal efficiencies dropped from 92% to 81%, while initial ammonium concentration decreased from 898 mg/L to 67 mg/L and from 773 mg/L to 149 mg/L, respectively.

Wang et al. (2007) showed that the presence of potassium, calcium and magnesium ions would compete with ammonium ions for ammonium exchange with the Chinese Na-modified zeolite. Of these ions, the presence of potassium had the strongest effect on the reduction of ammonium removal, followed by calcium. This is consistent with the results of another study by Farkaš et al. (2005), where both support the findings of reduction on ammonium removal. There is negligible VFA loss from the solution during the run of different K^+ ion tests (Fig. 3.7b).

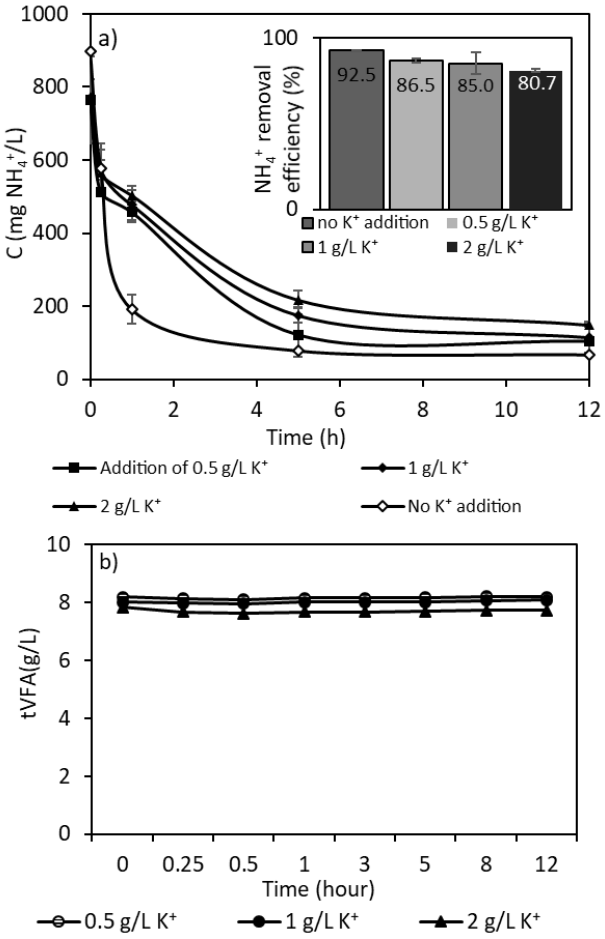

Figure 3.7 : Effect of K^+ ion on ammonium removal with ion exchange from sVFA solution

3.3.5 Ammonium removal from synthetic and AD-VFA effluent using fixed bed columns

Based on the results of different initial loadings batch experiments, an initial loading of 2.5 mg NH_4^+/g clinoptilolite was selected for full circulation (=batch) column experiments. In the data obtained in the Section 3.1 of isotherm experiments, the equilibrium capacity (q_e) that is evaluated through Langmuir isotherm was 26.8 mg/g in the pure solution and the capacity decreased to 11.3 mg/g due to the presence of other constituents in AD-VFA medium. In the experiments that is presented in Fig. 3.8, however, there was no difference between synthetic VFA and AD-VFA for ammonium removal. In this experimental stage, the removal of ammonium was investigated in fixed bed column using both AD-VFA and synthetic VFA solution with an initial tVFA concentration of 7.9 ± 0.8 and 5.9 ± 0.3 g/L, and ammonium concentrations of 2090 ± 36 and 2067 ± 41.6 mg NH_4^+/L, respectively. For a clearer comparison, ammonium removal and VFAs concentrations were analysed and

presented in Fig. 3.8 and Fig. 3.9, respectively. It can be observed that starting from an initial value around 2100 mg/L ammonium, which could come from the fermentation of protein- and/or urea-rich wastes such as animal manure, especially chicken manure, or slaughterhouse wastes, most of the ammonium content is removed within 12 hours. The results indicated that other ions present in AD-VFA did not decrease the ammonium removal percentages; in fact, the ammonium removal efficiency of 93.8% was almost comparable to that of synthetic VFA (92.6%) at a reaction time of 12 hours (Fig. 3.8b). Therefore, while a slight K^+ effect was observed in the batch experiments, K^+ showed no negative effect in these column experiments. Ion exchange process from AD-VFA solution allowed even higher ammonium removals even in the presence of other ions due to its large ion exchange capacity**.** It can be explained that the concentrations of other ions (K^+, Na^+, Mg^{2+}, Ca^{2+}, Fe^{2+}), were much lower than the ammonium concentration in real VFA medium and also their selectivity order was lower than that of ammonium. Table 3.2 shows that the concentration of the competing cation K^+ in the AD-VFA was 1554 ± 18.4 mg/L, while that of ammonium was 1955 ± 135 mg/L. The composition and the concentrations of the VFAs was stable during the 72-hour experiments (Fig.3.9).

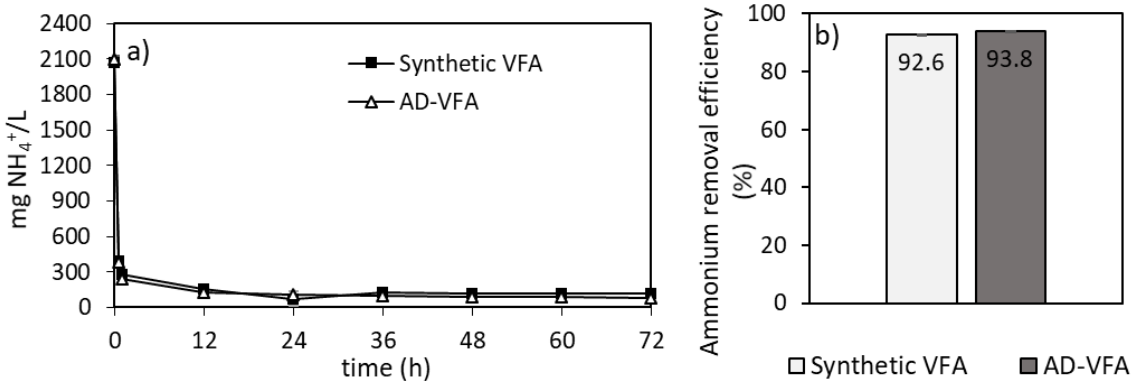

Figure 3.8 : Ammonium removal of clinoptilolite in sVFA and AD-VFA solutions (pH 6.8).

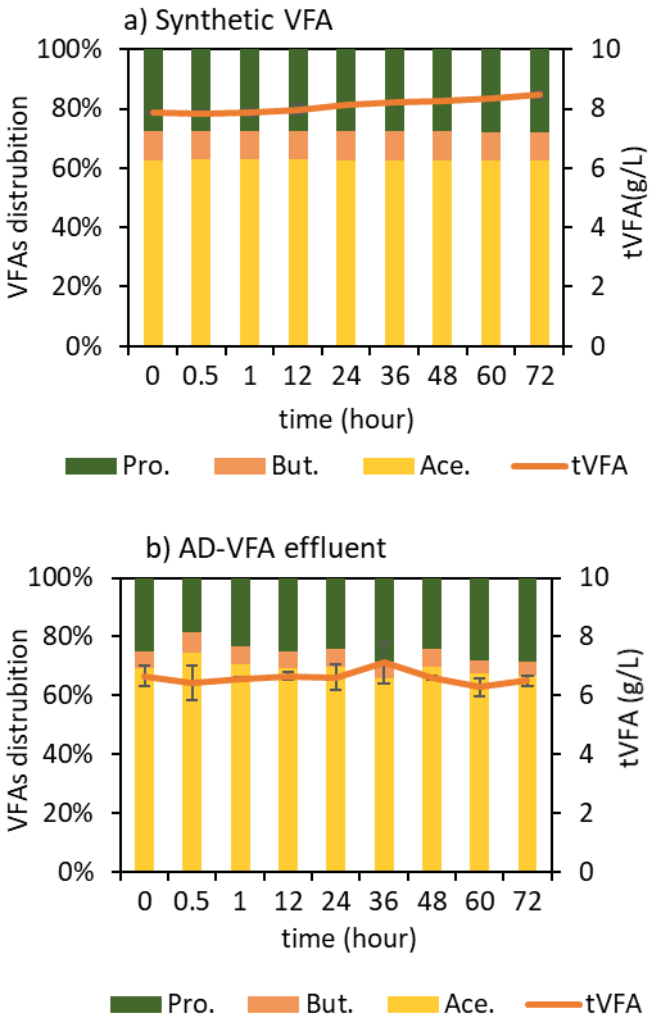

Figure 3.9 : VFA changes on (a) sVFA and (b) waste derived AD-VFA solution.

3.3.6 Preliminary breakthrough experiments for ammonium removal

In the last step of the study, in an attempt to gain a first overview, a preliminary breakthrough experiment with a solution of ammonium and sVFAs was performed with three different initial ammonium concentrations (selected based on expected ammonium concentration in the fermentation step). The number of bed volumes and number of hours processed at breakthrough point and surface concentration of the beds are used as performance indicators. Fig. 3.10 shows the results of the breakthrough experiment for ammonium obtained with initial ammonium concentrations of 570, 1135 and 2070 mg/L at a contact time of 16.6 minutes.

In this study, the extent of NH_4^+removal from the VFA solution aims not to interfere with further nitrogen removal application (denitrification). Ammonium concentrations above 60 mg/L affect nitrification negatively (Paśmionka et al., 2021). This leads to interference with whole nitrogen removal process. In the denitrification phase, the

ammonium content of the carbon source given to the environment may also enter the nitrification ammonium stream, affecting the ammonium concentration in the output stream. Hence, ammonium concentration at in the ion exchange effluent should be as low as possible by continuing to use of VFAs enriched effluent in nutrient removal so as not to cause external deterioration of ammonium removal. Thereby, the point where the C/C_0 value reached 0.01% was chosen as the breakthrough point in this study. This indicates that the column must be regenerated if the ammonium concentration at the outlet of the column is above this limit. Results showed that the breakthrough point where ammonium concentration first appeared in the effluent was reached faster at higher initial ammonium concentrations, indicating the need for further flow through studies. Increasing ammonium concentrations from 570 mg/L to 2070 mg/L resulted in sharper breakthrough curves and breakthrough for 2070 mg NH_4^+/L was almost immediate (3 BV or 0.8 h). However, for ammonium concentrations lower than 2070 mg/L, considerable amounts of ammonium removal, with a 99% (C/C_0=0.01%) removal efficiency for about 6 BV or 1.7 h and 13 BV or 3.6 h at 1135 and 570 mg NH_4^+/L, respectively. Under the experimental conditions employed, the removal of ammonium was practically completed at 1-4 h depending on the initial ammonium concentration in the VFA solution. Total ammonium removed is obtained calculating the area above the breakthrough curves and dividing this area by the amount of clinoptilolite in the column gives the mass ammonium adsorbed per unit mass of zeolite for the continuous system. The surface concentration of the clinoptilolite was calculated as 17.6, 21.2, 22.7 mg NH_4^+-N/g clinoptilolite for 570, 1135, 2070 mg NH_4^+/L initial concentrations, respectively. The liquid phase concentration decreased by 32%, 20% and 15% to 17.6, 21.2 and 22.7 mg NH_4^+/g clinoptilolite in the sVFA solution compared to pure ammonium solution. The ammonia concentration at the effluent of column starts increasing after 3 BV (0.8 h), 6 BV (1.7 h) and 13 BV (3.6 h) for the inlet ammonium concentrations of 2070, 1135 and 570 mg/L, respectively. As the inlet concentration of ammonium decreased, breakthrough occurs at later BVs as expected. Based on the preliminary results from breakthrough, effective ammonium removal with better performance can be expected at higher contact times by either increasing the height of the bed, using a larger/bigger column structure, or decreasing the flowrate.

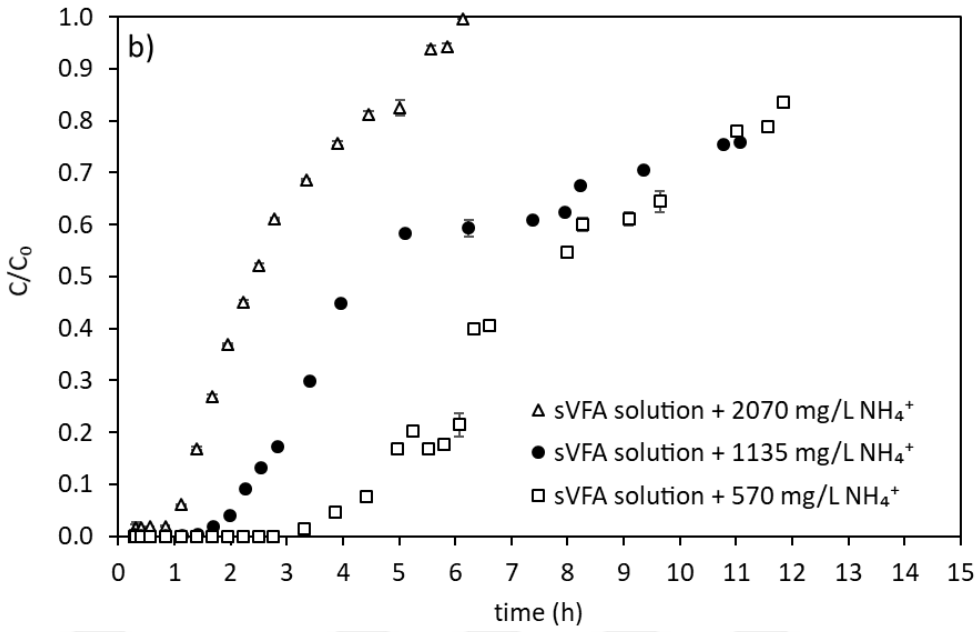

Figure 3.10 : Ammonium breakthrough curves in different concentrations of sVFA solution.

3.4 Conclusion

An application of the ion exchange process using a low-cost natural ion exchanger, clinoptilolite, was investigated on a laboratory scale for ammonium removal from synthetic and AD-VFA solutions. The results obtained provided valuable data for promoting biological nutrient removal processes in WWTPs as further implications with ammonium purified VFAs. Isotherm experiments showed the highest equilibrium capacity of 11.3 mg NH_4^+/g clinoptilolite from AD-VFA effluent with an equilibrium time of 12 hours. Ammonium removal of more than 92% from synthetic VFA and AD-VFA at a reaction time of 12 h was achieved by using a batch-mode operated clinoptilolite column. Breakthrough surface concentration per g of clinoptilolite that has been done on a mixture of synthetic VFAs and NH_4Cl (570, 1135 and 2070 mg NH_4^+/L) were determined to be between 17.6 to 22.7 mg NH_4^+/g clinoptilolite in breakthrough studies. Although an earlier breakthrough occurs at an ammonium concentration of 2070 mg/L, larger columns or lower flow rates need to be used, as well as further studies on the regeneration of the adsorbent and the recovery of the adsorbed ammonium need to be performed. The results obtained show selective removal of ammonium with minimal loss of VFAs from VFA-containing fermentation liquid produced during digestion of nitrogen-rich feedstock.

4. POTENTIAL OF FOOD WASTE-DERIVED VOLATILE FATTY ACIDS AS ALTERNATIVE CARBON SOURCE FOR DENITRIFYING MOVING BED BIOFILM REACTORS[3]

4.1 Introduction

Nutrient pollution is a well-documented widespread problem around the world, such as the Baltic Sea (Murray et al., 2019), the Mississippi River basin (Henderson et al., 2021; Mengistu et al., 2020), and Lake Taihu in China (Xu et al., 2015; Zhao et al., 2021). The two main nutrient pollutants in municipal wastewater are nitrogen and phosphorus, both of which are natural components of the ecosystem. However, uncontrolled accumulation of these nutrients, mostly due to human activities, leads to eutrophication of the receiving water bodies. This process favours the growth of algae and other aquatic plants, which in turn leads to a variety of negative effects such as extensive algal growth (algal blooms) and oxygen deficiency in the sea. Successful nutrient removal in wastewater treatment plants (WWTPs) is one of the keys to ensuring the environmental quality of receiving waters, indirectly protecting drinking water resources, and closing the loop by recycling used water for irrigation.

The treatment procedure in conventional WWTPs involves several processing stages. To remove sediments, organic debris, and nutrients from wastewater a combination of physical, chemical, and biological procedures and operations are applied. Preliminary, primary, secondary, and tertiary and/or advanced wastewater treatment are all names that are used to denote different levels of treatment (Burton & Stensel). While preliminary treatment removes big particles and grit from raw wastewater, primary treatment is to eliminate floatable and settable solids, and secondary treatment is to biologically remove soluble organics and eliminate refractory solids as sludge.

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[3]This chapter is based on: Sapmaz, T., Manafi, R., Mahboubi, A., Lorick, D., Koseoglu-Imer, D. Y., & Taherzadeh, M. J. (2022). Potential of food waste-derived volatile fatty acids as alternative carbon source for denitrifying moving bed biofilm reactors. Bioresource Technology, 364, 128046.

Secondary effluent undergoes nitrogen and phosphorous removal during tertiary treatment. Common nitrogen removal via sequential nitrification and denitrification (or activated sludge) can be performed at WWTPs using different technological approaches, including continuous flow activated sludge systems, sequencing batch reactors, fixed film (trickling filters, suspended plastic carriers, aerated filters), and granules. Nitrification is the oxidation of nitrite to nitrate that follows the biological oxidation of ammonium to nitrite done by nitrifiers, including nitrite-oxidizing bacteria and ammonia-oxidizing bacteria. Wastewater from the nitrification process subsequently passes through to the denitrification process. In denitrification, however, nitrate is used as an electron acceptor by heterotrophic denitrifying bacteria, so that N_2 is finally formed via series of gaseous nitric oxide intermediates (Rahimi et al., 2020). The problem with denitrification is that, unlike nitrification bacteria, which thrive on inorganic carbon sources such as CO_2, denitrifying bacteria require organic carbon sources for growth and energy acquisition. As most of the organic carbon source has been depleted during the initial treatment stages, denitrification often must be supplied with external organic carbon to comply with the allowable total nitrogen limits. In most cases, the lack of an easily biodegradable carbon source that can be used as an effective substrate by heterotrophic denitrifying bacteria is the limiting factor during denitrification (EPA, 2013). This *ex-situ* organic carbon source provision, preparation and addition inserts external loads to WWTPs, making them far from self-sustaining and sustainable.

Commercially available and commonly used external carbon sources for denitrification include mainly alcohols (i.e. methanol, ethanol), acetate or glycerine (Playchoom et al., 2011). However, purchasing, transporting and storage of the carbon source adds to process total costs, the price and availability of the carbon source can vary locally and seasonally, and most importantly fossil-based carbon sources such as methanol are controversial, questioning the environmental sustainability and circularity of the process (Cherchi et al., 2009). In addition, to ensure year-round access to the carbon source, large volumes are to be stored at the plant site, resulting in higher process footprints. Using industrial waste streams as a carbon source could be a potential alternative, however, the carbon source quality and consistency can fluctuate, affecting the WWT process.

As discharge limits become more stringent, existing WWTPs are challenged to cap operating costs as low as possible while enhancing effluent quality (Palatsi et al., 2021). Therefore, the choice of external carbon sources becomes of critical environmental and economic importance (EPA, 2013). Thus, it is important to seek new sustainable, low energy and capital demanding approaches in provision of low cost and renewable carbon sources for WWTPs.

The applicability of various electron donors for denitrification has been studied in recent decades. Literature revealed that, there is a lot of potential for recovering valuable chemicals like biomethane, bioethanol, and volatile fatty acids (VFAs) from industrial and municipal organic waste (Mahmoud et al., 2022). Due to the high levels of proteins, carbs, and lipids present, even low or negative value organic waste can further valorise valuable chemicals thanks to their high organic and biodegradable composition. It has been demonstrated that wastes with these qualities can be used to produce VFAs. VFAs are produced in the first two phases of anaerobic digestion process. Hydrolysis, acidogenesis, acetogenesis, and methanogenesis are the four steps of anaerobic digestion operation. The end of acidogenesis is marked by the microbial conversion of organic material into simpler monomers of VFAs. Since anaerobic digestion has a lower carbon footprint than other petrochemical routes, it is typically extracted as an intermediary from anaerobic digestion process to generate the VFAs, which is becoming more and more significant (Garcia-Aguirre et al., 2017). VFA can replace petrochemical derivatives because it can be converted into various platform chemicals. The market needs for the VFAs produced by acidogenic fermentation or digestion include carbon source alternative in biological nutrient removal processes.

There is a lot of waste produced globally along the food supply chains. Food waste is one of the significant wastes created because it accounts for a sizable portion of the organic waste produced globally (Wainaina, 2020). According to data by UNEP, 931 million tonnes of food waste were produced in 2019, with families producing 61% of it, food service producing 26%, and retail producing 13% (United Nations Environment Programme (2021)). One of the applications of mixed VFAs is the creation of value through usage as carbon source along with waste volume reduction. In the literature, there are records of food waste VFA effluents having been used biodegradable carbon source and thus electron donors to improve nitrogenous compounds removal performance (Feng et al., 2021; Kim et al., 2016; Yan et al., 2018;

Zhang et al., 2016b). All these studies clearly indicate the potential of using organic carbon already present in food waste streams. Although promising, the majority of the research detailed above is still in its early stages and is primarily limited to laboratory scale experiments. To the best of the authors' knowledge, there is no literature on the inclusion of an external carbon source from AD-VFAs against routinely utilized methanol to improve specifically post-denitrification systems in WWTPs.

The objective of this study was to evaluate the potentials of food waste-derived VFA-bearing anaerobic digestion effluent in the denitrification step of wastewater treatment and replacement of fossil-based conventional carbon sources. In this regard, the denitrification capacity of single VFAs together with VFA effluents from food waste (AD-VFA), and it's corresponding synthetically simulated VFA solution (sVFA) were compared to methanol for attached growth post denitrification in moving bed biofilm reactors (MBBRs). The effects of single VFAs (Acetic acid: AA, Propionic acid: PA, Butyric acid: BA, Valeric acid: VA, and Caproic acid: CA) provided based on chemical oxygen demand (COD) and molar weight basis were thoroughly investigated in this study. Denitrification rates, NO_3^--N removal efficiencies along with variations of NH_4^+-N, NO_3^--N, and NO_2^--N during the denitrification process have been determined and compared for all effects. The suitability of biodegradable food waste derived VFAs as electron donors for denitrification as well as their feasibility and performances have been fully explored in this study.

4.2 Materials and Methods

4.2.1 AD-VFA provision from food waste and characteristics of all carbon sources used

Carbon sources used in this study include one of the traditionally used carbon sources (methanol) and heat shock treated food waste through anaerobic digestion effluent (hereinafter referred to as AD-VFA). Methanol (≥99.8%, Sigma-Aldrich) was used as reference carbon source for the denitrification experiments.

AD-VFA from anaerobic digestion of food waste used in this study was produced as explained by Wainaina et al. (2020), and was utilized as carbon source for denitrification study. The AD-VFA was produced in a continuous stirred tank reactor with a working volume of 3.5 L, and the MBR housed a second generation Integrated

Permeate Channel (IPC) membrane panel. The IPC flat-sheet membrane was developed and produced by the Flemish Institute for Technological Research (VITO NV, Mol, Belgium). Hydrophilic polyether sulfone coated membrane on both sides of a spacer fabric were used to create a flat sheet membrane panel with a total effective area of 68.6 cm^2. The average pore size of the utilized membrane was 0.3m.

Heat shock pre-treatment, which enhances VFA generation, is one means of deactivating methanogens in which applied to food waste in the production stage of VFAs (Jomnonkhaow et al., 2021; Mondylaksita et al., 2021; Wainaina et al., 2019a). Methanogenesis must be blocked throughout the acidogenic digestion process in order to increase VFA production. Hence, the heat shock treatment was carried out in a water bath which was shaken at 110 rpm at 80 °C for 15 min. The MBR for the production of VFAs was operated in a similar design as described in (Jomnonkhaow et al., 2021). The micro filtered, particle-free and VFA-rich permeate was recovered from the anaerobic digestion process, which ran in a semi-continuous mode. After anaerobic digestion and MBR filtration, the recovered AD-VFAs were collected and stored at -18 ± 2 °C to be evaluated as an external carbon source for denitrification.

To be systematic, both naturally produced VFAs, and synthetically produced version with the synthetic compounds were considered. As for the synthetic VFAs, laboratory grade acetic, propionic, butyric, iso-butyric, valeric, iso-valeric and caproic acids were purchased from Sigma-Aldrich and the synthetic VFAs solutions (sVFA) were prepared by dissolving these acids in Mili-Q® water to represent AD-VFAs. Individual VFA types were also evaluated (AA, PA, BA, VA, and CA). The properties of carbon sources used are listed in Table 4.1.

Table 4.1 : Characteristics of carbon sources evaluated.

Parameters	AD-VFA	sVFA	Methanol
pH	5.38 ± 0.02	2.91 ± 0.02	-
sCOD (g/L)	14.5 ± 0.71	11 ± 0.00	1185
NH_4^+-N (mg/L)	380.0 ± 0.00	-	-
PO_4-P (mg/L)	99.1 ± 0.42	-	-
NO_3-N (mg/L)	3.2 ± 0.14	-	-
NO_2-N (mg/L)	0.01 ± 0.00	-	-
AA (g/L)	0.71 ± 0.01	0.7 ± 0.1	-
PA (g/L)	0.29 ± 0.04	0.3 ± 0.1	-
Ibu (g/L)	0.21 ± 0.00	0.2 ± 0.05	-
BA (g/L)	0.79 ± 0.19	0.8 ± 0.1	-
Iva (g/L)	0.07 ± 0.10	0.1 ± 0.1	-
VA (g/L)	0.21 ± 0.00	0.2 ± 0.05	-
CA (g/L)	2.09 ± 0.29	2.1 ± 0.1	-
tVFA (g/L)	4.37 ± 0.11	4.4 ± 0.2	-

4.2.2 Post denitrifying MBBR operation

The study was conducted on AnoxKaldnes K1 type carriers (AnoxKaldnes, Lund, Sweden) and wastewater originating from an MBBR type reactor from a municipal wastewater treatment plant (Gryaab, Gothenburg, Sweden), and having been transported to the laboratory for experiments and used for denitrification in this study within 24 h. The wastewater and carriers were collected at three different batches and the average pH, sCOD, NH_4-N, PO_4-P concentrations of denitrification influent wastewater was: pH: 6.46±0.21, sCOD (g/L): 38.00±13.08, NH_4^+-N (mg/L): <4, PO_4-P (mg/L): 0.52±0.04. A lab-scale system was built-up and operated with four identical glass reactors with a total capacity of 2L which were used for the evaluation of studied AD-VFAs as external carbon source in denitrification process. Each reactor had 1L AnoxKaldnes K1 type carriers that was measured with an Erlenmeyer flask and filled up to 1.8L with wastewater that was firstly nitrified in an aerated nitrification MBBR taken from inflowing wastewater to denitrification at the wastewater treatment plant. The carriers were cylindrical high density polyethylene cross-shaped cut out rings with a bulk surface area of 500 m^2/m^3 and a maximum filling ratio of 70%. Carriers measure 7 mm in depth and 10 mm in diameter.

The denitrification rate was controlled under anoxic conditions with an excess of NO_3, NH_4, PO_4 and a carbon source. Therefore, stock solutions of 1000 mg/L NH_4Cl, 50

mg/L KH_2PO_4 and 1000 mg/L KNO_3 were prepared. Nitrogen gas was purged in the reactors for 30 minutes before and during the experiments to ensure anoxic conditions. All reactors were fully mixed under anoxic conditions by placing them on a hot plate with magnetic stirrer (240 rpm) and duplicating for each carbon source tried. 100 ml of PO_4 stock solution and 10 ml of NH_4 stock solution were added to the reactors. After 30 minutes sparging 25 ml of NO_3 stock solution and carbon source was added and the denitrification test started. The amount/dosage of carbon source added usually denoted to the amount of COD required to remove each unit of NO_3. Therefore, a COD/N ratio of 9.5 was selected to the work in this study. The amount of each carbon source added was calculated before the denitrification experiments began. The temperature in the denitrification reactors was 15-18 °C. Temperature values were measured before and after denitrification. Methanol was used as the reference carbon source for all sets of experiments.

The denitrification rate (DNR) (Shrestha et al., 2009) was calculated as follows:

$$DNR\ (g\ NO_x/m^2/d) = \frac{k\ (mg/L/min)\ \text{x}\ 60\ (\text{min})\ \text{x}\ 24\ (h)\ \text{x}\ V_S\ (L)}{A_{carrier}\ (m^2)\ \text{x}\ 1000\ (mg)} \quad (4\ 1)$$

Where k, V_s and $A_{carrier}$ are:

k (mg/L/min) = NO_x removal rate

V_s (L) = V_0 (L) – (V_{sample}/2) – ($V_{carriers}$ (L) x bulk volume of the carriers (14%)

$A_{carrier}$ = $V_{carriers}$ (1L) x specific surface area of carriers (500 m^2/m^3)

Denitrification capacity was calculated as follows:

The amount of NO_3-N removed from the system in 60 minutes is estimated by subtracting the first minute's data from the concentration of the 60 minutes sample (4.2). The computed value for NO_3-N is deducted from the final 60 min NO_2-N concentration to get the denitrification capacity for NO_2-N. (4.3).

$$D_{cap}(NO_3 - N) = mg(NO_3 - N)\ (1\ min) - mg(NO_3 - N)(60\ min) = mgNO_3 - N\ \ removed/L/\ h \quad (4.2)$$

$$D_{cap}(NO_2 - N) = D_{cap}(NO_3 - N) - (NO_2 - N)(60\ min) = mgNO_2 - N\ removed/L/\ h \quad (4.3)$$

4.2.3 Analytical methods

During denitrification, samples were taken at regular intervals (1, 5, 10, 20, 30, 40, 50, and 60 min) to determine the content of nitrogenous compounds, COD, PO_4-P, and

pH. Samples were filtered through 1.6 μm glass microfiber filters before analysis. NANOCOLOR® tube tests were used to determine the concentrations of NO_3-N, NO_2-N, NH_4^+-N, PO_4-P and COD according to the manufacturer's instructions (MACHEREY-NAGEL GmbH & Co. KG, Germany). VFA profiles and concentrations were determined by gas chromatography (Agilent 7890 GC, Perkin-Elmer, Shelton, CT, USA) with hydrogen flame ionization detector and capillary column, using butanol solution (1 g/L) as internal standard. The VFA standards utilized ranged from 0.3125 to 10 g/L. Temperature and pH were routinely measured at the beginning and end of denitrification using a digital thermometer and pH electrode (S400, Mettler Toledo, Switzerland), respectively. All measurements were performed in parallel to obtain reliable results.

4.2.4 Statistical analysis

MINITAB® 17 (Minitab Ltd., Coventry, UK) was used to perform all statistical analyses. The one-way analysis of variance (ANOVA) was analyzed using a general linear model with a 95% confidence interval; statistical differences were discovered at p-value 0.05. Pairwise Tukey test comparisons were done to gain a better understanding of the level of variations between results obtained.

4.3 Results and Discussion

4.3.1 Denitrification using single VFAs as carbon source

Individual VFAs were added as a carbon source during denitrification, using the same C/N ratio of 9.5 and based on the same molecular weight (MW) of each acid, which corresponds to the advised methanol loading (C/N ratio of 9.5 equal to 5 mmol methanol), in series of batch assays. This set of experiments was performed to better understand how the composition of VFAs effluent would affect denitrification and to identify the rate and capacity of denitrification in terms of molecular size and the respective metabolic pathway of each VFAs.

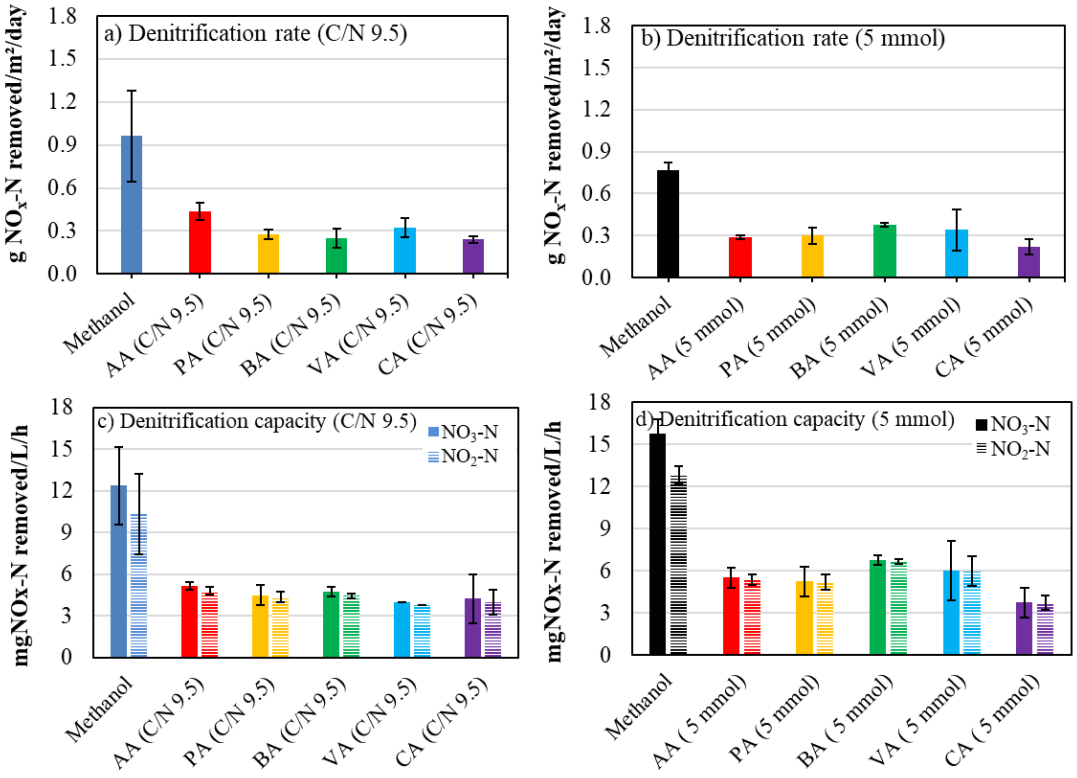

Figure 4.1 : a) Denitrification rate based on the same C/N ratio and b) the same molecular weight.

As can be seen in Figure 4.1, employing single acids resulted in noticeably lower denitrification rates and capacities than methanol (p-value < 0.05). For the denitrification test conducted based on the same C/N ratio, while methanol showed 0.96 g NO_x-N removed/m^2/day, acetic acid showed the highest rates of denitrification after methanol, with a value of 0.44 g NO_x-N removed/m^2/day. The performance of other acids was comparable, with values for propionic acid, butyric acid, valeric acid, and caproic acid, being respectively at 0.28, 0.25, 0.32, and 0.24 g NO_x-N removed/m^2/day (Figure 4.1a). However, the difference between single acids and between acetic acid and methanol is not statistically significant (p-value > 0.05). This shows that acetic acid is the preferred type VFA among all the acids studied, in compliance with the results by Elefsiniotis and Li (2006) who claimed faster utilization of acetic acid than propionic acid, resulting in preferred carbon source in terms of denitrification. This also is confirmed by Torresi et al. (2017), that the length of time it takes for a molecule to be digested and utilized in the denitrification process increases with its complexity. Theoretically, the denitrifying bacteria may consume smaller carbon source molecules more easily, which improves the removal efficiency.

As a result, low molecular weight organic substances such as acetic acid may be more ideal carbon sources (Domingos et al., 2009; Pelaz et al., 2018; Torresi et al., 2017).

The predominant use of VFAs can also be explained biologically through the metabolic pathway for the breakdown of the specific VFA. For example, a VFA with a more complicated metabolic pathway may have a lower rate of consumption. Since acetic acid is directly oxidized and converted to acetyl-CoA, its degradation follows a straightforward mechanism. On the other hand, the 3 and above carbon VFAs are either used by other, more complicated processes, or are going through numerous rounds of sequential oxidation, resulting in lower denitrification rates (Elefsiniotis et al., 2004).

Nevertheless, there was a variation in the denitrification rate when carbon sources were added at an equal mole (0.005 mol). The order of denitrification rate of the single VFAs in this case does not have a significant difference (p-value > 0.05 for all cases), which is different from previous studies (Elefsiniotis et al., 2004). Acetic acid, propionic acid, butyric acid, valeric acid, and caproic acid had mean denitrification rate values of 0.29, 0.30, 0.38, 0.34, and 0.22 g NO_x-N removed/m^2/day, while methanol had 0.77 g NO_x-N removed/m^2/day, respectively. These results disprove the notion that acetic acid is the preferred VFA for denitrification by demonstrating that there is a weak relationship between molecular complexity and denitrification rate. This relationship was also reflected in denitrification capacity. When the carbon source was based on the C/N ratio, 5.2 mg NO_3-N/L/h was removed by acetic acid. However, when balancing the carbon source by molecular weight, butyric acid had the highest overall value, with a value of 6.8 mg NO_3-N removed/L/h (Figure 4.1c and 4.1d).

The changes in the concentration of NO_3-N and NO_2-N are displayed in Figure 4.2a-f. To follow up on possible complete depletion of carbon sources, the values were recorded up to 120 minutes. All VFAs were consumed at a slow pace in the first 60 minutes, whereas the rate of NO_3-N decomposition accelerated after 60 minutes (Figure 4.2a-f). This initial lag might be interpreted as the microbial populations not being acclimated to the new carbon source provided. This claim is supported further by the fact that the rate of NO_3-N elimination increases with time. In all runs with methanol and individual VFAs, the NO_2-N concentration stayed below 5 mg/L throughout the experiment, showing that the NO_2-N created from the reduction of NO_3-N was used up right away due to the delayed conversion of NO_3 to NO_2 (Figure

4.2). Contrarily, in the run for methanol, which displayed rather rapid decrease in NO_3-N, however, a noticeable increase in NO_2-N were seen. Intriguingly, the rate of NO_2–N removal from the individual VFAs was much higher than that from methanol. As NO_2-N does not accumulate in the system, NO_2-N removal rates in all single acid tests are noticeably high. This is due to a slower but more balanced denitrification performance due to the NO_3 removal rate compared to methanol. This implies that VFAs outperformed methanol, in terms of NO_2–N reduction.

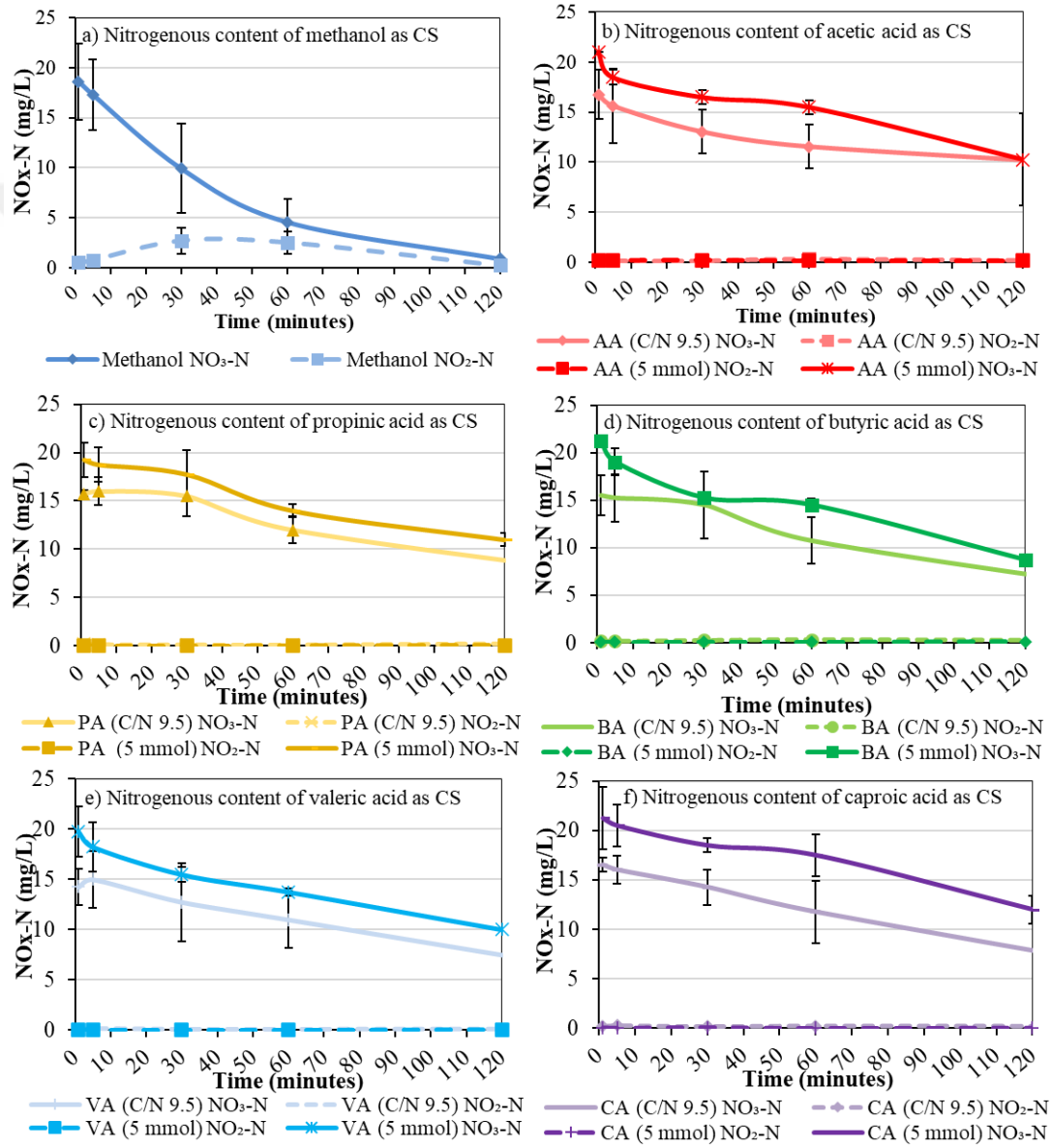

Figure 4.2 : Concentration of NO_3-N (solid line) and NO_2-N (dashed line) for a) methanol, b-f) single VFAs.

Table 4.2 shows that the acidity of the denitrification medium increased over time for some sets although denitrification is known to cause alkalinity. The extent of this rise

is dependent on the solution's ability to act as a buffer because nitrification, on the other hand, results in acidity (Albina et al., 2019). The observed increase in pH can be attributed to the purity of the added carbon source, which can influence this change. The largest pH shift is observed when VFAs are added on a molar basis as opposed to adding on a C/N basis, as larger amounts were added (Table 4.2) when the carbon source was molecular weight based (Nativ et al., 2021). Since acetic acid is the strongest acid of all VFAs tested (with a lowest pKa of 4.76) (Pervez et al., 2022), it caused the largest pH shift.

Table 4.2 : pH and temperature change during the test using single VFAs with C/N ratio of 9.5 and same molecular weight (5 mmol).

Carbon source	pH		Temperature	
	pH, 0 min	pH, 60 min	T (°C), 0 min	T (°C), 60 min
Methanol (C/N 9.5)	7.05±0.11	7.66 ±0.11	15.03±1.00	17.92±1.00
AA (C/N 9.5)	7.09±0.11	6.18±1.32	14.40±0.49	16.35±0.49
PA (C/N 9.5)	7.14±0.25	6.85±0.05	16.30±0.35	17.95±0.35
BA (C/N 9.5)	7.07±0.24	7.11±0.14	16.45±0.64	18.05±0.64
VA (C/N 9.5)	7.10±0.25	7.27±0.12	16.75±0.57	17.61±0.57
CA (C/N 9.5)	7.10±0.33	7.47±0.49	16.25±0.42	17.40±0.42
AA (5 mmol)	7.14±0.08	6.31±0.04	14.50±0.00	18.00±0.00
PA (5 mmol)	7.30±0.08	6.48±0.03	16.35±0.21	19.25±0.07
BA (5 mmol)	7.34±0.00	6.71±0.08	16.00±0.00	19.00±0.14
VA (5 mmol)	7.32±0.06	6.79±0.00	16.30±0.28	18.60±0.00
CA (5 mmol)	7.41±0.00	6.93±0.21	16.10±0.14	18.45±0.21

As shown in Table 4.3, the recorded values of nutrients remained relatively constant throughout the analysis. The test based on the same molecular weight shows the greatest differences in initial and final nutrient content. In the molecular weight-based tests and the C/N ratio-based tests, the initial ammonium levels varied from 10.0 to 18.3 mg/L and 6.0 to 8.2 mg/L, respectively. There were no significant changes in the detected phosphate content.

Table 4.3 : The change of nutrients concentration and added volume of carbon sources for individual VFAs experiments.

Carbon source (CS)	NH_4-N (mg/L)		PO_4-P (mg/L)		COD of CS (g/L)	Volume of CS added (µL)
	NH_4-N, 0 min	NH_4-N, 60 min	PO_4-P, 0 min	PO_4-P, 60 min		
Methanol (C/N 9.5)	8.3±1.3	8.8 ±0.0	3.4±1.7	2.1±1.3	1185	201
AA (C/N 9.5)	7.1±0.7	7.6±0.6	2.7±0.2	3.9±0.3	1122	212
PA (C/N 9.5)	6.0±2.3	7.7±0.4	3.1±0.2	3.3±0.3	1501	159
BA (C/N 9.5)	7.2±1.7	8.2±0.8	3.1±0.3	3.2±0.7	1750	136
VA (C/N 9.5)	8.1±0.7	8.6±0.3	3.0±0.4	3.5±0.7	1912	124
CA (C/N 9.5)	8.2±1.4	7.6±0.6	2.7±0.1	3.3±0.5	2043	117
AA (5 mmol)	10.0±0.0	9.5±0.7	1.2±0.0	2.6±0.1	1122	285
PA (5 mmol)	12.5±0.7	14.0±1.4	1.3±0.2	2.5±0.1	1501	373
BA (5 mmol)	13.3±0.4	12.5±0.7	1.3±0.1	2.5±0.0	1750	457
VA (5 mmol)	14.0±0.0	14.8±0.4	1.3±0.0	2.5±0.0	1912	544
CA (5 mmol)	18.3±3.2	20.0±2.8	2.5±0.9	2.7±1.0	2043	627

4.3.2 Denitrification using AD-VFA and sVFA as carbon source

The influence of using AD-VFA and sVFA as carbon sources on denitrification was examined using carriers acclimatized to methanol at Gryaab WWTP. Denitrification using methanol was also performed as a reference. In this section the denitrification potential of real VFA effluent (AD-VFA), sVFAs solution and methanol are compared.

The results of the denitrification assays are illustrated in Figure 4.3. As can be seen, when methanol was used as the carbon source the denitrification rate topped at 1.04 mg NO_x-N removed/m^2/day, while AD-VFA had 0.61 mg NO_x-N removed/m^2/day and sVFA had 0.57 mg NO_x-N removed/m^2/day. Each level mean in these results is in the same control level (not moderately significant, p value>0.05), demonstrating the AD-VFA and its synthetic counterpart (sVFA) as a potential carbon source for denitrification. Denitrification rate variations on methanol in terms of standard deviations may be caused by the microbial population and diversity of microorganisms carried by carriers even though duplications have been performed in the same day. The carriers for this experiment were obtained from Gryaab AB, where the post-denitrification process uses methanol as an external carbon source. As a result, the

microbial population has for long been adapted to methanol utilization. As asserted in literature the addition of a single carbon source restricts microbial diversity in denitrification in comparison to a carbon source with a variety of organic carbon-bearing components (Du et al., 2020). This could spark a conversation about how crucial microbial culture is when used in conjunction with a certain carbon source to achieve even higher denitrification rate. A certain carbon source metabolic pathway might also play a significant role. According to a study by Torresi et al. (2017), longer and more complex carbon sources require longer time to be metabolized and utilized in the denitrification process, which may explain why methanol has a greater rate of denitrification.

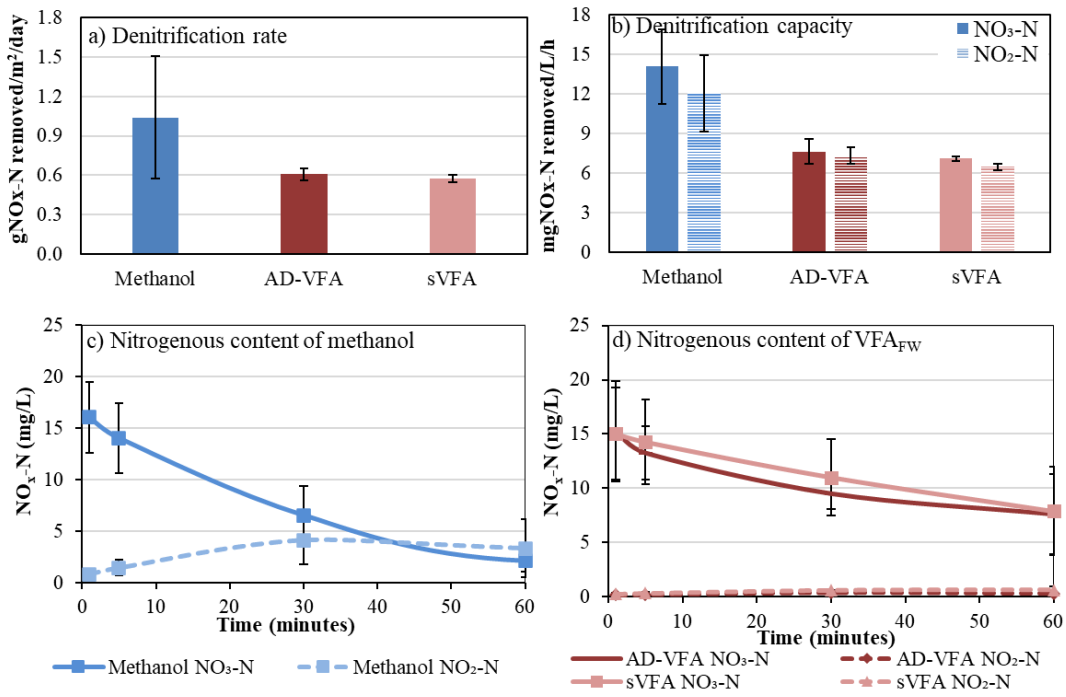

Figure 4.3 : a) Denitrification rate and b) denitrification capacity using AD-VFA, and sVFA, and methanol, c), and d), shows NO_3-N (solid line), and NO_2-N (dashed line) concentrations.

The total concentration of AD-VFA was 4.4 g/L and AD-VFA was composed of 16% acetic acid, 20% butyric acid, and 40% caproic acid. AD-VFA has a larger distribution of longer chain VFAs which can affect the pace of denitrification (Table 4.1 and Figure 4.3a). As can be seen in Table 4.1, AD-VFA also contained 380.0 mg/L ammonium, 99.1 mg/L phosphate. Regardless of the impact of impurities on denitrification, to meet nutrients discharge limits, the VFA effluent with the lowest nutrient content is favored. The sCOD/NO_3-N ratio of 9.5 was applied for all carbon sources, therefore the total

concentration of VFAs in the effluents affects the volume of effluent to be applied to reach similar sCOD loading and this defines the inflow of impurities.

Denitrification rates and capacities obtained for AD-VFA, and its synthetic counterpart (sVFA) were nearly equal (Figure 4.3a and 4.3b), supporting the claim that components other than VFAs in the AD-VFA effluent have no detrimental effects on denitrification performance. The nitrate and nitrite concentrations of the tests are plotted against time in Figure 4.3c and 4.3d. As can be seen in Figure 4.3c, even though methanol had a much faster nitrate removal rate compared to the VFAs, nitrite accumulation is the highest for methanol. However, removal of nitrite was substantially closer in rate to nitrate disappearance for the AD-VFA effluent. In this regard, the best result was achieved when AD-VFA was applied, where the amount of removed nitrate was quite similar to that of nitrite. This indicates that even though methanol has higher denitrification rate and capacity, mainly due to nitrate conversion, the VFAs were just as effective or better than methanol in eliminating nitrite. The lack of nitrite accumulation in VFA effluent lends credence to the claim that nitrite elimination in methanol and VFA effluent is equivalent.

The hypothesis that the composition of AD-VFA effluents plays a significant role in denitrification efficiency is further supported by the observation that nitrate removal performance improved over time for the AD-VFA. This work is comparable to a study on denitrification in which VFA filtrate/effluent was compared with other routinely utilized carbon sources (Kim et al., 2016), where denitrification was carried out using sludge as denitrification inoculum. In comparison to methanol and acetate, it was shown that VFAs had the highest potential for denitrification, while methanol had the slowest rate (Kim et al., 2016). However, the study conducted by Kim et al. (2016) employed denitrification sludge, whereas, in this work MBBR reactor was utilized. This would imply that when applied in combination with denitrification sludge, VFAs are a better carbon source for denitrification. The reason for this may be the use of a denitrifying inoculum in the form of suspended sewage sludge and not one immobilized on carriers. Sewage sludge is a well-studied inoculum largely used for biogas production, where VFAs are the intermediate product and are eliminated via beta-oxidation. This provides additional support for the idea that the microbial community and adaptation play a significant role in denitrification efficiency.

On the other hand, the study mentioned above discovered that the denitrification capabilities for VFAs and methanol were 2.78 mg nitrate-N removed/h and 0.82 mg nitrate-N removed/h, respectively. In comparison, significantly higher values of 10.6 mg nitrate-N removed/h and 7.6 mg nitrate-N removed/h were observed in this work for methanol and AD-VFA, respectively (Figure 4.3c and 4.3d). These values indicate that the different results are not due to the reactor type, as the MBBR denitrification showed much higher nitrate removal values compared to the denitrifying sludge used in the comparative study. This supports the argument that the lower denitrification efficiency of VFAs as carbon source is primarily due to the microbial community involved in the process and their biochemical reaction rates when using VFAs as electron donors.

In the denitrification experiments, as the reason was to see what effect the addition of carbon sources has on pH, pH was not adjusted, and it was controlled before and after denitrification as it is shown in Table 4.4. Although, denitrification functions best at neutral pH (range of pH 6-9), possible pH manipulation in WWTPs would result in high economic costs. In all assays the pH of denitrification ranged from 7.05 to 7.66, primarily reflecting the pH of the influent wastewater to the denitrification tanks. The final pH values (Table 4.4.) were all slightly higher than initial on the basis that denitrification reaction causes alkalinization (Ji et al., 2017). When the VFAs derived from anaerobic digestion applied, as they often have a neutral pH, which can mean that they do not have the same influence on the pH of the denitrification as synthetically produced pure VFAs have (Table 4.2).

Table 4.4 : pH, temperature, and nutrients change during the denitrification test using AD-VFA and sVFA.

Parameters	**Carbon sources**		
	Methanol	**AD-VFA**	**sVFA**
pH, 0 min	7.05±0.11	7.15±0.11	7.15±0.02
pH, 60 min	7.66±0.11	7.51±0.01	7.22±0.01
T (°C), 0 min	15.03±1.00	16.80±0.64	18.10±0.78
T (°C), 60 min	17.92±1.00	18.85±0.64	18.75±0.78
NH_4-N (mg/L), 0 min	8.3±1.3	11.0±4.2	7.8±3.9
NH_4-N (mg/L), 60 min	8.8 ±0.0	12.8±1.1	8.8±3.9
PO_4-P (mg/L), 0 min	3.4±1.7	4.1±0.5	3.8±0.7
PO_4-P (mg/L), 60 min	2.1±1.3	4.3±0.2	3.6±0.4
COD of CS (g/L)	1185	14.5	9.0
Volume of CS added (mL)	0.201	16.41	26.44

Regarding temperature, at the start of the experiment, the temperature was between 15 to 18 °C. This can be thought of as the average natural temperature during the denitrification process as the temperature of the incoming wastewater might vary from 7 to 25 °C due to seasonal changes in Sweden. According to a study by Elefsiniotis and Li (2006) on the effect of temperature on denitrification, regardless of temperature, none of the carbon sources evaluated demonstrated a tendency to lose denitrification efficiency throughout a range of temperatures studied (10-30 °C). The initial ammonium and phosphate content of the denitrification batches were different as an initial C/N ratio of 9.5 was to be reached on carbon sources containing different initial composition (Table 4.4). Additionally, compared to methanol, AD-VFA and sVFA as carbon source had a lower COD content, hence a larger volume of them was applied, resulting in a greater amount of unwanted nutrients. With an initial concentration of 11.0 mg/L ammonium and 4.1 mg/L phosphorus for AD-VFA compared to 7.8 mg/L ammonium and 3.8 mg/L phosphorus when the synthetic solution was employed. AD-VFA effluents, as expected, had the higher end nutrient concentrations (Table 4.1).

4.4 Conclusions

This study demonstrates that AD-VFA are promising carbon source that has the potential to replace methanol in denitrification. VFAs in WWTPs have been shown to reduce carbon footprints and enhance sustainability. Acetic acid is the preferred type among all the single acids. VFAs outperformed methanol in the removal of nitrite. With C/N ratio of 9.5 and denitrification rates of 0.61±0.04 and 0.57±0.03 g NO_x-Nremoved/m^2/day, respectively, AD-VFA and sVFA performed nearly as well as methanol (1.04±0.46 g NO_x-Nremoved/m^2/day). To demonstrate the viability of conventional application, the potential of using different VFAs, concentrating VFAs, and combining carbon sources must be researched further.

5. THE EFFECT OF SEQUENTIAL AND SIMULTANEOUS SUPPLEMENTATION OF WASTE-DERIVED VOLATILE FATTY ACIDS AND METHANOL AS ALTERNATIVE CARBON SOURCE BLEND FOR WASTEWATER DENITRIFICATION[4]

5.1 Introduction

An increase in the population almost always leads to an increase in key challenges worldwide. The protection of the aquatic environment and the conservation of surface waters are no exceptions (Elefsiniotis & Li, 2006). The rise in harmful nutrients in municipal wastewater has compelled policymakers to take an action and enact stringent legislation and regulations to prevent nutrient release. This is to avoid further issues such as eutrophication and the degradation of rivers and lakes. The treatment of contaminated wastewater is a critical step in preventing these issues. Surface water protection requires the application of mechanical, chemical, and biological wastewater treatment systems (Anderson et al., 2002). The objective of managing wastewater is two-fold, namely, to reduce the negative impacts on the environment and economy caused by its disposal, as well as to extract energy and resources from this waste flow. By doing so, we can create a more sustainable system that not only minimizes environmental damage but also provides economic benefits through increased resource efficiency (Cecconet & Capodaglio, 2022).

Biological nutrient removal, which removes nutrients such as nitrogen, and phosphorus, is one of the most important processes in a wastewater treatment plant (WWTP). The biological nutrient removal process often contains three consecutive stages that are phosphorus removal, nitrification, and denitrification (Swinarski et al., 2009). Nitrogen removal consists of two main steps of nitrification and denitrification. Nitrification is facilitated by specialized autotrophic bacteria, such as

[4]This chapter is based on: Sapmaz, T., Manafi, R., Mahboubi, A., Koseoglu-Imer, D. Y., & Taherzadeh, M. J. (2023). The Effect of Sequential and Simultaneous Supplementation of Waste-Derived Volatile Fatty Acids and Methanol as Alternative Carbon Source Blend for Wastewater Denitrification. Sustainability, 15(8), 6849.

ammonia-oxidizing bacteria and nitrite-oxidizing bacteria, that convert ammonium to nitrate under aerobic conditions (Metcalf et al., 2014). Following nitrification, the denitrification process then receives the wastewater and utilises heterotrophic microorganisms to convert nitrate to nitrogen gas via a sequence of biological reactions (Metcalf et al., 2014).

Fixed film post-denitrification processes, such as deep-bed denitrifying filters, fluidized bed reactors, submerged attached growth filters, and moving bed biofilm reactors, are likely to be installed in order to meet final total nitrogen criteria close to the technological limit (Akter et al., 2022).

The MBBR process, distinct from other fixed films systems, combines the benefits of both a normal activated sludge process and other biofilm processes by using free-floating polyethylene medium (carriers) to give significant surface area for attached biomass growth without the usage of an internal recycling. MBBRs have been utilized in industrial wastewater treatment since introduction in Norway in the late 1980s (Odegaard et al., 1999).

Pre-denitrification, post-denitrification, and combinations of the two have all been denitrification processes in which MBBRs have been employed. A combination configuration is used by the majority of plants, particularly those in northern Europe, which allows for operational flexibility in extreme cold climates to increase the nitrification/denitrification capacity in an existing plant.

However, by the time wastewater enters the post-denitrification process, it has been depleted of soluble organic material. Low carbon to nitrogen ratios (C/N) in wastewater are a common issue for WWTPs, reducing denitrification effectiveness. As a result, more carbon sources are needed for efficient denitrification, requiring external carbon source addition to elevate the C/N ratio to a suitable level for effective denitrification (Wei et al., 2012).

Conventionally, methanol, ethanol or acetate are used to substitute for the lack of carbon source in the denitrification process (Wei et al., 2012). Due to its effectivity, methanol is the most often used option for removing nitrate through denitrification (Dhamole et al., 2015). The use of methanol in conventional denitrification processes has been judged unsustainable due to its fossil-based production and associated costs, which can account for up to 70% of WWTP operating and maintenance costs

(MacDonald, 1990). Zhang et al. (2016a) tested differed carbon sources to find a greener and cheaper alternative. Although solid carbon sources such as paper, wood and straw were investigated, they were found to be less effective than methanol, resulting in very low denitrification rates. Previous studies have investigated alternative specific waste resources that show promising denitrification values (Cappai et al., 2004; Fernández-Nava et al., 2010; Rodriguez et al., 2007). It is known that heterotrophic nitrate reducers can also use organic acids as electron donors in addition to a variety of other carbon sources. Acetate, propionate, butyrate, valerate, and caproate collectively known as volatile fatty acids (VFAs), are additional substrates for denitrifiers (Pang & Wang, 2021). VFAs, are essential intermediates produced through acidogenic fermentation of organic waste. Hence, waste-derived VFAs are acknowledged as a sustainable and affordable external carbon source for biological nutrient removal processes (Feng et al., 2021).

Livestock manure is currently the greatest organic waste stream in most countries, with a variety of detrimental environmental effects. However, employing chicken manure as a readily available biomass for acidogenic fermentation process could reduce its negative impact on the environment while at the same time creating valuable end products like VFAs (Ngo et al., 2022). Not only animal manure but industrial waste streams have also shown a great potential for use in the production of VFAs, owing to their high concentrations of proteins, carbohydrates, and lipids. For example, the production of potato starch, particularly one of the common industries in southern Sweden, leaves behind a significant amount of potato pulp and liquor in which proteinaceous component of it is coagulated by steam treatment and separated using a decanter. The residual potato liquor—which primarily contains the soluble portion of the waste from the manufacturing of potato starch—is concentrated and forms the potato protein liquor (PPL) that still has 40% solids (Sar et al., 2022). It has been proven that the properties of such feedstocks are promising for VFA production (Sapmaz et al., 2023; Yin et al., 2022), which may then be employed as a carbon source in a denitrification process.

Although there are a number of reports on the application of VFAs for denitrification (Elefsiniotis & Li, 2006; Elefsiniotis et al., 2004; Lee et al., 2014; Mahmoud et al., 2022), application of waste-derived VFAs and along with methanol or in stepwise

manner as carbon source for post-denitrification biofilm process has not been studied in the literature according to the authors' knowledge.

In the current research, the feasibility and performance of VFAs originating from different waste resources of fermented potato protein liquor (PPL) effluent and chicken manure (CKM)- as electron donors in post denitrification process was evaluated. Moreover, the potential of sole addition and partial substitution of conventionally used carbon sources with VFA effluents was evaluated in order to propose solutions for potentially greener, more sustainable and fossil source-independent wastewater treatment process.

5.2 Materials and Methods

5.2.1 Carbon source characteristics

In this study, organic carbon sources/electron donors included both traditional carbon sources methanol and ethanol from the category of alcohols and heat shock treated various waste streams through anaerobic fermentation effluents namely chicken manure derived VFA effluent (AD-VFA_{CKM}) and potato protein liquor derived VFA effluent (AD-VFA_{PPL}) from the category of organic acids. Because methanol (≥99.8%, Sigma-Aldrich) and absolute ethanol (≥99.8%, VWR Chemicals) are routinely utilized carbon sources, they were used as reference carbon sources for denitrification tests.

To be thorough, synthetically produced VFAs ($sVFA_{CKM}$ and $sVFA_{PPL}$) were evaluated alongside the biologically produced AD-VFAs (AD-VFA_{CKM} and AD-VFA_{PPL}). AD-VFAs were created in prior from anaerobic fermentation of chicken manure (Yin et al., 2022) and potato protein liquor (Sapmaz et al., 2023). Membrane bioreactor (MBR) was operated in a similar design as described in (Jomnonkhaow et al., 2021) for the production of AD-VFA_{CKM} and AD-VFA_{PPL}. After anaerobic fermentation and MBR filtration, the recovered AD-VFAs were collected and stored at -18 ± 2 °C to be evaluated as an external carbon source for denitrification. As for the preparation of synthetic VFAs, solutions of AD-VFA effluents were mimicked using laboratory-grade acetic, propionic, butyric, iso-butyric, valeric, and caproic acids that were acquired from Sigma-Aldrich. For all denitrification tests, the total amount of carbon sources added was based on C/N ratio of 9.5 as recommended by Gryaab WWTP according to operational procedures.

The properties of each AD -VFAs and carbon sources used are listed in Table 5.1

Table 5.1 : Characteristics of evaluated carbon sources.

Parameters	Carbon sources					
	AD-VFA$_{CKM}$	**sVFA$_{CKM}$**	**AD-VFA$_{PPL}$**	**sVFA$_{PPL}$**	**Methanol**	**Ethanol**
pH	6.32 ± 0.02	2.99 ± 0.02	7.73 ± 0.02	2.97 ± 0.02	-	-
sCOD (g/L)	14.5 ± 0.71	9 ± 0.00	12.5 ± 0.00	8.75 ± 0.00	1185	1650
NH_4^+-N (mg/L)	2540.0 ± 28.28	-	485.0 ± 7.07	-	-	-
PO_4-P (mg/L)	1747.0 ± 15.56	-	33.0 ± 0.28	-	-	-
NO_3-N (mg/L)	20.8 ± 0.57	-	16.05 ± 0.21	-	-	-
NO_2-N (mg/L)	0.20 ± 0.00	-	0.31 ± 0.00	-	-	-
Acetic acid (g/L)	4.36 ± 0.02	4.4 ± 0.10	4.15 ± 0.09	4.1 ± 0.10	-	-
Propionic acid (g/L)	0.74 ± 0.01	0.7 ± 0.10	0.50 ± 0.19	0.5 ± 0.10	-	-
Iso-butyric acid (g/L)	0.26 ± 0.04	0.3 ± 0.10	0.13 ± 0.06	0.1 ± 0.10	-	-
Butyric acid (g/L)	1.03 ± 0.09	1 ± 0.10	0.66 ± 0.05	0.7 ± 0.10	-	-
Isovaleric acid (g/L)	0.02 ± 0.00	0.02±0.01	0.00 ± 0.00	0.0 ± 0.00	-	-
Valeric acid (g/L)	0.02 ± 0.00	0.02±0.01	0.02 ± 0.00	0.02±0.01	-	-
Caproic acid (g/L)	0.49 ± 0.01	0.5 ± 0.10	0.52 ± 0.01	0.5 ± 0.10	-	-
tVFA (g/L)	6.92 ± 0.08	7 ± 0.10	5.98 ± 0.07	6 ± 0.10	-	-

5.2.2 Post-denitrifying MBBR operation

A lab-scale moving bed biofilm reactor (MBBR) system was built-up and operated with four identical glass reactors the same way as described in *Sapmaz et al.* (Sapmaz et al., 2022b). Non-acclimated carriers (AnoxKaldnes^{TM} K1, internal specific surface area of 500 m^2/m^3) were used in post-denitrifying bench-scale MBBRs.

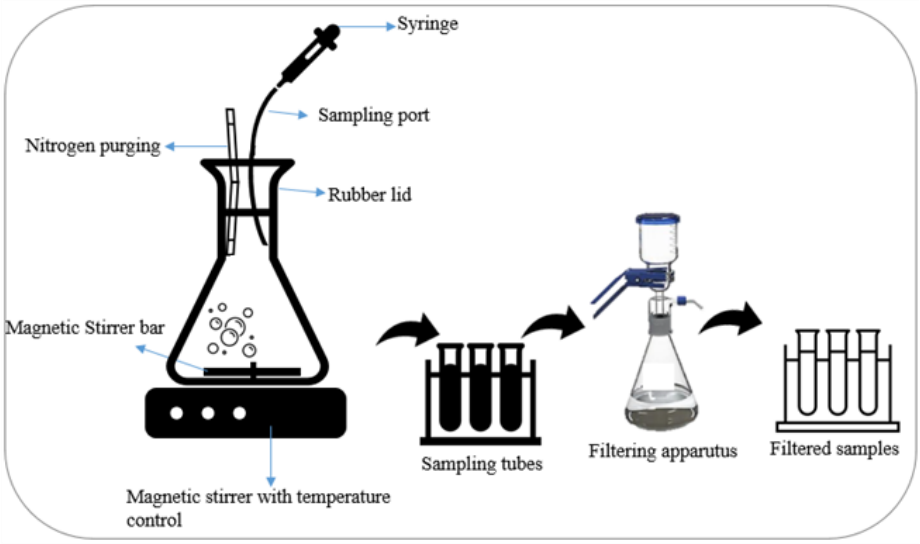

Figure 5.1 : Experimental schematic drawing of whole process.

The denitrification rate (DNR) (Shrestha et al., 2009) and denitrification capacity was calculated in the same way that was presented by *Sapmaz et al.* (2022b):

• Denitrification rate:

$$DNR\ (g\ NO_x/m^2/d) = \frac{k\ (mg/L/min) \times 60\ (\text{min}) \times 24\ (h) \times V_S\ (L)}{A_{carrier}\ (m^2) \times 1000\ (mg)} \quad (5.1)$$

Where k, V_s and $A_{carrier}$ are:

k (mg/L/min) = NO_x removal rate

V_s (L) = V_0 (L) − (V_{sample}/2) − ($V_{carriers}$(L) × bulk volume of the carriers (14%)

$A_{carrier}$ = $V_{carriers}$ (1L) × specific surface area of carriers (500 m^2/m^3)

• Denitrification capacity:

The amount of NO_3-N removed from the system in 60 minutes is estimated by subtracting the first minute's data from the concentration of the 60 minutes sample (2). The computed value for NO_3-N is deducted from the final 60 min NO_2-N concentration to get the denitrification capacity for NO_2-N. (3).

$$D_{cap}(NO_3 - N) = mg(NO_3 - N)\ (1\ min) - mg(NO_3 - N)(60\ min) = mgNO_3 - N\ \ removed/L/\ h \quad (5.2)$$

$$D_{cap}(NO_2 - N) = D_{cap}(NO_3 - N) - (NO_2 - N)(60\ min) = mgNO_2 - N\ removed/L/\ h \quad (5.3)$$

5.2.3 Sampling and analytical methods

Samples were obtained at regular intervals (1, 5, 10, 20, 30, 40, 50, and 60 minutes) during denitrification to measure the content of nitrogenous compounds, COD, PO_4-P, and pH. Before storing the samples for analysis, samples were filtered using 1.6 µm glass microfiber filters first and prior to kit analysis, they were filtered again with 0.45 µm syringe filters to remove turbid materials. NANOCOLOR® tube tests were used to measure the amounts of NO_3-N, NO_2-N, NH_4^+-N, PO_4-P and COD (MACHEREY-NAGEL GmbH & Co. KG, Germany) according to the manufacturer's instructions. Gas chromatography (GC) (Clarus 550, PerkinElmer, Norwalk, CT, USA) with a hydrogen flame ionization detector and capillary column (Elite-Wax ETR, 30 m × 0.32 mm × 1.00 µm, PerkinElmer, Norwalk, CT, USA) was used to calculate VFA profiles, with 1g/L butanol used as an internal reference. Temperature and pH were routinely measured at the start and end of denitrification with a digital thermometer and a pH electrode (S400, Mettler Toledo, Switzerland). To acquire trustworthy results, all measurements were carried out in parallel. The chemical analyses were performed in triplicate, and the denitrification study data were conducted in duplicate. Statistical significance of the obtained results was calculated using one-way analysis of variance (ANOVA, $p<0.05$). At the same time, all data points were calculated according to the mean and together with standard deviations. In statistical analyses, p-values of less than 0.05 were considered significant.

5.3 Results and Discussion

The supplied VFA effluents from anaerobic MBR of chicken manure and potato protein liquor substrates were tested as alternative carbon sources for enhanced nitrogen removal in wastewater treatment. AD-VFAs can replace the fossil-based and conventionally used methanol or ethanol in the wastewater denitrification process, potentially reducing carbon footprints and boosting sustainable development. To evaluate the denitrification potential of AD-VFA_{CKM} and AD-VFA_{PPL} and compare it to commonly used methanol and ethanol, this study first evaluated the impact of different waste-derived VFAs (AD-VFA_{CKM}, sVFA_{CKM} AD-$VFA_{PPL,}$ and sVFA_{PPL}), then evaluated partial substitution/replacement of methanol and ethanol with co-fed and sequential addition of AD-VFA_{PPL}.

5.3.1 Source of VFA effluents as the sole carbon source on denitrification

To explore the efficiency of proposed carbon sources as external carbon source for nitrogen removal, VFAs were added into lab-scale anoxic denitrification reactors, and commercial methanol was used as the control. For all denitrification tests, the total amount of carbon sources added was based on C/N ratio of 9.5. The effects of using AD effluents of PPL and CKM substrates as carbon sources on denitrification were investigated by using carriers provided by the WWTP that had previously been fed with methanol. This section of the study compares the denitrification potential of AD-VFA_{PPL}, AD-VFA_{CKM}, sVFA_{PPL}, sVFA_{CKM} solutions as the sole carbon source, and methanol as a reference.

The compositions of AD-VFAs were measured in this study (Table 5.1). There was no apparent variation in VFA effluents properties regarding VFA composition as it is shown in Table 5.1, indicating the viability of comparing these AD-VFAs for advanced nitrogen removal. The overall VFA concentration was roughly 6 g/L for VFA_{PPL} and 7 g/L for VFA_{CKM}, respectively. Acetic acid was the predominant product in both AD-VFAs and accounted for 69.4% and 63% of the total VFAs in AD-VFA_{PPL} and AD-VFA_{CKM}, respectively. Butyric and propionic acid made up 11% and 8.4% in AD-VFA_{PPL}, and 4.9% and 10.7% in AD-VFA_{CKM}, respectively (Table 5.1). Distribution of individual VFAs in the mixture influences the pace of denitrification as there is an utilization pattern (Elefsiniotis & Wareham, 2007). On the other hand, as shown in Table 5.1, there were differences in the concentrations of PO_4-P and NH_4^+-N in the AD-VFAs used. While AD-VFA_{PPL} contained only 485.0 mg/L ammonium and 33.0 mg/L phosphate, the values of these nutrients were 2540 mg NH_4^+/L and 1747 mg PO_4/L in the AD-VFA_{CKM} (Table 5.1). Because the C/N ratio of 9.5 was used, the total concentration of VFAs in the effluents impacts the volume of carbon source required for denitrification to achieve equivalent sCOD loading, which defines the impurity in the reactor. Depending on how or whether contaminants affect denitrification, and the process configuration of the WWTP, the VFA effluent with the lowest nutrient content may be preferable to avoid external deterioration and meet nutrient discharge standards (Sapmaz et al., 2022a).

The results of the denitrification assays are illustrated in Figure 5.1. When methanol was used as the carbon source, the denitrification rate topped at 1.06 mg NO_x-N removed/m^2/day, while AD-VFA_{PPL} and AD-VFA_{CKM} had 0.56 and 0.41 mg NO_x-N

removed/m^2/day, and $sVFA_{PPL}$ and $sVFA_{CKM}$ had 0.44 and 0.41 mg NO_x-N removed/m^2/day, respectively. Each level mean in this result is in the same control level (not moderately significant, p value>0.05), demonstrating the naturally occurred AD-VFAs from PPL and CKM and their synthetic counterpart; sVFAs, as a potential carbon source for denitrification.

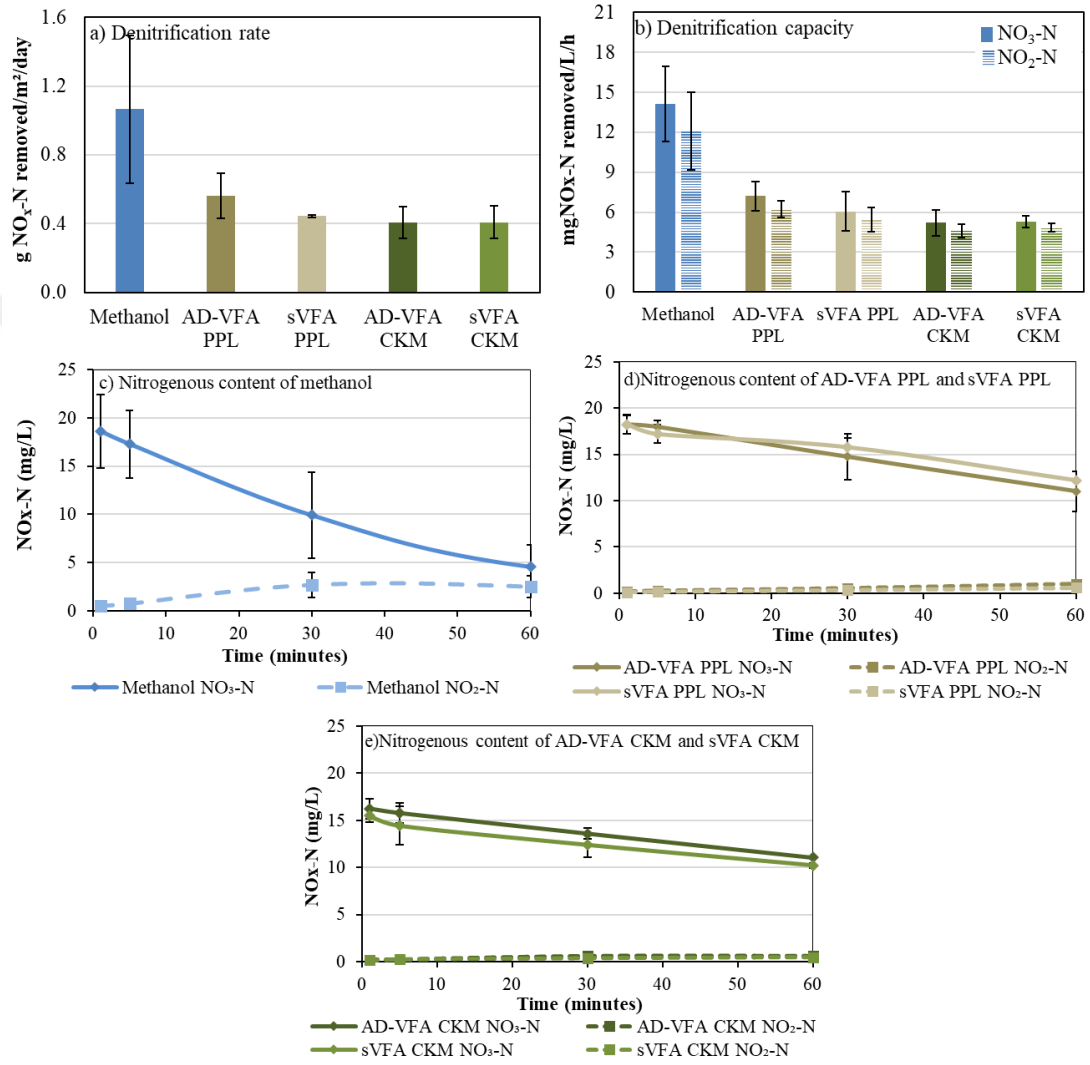

Figure 5.2 : (a) Denitrification rate (b) denitrification capacity (c-e) NO_x-N removal profiles over time.

Denitrification rate variations on methanol in terms of standard deviation may be caused by the microbial population and diversity of microorganisms carried by carriers that can differentiate during two consecutive days where experiments were conducted. The carriers for this experiment were obtained from the post-denitrification process that uses methanol as an external carbon source. Understanding the microbial culture of the denitrifying community is necessary to comprehend the denitrification rate theory (Barber & Stuckey, 2000). In order to achieve the highest possible

denitrification rates, it is believed that the link between bacterial culture and denitrification ability is crucial. More research is needed to get more firm results because few studies have been done on comparative analyses of various microbial cultures. In a study conducted on an anammox batch reactor that was analyzing the change in bacterial phyla following the addition of glucose as a carbon source, a wider variety of microbial cultures were seen when organic solids with various amounts of organic material were introduced. The microbial community's diversity was drastically decreased when glucose was added as the sole carbon source. This shows that only the bacteria that can metabolize a given carbon source becomes predominant in denitrification system (Du et al., 2020). The microbial population in the carriers that has been used in this study has for long been adapted to methanol utilization. Hence, the lower denitrification efficiency of AD-VFA$_{PPL}$ and AD-VFA$_{CKM}$ as carbon source is primarily due to the microbial community involved in the process and their biochemical reaction rates when using VFAs as electron donors. The yield and carbon utilization rates for VFAs were substantially lower than expected and lower than methanol as opposed to previously reported in (Kim et al., 2016; Liu et al., 2016; Mahmoud et al., 2022; Zhang et al., 2016a), indicating that suspended growth methods may have an advantage in terms of carbon utilization rates over attached growth processes. Higher biomass concentrations (with greater specific surface area) in smaller reactor volumes and shorter HRTs are generally advantages of attached growth methods over suspended growth; nevertheless, it is more difficult to transport substrates (mass transfer) to the biofilm as an electron donor due to diffusion and advection (Bill et al., 2009; Rittmann & McCarty, 2001). Furthermore, microbial culture adaption to a certain carbon source may be one of the reasons why methanol reaches even higher denitrification rate and capacity as unacclimated carriers were used in this study.

Denitrification rates and capacities obtained for AD-VFAs, and its synthetic counterparts (sVFAs) were nearly equal (Figure 5.1a and Figure 5.1b), supporting the claim that components other than VFAs in the AD-VFA effluent have no detrimental effects on denitrification performance. These results align with previous study that investigated the feasibility of using food waste fermentation liquid as the external carbon source (Feng et al., 2021). The ammonium nitrogen and phosphate in fermentation liquid did

not only cause any external deterioration of ammonium removal but also enhance the nutrient removal (Feng et al., 2021).

The nitrate and nitrite concentrations of the tests are plotted against time in Figure 5.1c-e. As can be seen in Figure 5.1c, even though methanol had a much faster nitrate removal rate compared to the VFAs, nitrite accumulation is the highest for methanol. However, removal of nitrite was substantially closer in rate to nitrate disappearance for the VFA_{PPL} and VFA_{CKM} (Figure 5.1d-e). In this regard, the best result was achieved when AD-VFA_{PPL} was applied, where the amount of removed nitrate was quite similar to that of nitrite. This suggests that the VFAs are more effective than methanol, owing primarily to nitrate conversion and particularly in reducing nitrite. This corresponds to a study done by Kim et al. (2016) that also observed faster removal of nitrite (in terms of reaction rate and lag length) with the fermentation filtrates as a denitrification carbon source among those tested (Kim et al., 2016). Therefore, hypothesis that the impurities even in AD-VFA_{CKM} did not cause deterioration in the denitrification efficiency is further supported by the observation of improved nitrate removal performance over time when AD-VFA_{PPL}, AD-VFA_{CKM}, $sVFA_{PPL}$ or $sVFA_{CKM}$ utilized.

Table 5.2 : pH, temperature, and nutrients change during the denitrification.

Carbon source used	Carbon sources (CS)				
	(Methanol)	**(AD-VFA_{PPL})**	**($sVFA_{PPL}$)**	**(AD-VFA_{CKM})**	**($sVFA_{CKM}$)**
pH, 0 min	7.05±0.11	6.94±0.03	7.03±0.06	7.10±0.14	7.16±0.25
pH, 60 min	7.66±0.11	7.71±0.15	7.37±0.41	7.40±0.28	7.43±0.49
T (°C), 0 min	15.02±1.00	15.70±0.57	15.85±0.78	15.70±0.99	15.45±1.77
T (°C), 60 min	17.52±1.00	17.70±0.57	17.55±0.78	18.00±0.99	18.15±1.77
NH_4-N, 0 min	8.3±1.3	11.8±0.3	7.6±0.6	19.0±0.8	8.1±0.6
NH_4-N, 60 min	8.8±0.0	12.3±1.8	7.7±1.2	17.4±3.0	8.5±0.7
PO_4-P, 0 min	3.4±1.7	3.1±3.2	2.1±1.9	4.3±1.2	3.0±0.4
PO_4-P, 60 min	2.1±1.3	3.3±1.9	2.2±1.2	5.7±2.4	3.3±0.2
COD of CS (g/L)	1185.0	12.5	8.8	15.5	11.0
Volume of CS added (mL)	0.201	19.04	27.20	16.41	21.64

In the denitrification experiments, the operating parameters such as pH and temperature were not adjusted. The reason was to see what effect the addition of carbon sources had on pH change and denitrification. Furthermore, the denitrification tests have been performed in a manner that could be similar to the traditional approach, with

the pH not being altered. The need to alter the pH in the WWTPs would result in high economic costs, hence it could be challenging.

Denitrification process function the best at neutral pH. As microbial denitrification is a biological respiration process uses various enzymes to reduce nitrogenous substances, all enzymes that function properly at an ideal pH are affected by the change of pH (Albina et al., 2019). As a result, the pH range for most denitrifying bacteria is between 7.5 and 9.5 (Albina et al., 2019). All experiments show that the pH of denitrification ranges from 6.94 to 7.71, primarily reflecting the pH of the influent wastewater to the denitrification tanks. The denitrification tests' final pH values (Table 5.2) were all slightly raised on the basis that denitrification reaction causes alkalinization, raising pH during the denitrification process (Albina et al., 2019; Ji et al., 2017). Although VFAs are weak acids, adding them as carbon source had little effect on the reactors' pH because all final pH values were close to the neutral pH of 7.

Regarding temperature, at the beginning of the experiment, the temperature was between 15.0 to 16.0 °C. This can be thought of as the average temperature that happens spontaneously during the traditional denitrification process as the temperature of the incoming wastewater might vary from 7 to 25 °C due to seasonal changes. Initial temperatures rose to around 18.0 °C for final temperature measurements and remained between 17.5 and 18.5 °C. As the temperature was not controlled, it is not clear from this study if temperature affects denitrification efficiency. However, according to a study by Elefsiniotis and Li (2006) on the effect of temperature on denitrification, regardless of temperature, none of the carbon sources evaluated, including VFA and methanol, demonstrated a tendency to lose denitrification efficiency throughout a range of temperatures studied (10-30 °C). This may suggest that the maintained temperature may not be necessary to demonstrate that one carbon source is more efficient than another; rather, maintaining a temperature within a closer range is enough to see results that are comparable to those obtained by maintaining the same temperature for all. In contrast to these, as the process originated in Scandinavia, emphasis has been placed on studying carbon sources (methanol and ethanol) at low temperatures in MBBRs as well. The use of methanol in pilot-scale denitrifying MBBRs at 10°C was examined by Rusten et al. (1997), and maximum denitrification rate of 1.4 g NO_3-N/m^2/day found with the 4.55 g COD_{used}/g NO_3-$N_{eq\ removed}$ ratio.

Similar experiments were conducted by Aspegren et al. (1998) at 16°C, and they reported a maximum denitrification rate of 2.0 g N/m^2/day with the ratio of 4-5 g COD/g NO_3-N_{eq}.

Analysis of the initial nutrients content of the denitrification experiments show a variation in the ammonium initial values. Ammonium-nitrogen and phosphate-phosphorus are the nutrients considered. To achieve the required C/N ratio of 9.5, the carbon source's sCOD value, which represents the amount of carbon source that must be added, is also presented in Table 5.2. Due to the different characteristics of the carbon sources utilized in denitrification, the starting nutrient concentration values displayed variances. Additionally, compared to methanol, AD-VFAs as carbon source had a lower COD due to the low VFA concentrations, hence a larger volume of them was needed to maintain the same C/N ratio of 9.5. When AD-VFA effluents were employed instead of a synthetically prepared solutions and methanol, a greater amount of nutrients was provided during denitrification due to the addition of higher volumes (19.04 ml for AD-VFA_{PPL}, 16.41 ml for AD-VFA_{CKM}). With an initial concentration of 19.0 mg/L ammonium and 4.3 mg/L phosphorus compared to 8.1 mg/L ammonium and 3 mg/L phosphorus when the $sVFA_{CKM}$ was employed, AD-VFA_{CKM} had the higher nutrient concentrations as shown by the initial characteristics (Table 5.2). Ammonium concentrations were around 8 mg/L and phosphorus concentrations were 2.1–3.4 mg/L when employing methanol and sVFAs (Table 5.2). A slight increase in concentrations was observed, but the change in ammonium and phosphorus concentrations was not significant and remained the same throughout the test. This could be due to the agitation that leads breaking up some of the biofilm to be broken up by the carriers, which would release some nutrients into the mixture.

5.3.2 Effect of co-fed carbon sources on denitrification

The goal of this stage of work is to analyze the effect of a mixed carbon source in a co-fed denitrification and its possibility to facilitate a partial substitution of conventionally used carbon sources. For this reason, denitrification test was analyzed observing the opportunity to mix various carbon sources together in a co-fed denitrification process. The mixture or partial replacement of methanol may indicate if it is possible to replace a portion of the added carbon source with VFA effluents and hint a way to adapt the MBBR denitrification carriers to the new carbon source by

gradually increasing the amount of VFA effluent parallel to decreasing the amount of methanol. Evaluated carbon sources in regards are ethanol, methanol and $sVFA_{PPL}$ and $AD\text{-}VFA_{PPL}$.

Denitrification rate for ethanol observed a value of 1.1 g NOx-N removed/m²/day with a denitrification capacity of 17.3 mg nitrate removed/L/h (Figure 5.2a and Figure 5.2b). The value reflects similarities with the denitrification efficiencies of methanol. One reason can be similar metabolic pathway in which both ethanol and methanol are alcohols and metabolised into their corresponding aldehydes (Schulz, 2002). However, the metabolic pathway of ethanol metabolites acetaldehyde into acetic acid which then follows a similar pathway that the VFAs undergo through beta oxidation. If then ethanol is comparted to VFAs one can observe a significantly better denitrification rate and denitrification capacity when using ethanol.

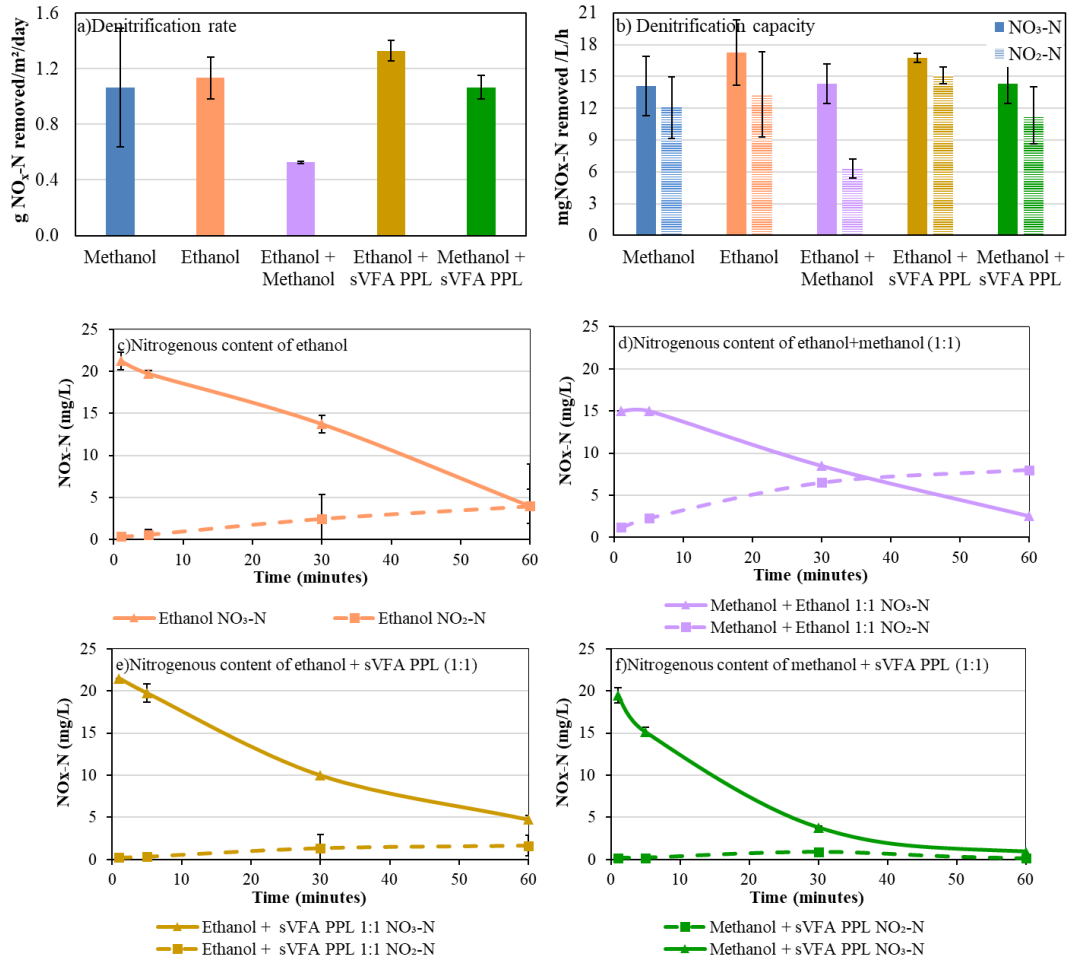

Figure 5.3 : (a) Denitrification rate (b) denitrification capacity (c-f) NO_x-N removal profiles over time for co-fed carbon sources.

The most efficient value observed under denitrification when using sole VFAs was observed at 0.56 g NO_x-N removed/m^2/day (Figure 5.1). One assumption of the limiting factor for VFAs as a carbon source can be the first steps of the biochemical pathway. Since ethanol and VFAs undergo beta oxidation, the limiting factor can be the first steps of the pathway for VFAs when they are metabolized into acyl adenylate. This can also reflect on the microbial diversity of the used carriers which do not have a well-established enzymatic network to metabolize VFA in which creates long lag phase to adapt to a certain carbon source. This in term reflected on the denitrification capacities negatively. When comparing methanol and ethanol in a 1:1 ratio on a C/N ratio of 9.5 (Figure 5.2) one can observe a denitrification rate of 0.53 g NO_x-N removed/m^2/day. This value is significantly lower than when using purely methanol or ethanol. When observing the denitrification capacity (Figure 5.2), a value of mg NO_2-N removed /L/h is obtained which is similar to what was observed when using pure methanol or ethanol. The reason for this is because of the high accumulation of NO_2-N when specifically using methanol purely or in combination. When ethanol was used a NO_2-N accumulation of 4 mg/L was observed, comparing that to the combined methanol and ethanol a value of 8 mg/L was observed. This indicates that when using methanol as a carbon source, a slower intermediate stage of NO_2-N conversion is observed in which effects the denitrification rate negatively. This also further strengthens the argument that VFAs can have a better NO_2-N conversion rate than methanol. This can be observed when methanol and $sVFA_{PPL}$ are combined. The denitrification rate is observed at 1.05 g NOx-N removed/m^2/day in a 1:1 ratio with a C/N of 9.5 and only 3 mg/L NO_2-N was accumulated (Figure 5.2). This value was similar to when using purely methanol and ethanol separately. This indicates that a co-digestion together with VFAs effluent can be beneficial, increasing the conversion rate of NO_2-N, than if only methanol is used.

The combination of carbon sources in a co-fed denitrification showed promising results. When ethanol and $sVFA_{PPL}$ was combined in a 1:1 ratio with a C/N ratio of 9.5, a denitrification rate of 1.4 NOx-N removed/m^2/day was obtained and a denitrification capacity of 16.8 mg NO_2-N removed /L/h NO_2-N removed. This value was the highest obtained denitrification rate and highlights the potential of co-digestion of denitrification. This indicates that when VFA effluent and ethanol are fed together, a higher denitrification efficiency is obtained. This may indicate that ethanol

and VFAs effluent have a boosting effect on each other. Another argument can be that a carbon source with diverse contents can be better utilized by the diverse microbial quality of the denitrifying microorganisms. These results show that when VFAs effluents are co-fed into a denitrification process, a high denitrification rate can be observed. This work can be related to what has been made and stated by He et al. (2020) in which used granular sludge as denitrifying media. They tested the effect of a mixture of different mixed carbon sources mixing acetate and sodium succinate and comparing this to using pure acetate or pure sodium succinate. This study stated that a mixture of carbon source for denitrification had a great impact on the denitrification rate and generally performed better than adding pure carbon source. This can strengthen the arguments that mixing VFAs together with methanol or ethanol can have boosting effects on denitrification efficiencies and further affect the denitrification rate positively. However, this study conducted simultaneous nitrification and denitrification with phosphorus removal in suspended growth process, which is a slightly different type of biological nutrient removal than suspended growth process, nevertheless the purpose of the carbon source is the same in both studies which validates the relevance of comparison. It is important to mention that the results did not only show improved denitrification but further eases the facilitation of integrating VFAs in a current conventional denitrification process. With the understanding that replacing a portion of the carbon source with VFAs effluents one can gradually increase the ratio of VFAs to an optimal ratio that suits the specific conventional process.

To further elaborate on denitrification and the effect of co-fed denitrification, a further set of tests were done comparing methanol with AD-VFA_{PPL} in a 1:1 ratio with a C/N ratio of 9.5 against methanol together with sVFA_{PPL} as it is shown inFigure 5.3. The reason for this is to evaluate if the portion that is set to be VFAs effluent in the carbon source mixture has any effect on the denitrification regarding other material existing in the effluent such as nutrients. This is done by comparing this to a carbon source mixture with the VFA portion being synthetic VFAs mixture.

Similar figures are shown for the denitrification efficiency of the mixed carbon sources. In comparison to the synthetic mixture, which had a denitrification rate of 1.03 g NO_x-N removed/m^2/day, the carbon source employing methanol and AD-VFA_{PPL} effluent had a denitrification rate of 1.07 g NO_x-N removed/m^2/day,

demonstrating that the denitrification rate is unaffected by contaminants in the actual effluent at the specified quantities (Figure 5.3). The mixture had identical nitrate and nitrite removal capabilities, with AD-VFA effluent mixtures having 18.75 and 18.65 and synthetic mixtures having 18.55 and 18.42, respectively (Figure 5.3).

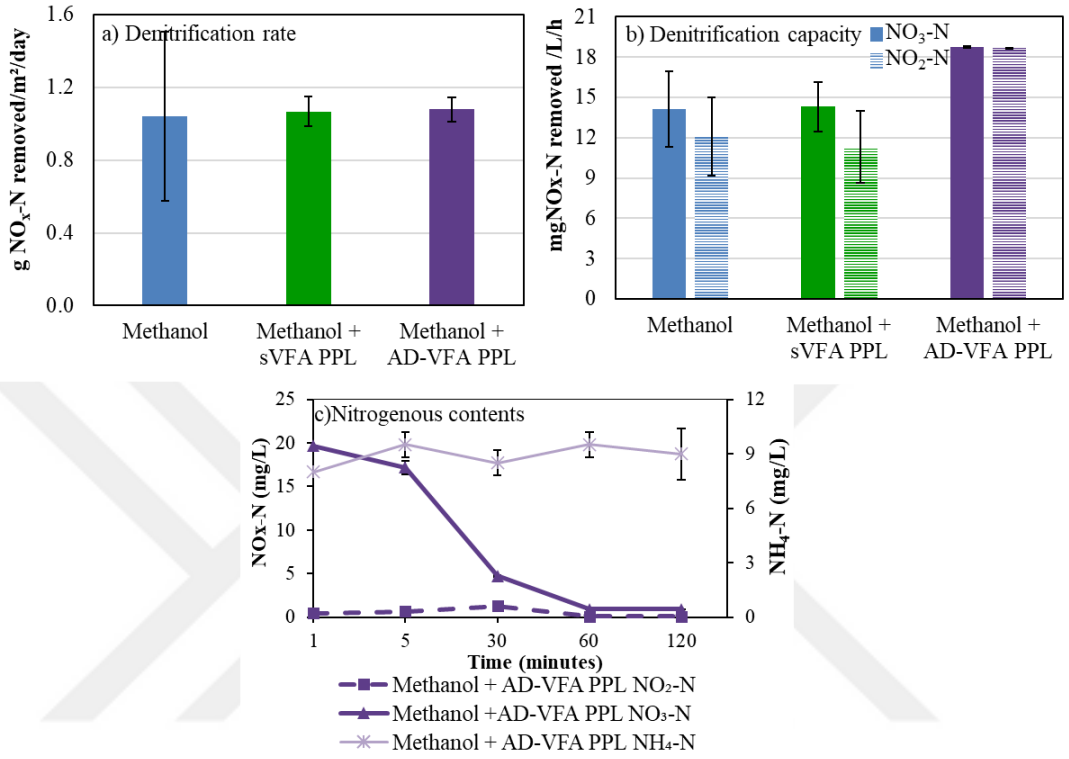

Figure 5.4 : (a) Denitrification rate (b) denitrification capacity (c-e) NO_x-N concentrations for methanol together with sVFA and AD-VFA.

The temperature, pH and nutrient contents all follow the same patterns as previously described in the section 3.1. The initial and final pH range from 6.98 to 7.7 and 7.33 to 7.71 respectively (Table 5.3). The temperatures range from 14.6 to 18.7 and 17.3 to 19.7 for initial and final respectively (Table 5.3). The ammonium content ranged from 9.5 -7.7 mg/L ammonium. The phosphorus content is generally lower than previous sets ranging from 2.3-3.4 mg/L (Table 5.3). The reason for this can be the quality of the wastewater collected from the nitrification outlet in the wastewater treatment plant. Duo to seasonal fluctuations of characteristics of the incoming wastewater to the denitrification tank may the cause of different values.

Similar pH values of 7.06 for methanol with AD-VFA$_{PPL}$ and 6.98 for methanol with synthetic sVFA$_{PPL}$ were observed with mixed carbon sources employing methanol and AD-VFA$_{PPL}$ combined. When employing methanol and AD-VFA$_{PPL}$ effluent or methanol and sVFA$_{PPL}$, a rise in pH was seen during denitrification for both mixed

carbon sources, with values of 7.71 and 7.51, respectively (Table 5.3). This indicates that denitrification took place in both experiments. The denitrification's ammonium content differed from the synthetic version in terms of initial values and the nutrients present in the AD-VFA effluent. The initial phosphate content for both seen to be similar (Table 5.3).

Table 5.3 : pH, temperature, and nutrients change during co-fed carbon sources.

Carbon source used	Carbon sources (CS)				
	(Ethanol)	(Ethanol + Methanol)	(Ethanol +sVFA$_{PPL}$)	(Methanol +sVFA$_{PPL}$)	(Methanol + AD-VFA$_{PPL}$)
pH, 0 min	7.7±0.02	7.44±0.00	6.99±0.21	6.98±0.01	7.06±0.03
pH, 60 min	7.38±0.38	7.72±0.00	7.33±0.01	7.51±0.03	7.71±0.01
T (°C), 0 min	14.6±0.7	14.8±0.0	14.8±0.78	18.3±0.26	18.7±0.07
T (°C), 60 min	17.4±0.7	17.9±0.00	18.45±0.8	19.7±0.07	19.5±0.07
NH_4-N, 0 min	7.1±1.8	9.5±0.1	7.7±1.2	7.8±0.4	9.5±0.7
NH_4-N, 60 min	8.5±2.1	10.0±1.3	8.1±1.0	7.0±0.0	9.5±0.7
PO_4-P, 0 min	2.5±0.3	2.4±0.3	3.6±0.2	1.7±0.1	2.0±0.1
PO_4-P, 60 min	2.6±0.2	2.3±0.2	2.8±0.3	1.2±0.0	1.8±0.0
COD of CS (g/L)	1650	1650 | 1185	1650 | 8.75	1185 | 8.75	1185 | 12.5
Volume of CS added (µL)	144	72 | 100.5	72 | 13,600	100.5 | 13,600	100.5 | 9520

5.3.3 Effect of sequential addition of co-fed carbon sources

To better understand the effect of combination, a mixture of carbon source containing methanol and AD-VFA$_{PPL}$ were set to be analyzed and added to the denitrification in a sequential manner. Hence, a sequential addition was set up to compare adding methanol first and the VFA effluent after 30 minutes and vice versa in order to better comprehend the idea of combined VFAs and the previously described potential boosting impact. The carbon sources were separately added first at the start of the denitrification and then again 30 minutes after denitrification to further analyze the effect of combination. The C/N ratio was 9.5. In the initial test, AD-VFA$_{PPL}$ effluent was employed as the initial carbon source addition, and methanol was added 30 minutes later (1.AD-VFA$_{PPL}$ + 2. Methanol). In the second test, methanol was supplied first, and then, 30 minutes later, AD-VFA$_{PPL}$ effluent (1. Methanol + 2. AD-VFA$_{PPL}$) was added.

Table 5.4 : pH, temperature, and nutrients during sequential addition of methanol and AD-VFA$_{PPL}$.

Parameters	Carbon sources (CS) used	
	1. AD-VFA$_{PPL}$ (0-30 mins) + 2. Methanol (30-60 mins)	**1. Methanol (0-30 mins) + 2. AD-VFA$_{PPL}$ (30-60 mins)**
pH, 0 min	7.25±0.03	7.29±0.01
pH, 60 min	7.82±0.06	7.82±0.01
T (°C), 0 min	18.3±0.71	18.15±0.21
T (°C), 60 min	19.70±0.14	19.85±0.07
NH_4-N (mg/L), 0 min	11.0±0.0	11.5±0.7
NH_4-N (mg/L), 60 min	11.0±1.4	11.0±1.4
PO_4-P (mg/L), 0 min	2.5±0.0	2.5±0.1
PO_4-P (mg/L), 60 min	2.3±0.1	2.2±0.1
COD of CS (g/L)	12.5 | 1185	1185 | 12.5
Volume of CS added (mL)	9.52 | 0.1005	0.1005 | 9.52

There was no discernible difference between the pH and temperature change. The same carbon sources were employed, but they were introduced at different periods during the denitrification process, resulting in negligible changes in the initial and final nutrient concentrations as well as pH and temperature variations (Table 5.4).

When AD-VFA$_{PPL}$ was added first and then methanol, the measured denitrification rate was equal to a value of 1.0 g NO_x-N removed/m^2/day and this value was 1.06 g NO_x-N removed/m^2/day when methanol was added before AD-VFA$_{PPL}$ (Figure 5.4a). Denitrification capacities were similar, removing 17.7 mg/L/h of nitrate when AD-VFA$_{PPL}$ was added first and then methanol was added afterwards, and 17.9 mg/L/h of nitrate when methanol was applied first and then AD-VFA$_{PPL}$. The nitrite levels show the most change. When AD-VFA$_{PPL}$ was added second, nitrate quickly decreased and reached a final value of 0.125 mg/L after 120 minutes, however when methanol was processed second, an accumulation of nitrite was visible at 1.6 mg/L (Figure 5.4b). This further supports the claim that VFAs are superior to methanol in the elimination of nitrite and that methanol may have some inhibitory effects on nitrite removal. Not only the alternative carbon sources needed to be increased to have more soluble substrates, but the adjustment strategy (mixed carbon sources) is required to increase the rate of nitrogen removal. Therefore, given that mixed carbon sources perform similarly to simple methanol and even better when compared in terms of nitrite removal rates, this emphasizes the potential for combined carbon sources to be widely

applicable. A reduced rate of denitrification was noticed for up to 30 minutes when AD-VFA$_{PPL}$ was applied first. A significantly greater rate of denitrification was seen with the addition of methanol. An accelerated rate of denitrification was seen when methanol was added first. Moreover, the denitrification rate significantly increased with the addition of AD-VFA$_{PPL}$. These results also lend credence to the idea that, when the rate of denitrification is considered, methanol and ethanol can speed up VFA metabolism.

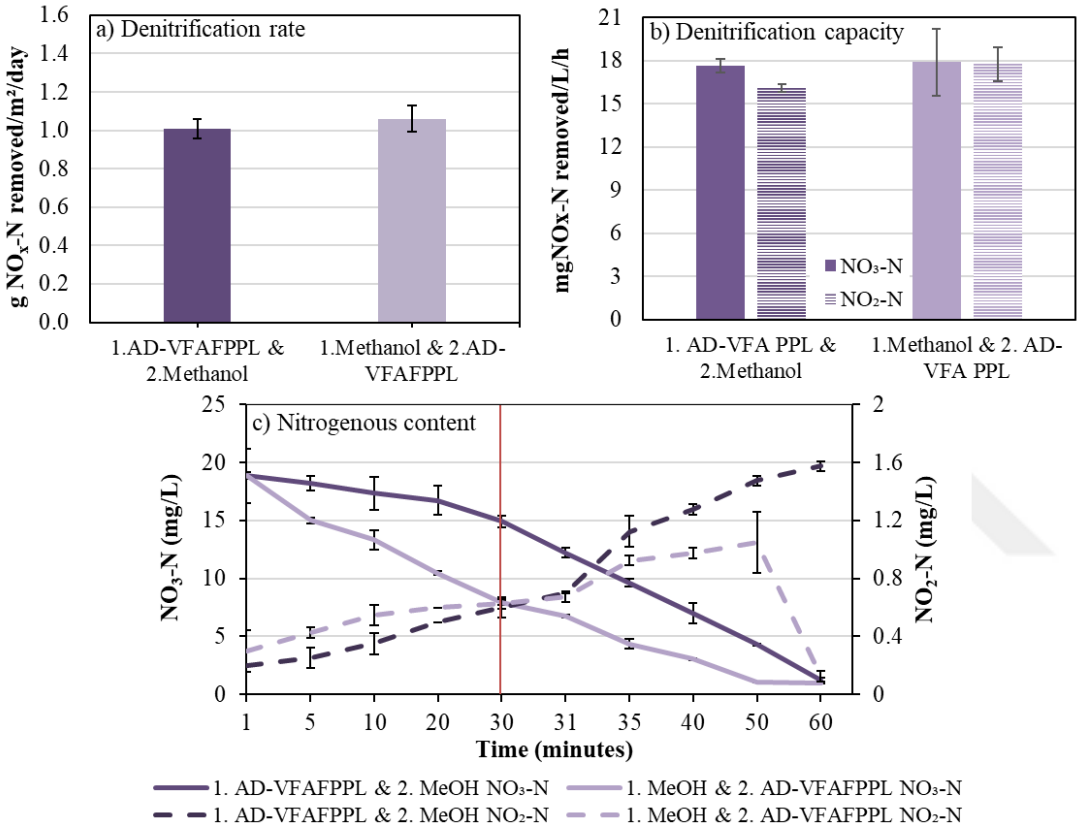

Figure 5.5 : (a) Denitrification rate and (b) denitrification capacity using sequential addition of VFA and methanol, (c) NO_x-N concentrations.

5.4 Conclusions

There is a considerable potential in the application of waste-derived VFAs as alternative carbon source for nitrogen removal in wastewater treatment process either by simultaneous application together with methanol or sequential addition. Animal excreta (chicken manure) or industrial side-streams (potato protein liquor fermentation effluent) stand out as large, organic sources for sustainable external carbon production. VFAs combined with methanol had a high denitrification rate and could be used more easily by denitrifiers than single VFAs. Furthermore, at the applied conditions

(C/N:9.5 and 17-18 °C), the addition of VFAs to methanol did not necessitate a lengthy acclimation period to acquire its full denitrification capacity. When the AD-VFA_{PPL} was introduced after 30 minutes post methanol addition, the denitrification capacity was superior in terms of NO_2-N reduction. The co-fed AD-VFA_{PPL} and methanol (1:1) test revealed highly promising denitrification rates, but more study needs be done to evaluate addition of alternative ratios as well as full scale applications. The MBBR denitrification study using VFAs derived from waste revealed that, fossil-based methanol could be successfully replaced to large extent with waste-derived VFAs to obtain the same nitrogen removal effectiveness as pure methanol during the post-denitrification process.

6. CONCLUSIONS AND RECOMMENDATIONS

The final chapter presents the primary conclusions drawn from this thesis. Finally, directions for future research are outlined.

6.1 Concluding Remarks

The results presented in this thesis suggest a promising opportunity for reducing the carbon footprint and promoting the sustainable development of wastewater treatment plants (WWTPs). In this thesis work, the feasibility of using waste-derived volatile fatty acids (VFAs) was evaluated as a potentially cost-effective and sustainable alternative carbon source in WWTP in a closed-loop cycle. The results of this study led to the following conclusions:

- Waste effluent resulting from FPPL has the potential to increase in value through acidogenic digestion in a membrane bioreactor (MBR), facilitating the production of valuable VFAs;
- A maximum product yield of 0.65 g-VFAs/gVSfed-FPPL and 0.54 g-VFAs/g-VSfed-FPPL was achieved in MBR1 and MBR2, respectively, with uncontrolled pH;
- Although methanol was superior in the conversion of NO_3^- to NO_2^- (methanol-adapted denitrifiers were used in the entire study), VFAs were in a better synchrony regarding the conversation of NO_3^- to NO_2^- and NO_2^- and N_2 in leading to reduced NO_2^- accumulation;
- Maximum denitrification rates obtained using anaerobic digestion AD-VFAs together with methanol (1:1 on chemical oxygen demand basis), i.e., 1.08 ± 0.07 g-NOx-Nremoved/m^2/day, were comparable with those obtained using methanol alone, i.e., 1.04 ± 0.46 g-NOx-Nremoved/m^2/day, indicating that the co-addition of VFAs together with methanol can potentially fully compete with pure methanol in performance;

- Unrefined waste-derived VFAs can support denitrification by replacing half of the carbon required by methanol; however; further upgrading of VFAs may be necessary to avoid secondary contamination in WWTP;
- Ion exchange process using clinoptilolite can be combined with the acidogenic fermentation of VFAs to purify the solution by removing its high ammonium content, avoiding the risk of secondary contamination when used as an external carbon source in the post-denitrification process;
- The rejection percentage (in mass) of total VFAs reached approximately 70% (concentration of VFAs increased from 6.6 to 35 g/L) with the nanofiltration process at the tested pH of 9, identified as a promising downstream processing option to enhance carbon content in VFA effluents.

However, many challenges still need to be addressed to develop effective VFAs for biological nitrogen removal, as described in the following section.

6.2 Future Work

Based on the results of this study, possible areas for future investigation and improvement are suggested to move relatively closer to the optimal use of VFAs derived from low-cost feedstocks as potential carbon sources for industrial-scale denitrification.

- Long-term pilot-scale acidogenic fermentation for VFAs to be used in continuous pilot-scale denitrification, along with a nitrification, and their techno-economic analysis and simulations must be carried out.
- Further research on acclimatized denitrifiers and full-scale denitrification process configurations with VFAs as carbon sources is required to optimize biological nitrogen removal.
- To better understand the biological mechanisms, in addition to the overall nitrogen removal performance, the microbial community structure, particularly the abundance of denitrifying genes in denitrifying reactors fed with VFAs needs to be investigated.

- A systematic investigation can be conducted to elucidate the microbial relationships between denitrification kinetics, NO_2^- accumulation, and N_2O emissions.
- Membrane selection for concentrating or separating VFAs is a complex process that presents numerous engineering challenges; therefore, additional membrane selection and membrane fouling research is required for downstream processing of VFAs.
- Comprehensive techno-economic evaluation, life cycle assessment and plant-wide modelling tool for the evaluation of different process configurations are also needed to reveal the feasibility of the proposed process and identify technology /market barriers and opportunities for improvement.

REFERENCES

Agnihotri, S., Yin, D.-M., Mahboubi, A., Sapmaz, T., Varjani, S., Qiao, W., Koseoglu-Imer, D.Y., Taherzadeh, M.J. (2022). A glimpse of the world of volatile fatty acids production and application: a review. *Bioengineered*, 13(1), 1249-1275.

Agriculture, S.B.o. (2022). Harvest of potatoes 2021. Preliminary statistics, (Ed.) S. Sweden, The Swedish Board of Agriculture's statistical reports.

Ahokas, M., Välimaa, A.-L., Lötjönen, T., Kankaala, A., Taskila, S., Virtanen, E. (2014). Resource assessment for potato biorefinery: Side stream potential in Northern Ostrobothnia. *Agronomy Research*, 12(3), 695-704.

Akter, J., Lee, J., Kim, I. (2022). A Fixed-Film Bio-Media Process Used for Biological Nitrogen Removal from Sewage Treatment Plant. *Nitrogen*, 3(3), 528-538.

Aktij, S.A., Zirehpour, A., Mollahosseini, A., Taherzadeh, M.J., Tiraferri, A., Rahimpour, A. (2020). Feasibility of membrane processes for the recovery and purification of bio-based volatile fatty acids: A comprehensive review. *Journal of Industrial and Engineering Chemistry*, 81, 24-40.

Albina, P., Durban, N., Bertron, A., Albrecht, A., Robinet, J.-C., Erable, B. (2019). Influence of hydrogen electron donor, alkaline pH, and high nitrate concentrations on microbial denitrification: a review. *International Journal of Molecular Sciences*, 20(20), 5163.

Allar, A.D. (2015). Processing Alternatives of Urine with Clinoptilolite for Indirect Use of Human Urine as Fertilizer (İnsan İdrarının Gübre Olarak Dolaylı Kullanımında Klinoptilolitle Muamele Ve Alternatifleri) (PhD Thesis) (in Turkish with an extended abstract in English) in: *Graduate School of Science and Engineering Technology*, Istanbul Technical University. Turkey (2015).

Anderson, D.M., Glibert, P.M., Burkholder, J.M. (2002). Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries*, 25(4), 704-726.

Aspegren, H., Nyberg, U., Andersson, B., Gotthardsson, S., la Cour Jansen, J. (1998). Post denitrification in a moving bed biofilm reactor process. *Water Science and Technology*, 38(1), 31-38.

Atasoy, M., Eyice, O., Schnürer, A., Cetecioglu, Z. (2019). Volatile fatty acids production via mixed culture fermentation: Revealing the link between pH, inoculum type and bacterial composition. *Bioresource technology*, 292, 121889.

Atasoy, M., Owusu-Agyeman, I., Plaza, E., Cetecioglu, Z. (2018). Bio-based volatile fatty acid production and recovery from waste streams: Current status and future challenges. *Bioresource technology*, 268, 773-786.

Balarak, D., McKay, G. (2021). Utilization of MWCNTs/Al_2O_3 as adsorbent for ciprofloxacin removal: equilibrium, kinetics and thermodynamic studies. *Journal of Environmental Science and Health, Part A*, 56(3), 324-333.

Barber, W.P., Stuckey, D.C. (2000). Nitrogen removal in a modified anaerobic baffled reactor (ABR): 1, denitrification. *Water research*, 34(9), 2413-2422.

Barłóg, P., Hlisnikovský, L., Kunzová, E. (2020). Effect of Digestate on Soil Organic Carbon and Plant-Available Nutrient Content Compared to Cattle Slurry and Mineral Fertilization. *Agronomy*, 10(3), 379.

Barreto, C.M., Garcia, H.A., Hooijmans, C.M., Herrera, A., Brdjanovic, D. (2017). Assessing the performance of an MBR operated at high biomass concentrations. *International Biodeterioration & Biodegradation*, 119, 528-537.

Bedaso, B. (2019). Volatile fatty acid production from co-fermentation of primary sludge and food waste without pH control.

Beler-Baykal, B., Allar, A.D., Bayram, S. (2011). Nitrogen recovery from source-separated human urine using clinoptilolite and preliminary results of its use as fertilizerB. Beler-Baykal et alNitrogen recovery from source-separated human urine. *Water Science and Technology*, 63(4), 811-817.

Beler-Baykal, B., Oldenburg, M., Sekoulov, I. (1996). The use of ion exchange in ammonia removal under constant and variable loads. *Environmental technology*, 17(7), 717-726.

Beler Baykal, B., Kocaturk, N.P., Allar, A.D., Sari, B. (2009). The effect of initial loading on the removal of ammonium and potassium from source-separated human urine via clinoptilolite. *Water Science and Technology*, 60(10), 2515-2520.

Bhandari, V.M., Yonemoto, T., Juvekar, V.A. (2000). Investigating the differences in acid separation behaviour on weak base ion exchange resins. *Chemical engineering science*, 55(24), 6197-6208.

Bill, K.A., Bott, C.B., Murthy, S.N. (2009). Evaluation of alternative electron donors for denitrifying moving bed biofilm reactors (MBBRs). *Water Science and Technology*, 60(10), 2647-2657.

Burton, F.L., Stensel, H.D. *Wastewater engineering: treatment and reuse*.

Cappai, G., Carucci, A., Onnis, A. (2004). Use of industrial wastewaters for the optimization and control of nitrogen removal processes. *Water Science and Technology*, 50(6), 17-24.

Cecconet, D., Capodaglio, A.G. (2022). Sewage sludge biorefinery for circular economy. *Sustainability*, 14(22), 14841.

Cesaro, A., Conte, A., Carrère, H., Trably, E., Paillet, F., Belgiorno, V. (2020). Formic acid pretreatment for enhanced production of bioenergy and biochemicals from organic solid waste. *Biomass and Bioenergy*, 133, 105455.

Chen, X. (2015). Modeling of experimental adsorption isotherm data. *information*, 6(1), 14-22.

Chen, Y., Liu, H., Zheng, X., Wang, X., Wu, J. (2017). New method for enhancement of bioenergy production from municipal organic wastes via regulation of anaerobic fermentation process. *Applied Energy*, 196, 190-198.

Cherchi, C., Onnis-Hayden, A., El-Shawabkeh, I., Gu, A.Z. (2009). Implication of using different carbon sources for denitrification in wastewater treatments. *Water Environment Research*, 81(8), 788-799.

Cincotti, A., Lai, N., Orrù, R., Cao, G. (2001). Sardinian natural clinoptilolites for heavy metals and ammonium removal: experimental and modeling. *Chemical Engineering Journal*, 84(3), 275-282.

Cooney, E.L., Booker, N.A., Shallcross, D.C., Stevens, G.W. (1999). Ammonia removal from wastewaters using natural Australian zeolite. I. Characterization of the zeolite. *Separation science and technology*, 34(12), 2307-2327.

Dai, X., Chen, Y., Zhang, D., Yi, J. (2016). High-solid anaerobic co-digestion of sewage sludge and cattle manure: The effects of volatile solid ratio and pH. *Scientific reports*, 6(1), 1-10.

De Groof, V., Coma, M., Arnot, T., Leak, D.J., Lanham, A.B. (2021). Selecting fermentation products for food waste valorisation with HRT and OLR as the key operational parameters. *Waste Management*, 127, 80-89.

den Boer, E., Łukaszewska, A., Kluczkiewicz, W., Lewandowska, D., King, K., Reijonen, T., Kuhmonen, T., Suhonen, A., Jääskeläinen, A., Heitto, A. (2016). Volatile fatty acids as an added value from biowaste. *Waste management*, 58, 62-69.

Dhamole, P.B., D'Souza, S., Lele, S. (2015). A review on alternative carbon sources for biological treatment of nitrate waste. *Journal of The Institution of Engineers (India): Series E*, 96(1), 63-73.

Domingos, S., Boehler, K., Felstead, S., Dallas, S., Ho, G. (2009). Effect of external carbon sources on nitrate removal in constructed wetlands treating industrial wastewater: woodchips and ethanol addition, Nova Science Publishers.

Dong, S., Sartaj, M. (2016). Statistical analysis and optimization of ammonia removal from landfill leachate by sequential microwave/aeration process using factorial design and response surface methodology. *Journal of environmental chemical engineering*, 4(1), 100-108.

Doyen, W., Mues, W., Molenberghs, B., Cobben, B. (2010). Spacer fabric supported flat-sheet membranes: A new era of flat-sheet membrane technology. *Desalination*, 250(3), 1078-1082.

Doyen, W., Van Hoof, E., Molenberghs, B., Dotremont, C. (2003). A new approach to pilot installation automation, the ingeneous solution you have been looking for, European Desalination Society Newsletter, pp. 4-7.

Du, S., Ya, T., Zhang, M., Zhu, M., Li, N., Liu, S., Wang, X. (2020). Distinct microbial communities and their networks in an anammox coupled with sulfur autotrophic/mixotrophic denitrification system. *Environmental Pollution*, 262, 114190.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A.t., Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical chemistry*, 28(3), 350-356.

Elefsiniotis, P., Li, D. (2006). The effect of temperature and carbon source on denitrification using volatile fatty acids. *Biochemical Engineering Journal*, 28(2), 148-155.

Elefsiniotis, P., Wareham, D.G. (2007). Utilization patterns of volatile fatty acids in the denitrification reaction. *Enzyme and Microbial Technology*, 41(1-2), 92-97.

Elefsiniotis, P., Wareham, D.G., Smith, M.O. (2004). Use of volatile fatty acids from an acid-phase digester for denitrification. *Journal of biotechnology*, 114(3), 289-297.

EPA. (2013). External Carbon Sources for Nitrogen Removal. in: *Environmental Protection Agency Office of Wastewater Management: Washington, DC, USA*.

Eregowda, T., Rene, E.R., Rintala, J., Lens, P.N.L. (2020). Volatile fatty acid adsorption on anion exchange resins: kinetics and selective recovery of acetic acid. *Separation Science and Technology*, 55(8), 1449-1461.

Fang, W., Zhang, X., Zhang, P., Wan, J., Guo, H., Ghasimi, D.S.M., Morera, X.C., Zhang, T. (2020). Overview of key operation factors and strategies for improving fermentative volatile fatty acid production and product regulation from sewage sludge. *Journal of Environmental Sciences*, 87, 93-111.

Farkaš, A., Rožić, M., Barbarić-Mikočević, Ž. (2005). Ammonium exchange in leakage waters of waste dumps using natural zeolite from the Krapina region, Croatia. *Journal of Hazardous Materials*, 117(1), 25-33.

Federation, W.E., Association, A.P.H. (2005). Standard methods for the examination of water and wastewater. *American Public Health Association (APHA): Washington, DC, USA*.

Feng, X.-C., Bao, X., Che, L., Wu, Q.-L. (2021). Enhance biological nitrogen and phosphorus removal in wastewater treatment process by adding food waste fermentation liquid as external carbon source. *Biochemical Engineering Journal*, 165, 107811.

Fernández-Nava, Y., Marañón, E., Soons, J., Castrillón, L. (2010). Denitrification of high nitrate concentration wastewater using alternative carbon sources. *Journal of Hazardous Materials*, 173(1), 682-688.

Fradinho, J.C., Oehmen, A., Reis, M.A.M. (2014). Photosynthetic mixed culture polyhydroxyalkanoate (PHA) production from individual and mixed volatile fatty acids (VFAs): Substrate preferences and co-substrate uptake. *Journal of Biotechnology*, 185, 19-27.

Frølund, B., Palmgren, R., Keiding, K., Nielsen, P.H. (1996). Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water research*, 30(8), 1749-1758.

Fufachev, E.V., Weckhuysen, B.M., Bruijnincx, P.C.A. (2020). Toward Catalytic Ketonization of Volatile Fatty Acids Extracted from Fermented Wastewater by Adsorption. *ACS Sustainable Chemistry & Engineering*, 8(30), 11292-11298.

Fujihara, T., Maeda, S., Shem, M.N. (2002). The effect of ruminal infusion of acetic, propionic and butyric acid on eating, rumination behaviour and feed utilization in sheep fed on urea-supplemented roughage. *Journal of Animal and Feed Sciences*, 11(1), 91-108.

Garcia-Aguirre, J., Aymerich, E., de Goñi, J.G.-M., Esteban-Gutiérrez, M. (2017). Selective VFA production potential from organic waste streams: assessing temperature and pH influence. *Bioresource technology*, 244, 1081-1088.

Griebe, T., Nielsen, P.H. (1995). Enzymatic activity in the activated-sludge floc matrix. *Applied microbiology and biotechnology*, 43(4), 755-761.

Guida, S., Conzelmann, L., Remy, C., Vale, P., Jefferson, B., Soares, A. (2021). Resilience and life cycle assessment of ion exchange process for ammonium removal from municipal wastewater. *Science of The Total Environment*, 783, 146834.

Guo, X.M., Trably, E., Latrille, E., Carrère, H., Steyer, J.-P. (2010). Hydrogen production from agricultural waste by dark fermentation: a review. *International journal of hydrogen energy*, 35(19), 10660-10673.

He, Q., Song, J., Zhang, W., Gao, S., Wang, H., Yu, J. (2020). Enhanced simultaneous nitrification, denitrification and phosphorus removal through mixed carbon source by aerobic granular sludge. *Journal of hazardous materials*, 382, 121043.

Hedström, A. (2001). Ion exchange of ammonium in zeolites: a literature review. *Journal of environmental engineering*, 127(8), 673-681.

Henderson, K.A., Murdock, J.N., Lizotte Jr, R.E. (2021). Water depth influences algal distribution and productivity in shallow agricultural lakes. *Ecohydrology*, 14(6), e2319.

Huang, W., Huang, W., Yuan, T., Zhao, Z., Cai, W., Zhang, Z., Lei, Z., Feng, C. (2016). Volatile fatty acids (VFAs) production from swine manure through short-term dry anaerobic digestion and its separation from nitrogen and phosphorus resources in the digestate. *Water research*, 90, 344-353.

Inglezakis, V.J., Hadjiandreou, K.J., Loizidou, M.D., Grigoropoulou, H.P. (2001a). Pretreatment of natural clinoptilolite in a laboratory-scale ion exchange packed bed. *Water Research*, 35(9), 2161-2166.

Inglezakis, V.J., Papadeas, C.D., Loizidou, M.D., Grigoropoulou, H.P. (2001b). Effects of pretreatment on physical and ion exchange properties of natural clinoptilolite. *Environmental technology*, 22(1), 75-82.

Ishii, S.K.L., Boyer, T.H. (2015). Life cycle comparison of centralized wastewater treatment and urine source separation with struvite precipitation: Focus on urine nutrient management. *Water research*, 79, 88-103.

Jankowska, E., Chwiałkowska, J., Stodolny, M., Oleskowicz-Popiel, P. (2015). Effect of pH and retention time on volatile fatty acids production during mixed culture fermentation. *Bioresource Technology*, 190, 274-280.

Ji, J., Peng, Y., Wang, B., Wang, S. (2017). Achievement of high nitrite accumulation via endogenous partial denitrification (EPD). *Bioresource technology*, 224, 140-146.

Jomnonkhaow, U., Uwineza, C., Mahboubi, A., Wainaina, S., Reungsang, A., Taherzadeh, M.J. (2021). Membrane bioreactor-assisted volatile fatty acids production and in situ recovery from cow manure. *Bioresource Technology*, 321, 124456.

Jordbruksverket. (2022). Harvesting of agricultural plants, Vol. 2022, Jordbruksverkets.

Jorgensen, T.C., Weatherley, L.R. (2006). Continuous removal of ammonium ion by ion exchange in the presence of organic compounds in packed columns. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, 81(7), 1151-1158.

Judd, S. (2010). *The MBR book: principles and applications of membrane bioreactors for water and wastewater treatment*. Elsevier.

Karadag, D., Tok, S., Akgul, E., Turan, M., Ozturk, M., Demir, A. (2008). Ammonium removal from sanitary landfill leachate using natural Gördes clinoptilolite. *Journal of hazardous materials*, 153(1-2), 60-66.

Kim, H., Kim, J., Shin, S.G., Hwang, S., Lee, C. (2016). Continuous fermentation of food waste leachate for the production of volatile fatty acids and potential as a denitrification carbon source. *Bioresource technology*, 207, 440-445.

Kithome, M., Paul, J.W., Lavkulich, L.M., Bomke, A.A. (1998). Kinetics of ammonium adsorption and desorption by the natural zeolite clinoptilolite. *Soil Science Society of America Journal*, 62(3), 622-629.

Klingspohn, U., Vijai Papsupuleti, P., Schügerl, K. (1993). Production of enzymes from potato pulp using batch operation of a bioreactor. *Journal of Chemical Technology & Biotechnology*, 58(1), 19-25.

Kocatürk, N.P., Baykal, B.B. (2012). Recovery of plant nutrients from dilute solutions of human urine and preliminary investigations on pot trials. *CLEAN–Soil, Air, Water*, 40(5), 538-544.

Kuktaite, R., Newson, W.R., Rasheed, F., Hedenqvist, M.S., Johansson, E. (2015). How the unuseful can be turned into sustainable and useful: Novel potato protein bioplastics with unusual strength.

Lee, W.S., Chua, A.S.M., Yeoh, H.K., Ngoh, G.C. (2014). A review of the production and applications of waste-derived volatile fatty acids. *Chemical Engineering Journal*, 235, 83-99.

Lei, L., Li, X., Zhang, X. (2008). Ammonium removal from aqueous solutions using microwave-treated natural Chinese zeolite. *Separation and purification Technology*, 58(3), 359-366.

Li, B.-Y., Xia, Z.-Y., Gou, M., Sun, Z.-Y., Huang, Y.-L., Jiao, S.-B., Dai, W.-Y., Tang, Y.-Q. (2021). Production of volatile fatty acid from fruit waste by anaerobic digestion at high organic loading rates: Performance and microbial community characteristics. *Bioresource Technology*, 126648.

Li, X.Y., Yang, S.F. (2007). Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water research*, 41(5), 1022-1030.

Li, Y., Zhang, X., Xu, H., Mu, H., Hua, D., Jin, F., Meng, G. (2019). Acidogenic properties of carbohydrate-rich wasted potato and microbial community analysis: effect of pH. *Journal of bioscience and bioengineering*, 128(1), 50-55.

Liu, F., Tian, Y., Ding, Y., Li, Z. (2016). The use of fermentation liquid of wastewater primary sedimentation sludge as supplemental carbon source for denitrification based on enhanced anaerobic fermentation. *Bioresource Technology*, 219, 6-13.

Liu, H., Dong, Y., Wang, H., Liu, Y. (2010). Ammonium adsorption from aqueous solutions by strawberry leaf powder: Equilibrium, kinetics and effects of coexisting ions. *Desalination*, 263(1-3), 70-75.

Liu, H., Han, P., Liu, H., Zhou, G., Fu, B., Zheng, Z. (2018). Full-scale production of VFAs from sewage sludge by anaerobic alkaline fermentation to improve biological nutrients removal in domestic wastewater. *Bioresource technology*, 260, 105-114.

Lotz, M., Fröhlich, R., Matthes, R., Schügerl, K., Seekamp, M. (1991). Bakers' yeast cultivation on by-products and wastes of potato and wheat starch production on a laboratory and pilot-plant scale. *Process biochemistry*, 26(5), 301-311.

Lu, Y., Zhang, Q., Wang, X., Zhou, X., Zhu, J. (2020). Effect of pH on volatile fatty acid production from anaerobic digestion of potato peel waste. *Bioresource Technology*, 316, 123851.

Ma, H., Liu, H., Zhang, L., Yang, M., Fu, B., Liu, H. (2017). Novel insight into the relationship between organic substrate composition and volatile fatty acids distribution in acidogenic co-fermentation. *Biotechnology for biofuels*, 10(1), 1-15.

MacDonald, D.V. (1990). Denitrification by Fluidized Biofilm Reactor. *Water Science and Technology*, 22(1-2), 451-461.

Magdalena, J.A., Greses, S., González-Fernández, C. (2019). Impact of organic loading rate in volatile fatty acids production and population dynamics using microalgae biomass as substrate. *Scientific reports*, 9(1), 1-11.

Mahmoud, A., Hamza, R.A., Elbeshbishy, E. (2022). Enhancement of denitrification efficiency using municipal and industrial waste fermentation liquids as external carbon sources. *Science of The Total Environment*, 816, 151578.

Mei, R., Narihiro, T., Nobu, M.K., Liu, W.T. (2016). Effects of heat shocks on microbial community structure and microbial activity of a methanogenic enrichment degrading benzoate. *Letters in applied microbiology*, 63(5), 356-362.

Meng, F., Zhang, S., Oh, Y., Zhou, Z., Shin, H.-S., Chae, S.-R. (2017). Fouling in membrane bioreactors: An updated review. *Water research*, 114, 151-180.

Mengistu, S.G., Golden, H.E., Lane, C.R., Christensen, J.R., Wine, M.L., D'Amico, E., Prues, A., Leibowitz, S.G., Compton, J.E., Weber, M.H. (2020). Wetland flowpaths mediate nitrogen and phosphorus concentrations across the Upper Mississippi River Basin. *JAWRA Journal of the American Water Resources Association*.

Metcalf, E., Abu-Orf, M., Bowden, G., Burton, F., Pfrang, W., Stensel, H., Tchobanoglous, G., Tsuchihashi, R. (2014). AECOM (2014) Wastewater engineering: treatment and resource recovery, New York: McGraw-Hill.

Metcalf, L., Eddy, H.P., Tchobanoglous, G. (1991). *Wastewater engineering: treatment, disposal, and reuse*. McGraw-Hill New York.

Mondylaksita, K., Ferreira, J.A., Budhijanto, W., Niklasson, C., Taherzadeh, M.J., Millati, R. (2021). Enhanced Volatile Fatty Acid Production from Oil Palm Empty Fruit Bunch through Acidogenic Fermentation—A Novel Resource Recovery Strategy for Oil Palm Empty Fruit Bunch. *Fermentation*, 7(4), 263.

Murray, C.J., Müller-Karulis, B., Carstensen, J., Conley, D.J., Gustafsson, B.G., Andersen, J.H. (2019). Past, present and future eutrophication status of the Baltic Sea. *Frontiers in Marine Science*, 6, 2.

Nativ, P., Gräber, Y., Aviezer, Y., Lahav, O. (2021). A Simple and accurate approach for determining the vfa concentration in anaerobic digestion liquors, relying on two titration points and an external inorganic carbon analysis. *ChemEngineering*, 5(2), 15.

Ngo, T., Shahsavari, E., Shah, K., Surapaneni, A., Ball, A.S. (2022). Improving bioenergy production in anaerobic digestion systems utilising chicken manure via pyrolysed biochar additives: A review. *Fuel*, 316, 123374.

Odegaard, H., Rusten, B., Siljudalen, J. (1999). The development of the moving bed biofilm process- from idea to commercial product. *European water management*, 2(3), 36-43.

Osman, A.I., Mehta, N., Elgarahy, A.M., Al-Hinai, A., Al-Muhtaseb, A.a.H., Rooney, D.W. (2021). Conversion of biomass to biofuels and life cycle assessment: a review. *Environmental Chemistry Letters*, 19(6), 4075-4118.

Paes, J.L., Alves, T., da Silva, L.D.B., Marques, A.d.S., Dias, V.R.S. (2020). Use of inoculum in biodigesters with cattle manure under conventional and organic production systems. *Engenharia Agrícola*, 40, 146-153.

Palatsi, J., Ripoll, F., Benzal, A., Pijuan, M., Romero-Güiza, M.S. (2021). Enhancement of biological nutrient removal process with advanced process control tools in full-scale wastewater treatment plant. *Water Research*, 200, 117212.

Pang, Y., Wang, J. (2021). Various electron donors for biological nitrate removal: A review. *Science of the total environment*, 794, 148699.

Parchami, M., Wainaina, S., Mahboubi, A., I'Ons, D., Taherzadeh, M.J. (2020). MBR-assisted VFAs production from excess sewage sludge and food waste slurry for sustainable wastewater treatment. *Applied Sciences*, 10(8), 2921.

Paśmionka, I.B., Bulski, K., Herbut, P., Boligłowa, E., Vieira, F.M.C., Bonassa, G., Bortoli, M., Prá, M.C.d. (2021). Toxic Effect of Ammonium Nitrogen on the Nitrification Process and Acclimatisation of Nitrifying Bacteria to High Concentrations of NH4-N in Wastewater. *Energies*, 14(17), 5329.

Pelaz, L., Gómez, A., Letona, A., Garralón, G., Fdz-Polanco, M. (2018). Nitrogen removal in domestic wastewater. Effect of nitrate recycling and COD/N ratio. *Chemosphere*, 212, 8-14.

Pervez, M.N., Mahboubi, A., Uwineza, C., Sapmaz, T., Zarra, T., Belgiorno, V., Naddeo, V., Taherzadeh, M.J. (2022). Feasibility of nanofiltration process for high efficient recovery and concentrations of food waste-derived volatile fatty acids. *Journal of Water Process Engineering*, 48, 102933.

Playchoom, C., Pungrasmi, W., Powtongsook, S. (2011). Effect of carbon sources and carbon/nitrogen ratio on nitrate removal in aquaculture denitrification tank. *Environ. Chem*, 1, 307-311.

Rahimi, S., Modin, O., Mijakovic, I. (2020). Technologies for biological removal and recovery of nitrogen from wastewater. *Biotechnology Advances*, 43, 107570.

Rajput, A.A., Sheikh, Z. (2019). Effect of inoculum type and organic loading on biogas production of sunflower meal and wheat straw. *Sustainable Environment Research*, 29(1), 1-10.

Ramos-Suarez, M., Zhang, Y., Outram, V. (2021). Current perspectives on acidogenic fermentation to produce volatile fatty acids from waste. *Reviews in Environmental Science and Bio/Technology*, 1-40.

Rebecchi, S., Pinelli, D., Bertin, L., Zama, F., Fava, F., Frascari, D. (2016). Volatile fatty acids recovery from the effluent of an acidogenic digestion process fed with grape pomace by adsorption on ion exchange resins. *Chemical Engineering Journal*, 306, 629-639.

Reddy, M.V., Amulya, K., Rohit, M.V., Sarma, P.N., Mohan, S.V. (2014). Valorization of fatty acid waste for bioplastics production using Bacillus tequilensis: Integration with dark-fermentative hydrogen production process. *international journal of hydrogen energy*, 39(14), 7616-7626.

Rengaraj, S., Yeon, K.-H., Moon, S.-H. (2001). Removal of chromium from water and wastewater by ion exchange resins. *Journal of hazardous materials*, 87(1-3), 273-287.

Reyhanitash, E., Kersten, S.R.A., Schuur, B. (2017). Recovery of volatile fatty acids from fermented wastewater by adsorption. *ACS sustainable chemistry & engineering*, 5(10), 9176-9184.

Rigout, S., Hurtaud, C., Lemosquet, S., Bach, A., Rulquin, H. (2003). Lactational effect of propionic acid and duodenal glucose in cows. *Journal of Dairy Science*, 86(1), 243-253.

Rittmann, B.E., McCarty, P.L. (2001). *Environmental biotechnology: principles and applications*. McGraw-Hill Education.

Rizzioli, F., Battista, F., Bolzonella, D., Frison, N. (2021). Volatile Fatty Acid Recovery from Anaerobic Fermentate: Focusing on Adsorption and Desorption Performances. *Industrial & Engineering Chemistry Research*, 60(37), 13701-13709.

Rodriguez, L., Villasenor, J., Fernández, F. (2007). Use of agro-food wastewaters for the optimisation of the denitrification process. *Water science and technology*, 55(10), 63-70.

Rota, M. (2021). Product information sheet.

Rusten, B., Wien, A., Skjefstad, J. (1997). Spent aircraft deicing fluid as external carbon source for denitrification of municipal wastewater: From waste problem to beneficial use. *51. INDUSTRIAL WASTE CONFERENCE.* pp. 359-370.

Sambusiti, C., Saadouni, M., Gauchou, V., Segues, B., Leca, M.A., Baldoni-Andrey, P., Jacob, M. (2020). Influence of HRT reduction on pilot scale flat sheet submerged membrane bioreactor (sMBR) performances for Oil&Gas wastewater treatment. *Journal of Membrane Science*, 594, 117459.

Sapmaz, T., Mahboubi, A., Taher, M.N., Beler-Baykal, B., Karagunduz, A., Taherzadeh, M.J., Koseoglu-Imer, D.Y. (2022a). Waste-derived volatile fatty acid production and ammonium removal from it by ion exchange process with natural zeolite. *Bioengineered*, 13(6), 14751-14769.

Sapmaz, T., Manafi, R., Mahboubi, A., Lorick, D., Koseoglu-Imer, D.Y., Taherzadeh, M.J. (2022b). Potential of food waste-derived volatile fatty acids as alternative carbon source for denitrifying moving bed biofilm reactors. *Bioresource Technology*, 364, 128046.

Sapmaz, T., Uwineza, C., Mahboubi, A., De Wever, H., Koseoglu-Imer, D.Y., Taherzadeh, M.J. (2023). Towards maximum value creation from potato protein liquor: volatile fatty acids production from fungal cultivation effluent. *Biomass Conversion and Biorefinery*.

Sar, T., Larsson, K., Fristedt, R., Undeland, I., Taherzadeh, M.J. (2022). scale production of protein-rich fungal biomass from potato protein liquor for use as innovative food and feed products. *Food Bioscience*, 47, 101637.

Schügerl, K., Rosen, W. (1997). Investigation of the use of agricultural byproducts for fungal protein production. *Process biochemistry*, 32(8), 705-714.

Schulz, H. (2002). Oxidation of fatty acids in eukaryotes. in: *New comprehensive biochemistry*, Vol. 36, Elsevier, pp. 127-150.

Setlow, P. (2014). Germination of spores of Bacillus species: what we know and do not know. *Journal of bacteriology*, 196(7), 1297-1305.

Shewa, W.A., Hussain, A., Chandra, R., Lee, J., Saha, S., Lee, H.-S. (2020). Valorization of food waste and economical treatment: Effect of inoculation methods. *Journal of Cleaner Production*, 261, 121170.

Shrestha, A., Riffat, R., Bott, C., Takacs, I., Stinson, B., Peric, M., Neupane, D., Murthy, S. (2009). Denitrification stoichiometry and kinetics of moving bed biofilm reactor. *Proceedings of the Water Environment Federation*, 2009(4), 153-165.

Souza Filho, P.F., Brancoli, P., Bolton, K., Zamani, A., Taherzadeh, M.J. (2017a). Techno-economic and life cycle assessment of wastewater management from potato starch production: Present status and alternative biotreatments. *Fermentation*, 3(4), 56.

Souza Filho, P.F., Zamani, A., Taherzadeh, M.J. (2017b). Production of edible fungi from potato protein liquor (PPL) in airlift bioreactor. *Fermentation*, 3(1), 12.

Srisowmeya, G., Chakravarthy, M., Devi, G.N. (2020). Critical considerations in two-stage anaerobic digestion of food waste–A review. *Renewable and Sustainable Energy Reviews*, 119, 109587.

Sun, J., Zhang, L., Loh, K.-C. (2021). Review and perspectives of enhanced volatile fatty acids production from acidogenic fermentation of lignocellulosic biomass wastes. *Bioresources and Bioprocessing*, 8(1), 1-21.

Swinarski, M., Makinia, J., Czerwionka, K., Chrzanowska, M., Drewnowski, J. (2009). Comparison of the effects of conventional and alternative external carbon sources on enhancing the denitrification process. *Water environment research*, 81(9), 896-906.

Sydney, E.B., Novak, A.C., Rosa, D., Medeiros, A.B.P., Brar, S.K., Larroche, C., Soccol, C.R. (2018). Screening and bioprospecting of anaerobic consortia for biohydrogen and volatile fatty acid production in a vinasse based medium through dark fermentation. *Process Biochemistry*, 67, 1-7.

Taherzadeh, M.J. (2019). Bioengineering to tackle environmental challenges, climate changes and resource recovery. *Bioengineered*, 10(1), 698-699.

Takahashi, S., Tanifuji, K., Shiell, K., Fatehi, P., Jahan, M.S., Ohi, H., Ni, Y. (2013). Removal of acetic acid from spent sulfite liquor using anion exchange resin for effective xylose fermentation with Pichia stipitis. *BioResources*, 8(2), 2417-2428.

Tampio, E.A., Blasco, L., Vainio, M.M., Kahala, M.M., Rasi, S.E. (2019). Volatile fatty acids (VFAs) and methane from food waste and cow slurry: Comparison of biogas and VFA fermentation processes. *GCB Bioenergy*, 11(1), 72-84.

Torresi, E., Casas, M.E., Polesel, F., Plósz, B.G., Christensson, M., Bester, K. (2017). Impact of external carbon dose on the removal of micropollutants using methanol and ethanol in post-denitrifying Moving Bed Biofilm Reactors. *Water Research*, 108, 95-105.

United Nations Environment Programme (2021). Food Waste Index Report 2021. Nairobi.

Valdez-Vazquez, I., Poggi-Varaldo, H.M. (2009). Hydrogen production by fermentative consortia. *Renewable and sustainable energy reviews*, 13(5), 1000-1013.

Wainaina, S. (2020). Developing a food waste-based volatile fatty acids platform using an immersed membrane bioreactor, Högskolan i Borås.

Wainaina, S., Awasthi, M.K., Horváth, I.S., Taherzadeh, M.J. (2020). Anaerobic digestion of food waste to volatile fatty acids and hydrogen at high organic loading rates in immersed membrane bioreactors. *Renewable Energy*, 152, 1140-1148.

Wainaina, S., Lukitawesa, Kumar Awasthi, M., Taherzadeh, M.J. (2019a). Bioengineering of anaerobic digestion for volatile fatty acids, hydrogen or methane production: a critical review. *Bioengineered*, 10(1), 437-458.

Wainaina, S., Parchami, M., Mahboubi, A., Horváth, I.S., Taherzadeh, M.J. (2019b). Food waste-derived volatile fatty acids platform using an immersed membrane bioreactor. *Bioresource technology*, 274, 329-334.

Wang, Y.F., Lin, F., Pang, W.Q. (2007). Ammonium exchange in aqueous solution using Chinese natural clinoptilolite and modified zeolite. *Journal of hazardous materials*, 142(1-2), 160-164.

Weber Jr, W.J. (1972). Ion exchange. *Physicochemical Process for Water Quality Control*, 268.

Wei, Y., Ji, M., Li, R., Qin, F. (2012). Organic and nitrogen removal from landfill leachate in aerobic granular sludge sequencing batch reactors. *Waste Management*, 32(3), 448-455.

Xu, H., Paerl, H.W., Qin, B., Zhu, G., Hall, N.S., Wu, Y. (2015). Determining critical nutrient thresholds needed to control harmful cyanobacterial blooms in eutrophic Lake Taihu, China. *Environmental science & technology*, 49(2), 1051-1059.

Yan, F., Jiang, J., Zhang, H., Liu, N., Zou, Q. (2018). Biological denitrification from mature landfill leachate using a food-waste-derived carbon source. *Journal of environmental management*, 214, 184-191.

Yin, D.-m., Mahboubi, A., Wainaina, S., Qiao, W., Taherzadeh, M.J. (2021). The effect of mono-and multiple fermentation parameters on volatile fatty acids (VFAs) production from chicken manure via anaerobic digestion. *Bioresource Technology*, 330, 124992.

Yin, D.M., Uwineza, C., Sapmaz, T., Mahboubi, A., De Wever, H., Qiao, W., Taherzadeh, M.J. (2022). Volatile Fatty Acids (VFA) Production and Recovery from Chicken Manure Using a High-Solid Anaerobic Membrane Bioreactor (AnMBR). *Membranes*, 12(11), 1133.

Yuan, Y., Hu, X., Chen, H., Zhou, Y., Zhou, Y., Wang, D. (2019). Advances in enhanced volatile fatty acid production from anaerobic fermentation of waste activated sludge. *Science of the Total Environment*, 694, 133741.

Yurtoğlu, A. (2007). Effects of Mineralogical Properties of Turkish Clinoptilolites on Ammonium Removal Capacity by Ion Exchange (Türk Klinoptilolitlerinin Mineralojik Özelliklerinin Iyon Degisimi Yöntemiyle Amonyum Giderim Kapasitesi Üzerindeki Etkileri) (MSc Thesis) (in Turkish with an extended abstract in English) in: *Graduate School of Science and Engineering Technology*, Istanbul Technical University.

Zhang, H., Jiang, J., Li, M., Yan, F., Gong, C., Wang, Q. (2016a). Biological nitrate removal using a food waste-derived carbon source in synthetic wastewater and real sewage. *Journal of Environmental Management*, 166, 407-413.

Zhang, L., Tsui, T.-H., Loh, K.-C., Dai, Y., Tong, Y.W. (2022). Acidogenic fermentation of organic wastes for production of volatile fatty acids. in: *Biomass, Biofuels, Biochemicals*, Elsevier, pp. 343-366.

Zhang, Q., Lu, Y., Zhou, X., Wang, X., Zhu, J. (2020). Effect of different vegetable wastes on the performance of volatile fatty acids production by anaerobic fermentation. *Science of the Total Environment*, 748, 142390.

Zhang, Y., Wang, X.C., Cheng, Z., Li, Y., Tang, J. (2016b). Effect of fermentation liquid from food waste as a carbon source for enhancing denitrification in wastewater treatment. *Chemosphere*, 144, 689-696.

Zhao, Z., Huang, C., Meng, L., Lu, L., Wu, Y., Fan, R., Li, S., Sui, Z., Huang, T., Huang, C. (2021). Eutrophication and lakes dynamic conditions control the endogenous and terrestrial POC observed by remote sensing: Modeling and application. *Ecological Indicators*, 129, 107907.

Zhu, Z., Zhao, Y., Guo, Y., Zhang, R., Pan, Y., Zhou, T. (2021). A novel additional carbon source derived from rotten fruits: Application for the denitrification from mature landfill leachate and evaluation the economic benefits. *Bioresource Technology*, 334, 125244.

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- Tuğba Sapmaz Wikström graduated as an environmental engineer from the Environmental Engineering Department of Istanbul Technical University in 2015.
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PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:

Journal papers

- **Sapmaz, T.**, Uwineza, C., Mahboubi, A., De Wever, H., Koseoglu-Imer, D. Y., & Taherzadeh, M. J. (2023). Towards maximum value creation from potato protein liquor: Volatile fatty acids production from fungal cultivation effluent. ***Biomass Conversion and Biorefinery***, 1–14.
- **Sapmaz, T.**, Mahboubi, A., Taher, M. N., Beler-Baykal, B., Karagunduz, A., Taherzadeh, M. J., & Koseoglu-Imer, D. Y. (2022). Waste-derived volatile fatty acid production and ammonium removal from it by ion exchange process with natural zeolite. ***Bioengineered***, 13(6), 14751–14769.
- **Sapmaz, T**., Manafi, R., Mahboubi, A., Lorick, D., Koseoglu-Imer, D. Y., & Taherzadeh, M. J. (2022). Potential of food waste-derived volatile fatty acids as alternative carbon source for denitrifying moving bed biofilm reactors. ***Bioresource Technology***, 364, 128046.
- **Sapmaz, T.**, Manafi, R., Mahboubi, A., Koseoglu-Imer, D. Y., & Taherzadeh, M. J. (2023). The Effect of Sequential and Simultaneous Supplementation of Waste-Derived Volatile Fatty Acids and Methanol as Alternative Carbon Source Blend for Wastewater Denitrification. ***Sustainability***, 15(8), 6849.

Conference participations

- **Sapmaz, T.**, Uwineza, C., Mahboubi, A., De Wever, H., Koseoglu-Imer, D. Y., & Taherzadeh, M. J. Towards maximum value creation from potato protein liquor: volatile fatty acid production from the effluent of fungal cultivation [Poster presentation, online]. The 9th International Conference on Sustainable Solid Waste Management, Corfu, Greece, 15-18 June 2022.
- **Sapmaz, T.**, Manafi, R., Mahboubi, A., Lorick, D., Koseoglu-Imer, D. Y., & Taherzadeh, M. J. Application of food waste-derived volatile fatty acids as alternative carbon source for post-denitrification process in municipal wastewater treatment [Oral presentation]. The 10th International Conference on Sustainable Solid Waste Management, Chania, Greece, 21-24 June 2023.
- **Sapmaz, T.**, Mahboubi, A., Koseoglu-Imer, D. Y., & Taherzadeh, M. J. A sustainable biorefinery approach: Investigating the potential of waste-derived volatile fatty acids as alternative carbon source in wastewater treatment [Oral presentation]. FULLRECO4US, International Sustainable Resource Recovery Strategies Toward Zero Waste, Istanbul, Türkiye, 13-15 September 2023.

OTHER PUBLICATIONS, PRESENTATIONS AND PATENTS:

- Agnihotri, S., Yin, D. M., Mahboubi, A., **Sapmaz, T.**, Varjani, S., Qiao, W., Koseoglu-Imer D. Y., & Taherzadeh, M. J. (2022). A glimpse of the world of volatile fatty acids production and application: a review. ***Bioengineered***, 13(1), 1249–1275.
- Yin, D. M., Uwineza, C., **Sapmaz, T.**, Mahboubi, A., De Wever, H., Qiao, W., & Taherzadeh, M. J. (2022). Volatile fatty acids (VFA) production and recovery from chicken manure using a high-solid anaerobic membrane bioreactor (AnMBR). ***Membranes***, 12(11), 1133.

- Pervez, M. N., Mahboubi, A., Uwineza, C., **Sapmaz, T.**, Zarra, T., Belgiorno, V., Naddeo, V., Taherzadeh, M.J. (2022). Feasibility of nanofiltration process for high efficient recovery and concentrations of food waste-derived volatile fatty acids. ***Journal of Water Process Engineering***, 48, 102933.
- Taher, M. N., Al-Mutwalli, S. A., **Sapmaz, T.**, Koseoglu-Imer, D.Y. (2021). Chapter 27 - Potentials and performance of biological processes for treatment of pharmaceuticals and personal care products in wastewater. In: The Future of Effluent Treatment Plants, (Eds.) M. Shah, S. Rodriguez-Couto, K. Mehta, Elsevier, pp. 523–550.
- Koseoglu-Imer, D.Y., Topuz, E., **Sapmaz, T**., Al-Mutwalli, S.A., Ikizoglu, B., Taher, M.N., Pala, B., Aydiner, C. (2023). Emerging Organic Compound (EOC) Removal from Water and Wastewater Using Innovative Technologies and Materials. In: Cost-efficient Wastewater Treatment Technologies: Engineered Systems, (Eds.) M. Nasr, A.M. Negm, Springer International Publishing. Cham, pp. 379–419.