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# PRODUCTION AND RECOVERY OF VALUABLE BIOPRODUCTS THROUGH ANAEROBIC FERMENTATION OF ORGANIC WASTE

**THESIS SUBMITTED**
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presented by

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*A mi familia*

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# ABSTRACT

This thesis explored controlled anaerobic fermentation processes to gain insights into the feasibility of treating different sources of organic waste to obtain valuable fermentation bioproducts, such as volatile fatty acid (VFA) and additional bioproducts. Anaerobic fermentation will constitute an innovative approach to recover value from organic waste in future biorefineries and contribute to the Circular Economy.

The VFA production was assessed by performing laboratory-scale experiments where the acidogenic potential of different agro-industrial and urban waste was evaluated. These tests provided a valuable methodology to understand the process chemistry, where the impact of process variables, i.e. temperature and pH, was evaluated. Besides, these tests provided valuable information for subsequent up-scaling.

Mixed culture VFA production tests were up-scaled in a pilot-scale platform, where the results of laboratory-scale tests could be reproduced. Herein, co-fermentation of substrates of complementary nature showed potential benefits to enhance the acidogenic fermentation process.

The VFA production process was evaluated in a continuous mode of operation at pilot-scale. Herein, different process variables were optimised, i.e. the pH and the hydraulic retention time. Organic Fraction of Municipal Solid Waste (OFMSW) showed potential benefits as co-substrate, which enabled to boost the VFA productivity and enrich the fermentation composition. Continuous acidogenic fermentation was a feasible alternative, where stable VFA processes could be obtained. Additionally, ultrafiltration technology was adequate to recover a VFA rich permeate.

During the last phase of the thesis, the downstream of fermentation bioproducts was evaluated by assessing the performance of a novel membrane-based technology, namely forward osmosis (FO).

During the downstream process, other value-added fermentation bioproducts beyond VFA were evaluated, such as succinic acid, lactic acid and ethanol. These consist of valuable bioproducts that can be obtained from controlled anaerobic fermentation processes, for example using pure bacterial strains and different sources of organic waste, such as third generation biomass.

The performance of different types of FO membranes, i.e. cellulose triacetate flat sheet membranes and thin film composite hollow fibre membranes showed the potential of FO technology as a downstream technology to up-concentrate the fermentation bioproducts, enabling high rates of water removal and high solute concentration factors. The application of FO with synthetic binary mixtures of target chemicals enabled to obtain low volume and high concentrated solutions, which might constitute an essential step for the final downstream step and bioproduct recovery.

The application of FO membranes was validated with real fermentation mixtures obtained from pure culture fermentation tests, which were performed with the OFMSW and macroalgae substrates. The application of FO with the real fermenttiaon broths, confirmed the potential of the technology as a novel application to upgrade target chemicals. This may raise the interest of the private sector by becoming a core technology of future downstream processes in the biorefinery context and by opening new market opportunities.

Overall, this thesis covered the whole picture of fermentation processes that could be performed in future biorefineries, from laboratory scale experiments to pilot-scale, by treating different sources of organic waste and exploring a potential downstream technology.

# RESUMEN

El objetivo de la presente tesis ha sido explorar la tecnología de fermentación anaerobia para la obtención de productos de valor añadido, tales como los ácidos grasos volátiles (AGV) a partir de diferentes corrientes de residuos orgánicos. La producción de AGV mediante fermentaciones anaerobias controladas podría constituir una solución innovadora para recuperar compuestos de valor añadido a partir de residuos orgánicos, los cuales constituyen un problema ambiental y de gestión, y contribuir de este modo al modelo de Economía Circular.

En primer lugar, se evaluó el potencial acidogenico de diferentes corrientes de residuos de origen agro-industrial y urbano. Estos ensayos proporcionaron una metodología valida para el posterior escalado en planta piloto. A su vez, se analizó el efecto de las principales variables de proceso, como el pH y la temperatura, tanto en el rendimiento de la fermentación como en la bioquímica del proceso.

A continuación, se realizó el escalado de la producción de AGV, a partir de cultivos mixtos, en una planta piloto automatizada. Durante los ensayos en modo batch, se pudieron reproducir los principales valores obtenidos en los ensayos acidogenicos previos. La co-fermentación de residuos de naturaleza complementaria se identificó como un aspecto clave para establecer sinergias y mejorar el proceso fermentativo.

En tercer lugar, se evaluó el proceso de producción de AGV en modo continuo de operación en planta piloto. Durante los ensayos, se testearon diferentes condiciones de pH y tiempo de retención hidraúlico para optmizar el proceso fermentativo. Mediante la adición de la fracción orgánica de residuos sólidos urbanos durante la fermentación de lodo de depuradora, se consiguió mejorar la productividad del proceso y obtener mezclas de mayor riqueza en composición de AGV. A su vez, la tecnología de ultrafiltración se identificó como una alternativa adecuada para obtener fracciones líquidas enriquecidas en AGV.

Finalmente, en la última parte de la tesis, se ahondó en el proceso de separación de las mezclas obtenidas mediante fermentación anaerobia, con el fin de facilitar la obtención de los bioproductos con aplicación en el mercado. Para ello, se analizó la viabilidad de una técnica avanzada de separación de membrana, como la osmosis directa.

Durante este periodo, se analizaron otros productos de interés más allá de los AGV, que pueden ser obtenidos a partir de fermentaciones anaerobias controladas, como el ácido láctico, el ácido succinico y el etanol. Estos consituyen productos de alto valor añadido y se han identificado como “platform chemicas” por ejemplo en el caso del ácido succínico y el ácido láctico. Estos bioproductos pueden ser obtenidos a partir de corrientes residuals alternativas, como la biomasa de tercera generación (e.g. macroalgas).

Se realizaron ensayos de osmosis directa con membranas planas de triacetato de celulosa y tubulares poliméricas con proteinas biomiméticas integradas, donde se testearon mezclas binarias sintéticas de los compuestos mencionados. Los resultados evidenciaron el elevado potencial de la tecnología de osmosis directa para concentrar los compuestos objetivo del studio, donde se pudieron eliminar cantidades elevadas de agua. Esto permitió obtener mezclas de volumen reducido y con factores de concentración elevados de soluto. De acuerdo a los resultados obtenidos, la tecnología de osmosis directa prodría convertirse en un paso clave en la purificación de compuestos obtenidos mediante fermentación anaerobia en biorefinería.

Los resultados obtenidos con las mezclas sintéticas de los compuestos objetivo de estudio, fueron validados con mezclas de fermentación reales donde se obtuvieron ácido succinico, ácido láctico y etanol a partir de la fermentación realizada con cultivos microbianos puros, y donde se emplearon FORSU y macroalgas como residuos. Se confirmó el potencial de la tecnología de ósmosis directa como una applicación innovadora de las membranas para concentrar compuestos obtenidos en procesos de fermentación anaerobios. De acuerdo a los resultados obtenidos, se prevé que esta solución tecnológica podría despertar el interés del sector privado y abrir la posibilidad a nuevos mercados.

La presente tesis, permitió cubrir el proceso integro de fermentación anaerobia, desde un enfoque de biorefinería. Con un carácter experimental, se realizaron ensayos fermentativos a pequeña escala y en planta piloto, y se dieron los primeros pasos para procesar y separar las mezclas fermentadas obtenidas mediante la aplicación de una tecnología innovadora de separación por membrana.

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# NOTATION AND ABBREVIATIONS

## Abbreviations

| | |
|---|---|
| AD | Anaerobic digestion |
| An-MBR | Anaerobic membrane bioreactor |
| CE | Circular economy |
| CG | Crude glycerol |
| COD | Chemical oxygen demand |
| CSTR | Continuous stirred tank reactor |
| CTA | Cellulose triacetate |
| DA | Degree of acidification |
| EER | Earth energy renewables |
| FID | Flame ionization detector |
| F:M | Food to microorganism ratio |
| FNA | Free nitrous acid |
| FS | Filtration system |
| FlS | Flat sheet |
| FO | Forward osmosis |
| GC | Gas chromatograph |
| HAc | Acetic acid |
| HBut | Butyric acid |
| HF | Hollow fibre |
| HPr | Propionic acid |
| HLac | Lactic acid |
| HSuc | Succinic acid |
| HVal | Valeric acid |
| HRT | Hydraulic retention time |

| | |
|---|---|
| ICP | Internal concentration polarization |
| Iso-but | Iso butyric acid |
| Iso-val | Iso valeric acid |
| $J_w$ | Water flux |
| $K_h$ | Hydrolisis rate constant |
| LBR | Leaching bed reactor |
| LCA | Life cycle assessment |
| LCCA | Life-cycle cost analysis |
| MBM | Meat and bone meal |
| MFC | Microbial fuel cell |
| MSW | Municipal solid waste |
| MW | Molecular weight |
| OFMSW | Organic fraction of municipal solid waste |
| OLR | Organic loading rate |
| PA | Partial alkalinity |
| PHA | Polyhydroxialkanoate |
| PHB | Polyhydroxibutyrate |
| PHV | Polyhydroxivalerate |
| PLA | Polylactic acid |
| $P_{ww}$ | Paper mill wastewater |
| SCF | Solute concentration factor |
| sCOD | Soluble chemical oxygen demand |
| SRT | Solid retention time |
| SS | Sewage sludge |
| $S_{ww}$ | Slaughterhouse wastewater |
| TAN | Total ammonium nitrogen |
| TBME | Tert-butyl-metyl-ether |
| tCOD | Total chemical oxygen demand |
| TA | Total alkalinity |
| TFC | Thin film composite |
| TKN | Total Kjeldahl nitrogen |
| TS | Total solids |
| TSS | Total suspended solids |
| VAM | Vynil acetate monomer |
| VS | Volatile solids |

| | |
|---|---|
| VSS | Volatile suspended solids |
| WAS | Waste activated sludge |
| WCF | Water concentration factor |
| WWTP | Wastewater treatment plant |
| $W_{ww}$ | Winery wastewater |

# CHAPTER 1

# INTRODUCTION

## 1.1 MOTIVATION OF THE THESIS

The increasing world population, the fast economic growth and the high level of technology development are leading to the generation of vast amounts of waste, the depletion of natural resources and adverse effects on nature and climate change. Global temperature rise, warming oceans, polluted cities and extreme weather events (NASA, 2019) are consequences of this accelerated development.

However, these effects are also increasing the awareness on the importance of resource recovery, energy minimisation and increasing the sustainability of industrial production processes.

Sustainability is defined as the “quality of not being harmful to the environment or depleting natural resources, and thereby supporting the long term ecological balance”. Therefore, sustainable development should be prioritised in the short term future, avoiding the rapid consumption of non-renewable fossil fuels and resources (petroleum, natural gas, coal and minerals).

Pearce and Turner, two British environmental economists, introduced the concept of Circular Economy (CE) in 1989 (Pearce and Turner, 1990). This economic model has been recognised as part of the solution for achieving a sustainable development and is rising a continuous interest among local policymakers, industry and other social stakeholders. The concept of a circular economy is based on the principle of an economic activity that builds and rebuilds the overall system health (Ellen MacArthur Foundation, 2019). Herein, eco-innovation and sustainable design strategies become key aspects of closing the loop of the product's lifecycle, getting valuable products from waste streams and promoting environmental resilience (Scheel *et al.*, 2016). Recent definitions of the circular economy include a multi-level vision, on micro, meso and macro-scale levels and the importance of sustainable business models (Kirchherr *et al.*, 2017). Among the four main aspects highlighted in the concept of CE by Pietro-Sandoval *et al.* (2018), the authors stated the importance of recirculation of resources and energy and the recovery of value from waste.

In 2015, countries adopted the 2030 agenda of Sustainable Development and the Sustainable Development Goals (United Nations, 2019). In addition, Europe has shown a strong commitment to adopt measures to develop a CE model. Concretely, the European Commission published “Closing the loop, an EU action plan for the Circular Economy” (COM 2015, 614) which contributes to goal nº 12 of Responsible Production and Consumption of the Development Goals. Following the European standards, at a regional level, the Basque Gonverment has showed a firm commitment to promote the necessary measures to make a transition towards a Circular Economy, which will create a positive social and economic impact, by for example creating new market and job opportunities.

Herein, waste management is a key aspect and necessary to be included within the model of a Circular Economy. Regarding waste, recently, the Directive (EU) 2018/851 made amendments on the important Waste Framework Directive 2008/98/EC contributing to the transformation of the perception of waste towards a valuable resource, where it is highlighted the need to reutilize and recycle the municipal solid waste.

All these, gather important drivers which are required to help change the perception of waste, and likely modify social habits related to consumer goods and waste generation in the coming years, contributing to a more sustainable economic model.

Interestingly, organic waste can be treated for the production of valuable resources. The classical approach to get value from organic waste stream was conducted by biomethanization or conventionally known as anaerobic digestion (AD). Biomethanization of organic waste enables the production of renewable bioenergy and a digestate that can be valorised as a fertiliser (Li *et al.*, 2019). AD is a mature technology that has been adapted widely to treat different sources of organic waste, by different reactor configurations, such as full mix, biofilm, UASB, plug flow etc. and by optimizing the process, i.e. using different pretreatment methods, pure biological cultures, or chemical additives, and trying different process configurations. (Mao *et al.*, 2015).

A second approach to get value from organic waste relies in the biorefinery concept. A biorefinery is analogous to an oil-based refinery which uses biomass coming from the different origin as feedstock. Herein, different biorefinery platforms exist, i.e. sugar, thermochemical (syngas) and carbon-rich chains (Kin e*t al.*, 2018). For example, the sugar platform uses lignocellulosic biomass for ethanol production while the syngas platform uses different sources of dry biomass, i.e. agricultural residues, or by-products from the food industry for the production of syngas (CO + $H_2$) which can be converted to a variety of fuels ($H_2$, Fischer – Tropsh (FT) diesel, gasoline) and chemicals (methanol, urea).

In addition, another platform was proposed by Agler *et al.* (2011), the Volatile Fatty Acid (VFA) platform. VFA consist of a mixture of acids containing between C2 – C6, i.e. acetic, propionic, butyric and iso-butyric, and valeric and isovaleric acids. VFA constitute essential intermediates of conventional AD processes when methanogenesis is inhibited (Li *et al.*, 2019).

During the hydrolysis, the extracellular enzymes excreted by fermentative bacteria contribute to degrade macromolecules and organic polymers into simpler monomers, and additionally, some insoluble particles are solubilised in the medium (Bastidas-Oyanedel *et al.*, 2015). Herein, lipids, polysaccharides and proteins that compose the cellular mass are degraded into long chain fatty acids, monosaccharides and amino acids, which are soluble in water. The enzymes involved during the hydrolysis are present in the bulk solution or attached to the microbial cell (Tong and McCarty, 1991). The particle size, the pH, the production of enzymes and the diffusion and adsorption of enzymes to particles are the main parameters affecting the hydrolysis (Zhou *et al.*, 2017).

In a mixed culture fermentation process, during the acidogenesis, a heterogeneous microbial population of facultative and obligate anaerobic bacteria degrade the dissolved organic matter within a series of oxidation-reduction reactions, into volatile fatty acids, consisting mainly of acetic acid, propionic acid and butyric acid, and other minor VFA and alcohols (Louis and Flint, 2009). Acidogenesis is mainly affected by the process pH, the hydrogen transfer between species, the Hydraulic Retention Time (HRT) and the acclimation of the anaerobic culture.

Finally, during the acetogenesis step the oxidation of fermentation products into $H_2$, $CO_2$ and acetate occurs. Homoacetogenesis can also lead to the production of acetate as the product from $H_2$ and $CO_2$, which is less favourable thermodynamically than methane and sulphate production. Additionally, syntrophic acetogenesis involves the anaerobic oxidation of propionate and butyrate to acetate and $H_2$, which is inhibited with a high presence of $H_2$ (Angelidaki *et al.*, 2011).

Anaerobic fermentation is a complex process, where the fermentation outcome and VFA composition are highly dependent on the combined effects and the interactions of the microorganisms present, the process parameters employed and the microenvironment that is generated in the process. Specifically, the process is affected by the substrate and composition in terms of carbohydrates, proteins and lipids, the inoculum, the pH, the temperature, the configuration of the reactor, the Organic Loading Rate (OLR), the hydraulic retention time, the operation mode, as well as the headspace $H_2$ pressure (Zhou *et al.*, 2017).

Herein, pyruvate is the pivotal metabolite product, which is converted to the full range of VFA and other metabolites. The pyruvate conversion depends on substrate concentrations, on the cellular redox balance and enzyme activities which control the activities of competing pathways. Additionally, fluxes in the metabolic network are controlled by kinetic and/or thermodynamic limitations. Kinetic limitations occur when the rate of substrate utilisation controls the pathway of product formation, and all the reactions are thermodynamically favourable. Contrarily, thermodynamic control occurs when Gibbs energy change of the reaction is close to thermodynamic equilibrium.

One significant advantage of the VFA platform is that the carboxylic acids can be obtained by employing mixed culture inoculum. In a mixed microbial culture many organisms exist in a competitive environment and factors affecting the product mixture are less understood (Tamudo *et al.*, 2008). This is an essential aspect since no sterilisation methods are required to culture-specific microorganisms compared to other biological processes, making the process more economically favourable and cost-competitive (Strazzera *et al.*, 2018).

The VFA platform can use different types of waste stream, including agro-industrial waste and wastewater, urban waste and other types of lignocellulosic and marine waste (e.g. macroalgae).

**Table 1. 1** VFA market, derived products and applications

| VFA market share | Products | Application |
|---|---|---|
| **Acetic acid**<br>• Market size: 3,500.000 t/year<br>• Price: 800 $/ton<br>• Growth rate: 4.3% year | • Esters | • Chemical industry: production of inks, paints, adhesives, coating. |
| | • Solvents | • Chemical industry |
| | • Calcium, magnesium, potassium acetate salts | • De-icing roads and heat exchange fluids |
| | • Vinegar | • Food industry |
| | • Vynil acetate | • Production of polymers |
| **Propionic acid**<br>• Market size: 180.000 t/year<br>• Price: 2500 $/ton<br>• Growth rate: 15.1% year | • Cellulose acetate propionate | • Production of plastics |
| | • Esters | • Pharmaceutical industry, cosmetics |
| | • Others | • Antifingicide for animal feed<br>• Chemical industry: printing inks, varnishes |
| | • Salts | • Food and grain preservatives, herbicides |
| **Butyric acid**<br>• Market size: 30.000 t/year<br>• Price: 1700 $/ton<br>• Growth rate: 2.7% year | • Cellulose acetate butyrate | • Synthesis of plastics |
| | • Esters<br>• Salts | • Production of fruit like fragance, cosmetics industry |
| | • Others | • Flavour additive for animal feed<br>• Chemical industry<br>• Pharmaceutical industry – drug preparation |
| **Valeric acid**<br>• Market size: 10.036 t/year<br>• Price: 2600 $/ton<br>• Growth rate: 3.84% | • Esters | • Food and cosmetics: production of flavor and perfume.<br>• Lubricants<br>• Agricultural chemicals, plasticizers, and vynil stabilizers |

Mixed VFA resulting from the VFA platform can be used in novel applications, such as polyhydroxyalkanoates production, electricity generation in microbial fuel cells, biodiesel production and nutrient removal in wastewater treatment facilities (Bengtsson *et al.*, 2017, Ryu *et al.*, 2013, Park *et al.*, 2017, Zhang *et al.*, 2016). Additionally, the pure form of the chemicals consist of valuable industrial products which can be further processed into other value-added chemicals, e.g. ketones, esters, alcohols and polymers (Table 1. 1) (Atasoy *et al.*, 2018, Kim *et al.*, 2018).

Besides the VFA platform, pure culture anaerobic fermentation can lead to the production of value-added bioproducts, such as succinic and lactic acid. Among different examples, these bioproducts can be produced from carbohydrate rich biomass, such as macroalgae and *Actinobacillus Succinogens* strain for succinic acid, and the organic fraction of municipal solid waste and *Lactobacillus delbrueckii* strain for lactic acid. Both chemicals were identified as top platform chemicals (Datta and Henry, 2006, U.S. Deparment of Energy, 2004) and are utilized in a wide range of commercial applications. Within the biodegradable polymer field, succinic acid can be polymerized with 1, 4 butanediol to produce polybutyilene, a bioplastic that is expected to have a high economic growth in the coming years (Sreedevi *et al.*, 2014). In addition, lactic acid can be used to produce polylactic acid, a biodegradable and biocompatible aliphatic polyester (Vandenberghe *et al.*, 2018).

The current challenge for obtaining pure forms of marketable VFA or additional value-added bioproducts resides in the separation/purification step, e.g. the mixture of VFA produces azeotropic mixtures with water. Despite the promising efforts in the field, there is a technical challenge to be overcome in the coming years, to find sustainable and cost-effective solutions to extract these value-added products from the fermentation broth (Atasoy *et al.*, 2018, Rebecchi *et al.*, 2016, Reyhanitash *et al.*, 2017). The current state of the art is focusing on developing laboratory-scale solutions for VFA extraction, such as liquid-liquid extraction, membrane separation solutions, i.e. electrodialysis, pervaporation or nanofiltration, adsorption and ion exchange resins. The feasibility of these technologies will depend on the applicability at the pilot/demonstration scale and the costs associated with the recovery process.

Regarding the downstream of fermentation products, an interesting aspect resides in increasing the concentration of the bioproducts obtained thorugh anaerobic fermentation, which might be highly beneficial for the subsequent recovery step.

This might be feasible by applying a low-pressure membrane process, such as forward osmosis technology.

Considering the current challenges and the state of the art, further scientific studies and new solutions are required to cover the whole spectrum of an integrated fermentation-separation process, which could launch for example the VFA platform to the market.

Currently, the VFA platform is in the demonstration stage. Beyond the purification step, research efforts are also directing towards improving the pretreatment steps, which optimise the use of chemicals and energy, to improve the yield and productivity of the fermentation process and to better control the bacterial community for selective VFA production.

There are some companies, i.e. Earth Energy Renewables (EER) in the US and Aquiris in EU, which are trying to develop and commercialize the VFA platform. Other companies or institutions include MIT, Kaist and the Texas A&M Unversity (Kim *et al.*, 2018).

For example, EER is working towards the up-scaling of the VFA platform with a demonstration facility of 8 tonnes day$^{-1}$, to serve the fragance and flavour industry. Interestingly, water boards and local authorities in the Netherlands are working in the PHARIO project 2.0 intending to develop a demonstration facility that will produce between 1,000 – 3,000 kg PHA/day (Bio-based, 2018). Promising results were obtained in the first period during 2015, where 1 kg of PHA was obtained per week in the WWTP of Bath.

Overall, anaerobic fermentation constitutes a promising approach that can be used for the valorisation of organic waste. This sustainable approach will contribute to reduce the environmental impact through obtaining valuable bioproducts in the form of pure chemicals and mixtures that can be further valorised. Thus, contributing not only to fulfil the environmental regulations but also to make a significant contribution to the bio-based economy.

## 1.2 OBJECTIVE OF THE THESIS

The primary objective of this thesis was to gain deep insights into new solutions to recover value from organic waste, by exploring controlled anaerobic fermentation processes. Concretely, mixed culture anaerobic fermentation processes were explored, to obtain VFA from different sources of organic waste from the urban and agro-industrial origin. These included wastewater from industry, namely winery, slaughterhouse, crude glycerol and paper mill wastewaters; different sources of sludge, such as sewage sludge from WWTP and sludge from the dairy industry; and solid waste from the meat industry and the organic fraction of municipal solid waste. It was aimed to gain insights into the VFA production process, by evaluating the influence of process parameters at both laboratory and pilot-scale. Additionally, a second objective was to assess the separation mechanisms of the fermentation mixture by using advance membrane technologies, i.e. ultrafiltration, and forward osmosis technology. In addition, it was intented to explore the production of other valuable fermentation products, i.e. lactic acid, succinic acid and ethanol (as secondary fermentation product obtained from lactic acid fermentation).

The primary objective was fulfilled by achieving the following partial objectives:

- Assessing the acidogenic potential at laboratory-scale with organic waste streams from different agro-industrial and urban origin.

    - The selection of the abovementioned agro-industrial and urban waste streams and wastewater for the evaluation of their acidogenic potential.
    - Analysis of the pH at acidic and alkaline conditions on the fermentation process of the organic waste considered.
    - Analysis of the temperature at acidic and alkaline conditions on the fermentation process, the fermentation yield and product distribution.

- Up-scaling of the VFA production process by performing batch experiments in a pilot-scale anaerobic fermentation system.

    - The development of fermentation tests in batch mode of operation with selected agro-industrial waste, namely, winery wastewater, meat and bone meal and sewage sludge, from the previous laboratory tests.
    - Assessment of co-fermentation experiments with sewage sludge as a model substrate.

- Optimisation of the mixed culture fermentation process during continuous acidogenic processes performed in a pilot-scale system.

  - Determination of opitmial pH and hydraulic retention time during sewage sludge continuous fermentation tests.
  - Enhancement of continuous sewage sludge alkaline fermentation by the addition of organic fraction of municipal solid waste as co-substrate.
  - Assessment of ultrafiltration technology as a first step to recover the VFA rich permeate.

- Assessing the potential of Forward Osmosis technology for the recovery of valuable fermentation products.

  - Evaluation of cellulose triacetate flat sheet and thin film composite hollow fibre forward osmosis membranes with synthetic binary mixtures containing succinic acid, lactic acid, acetic acid, ethanol and deionized water.
  - Evaluation of feed solution pH effect in the main parameters governing the forward osmosis process.

- Application and evaluation of forward osmosis membranes with real fermentation broths.

  - Performance of pure-culure fermentation tests to produce succinic acid, lactic acid and ethanol fermentation broths.
  - Evaluation of draw solution concentration in the main parameters governing the forward osmosis process.

## 1.3 CONTENTS OF THE THESIS

The contents of the present Thesis are distributed into seven chapters, as detailed below:

**Chapter 1** (introduction) introduces the main motivation of this thesis. The objectives that were pursued are listed, and the summary of the content of each chapter is presented.

**Chapter 2** (Selective VFA potential of organic waste streams: assessing temperature and pH influence), describes the first results that were obtained during controlled fermentation tests performed with organic waste streams of different agro-industrial and urban origin. This study was a preliminary assessment of the potential of each waste stream for VFA production, where different process conditions were evaluated, i.e. the temperature and pH. This study enabled to obtain the necessary knowledge to up-scale the fermentation tests.

**Chapter 3** (From sewage sludge and agri-food waste to VFA: individual acid production potential and up-scaling). This chapter describes the following step of up-scaling, where the fermentation step was conducted with selected waste streams from the previous screening tests. Monofermentation tests were performed with sewage sluge, winery wastewater and meat and bone meal substrates, and the results were compared with the previous laboratory-scale tests. Finally, a co-fermentation scenario was evaluated by combining different waste streams with sewage sludge.

**Chapter 4** (Continuous acidogenic fermentation: narrowing the gap between laboratory testing and industrial application). This study explored the potential of continuous acidogenic fermentation tests, performed with sewage sludge as model substrate at pilot-scale. Herein, the start-up methodology was evaluated. Additionally, the effect of pH and HRT were assessed on the fermentation yield and VFA distribution. The potential of the organic fraction of municipal waste was evaluated in a co-fermentation scenario, and ultrafiltration technology was assessed to obtain a VFA rich permeate. This chapter intended to evidence the potential of mixed culture acidogenic fermentation process, as a robust solution for bioproduct recovery. Additionally, the process was enhanced by the addition of OFMSW cosubstrate to sewage sludge to obtain a high-rate VFA process.

Regarding the need to explore downstream processes to recover fermentation products, **Chapter 5** (Forward Osmosis technology for downstream recovering of valuable acidogenic fermentation bioproducts) analysed the potential of Forward Osmosis (FO) technology to up-concentrate binary synthetic mixtures containing value-added bioproducts that can be obtained from an anaerobic fermentation process. Herein, acetic acid was tested as model VFA. Additionally, other valuable fermentation bioproducts were introduced. Concretely, succinic acid, lactic acid and ethanol. These are valuable fermentation products that are presented in the Chapter, which have attracted research interest in recent years and which can be obtained from additional sources of waste.

**Chapter 6** (Recovery of succinic acid, lactic acid and ethanol through forward osmosis membranes) validated the application of FO membranes with real fermentation broths. The target chemicals were obtained from the fermentation of *Saccharina Lattisima* macroalgae and biopulp (OFMSW) as substrate. Herein, *Actinobacillus Succinogens* and *Lactobacillus delbrueckii* bacterial strains were employed. The forward osmosis tests were performed with the soluble fraction of the fermentation broths with different concentration of NaCl draw solution, i.e. 1.5 and 5M. The results confirmed the potential of the FO technology to upgrade the target chemicals.

**Chapter 7** (Conclusion and future research lines). This chapters highlights the main conclusions of this thesis and proposes further research lines.

**Appendix A**: Publications generated.

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# CHAPTER 2

# SELECTIVE VFA PRODUCTION POTENTIAL FROM ORGANIC WASTE STREAMS: ASSESSING TEMPERATURE AND PH INFLUENCE

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This chapter has been published in:

*Garcia-Aguirre J., Aymerich E., González Mtnez. de Goñi J., Esteban-Gutiérrez M., 2017. Selective VFA production potential from organic waste streams: Assessing temperature and pH influence. Bioresource Technology, 244, 1081-1088.*

*Part of the results were presented in an oral presentation at "Firt international conference on Bioenergy and Climate Change", June 2016, Soria, Spain.*

## 2.1 GRAPHICAL ABSTRACT

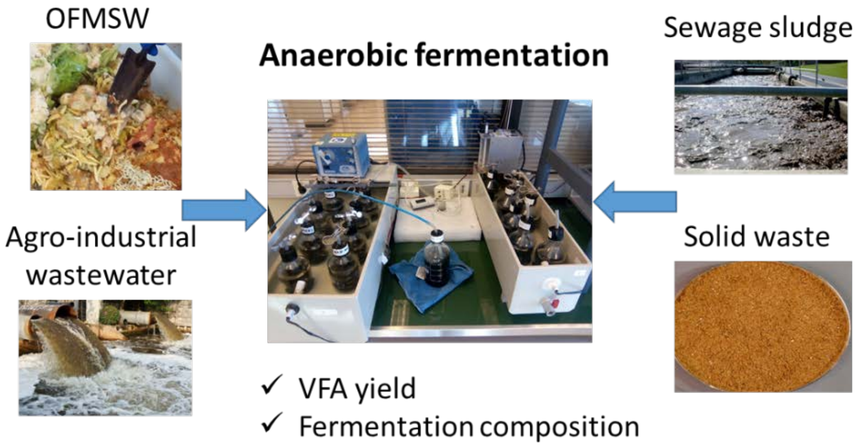

**Figure 2. 1.** Graphical abstract

## 2.2 ABSTRACT

This study explored the volatile fatty acid (VFA) production potential of seven waste streams from urban and agroindustrial sources. For that purpose, batch assays were performed under acidic (pH 5.5) and alkaline (pH 10) conditions at both mesophilic (35 °C) and thermophilic (55 °C) temperature. Overall, the VFA yield was influenced by temperature, and it was positively affected by pH, ranging between 220 and 677 mg COD g$^{-1}$ COD$_{fed}$ for liquid waste streams and between 127 and 611 mg COD g$^{-1}$ COD$_{fed}$ for solid waste streams and urban sludge. The highest VFA concentration and highest VFA/sCOD ratio was obtained during the organic fraction of municipal solid waste (OFMSW) fermentation, with 8,320 mg COD L$^{-1}$ and 94% at alkaline pH and mesophilic temperature. The results of this study suggest that selective VFA production, i.e. via propionic, butyric and acetic acid production, might be feasible for scaling-up purposes with specific waste streams by adjusting the process parameters.

## 2.3 INTRODUCTION

In the current context of changing waste policy within the framework of the circular economy, a need to re-orientate waste management in the mid-term is anticipated. Recently, the EU published "Closing the loop – An EU action plan for the Circular Economy" (COM (2015) 614) (European Commission, 2015), which is a step forward in relation to the previous Landfill Directive 1999/31/EC and the Waste Framework Directive 2008/98/EC in that it includes specific measures for addressing waste management and promoting the reuse of materials. In addition, the perception of waste has changed from an unwanted product associated with considerable treatment and disposal costs to a valuable resource.

Anaerobic digestion (AD) has been widely implemented to get value-added methane from organic waste, i.e. sludge from wastewater treatment plants (WWTP) or agricultural bio-waste. As the literature has pointed out, despite the maturity of the technology, its implementation relies on the economic subsidies that are required in order to achieve cost-effective AD processes (Kleerebezem *et al.*, 2015). Moreover, in some EU countries (i.e. Spain) the current unstable biogas market may be detrimental to successful AD implementation, and this state of affairs may open the door to a re-orientation of how organic waste is managed.

One way in which organic waste might better managed and reused is by transforming AD technology into volatile fatty acid (VFA) production. In recent years, volatile fatty acids have attracted research interest as value-added end products, which can be synthesized from heterogeneous organic waste streams in mixed culture engineered hydrolysis and fermentation processes. VFA may constitute one of the main platforms in new biorefineries, which will be used to produce biofuels and valuable chemicals by transforming biomass-based feedstock (Satinder *et al.*, 2017).

VFAs present a wide range of applications, e.g. VFAs can be exploited individually as valuable industrial chemicals in the production of cosmetics, in petrochemical synthesis, in the pharmaceutical industry and in the food and beverage industry (Zacharof and Lovitt, 2013). Additionally, VFA production by means of mixed culture fermentation has created an opportunity for novel applications, such as biodegradable polymer (PHA) production (Shen *et al.*, 2014), nutrient removal in wastewater treatment plants (Lim *et al.*, 2008; Li *et al.*, 2015), chain elongation (Cabrera-Rodriguez *et al.*, 2017), bioenergy production in microbial fuel cells (MFC) (Cavdar *et al.*, 2011) or bulk fuel and solvent production (Agler *et al.*, 2011).

VFA production from organic waste was addressed in a recent study (Lee *et al.*, 2014). The influence of operational parameters i.e. pH, temperature, retention time and organic loading rate, has been studied by several authors (Jiang *et al.*, 2013, Hao and Wang, 2015, Jankowska *et al.*, 2015, Maspolim *et al.*, 2015, Wang *et al.*, 2016a). Based on previous findings, the operational parameters largely determine which reactions are thermodynamically feasible and which microorganisms can grow more effectively. This, in turn, influences the selective mechanisms for VFA production. However, the influence of process parameters in the VFA production needs further research and understanding prior to full-scale implementation.

Furthermore, it is assumed that a cost-effective VFA production process should use mixed microbial cultures to handle the diverse nature of different organic waste streams (Agler *et al.*, 2011). Depending on the readily fermentable organic matter, different substrates may be adequate for VFA production. The use of a specific waste stream will depend on locally available resources and on the niche market that may arise for VFA application.

Despite the fact that the organic fraction of municipal solid waste (OFMSW) and sewage sludge have been a focus of study (Xiong *et al.*, 2012, Jiang *et al.*, 2013, Pittman and Steinmetz, 2013, Yin et al., 2016, Chen *et al.*, 2017), the literature devoted to comparing the acidogenic potential of other organic waste streams from urban and agroindustrial sectors is limited. Among the few studies that have been done, Silva et al. (2013) compared different agroindustrial waste streams for VFA production. Likewise, Shen *et al*. (2014) compared five waste streams for poly(hydroxybutyrate and hydroxyvalerate) production. However, during these studies the process parameters were not modified. Still, there is a gap in the information on VFA production from different organic waste streams from agroindustrial sources and the optimization of process parameters for VFA production. Further research may assess how different types of organic waste affect the fermentation process, which will provide valuable information to the carboxylate industry in order to develop a future VFA platform.

The objective of this study was to evaluate the VFA potential of seven waste streams (liquid, sludge and solid) from urban and agroindustrial sources, which pose an environmental threat and bring up issues of handling. The aim was assess the influence of the type of waste and the process parameters on the composition of the fermentation, the VFA yield and the VFA/sCOD ratio attained. For that purpose, batch assays were performed under acidic and alkaline conditions at both mesophilic and thermophilic temperature. In addition, the feasibility of a selective VFA production based on process parameter control was assessed.

## 2.4 MATERIAL AND METHODS

### 2.4.1. Waste streams and inoculum

In this study, different waste streams were assessed for VFA production.The analysed streams consisted of four liquid effluents from industry: slaughterhouse wastewater (Sww), paper mill wastewater (Pww), winery wastewater (Www) and crude glycerol (CG); sewage sludge (SS); and two solid waste streams, namely the organic fraction of municipal solid waste (OFMSW) and meat and bone meal (MBM). Sww was collected from a slaughterhouse, Pww was obtained from a Kraft paper mill, Www was collected from the effluent discharged at a winery waste valorization plant, and CG was obtained at a biodiesel production plant. SS was obtained from the urban WWTP in San Sebastian (Spain). The OFMSW consisted of food waste collected at a university canteen, and the MBM was obtained from a meat waste processing plant. The main characteristics of the substrates used in the present study are shown in Table 2. 1. Regarding the nature of organic waste, $W_{ww}$, $P_{ww}$ and OFMSW were rich in carbohydrates while the $S_{ww}$, MBM and SS were rich in proteinaceous content (data not shown). CG consists of a polypol containing mainly glycerol, methanol and water.

**Table 2. 1.** Characteristics of the organic waste streams and the inoculum used in the present study.

| | $S_{WW}$ | $P_{WW}$ | $W_{WW}$ | CG | SS | OFMSW | MBM | Inoc. |
|---|---|---|---|---|---|---|---|---|
| pH | 7.49 | 5.86 | 5.31 | 4.4 | 6.28 | 5.25 | 6.12 | 7.44 |
| TS (%) | 0.33 | 0.62 | 8.92 | 17.6 | 5.72 | 30.36 | 99.21 | 3.28 |
| VS (%) | 0.27 | 0.27 | 6.44 | 7.88 | 4.46 | 28.72 | 68.07 | 2.03 |
| VS/TS | 0.82 | 0.43 | 0.72 | 0.45 | 0.78 | 0.95 | 0.69 | 0.62 |
| tCOD (mg $O_2$ L$^{-1}$) | 4535 | 5803 | 95756 | 203137 | 66281 | 1370* | 1017* | 26538 |
| sCOD (mg $O_2$ L$^{-1}$) | 1064 | 4596 | 37261 | 199710 | 2499 | N.A. | N.A. | 3051 |
| TAN (mg N L$^{-1}$) | 28 | 6.3 | 196 | 169 | 1250 | N.A. | N.A. | 1847 |

*mg $^{-1}$g ST

Digested sludge was used as inoculating media for all the waste streams analysed in the present study. This was obtained from the anaerobic digester at the above-mentioned urban wastewater treatment plant. The inoculum was collected before each experimental assay to avoid degradation during extended storage. All waste samples and inoculum were stored at 5 °C prior to use.

### 2.4.2. Batch fermentation test set-up

The acidogenic fermentation tests were performed in duplicate by means of 10-day batch assays in 500 mL Pyrex bottles in acidic (pH 5.5) and alkaline (pH 10) conditions. Both mesophilic (35 °C) and thermophilic (55 °C) temperature ranges were tested. Control tests were performed with each waste stream during each experimental condition tested to assess the acidogenic potential and to calculate the net VFA production of the sample streams. The latter consisted of fermentation assays containing solely anaerobic digested inoculum as fermentation substrate. The volume of substrate was adjusted to an initial tCOD (total chemical oxygen demand) concentration of 10,000 mg $O_2$ $L^{-1}$ in each reactor (Shen *et al.*, 2014), whereas the volume of inoculum was adjusted in order to provide 5,000 mg VSS (volatile suspended solids) $L^{-1}$. As an exception, the initial COD was adjusted to 4,000 mg $O_2$ $L^{-1}$ and the volume of inoculum was adjusted to 2,000 mg VSS $L^{-1}$ for $S_{ww}$ and $P_{ww}$ liquid effluents, due to their lower COD content.

In order to avoid methanogenic activity by microorganisms, the pH of the mixtures was adjusted initially to acidic values, at pH 5.5, (Yu *et al.*, 2008, Lim *et al.*, 2008, Kim *et al.*, 2016) or alkaline values, i.e. pH 10 (Zhao *et al.*, 2015, Jankowska *et al.*, 2015). Either pH values below 6 and above 8 may lead to low or negligible biogas production (Maspolim *et al.*, 2015). In addition, as previously suggested, different pH may be optimal for VFA production, i.e. pH 5.5–6 for the OFMSW (Jiang *et al.*, 2013, Lim *et al.*, 2008), 5.25–6 for industrial wastewaters (Bengtsson *et al.*, 2008) and alkaline pH for SS (Maspolim *et al.*, 2015). A pH meter (CRISON GLP 21+) was used to measure the pH and to adjust it, either to acidic values or alkaline values. Both hydrochloric acid 4 N and sodium hydroxide 4 were used for pH adjustment. Distilled water was used to adjust the volume of the bottles to 400 mL. During the entire fermentation period the pH in the bottles remained uncontrolled. Nitrogen gas was flushed into the bottles for 1 min to remove any residual oxygen, and the bottles were then sealed with rubber stoppers. The reactors were placed in a thermostatic incubator at both temperature conditions.

At the beginning of the tests, a full characterization of the waste streams and of the initial mixture was performed. The pH evolution was monitored daily, and samples were extracted daily to determine the VFA composition of the fermentation broth which were measured from the soluble fraction, i.e. the sCOD (soluble chemical oxygen demand) fraction, during the 10-day incubation period. In addition, biogas production and composition were monitored daily. A final characterization of the fermentation broth was conducted to analyse the composition of the fermentation mixtures.

### 2.4.3. Data analyses

The extent of hydrolysis of the waste streams were calculated according to Wu *et al.,* (2015) and Chen *et al.,* (2017), as shown in Eq. (1). However, in this study, the contribution of the $COD_{H_2}$ was not considered. Eq. (2) shows the acidification extent, which represents the readily fermentable matter present in the soluble fraction of the final fermentation broth.

$$\text{Extent of Hydrolysis (\%)} = \frac{COD_{CH4} + sCOD_{final} - sCOD_{initial}}{tCOD_{initial} - sCOD_{initial}} \tag{2.1}$$

$$\text{Acidification extent} = \frac{COD_{VFA}}{sCOD_{final}} \cdot 100 \tag{2.2}$$

$COD_{CH4}$ represents the amount of COD converted into methane gas, expressed as COD equivalents, by considering the ratio of 4 g COD/ g $CH_4$. The amount of VFA in Eqs. (2.1) and (2.2) is represented in COD equivalents of the final mixture, where the following coefficients for VFA conversion were used: 1.07 g COD/g acetate, 1.51 g COD/g propionate, 1.81 g COD/g butyrate and 2.04 g COD/g valerate (Eastman and Ferguson, 1981). In order to assess the acidification potential of the organic waste streams, the total VFA production was considered as the sum of each individual acid. Likewise, the net VFA production was considered to be the final VFA production minus the initial VFA present in the waste streams. The specific VFA yield was calculated based on the total VFA production, expressed as COD equivalents per gram of COD fed.

$$\text{VFA yield} = \frac{COD_{VFA}}{g\ COD_{fed}} \cdot 100 \tag{2.3}$$

### 2.4.4. Analytical methods

Analyses of the total fraction were performed directly over raw samples of the organic streams under study. The soluble fraction was obtained by means of centrifugation at 12,000 rpm for 15 min, and the supernatant was filtered (Millipore 0.7 μm filters). TS (total solids), VS (volatile solids), tCOD (total chemical oxygen demand), sCOD (soluble chemical oxygen demand), TAN (total ammonia nitrogen) and pH were determined according to Standard Methods, 21st Edition (APHA, 2005). The samples of the solid streams were processed according to their characteristics by ensuring the repeatability of the method. The output performance of the batch tests was checked by analyzing the soluble or filtered fraction of the fermentation broth and by checking the biogas production. A sample of 3 mL was extracted daily from each bottle, without opening the bottles and changing the biogas atmosphere. A filtered or soluble fraction was obtained for VFA analysis by centrifuging the 3 mL sample at 12,000 rpm for 20–30 min followed by vacuum filtration (Millipore 0.7 μm filters). The biogas pressure of the bottles was monitored daily with a pressure sensor (IFM electronic, PN 20279). A sample of biogas was obtained with a syringe to perform the composition analysis, which was carried out with a GC-TCD HP6890 (column SUPELCO 60/80 Carboxen, Ref. 10001-2390-U). After biogas composition measurement, the pressure was released from the bottles.

Individual VFA concentration (acetic, propionic, isobutyric, butyric, isovaleric and valeric acid) was determined by means of an Agilent GC- 6890 gas chromatograph equipped with a FID (flame ionization detector) and a capillary column (DB-FFAP, 30 m × 0.25 mm i.D., 0.25 nm film; Agilent J & W: Ref. 122-3232E). The method used to obtain the sample for VFA determination consisted of processing the filtered fraction via a liquid-liquid extraction with an organic solvent, i.e. TBME (tert-butyl-methyl-ether). Pivalic acid was used as an internal standard solution.

## 2.5 RESULTS AND DISCUSSION

### 2.5.1. VFA production

As Figure 2. 2 shows, VFA evolution was monitored for each waste stream in all pH and temperature conditions, and it was expressed as $COD_{eq}$ $L^{-1}$. Additionally, during the tests low biogas production was observed, especially when operating in alkaline conditions at both temperature ranges, which accounted for 2.5–271 NmL with 1.5–10.18% of $CH_4$. As previously reported, pH 10 may contribute to lower the activity of methanogens (Zhang *et al.*, 2009). Besides, during the alkaline tests, despite the progressive pH drop (data not shown), the final pH values remained in alkaline conditions, i.e. pH > 8. Biogas production was slightly higher when operating at an acidic pH at both 35 °C and 55 °C, ranging within 4.8–688 NmL with 1.03–13.71% of $CH_4$. Overall, the biogas production monitoring confirmed that the methanogenic activity was low, except for SS and CG fermentation at acidic pH and mesophilic temperature, where the accumulated biogas accounted for 1,463 and 1,695 NmL, with a $CH_4$ content of 33–35%. This might be related to the dynamic change of pH during the fermentation period, which tended to increase with time for both waste, achieving values up to 6.3, and likely favouring the proliferation of acetoclastic and hydrogenotrophic methanogens, which can grow beyond their optimal pH range, i.e. 6.8–7.2.

VFA production varied depending on the substrate as well as the process parameters, i.e. temperature and pH. In alkaline conditions, the solid waste streams, namely MBM and OFMSW, showed the highest VFA concentration, with values up to 4,922 and 8,320 mg $COD_{eq}$ $L^{-1}$ under thermophilic and mesophilic temperature and alkaline pH, respectively (Figure 2. 2 f and e). With regard to liquid waste streams, CG and $W_{ww}$ achieved the highest VFA concentrations at alkaline pH, with 4,257 and 4,211 mg $COD_{eq}$ $L^{-1}$ at 35 °C and 55 °C, respectively (Figure 2. 2c and b). 4,014 mg $COD_{eq}$ $L^{-1}$ were obtained from SS at alkaline pH and thermophilic temperature. Other waste streams produced lower VFA levels i.e. $S_{ww}$ up to 1,461. However, the initial COD added to the bottles relative to the other waste streams was lower. During $P_{ww}$ fermentation, the initial VFA present in the substrate accounted for 66–86% of the final VFA production. Due to this, the acidification attained by this waste stream was lower compared to the other organic waste acidification. Indeed, from the composition of the $P_{ww}$ one could think on this waste stream as an adequate substrate for VFA recovery.

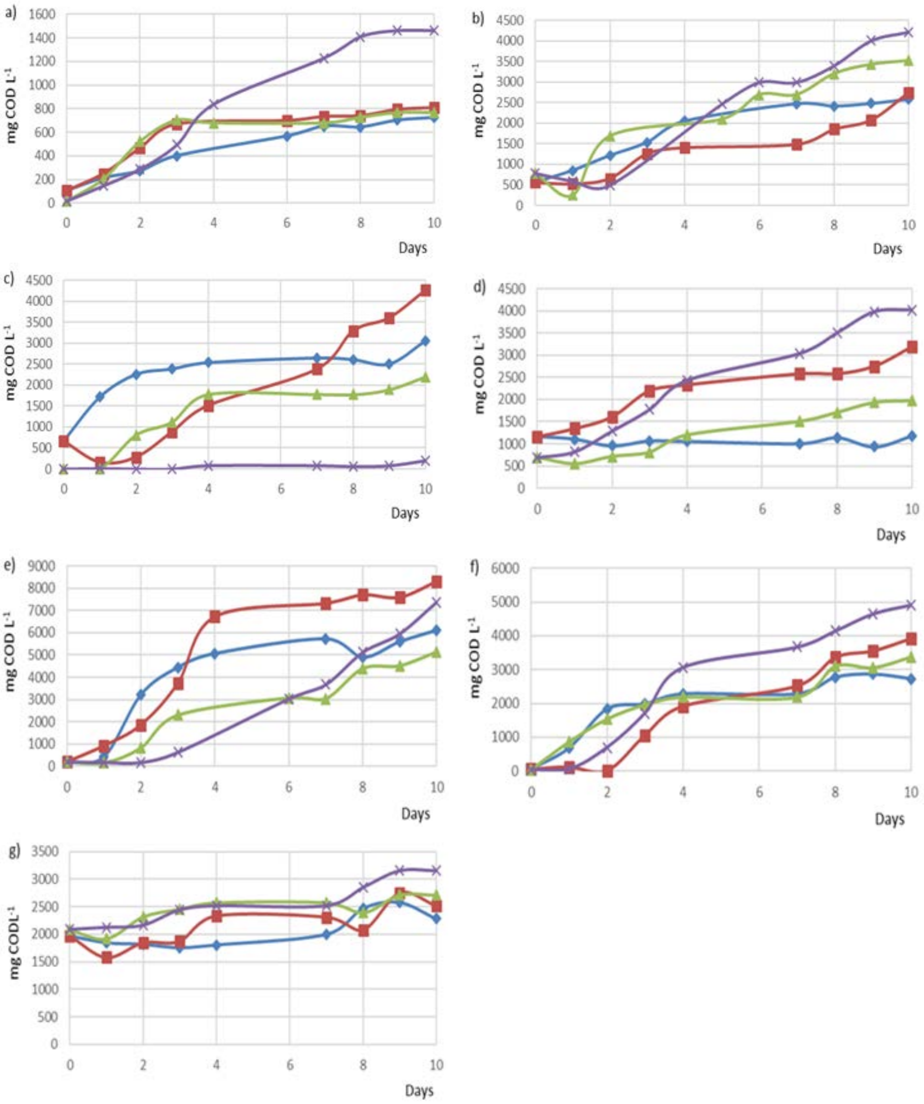

**Figure 2. 2**. VFA production profile in acidic and alkaline conditions and mesophilic and thermophilic temperature for $S_{ww}$ (a), $W_{ww}$ (b), CG (c), SS (d), OFMSW (e), and MBM (f).

By the end of the fermentation period, the maximum VFA production had been reached at acidic conditions for all substrates under study, although it seems probable that the acidification might not have been completed by the 10th day of fermentation in alkaline fermentation tests conducted with CG, $W_{ww}$, OFMSW and MBM.

Regarding maximum VFA concentration, differing trends were observed among the different waste streams. The VFA production profiles suggest that VFA production kinetics were favoured in acidic conditions and mesophilic temperature, where the maximum VFA concentration was achieved faster. When changing the pH from acidic to alkaline conditions, the fermentation kinetics seemed to slow down, despite the fact that higher VFA concentrations were attained. This might be related to the effect of NaOH on the media promoting the disintegration and solubilisation of particulate matter, and thus retarding the subsequent VFA production process (Yu *et al.*, 2008). An initial lag phase of 2 days was observed during the OFMSW (pH 10, 55 °C), MBM and CG (pH 10, 35 °C) and $W_{ww}$ (pH 10, 35 °C and 55 °C) fermentation tests. Besides, due to the alkaline environment an acclimation period may be needed by the biomass to adapt to the substrate. Silva *et al.* (2013) reported an initial lag phase during CG fermentation. Interestingly, despite the fact that some waste streams, namely CG, $S_{ww}$ and CG, showed a continuous acidification profile, other waste streams showed a sequential fermentation trend. For example, MBM, SS and $W_{ww}$ showed an initial VFA production phase, followed by a lag phase and a final production phase. A similar VFA production profile was reported by Pittman and Steinmetz (2013) during sewage sludge fermentation which may be related to the specific nature of the waste streams.

Depending on the kinetics of the fermentation processes, different reaction times were required by the waste streams to yield over 70% of the total VFA production. Likewise, the nature of each waste stream in addition to the modifications of fermentation conditions directly influenced the microorganisms promoted in the fermentation broth, and thus the performance of the acidogenic step (Hao and Wang, 2015, Maspolim *et al.*, 2015, Ma *et al.*, 2016). On the 3rd day of fermentation, 83% and 92% of the final VFA production had been reached with $S_{ww}$ and $P_{ww}$ at pH 10 and 35 °C and pH 5.5 at 55 °C, respectively. CG and $W_{ww}$ required 4 days to attain 81% and 79% of total VFA production both at pH 5.5 and 55 °C. Likewise, 4 days were required during MBM and OFMSW to achieve 83% of total VFA production at acidic conditions and mesophilic temperature which is in line with the results of Jiang *et al.* (2013). Similarly, 4 days were required during SS fermentation to produce 73% of total VFA at pH

10 and 35 °C (Pittman and Steinmetz, 2013). Thus, depending on the process parameter, 3–4 days could be used as reference retention time values as a starting point in large-scale continuous acidogenic fermentation processes.

### 2.5.2. Hydrolysis, acidification extent and VFA yield

During anaerobic fermentation, part of the particulate COD is solubilized and hydrolyzed via hydrolytic enzymes excreted by microorganisms, resulting in higher amounts of sCOD. A fraction of sCOD is transformed into gaseous phase mainly in the form of $H_2$ and $CO_2$, when the methanogenic activity of microorganisms is adequately inhibited, i.e. with pH control. Thus, optimizing this step is important for optimal VFA production.

As Table 2. 2 illustrates, the process parameters exerted a clear effect on the solid waste and sewage sludge hydrolysis and thus on the extent of acidification. Increasing the temperature and pH enhanced the hydrolysis due to the higher solubilization of the particulate COD. Overall, the pH had a greater influence on the extent of hydrolysis, likely due to the abiotic effect of NaOH dosing as previously suggested by Yu *et al.* (2008). The alkaline shock may have acted as a pre-treatment step by enhancing the hydrolysis rate constant ($K_h$), thus resulting in higher release of soluble carbohydrates and proteins (Ma *et al.*, 2016), which was especially significant in proteinaceous-rich waste streams, i.e. SS and MBM. Nonetheless, this did not necessarily translate into higher VFA production and a higher acidification extent. As shown in Figure 2. 3, during MBM fermentation the VFA/sCOD ratio was reduced to 52–53% when operating at alkaline pH. As suggested above, the strong alkaline conditions may be detrimental for the activity of acidogenic microorganisms. Hence, despite the higher hydrolysis and the enhanced VFA yield, lower acidification was observed (Chen *et al.*, 2017). This may be explained by the fact that a fraction of soluble protein and carbohydrates may remain in the fermentation broth (Khiewwijit *et al.*, 2015). Besides, at alkaline conditions other chemical species such as alcohols may be present in low concentrations (Jankowska *et al.*, 2015). During SS degradation, the cell walls of the sludge were broken down, which resulted in a substantial release of intracellular organic matter, with the highest VFA/sCOD ratio of 50% having been obtained at alkaline conditions. Likewise, a fraction of soluble protein and carbohydrates may remain unchanged.

**Table 2. 2.** Extent of hydrolysis of solid waste and sludge

| | Extent of hydrolysis (%) | | | |
|---|---|---|---|---|
| Type of waste | 35 ºC | | 55 ºC | |
| | pH 5.5 | pH 10 | pH 5.5 | pH 10 |
| SS | 10 | 31 | 19 | 40 |
| OFMSW | 31 | 40 | 30 | 39 |
| MBM | 16 | 49 | 30 | 54 |

Increasing the temperature had different effects during the fermentation of the waste streams. According to Figure 2. 2 with some waste streams i.e. $S_{ww}$, $P_{ww}$, $W_{ww}$, the increase in temperature turned out to be beneficial for attaining higher VFA concentrations (Hao and Wang, 2015). With other waste streams though, i.e. MBM a slight improvement of VFA production was observed at thermophilic range. Besides, the temperature increase led to lower VFA concentrations at both pH conditions during OFMSW fermentation. This might be due to the fact that OFMSW presents particulate COD mainly in the form of macromolecules, with almost no cell content, as opposed to other streams such as sewage sludge.

According to Figure 2. 3 the VFA/sCOD ratio of liquid waste varied among the substrates and depended on the process parameters. A high acidification extent was obtained during $S_{ww}$ fermentation, with 75% at pH 10 and 55 °C, followed by $W_{ww}$, with 69% at pH 5.5 and 55 °C. Remarkably, increasing the temperature did not translate into a higher VFA/sCOD ratio for $P_{ww}$, $W_{ww}$, SS and MBM fermentation at alkaline pH, likely due to the unchanged fraction of soluble proteins and carbohydrates that remained in the fermentation broth. A moderate acidification extent was reported during CG fermentation, where 32–47% of VFAs were obtained in the sCOD. Indeed, alkaline conditions and thermophilic temperature turned out to be detrimental during CG fermentation, where a low VFA/sCOD ratio of only 11% was observed. Silva *et al.* (2013) also reported low acidification during CG fermentation.

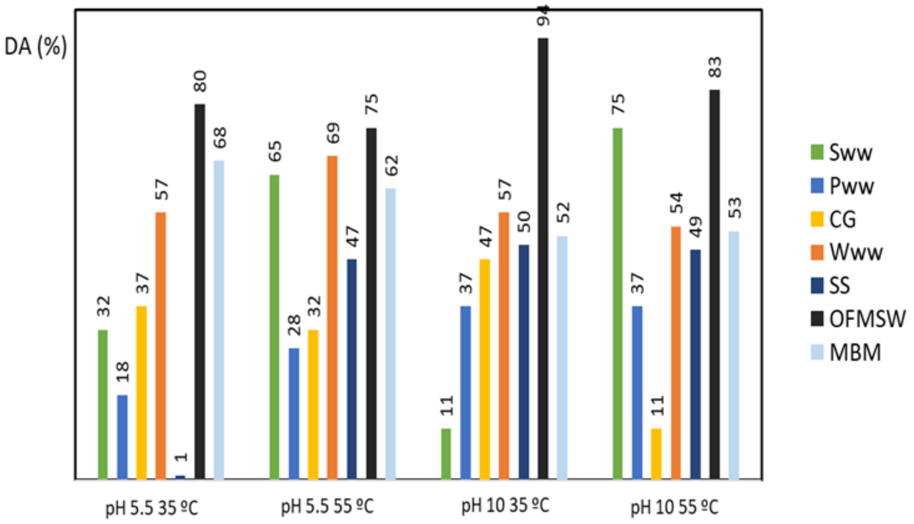

**Figure 2. 3**. Extent of acidification attained.

This may be explained by the low acclimation of the biomass to CG, which impaired the proliferation of acidogenic microorganisms, probably due to the presence of other soluble compounds such as methanol. Likewise, the specific nature and composition of OFMSW enabled to attain the highest acidification extent with 75–94% in all the conditions tested which is in line with the results from Jiang *et al.* (2013).

Figure 2. 4 summarizes the VFA yields in terms of the mg $COD_{eq}$ $g^{-1}$ $COD_{fed}$ of the organic waste that have been analysed in the present study. Overall, increasing the pH to alkaline values enhanced the VFA yield. Alkaline pH results beneficial for the solubilisation of fats, and degradation of soluble proteins and carbohydrates, also preventing the growth of both hydrogenotrophic and acetoclastic methanogens and thus enriching alkali tolerant conditions. During liquid waste fermentation (i.e. $S_{ww}$, $P_{ww}$, $W_{ww}$ and CG), the VFA yield accounted for 127–273 mg $COD_{eq}$ $g^{-1}$ COD. Furthermore, increasing the temperature was beneficial, with values increasing up to 268–677 mg $COD^{eq}$ $g^{-1}$ COD. Similarly, sludge and solid waste streams (i.e. MBM and OFMSW) performed better at alkaline pH and yielded values within 190–611 mg $COD_{eq}$ $g^{-1}$ COD. Specifically, the SS fermentation yielded 476 mg COD $g^{-1}$ VSS at alkaline conditions and thermophilic temperature, which was higher than the values obtained by Zhang *et al.* (2009), of 368 mg VFA $g^{-1}$ VSS. In addition, previous studies highlighted

that acidic pH may be optimal for VFA production from OFMSW (Lim *et al.*, 2008, Jiang *et al*., 2013). However, in this study better VFA yield was obtained at alkaline pH, which is in line with the results of Dahiya *et al.* (2015). Interestingly, recent research highlighted the influence of ammonium release and the higher free ammonia (FA) content on the VFA accumulation and pH drop at alkaline fermentation (Wang *et al.*, 2017). The results of this study showed that the higher the temperature, the ammonium release was enhanced at alkaline conditions for all feedstock except CG fermentation. During OFMSW fermentation the highest ammonium release was observed at alkaline pH and thermophilic temperature with concentrations up to 929 mg N $L^{-1}$ (3.75-fold increase from its initial TAN content). However, the VFA yield during the OFMSW fermentation was lowered in the thermophilic range, which is in line with the results from Jiang *et al.* (2013), where VFA production was reduced from 0.38 to 0.14 mg VFA $g^{-1}$ $VS_{fed}$. During MBM fermentation, increasing the temperature resulted in 1.74–2.06 fold increase in the TAN content and it had a negligible effect on the final VFA yield, where there was only a 4–5% increase. From this results, it could not be confirmed a clear correlation in the TAN release and subsequent VFA accumulation at alkaline pH. Overall, as Figure 2. 4 shows, $S_{ww}$ produced the highest VFA yield at alkaline conditions and thermophilic temperature (677 mg $COD_{eq}$ $g^{-1}$ COD), followed by the OFMSW at mesophilic temperature (611 mg $COD_{eq}$ $g^{-1}$ COD).

In addition, recent works reported that applying pretreatment methods could improve the VFA yield. Interestingly, alkaline fermentation was enhanced by using free nitrous acid (FNA) prior to alkaline addition (Zhao *et al.*, 2015). FNA enabled to increase the cell disruption of waste activated sludge and to stimulate the activities of key enzymes responsible for hydrolysis and acidification (Li *et al.*, 2016). This pretreatment may allow shorten the fermentation time, leading to an enhanced VFA production. Wang *et al.* (2016b) obtained over a two-fold increase in acetic acid yield and over 85.9% of protein hydrolysis was derived from sludge protein fermentation by applying a combined heat and alkaline pre-treatment at 120 °C for 30 min, and pH 12 for 24 h. Likewise, it has also been suggested that microaerobic conditions could improve protein hydrolysis and subsequent VFA yield (Yin *et al.*, 2016).

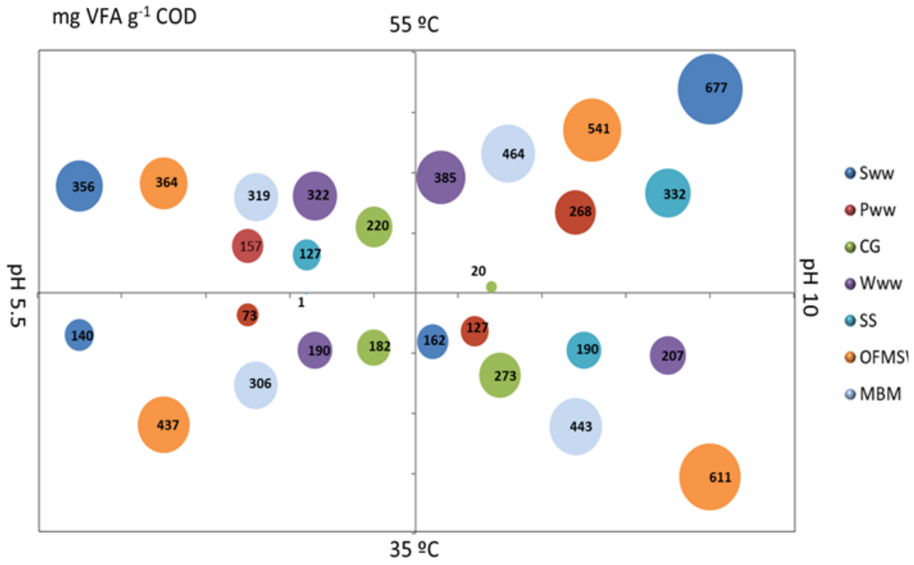

**Figure 2. 4**. VFA yield in all the temperature and conditions tested

### 2.5.3. VFA composition

The evolution of individual VFA, i.e. acetic, propionic, butyric and iso-butyric, valeric and iso-valeric acids, showed different fermentation patterns depending on the waste stream and process condition (data not shown). According to, the composition of the fermentation broth on the 10th day of incubation shows that VFA distribution was notably affected by pH and temperature

Overall, the process temperature and especially the pH had an effect on the final product distribution (Lee *et al.*, 2014). Under acidic conditions the predominant fermentation products were acetic acid and longer chain products, i.e. propionic and butyric acids, which are common primary fermentation products (Agler *et al.*, 2011), while isobutyric, n-valeric and iso-valeric acids appeared to a lesser extent (Figure 2. 5). In contrast, in alkaline conditions acetic acid was promoted to a greater extent, which may be expected in the alkaline range due to the predominance of phosphoroclastic degradation pathway (Dahiya *et al.*, 2015).

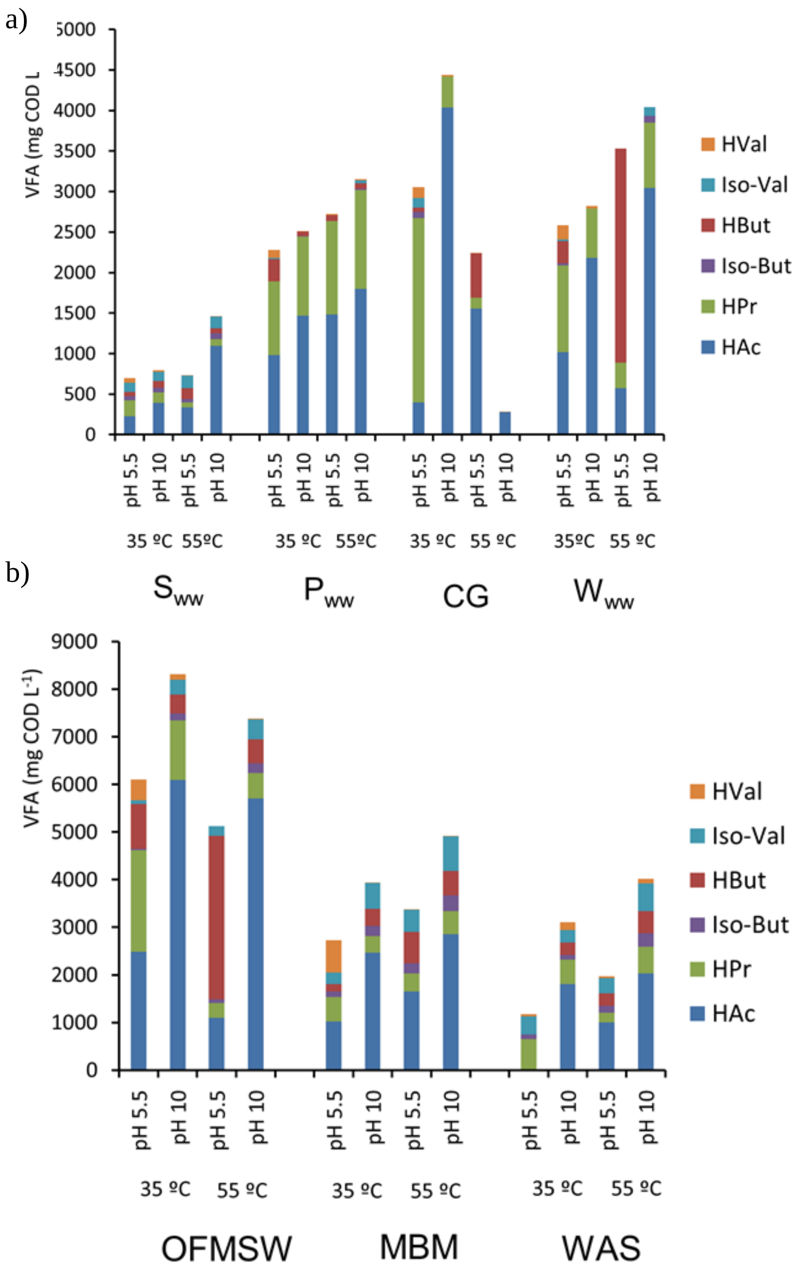

**Figure 2. 5**.VFA composition of liquid waste streams (a) and solid waste and sludge streams (b)

During CG fermentation, in acidic conditions and mesophilic temperature the primary fermentation product was propionic acid, accounting for 74% of the fermentation mixture, with 2271 mg $COD_{eq}$ $L^{-1}$. The metabolism of glycerol was similar to glucose through the glycerol pyruvate VFA fermentation pathway (Yin *et al.,* 2016). Kosmider *et al.* (2010) highlighted the possibility of using crude glycerol for propionic acid production. In contrast, Silva *et al.* (2013) reported a higher proportion of acetic and butyric acid during CG fermentation, although in their study the pH remained higher than 7. Likewise, during $P_{ww}$ fermentation propionic acid was promoted, which accounted for 39–42% of the VFA mixture in all the process conditions tested. Bengtsson *et al.* (2008) also reported acetic and propionic acids as the main components during the acidogenic fermentation of different paper mill wastewaters at pH 6.

The $S_{ww}$, SS and MBM substrates showed a similar fermentation composition with a predominance of acetic acid, which was 32–75% of the VFA, followed by a mixture of primary fermentation products, i.e. propionic (5–27%) and butyric acids (4–20%). Interestingly, MBM produced significant amounts of valeric acid, with values up to 681 mg $COD_{eq}$ $L^{-1}$ at pH 5.5 and 35 °C. In the MBM fermentation test, iso-valeric acid remained within 239–731 mg $COD_{eq}$ $L^{-1}$, with the highest values obtained at pH 10 and 55 °C. To the best of the authors' knowledge, there are no previous studies on the fermentation of $S_{ww}$ and MBM streams for VFA production. Similarly, SS fermentation produced iso-valeric acid, i.e. 580 mg $COD_{eq}$ $L^{-1}$ at alkaline conditions and thermophilic temperature. During $S_{ww}$ fermentation, iso-valeric acid accounted for 10–21% of the VFA, with a range of 112–157 mg $COD_{eq}$ $L^{-1}$. In terms of the results for $W_{ww}$ and OFMSW, both substrates showed a similar fermentation trend. A mixture of acetic and propionic acid was favoured at acidic conditions and 35 °C, with 1,071 mg $COD_{eq}$ $L^{-1}$ of propionic acid (41%) during $W_{ww}$ fermentation and 2,129 mg $COD_{eq}$ $L^{-1}$ (35%) during OFMSW fermentation. In contrast, increasing the temperature to the thermophilic range while keeping acidic conditions resulted in a product shift towards butyric acid. Butyric acid accounted for 75% of the VFA during $W_{ww}$ fermentation, with 2,643 mg $COD_{eq}$ $L^{-1}$. Similarly, 3,437 mg $COD_{eq}$ $L^{-1}$ of butyric acid were produced during OFMSW fermentation, which accounted for 69% of the fermentation broth.

### 2.5.4. Selective VFA production potential

Zheng *et al.* (2015) reported that different metabolic routes might be promoted by altering the process conditions. In addition, the selection of the waste stream can change the microbial community and change the dominant species in the anaerobic digestion process (Shen *et al.*, 2014, Chen *et al.*, 2017). The results of this study suggest that the selection of the waste stream in addition to the process parameters could offer a means for controlling the final VFA production and obtaining the desired products, which may be valuable for future applications.

During CG fermentation, modification of the process pH offered a means for controlling the final VFA production in the fermentation broth. While propionic acid was promoted in the acidic range, acetic acid was produced when increasing the pH to the alkaline range, with 91% of the fermentation broth. Moreover, the choice of process parameters offered a means for controlling OFMSW and $W_{ww}$ product distribution. The fermentation of these carbohydrate rich waste streams promoted propionic acid production in acidic conditions and mesophilic temperature, while butyric acid was favoured in the thermophilic range. A similar product shift was reported by Jiang *et al.* (2013) during OFMSW fermentation. Again, in alkaline conditions both substrates led to the production of acetic acid (i.e. 75–77%) and propionic acid (7–22%) to a lesser extent. In contrast, controlling the process parameters had a smaller effect during the fermentation of proteinaceous-rich SS, $S_{ww}$ and MBM waste streams. During $S_{ww}$ fermentation, acetic acid accounted for 90% in alkaline conditions and thermophilic temperature. In fact, Ma *et al.* (2016) recently reported that a higher pH may result in the loss of microbial richness, while increasing the temperature may favour the proliferation of specific microorganisms, i.e. the proliferation of homoacetogens in thermophilic conditions (Hao and Wang, 2015). Thus, higher amounts of acetic acid may be expected in higher pH and thermophilic temperature conditions. In addition, these proteinaceous rich waste streams led to significant amounts of valeric and iso-valeric acids (Cavinato *et al.,* 2017), which may be desirable value-added products. The latter are end products resulting from the decomposition of proteinaceous matter via Stickland type reactions (Liu *et al.,* 2011). Maspolim *et al.* (2015) also reported significant amounts of iso-valeric acids during sewage sludge fermentation.

Despite the lower VFA yields in specific waste streams and process conditions, i.e. during CG fermentation in acidic conditions and mesophilic temperature, these may be valuable for specific applications. For example, a mixture of propionic and acetic acid may be desirable for denitrification purposes (Li *et al.,* 2015) in urban WWTPs. Likewise, regarding PHA production, while acetic acid may promote the homopolymer PHB, other acids such as propionic and valeric acid may promote the production of P(HB/HV), and the composition of the PHA may be directly linked to the VFA composition of the waste (Shen *et al.*,2014).

Overall, the results of this study show that the combined effect of process variables in addition to the substrate type most likely modify the thermodynamics of the fermentation process and thus lead to different metabolic pathways for carbohydrate, protein and lipid degradation. The product spectrum of CG and carbohydrate-rich waste streams, i.e. OFMSW and $W_{ww}$, seems easier to control while the process parameters exert a smaller effect on proteinaceous feedstock. The results of this study suggest that acetic acid production should be promoted by adjusting the pH to alkaline values. Likewise, propionic and butyric up-scaling can be attained by selecting carbohydrate-rich feedstock, i.e. OFMSW and $W_{ww}$, and by means of process temperature control. While propionic acid can be promoted in mesophilic conditions during $W_{ww}$ fermentation or CG fermentation, butyric acid may be promoted when operating in the thermophilic range. In addition, valeric and iso-valeric acids may be expected by means of proteinaceous waste fermentation, i.e. SS, MBM and $S_{ww}$.

Due to the differences observed, there is not an ideal waste stream for VFA production. Based on VFA yield, the VFA/sCOD ratio and the product spectrum obtained, OFMSW, $S_{ww}$, MBM, CG and $W_{ww}$ all seem to be interesting waste substrates that could be used in future biorefineries. However, the selection of the waste will depend on the locally available resources, i.e. the large amounts of OFMSW or sewage sludge generated worldwide, and on the scope of application. Besides, if the fermentation is combined with a proper downstream process, this may lead to the recovery of individual VFA. Current research is being carried out on efficient and cost-effective VFA recuperation processes, such as anion exchange resins or membrane separation (Cabrera-Rodriguez *et al.*, 2017, Trad *et al.*, 2015). Further research could be conducted regarding the co-fermentation of waste with complementary characteristics, i.e. waste activated sludge and food waste (Zhao *et al.*, 2016). Recently, Wu *et al.* (2016) addressed the co-fermentation of food waste and sewage sludge without pH control, obtaining positive results in terms of waste hydrolysis and VFA production yield, which were enhanced by 63% and 83%, respectively,with values up to 867 mg COD $g^{-1}$ VS. Moreover, co-fermentation turned out to be positive in the enrichment of hydrolytic and acidogenic bacteria.

## 2.6 CONCLUSIONS

The results of this study highlight the influence of process parameters, i.e. process pH and temperature, in the VFA production yield and the extent of acidification of different organic waste streams. According to the results, VFA production could be directed towards desired products that are appropriate for the scope of application by means of waste stream selection and process parameter control. These results give valuable information for acidogenic process design and for the valorization of specific waste streams in the urban sector and in the agroindustrial scenario by means of VFA production.

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# CHAPTER 3

# FROM SEWAGE SLUDGE AND AGRI-FOOD WASTE TO VFA: INDIVIDUAL ACID PRODUCTION POTENTIAL AND UP-SCALING

The concent of this Chapter contains the publication:

*Esteban Gutiérrez., M., Garcia-Aguirre, J., Irizar, J., Aymerich, E., 2018. From sewage sludge and agri-food waste to VFA: individual acid production potential and up-scaling. Waste Managent 77, 203-212.*

*The results were presented in an oral presentation at the IWA Specialist Conference on sludge Management (SludgeTech), July 2017, London, UK.*

## 3.1 GRAPHICAL ABSTRACT

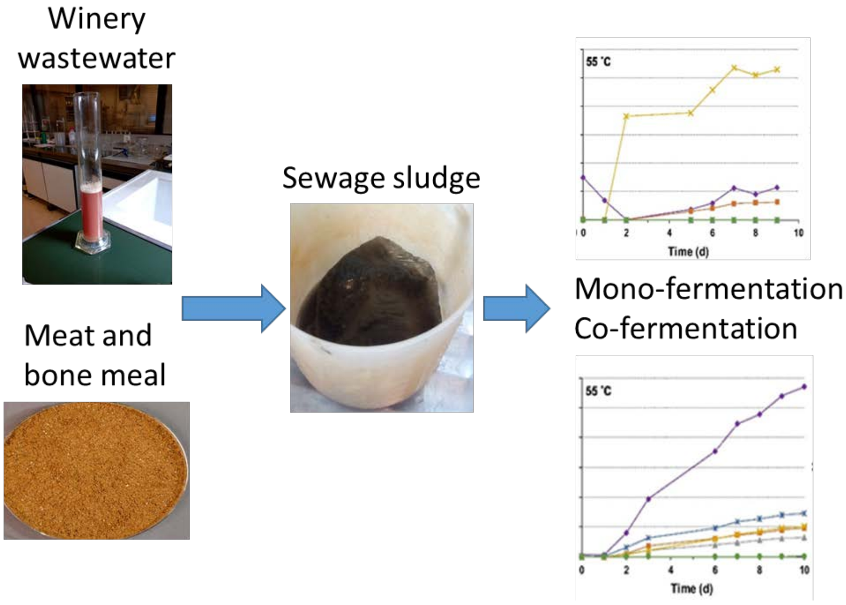


**Figure 3. 1**. Graphical abstract.

## 3.2 ABSTRACT

Volatile fatty acid (VFA) production through anaerobic fermentation may constitute an innovative solution for organic waste management within the context of circular economy. In the present study, the evolution of individual VFA during laboratory-scale fermentation of sewage sludge (SS), winery wastewater ($W_{ww}$) and meat and bone meal (MBM) was assessed, focusing on the effect of pH (5.5 and 10) and temperature (35 and 55 °C). Up-scaling of the fermentation process was evaluated in batch operation. The latter showed that specific VFA could be produced, giving similar individual evolution to lab-scale testing. To be precise, acetic acid percentage ranged within 30–65% and increased up to 5,900 mg $O_2$ $L^{-1}$ during SS fermentation at 55 °C and pH 9. In addition, 60% butyric acid was reached during $W_{ww}$ acid fermentation at 55 °C, which corresponded to 6,670 mg $O_2$ $L^{-1}$ concentration in the fermentation broth. Regarding valeric acid, over 20% proportion and 2,700 mg $O_2$ $L^{-1}$ were reached in MBM acid fermentation at 35 °C. Finally, iso-valeric maximum level ranged within 15–17% in SS alkaline fermentation at 55 °C, which represented a

concentration close to 2,000 mg $O_2$ $L^{-1}$. Interestingly, co-fermentation of agri-food waste and SS at thermophilic temperature and alkaline pH, boosted the VFA concentration 1.7–2 fold, which suggests that anaerobic co-fermentation of substrates from different nature could give promising outcomes in full-scale operation.

## 3.3 INTRODUCTION

In the last decades, modifications in environmental legislation in the European Union have promoted sewage sludge valorisation in urban wastewater treatment plants (WWTP). Consequently, anaerobic digestion (AD) has been widely implemented as an on-site management solution for sludge. Nonetheless, it is still quite common the final disposal of sludge in landfills or land application to occur without stabilization, without hygienization or without recovering energy. The latter contradicts the recently published “Closing the Loop – an EU action plan of the Circular Economy” which goes a step forward the previous waste legislation by including specific measures to address waste management and to promote the reuse of materials (Com (2015), 614). In the context of new waste policies and an unstable biogas market, which can be drastically affected by new legislation such as the Spanish law RD 900/2015, it can be foreseen that adaptation of waste management strategies in the framework of circular economy is likely to be undertaken by employing innovative solutions. A feasible strategy towards the implementation of circular economy could be transforming widely implemented AD for biogas production into volatile fatty acid (VFA) fermentation.

VFA are added value molecules that can be used for many applications such as (i) a carbon source for biopolymer production (i.e. PHA) or biological nutrient removalin WWTP, (ii) precursors for biodiesel and other fuels, and (iii) chemical building blocks for the production of pharmaceuticals, and solvents. Regarding market size for the main VFA (i.e. 2–5 carbon atoms), the share for acetic acid is the biggest one, followed by propionic acid, and then butyric acid, presenting values up to 3,500,000 t/year, 180,000 t/year and 30,000 t/year, respectively (Zacharof and Lovitt, 2013). In contrast, the market price is the highest for butyric acid, followed by propionic acid; acetic acid is the product with the lowest market price compared to other VFA. Interestingly, the world market for bio-products is estimated to increase, for 2020, more than 5 times the market size in 2010, reaching 515 billion € (Jankowska *et al.*, 2017). Therefore, despite the differences in market size, the exploitation of all VFA could be profitable if efficient production solutions are implemented. In this respect, it is remarkable that although the main synthesis route for VFA worldwide is currently based on the chemical transformation of petrochemicals, their biological synthesis has recently gained interest due to the above-mentioned policy framework and to the urgent need to diminish dependency on non-renewable fossil fuels. VFA can be synthesized from organic waste streams in mixed culture engineered fermentation processes, and thus they may constitute one of the main products of novel biorefineries, which will most likely be oriented towards the production of biofuels and valuable chemicals by transforming biomass-based feedstock (Satinder *et al.*, 2017).

According to Zhou *et al.* (2017), metabolic routes in anaerobic fermentation conditions may greatly influence the final VFA distribution in the fermentation broth, i.e. acetate/ethanol fermentation, propionate fermentation, butyrate fermentation, mixed acid type fermentation, lactate type fermentation and homoacetogenic fermentation. Moreover, the anaerobic metabolism in mixed culture media is inherently linked to the experimental conditions, namely pH, temperature, organic loading rate (OLR), inoculum source, retention time, and operating mode. Sewage sludge anaerobic fermentation for VFA production has been addressed in the literature by several authors in the last decade (Lee *et al.*, 2014, Maspolim *et al.*, 2015, Ma *et al.*, 2016, Wu *et al.*, 2017). The influence of pH, temperature and retention time in the acidogenic fermentation of sewage sludge has been a major subject of study. On the one hand, it has been reported that the pH values for VFA production lies within the range 5.25–11, although it may be changed depending on the kind of substrate to be fermented (Jankowska *et al.*, 2017). On the other hand, according to recent studies, alkaline fermentation can be considered a promising technology for VFA recovery (Liu *et al.*, 2011, Jankowska *et al.*, 2015, Fang *et al.*, 2017). Regarding the effect of temperature, Hao and Wang (2015) stated that thermophilic conditions lead to higher VFA accumulation. VFA production yield and degree of acidification degree have been addressed in the afore-mentioned studies, while individual VFA production has been analysed to a lesser extent. The influence of pH, substrate complexity and retention time on VFA production potential and individual VFA of various organic streams has been recently discussed (*Fang et al.*, 2017, Jankowska *et al.*, 2017).

To the author's knowledge, little literature is available dealing with up-scaling and pilot-scale VFA production by means of anaerobic fermentation. Longo *et al.* (2015) studied the alkaline fermentation of sewage sludge in a reactor of 500 L followed by a filtration step. Additionally, den Boer *et al.* (2016) obtained promising results for VFA production optimization, employing a co-culture of microorganisms with kitchen biowaste and potato peels in order to produce short and medium chain fatty acids, under microaerobic conditions. Nonetheless, further studies are required for VFA production optimization prior to full-scale implementation.

The main objective of the present study was to evaluate the anaerobic fermentation of sewage sludge and agri-food waste for the production of a VFA-rich by-product. Firstly, the study aimed to assess and compare the individual VFA production potential from urban sewage sludge (SS) and two primary organic streams from the Spanish agri-food industry, namely winery wastewater ($W_{ww}$) and meat and bone meal (MBM). For that purpose, individual VFA production profile was assessed in batch stage laboratory assays under acid pH and alkaline pH (i.e., 5.5 and 10) and different temperature conditions (i.e., 35 °C and 55 °C). Secondly, the present research aimed to evaluate the effect of up-scaling on the fermentation products of the afore-mentioned organic waste streams, comprising both mono-fermentation assays and two co-fermentation scenarios, namely SS + $W_{ww}$ and SS + MBM.

## 3.4 MATERIAL AND METHODS

### 3.4.1. Organic waste streams

In this study the waste streams analysed consisted of (i) SS collected from the thickener of a municipal WWTP in San Sebastian (Spain) which performs a high-load biological treatment, and two biowaste streams, namely (ii) $W_{ww}$ collected from an industrial plant which obtains value added products (i.e. alcohol, tartrate, grape seed oil, and enocianin) from the raw waste generated in wine production (mainly marks, lees, scrape and wine) and (iii) MBM from a meat waste valorisation plant which processes meat waste from the slaughterhouse industry.

**Table 3. 1.** Characteristics of the organic waste streams and the inoculum used in the present study.

| | SS | $W_{ww}$ | MBM | Inoculum |
|---|---|---|---|---|
| pH | 6.2 ± 0.1 | 5.3 ± 0.2 | NA | 7.45 ± 0.2 |
| TS (%) | 5.3 ± 0.9 | 9.1 ± 0.46 | 99.2 | 3.2 ± 0.3 |
| VS (%) | 4.1 ± 0.6 | 6.5 ± 0.61 | 68.1 | 2.0 ± 0.2 |
| tCOD (mg $O_2$ $L^{-1}$) | 56,457 ± 10,697 | 97,170 ± 5864 | 1016[a] | 27,205 ± 3254 |
| sCOD (mg $O_2$ $L^{-1}$) | 2776 ± 697 | 38,860 ± 7457 | n.a. | 1882 ± 684 |
| TAN (mg N $L^{-1}$) | 152 ± 33 | 224 ± 97 | n.a. | 1922 ± 296 |
| TKN (mg N $L^{-1}$) | 4262 ± 1314 | 3,108 ± 436 | 86[b] | n.a. |

[a] mg $O_2$ $g^{-1}$ TS
[b] mg N $g^{-1}$ TS

The latter were collected from industrial plants located in the Northern Spain. Both $W_{ww}$ and MBM were regarded as interesting waste streams for VFA recovery, due to the importance of the wine and meat sector in the Spanish agri-food industry (Cesce, 2016). In addition, to the author’s knowledge there is a lack of literature available regarding their anaerobic fermentation. The main characteristics of the substrates used in the present study are shown in

Table 3. **1**. Digested sludge from the anaerobic digester of the above mentioned municipal WWTP was used as inoculum and as a source of active acidogenic biomass. All waste samples and inoculum were stored at 5 °C prior to use.

### 3.4.2. Analytical methods

Acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid were determined on the filtered fraction with an Agilent GC–6890 gas chromatograph equipped with a FID (Flame Ionization Detector) and a capillary column (DB-FFAP, 30 m × 0.25 mm i.d., 0.25 μm film, Agilent J&W: ref. 122-3232E). The filtered fraction was obtained by means of centrifugation (4,000 rpm, 5–10 min) followed by vacuum filtration (Whatman 1.5 μm) and it was processed by liquid–liquid extraction with an organic solvent (Ter-Butil-Metyl-Eter) in order to obtain the sample for VFA determination. Pivalic acid was used as an internal standard solution.

The VFA results were expressed as g COD $g^{-1}$ acid, with the following conversion rates: 1.07 g COD $g^{-1}$ acetate, 1.51 g COD $g^{-1}$ propionate, 1.81 g COD $g^{-1}$ butyrate and 2.04 g COD $g^{-1}$ valerate (Eastman and Ferguson, 1981).

pH, Total Solids (TS), Volatile Solids (VS), Total Ammonia Nitrogen (TAN), Total Kjeldahl Nitrogen (TKN), and Chemical Oxygen Demand (COD) were measured according to Standard Methods 21st Edition (APHA, 2005). Soluble COD (sCOD) and TAN were measured by using filtered fraction, whereas total COD (tCOD) was determined by using raw substrate. pH measurements were conducted by means of a pH probe (Cryson GLP 21+).

Biogas production and biogas composition analyses (GC-TCD HP6890, column SUPELCO 60/80 Carboxen, Ref. 10001-2390-U) were carried out on a daily basis in order to check the methane production in laboratory-scale batch tests and also during pilot-scale fermentation as well.

### 3.4.3. Laboratory-scale fermentation tests

The acidogenic fermentation tests were performed in duplicate by means of 10-day batch assays in 500 mL Pyrex bottles, at acid (pH 5.5) and alkaline (pH 10) conditions. Both mesophilic (35 °C) and thermophilic (55 °C) temperatures were tested. Control tests were performed to assess the acidogenic potential of the inoculum and to calculate the net VFA production of the waste streams.

The volume of substrate that was added in each reactor was adjusted to an initial tCOD of 10,000 mg $O_2$ $L^{-1}$, based on Shen *et al.* (2014), and the inoculum concentration was set to 5,000 mg of VSS (Volatile Suspended solids) $L^{-1}$ (Field *et al.*, 1988).

The pH was adjusted initially to 5.5 and 10 to prevent the methanogenic activity of microorganisms by dosing HCl 4 N and NaOH 4 N, respectively. Exceptionally, the pH was adjusted once during fermentation of $W_{ww}$ at 55 °C under acid conditions and alkaline conditions on the 3rd day and 8th day, respectively. Nitrogen gas was flushed to the bottles for 1 min to remove any residual oxygen, and the bottles were then sealed with rubber stoppers. The reactors were placed in a thermostatic incubator at both temperature conditions.

The pH evolution was monitored daily and TAN was measured at the beginning and at the end of the fermentation tests. Liquid samples were extracted to determine the VFA composition on a daily basis during the 10-day incubation period. The incubation period was settled based on previous work in the literature (Pittman and Steinmetz, 2013, Jankowska *et al.*, 2015). Additionally, increasing the treatment time beyond 10 days had a minimum effect during SS fermentation at pH 10 (data not shown). In addition, biogas production and composition were monitored daily. A final characterisation of the fermentation broth was conducted to analyse the composition of the fermentation mixtures.

### 3.4.4. Pilot-scale fermentation tests

Fermentation tests were up-scaled in an 80 L stainless steel completely stirred tank reactor (CSTR) (Figure 3. 2) operated in batch mode with 50 L working volume. The pilot plant was equipped with a PT100 temperature probe and an electrical heater in order to automatically control the temperature of the batch. In addition, a pH sensor was installed to manually adjust this variable during each test. Biogas production was also monitored by means of a biogas flow-meter, while the fraction of methane in the gas headspace was analysed daily.

Since the substrate to inoculum ratio has been reported to have a significant impact on anaerobic digestion, different substrate to inoculum ratios were evaluated, i.e. 1:3, 1:1 and 3:1 (v/v), in order to settle the food to microorganism (F:M) ratio prior to up-scaling. For that purpose, a laboratory-scale experimental platform, which was similar to the one used in the above batch assays, was used. The testing conditions (i.e. T and pH) were adjusted initially according to the results from previous laboratory-scale fermentation tests. SS and $W_{ww}$ were used separately and they were mixed with a given volume of inoculum, for example, 200 mL of SS and 200 mL of digested sludge to get a final F:M of 1:1. Concretely, the volume adjustment fixed the VS/VS ratio to 0.7, 2.1 and 6.1 for SS and 1.1, 3.2 and 9.7 during $W_{ww}$ fermentation test. Nitrogen gas was flushed to the assay bottles to remove any residual oxygen. Based on the optimal results that were obtained in the aforementioned experiments, the initial F: M ratio was set at 1:1.

**Figure 3. 2**. Picture of the 80 L CSTR pilot-scale reactor that was used in batch mode for up-scaling.

The experimental conditions for the fermentation tests at pilot-scale are shown in Table 3. 2**.**

Table 3. 2**.** Pilot-scale fermentation tests: experimental conditions.

| Test | Waste stream | F:M | pH | pH control | Temperature (ºC) |
|---|---|---|---|---|---|
| Mono-fermentation | 1 SS | 1:1 | 10 | Uncontrolled | 55 |
| | 2 SS | 1:1 | 9 | Manual-adjustment | 55 |
| | 3 $W_{ww}$ | 1:1 | 5.5 | Uncontrolled | 55 |
| | 4 MBM | 1:1 | 5.5 | Uncontrolled | 35 |
| Co-fermentation | 5 $W_{ww}$:SS | (0.5:0.5):1 | 9 | Manual-adjustment | 55 |
| | 6 MBM:SS | (0.1:0.9):1 | 9 | Manual-adjustment | 55 |

[a] NaOH dosing when pH dropped below 8 on the 3rd day.

[b] NaOH dosing when pH dropped below 8 on the 2nd day.

[c] NaOH dosing when pH dropped below 8 on the first day

The pH was adjusted initially and controlled during the tests. During the alkaline tests NaOH 16 N was added when the pH dropped below 8 and readjusted to 9. Nitrogen gas was flushed to avoid aerobic conditions. The temperature was adjusted to the desired range and it was controlled by a thermal resistance governed by a MONOPID controller. During co-fermentation tests, the co-substrate to sludge ratio in terms of SV was kept equal on both assays, i.e. 1.28.

## 3.5 RESULTS AND DISCUSSION

### 3.5.1. Laboratory-scale fermentation tests

VFA production, pH evolution and ammonia release for anaerobic fermentation of SS, $W_{ww}$ and MBM are discussed in this section. Overall, the biogas production remained low, with a methane content below 15%.

#### 3.5.1.1. Fermentation tests under acid pH

Overall, the major VFA at pH 5.5 was acetic acid, as shown in previous research (Maspolim *et al.*, 2015). However, significant differences were observed, which are discussed below.

Firstly, during SS mesophilic fermentation a drastic consumption of acetic acid was observed, whereas propionic acid and isovaleric acid reached values of 624 mg $O_2$ $L^{-1}$ and 381 mg $O_2$ $L^{-1}$, respectively. At 55 °C, the main fermentation product was acetate, which increased sharply from the 4th day on, reaching a final concentration close to 1,000 mg $O_2$ $L^{-1}$. The increase in acetate concentration at 55 °C is in line with the findings by to Hao and Wang (2015), who stated that thermophilic temperature turned out to be beneficial in boosting VFA production. As for the rest of the VFA, their concentration was kept below 400 mg $O_2$ $L^{-1}$. Nonetheless, one must bear in mind that pH and ammonia evolved differently in SS fermentation. pH was kept above 6 at 35 °C, while in it was below 6 during the entire thermophilic assay. Ammonia release went up to 899 mg N $L^{-1}$ and 930 mg N $L^{-1}$ at 35 °C and 55 °C, respectively. Therefore, the results for acid SS fermentation might be indicative of the fact that pH values within the range 6–6.5 favour the accumulation of VFA other than acetic acid.

Secondly, it can be seen that temperature exerted a shift towards butyrate production in $W_{ww}$ fermentation (Figure 3. 3b) which was also reported by Jiang *et al.* (2013) when increasing the temperature to thermophilic range. The butyric acid level increased sharply, reaching 1,880 mg $O_2$ $L^{-1}$ in 48 h, and it accumulated until it reached 2,640 mg $O_2$ $L^{-1}$. The results of this test possibly reveal that a butyrate type fermentation was set as the major acidogenic route. Similarly, other researcher have reported butyrate type metabolic pathway in mesophilic acid fermentation (pH 5–6) of olive oil mill wastewaters (Ntaikou *et al.*, 2014) and fruit and vegetable waste (Wu *et al.*, 2016).

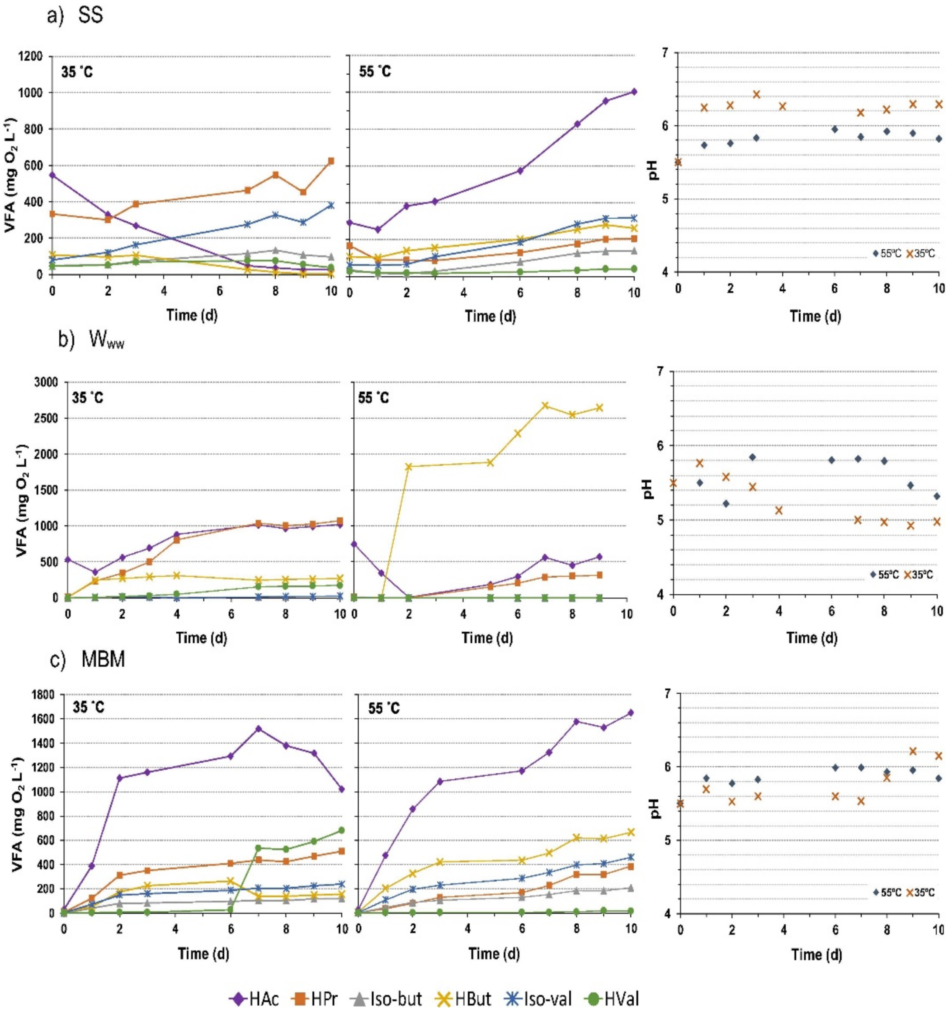


**Figure 3. 3**.Individual VFA profile and pH evolution at mesophilic and thermophilic temperature during batch scale tests in acid conditions (pH 5.5): SS (a), $W_{ww}$ (b) and MBM (c)

However, in this study, $W_{ww}$ fermentation at 35 °C was almost equally dominated by acetic acid and propionic acid, which increased until reaching a maximum concentration close to 1,000 mg $O_2$ $L^{-1}$ on the 7th day. With regard to the pH, it dropped progressively to values close to 5 at 35 °C, thus setting optimal conditions for an acetate/ethanol type fermentation, as stated by Zhou *et al.* (2017). Nonetheless, at 55 °C, pH decreased initially and it remained close to 6 from day 3th to 8th. Therefore, it can be stated that pH values within the range 5–5.5 and 35 °C could be beneficial for producing a fermentation broth with similar amounts of acetic acid and propionic acid, whereas butyric acid would be expected to be the major VFA for thermophilic temperature and pH close to 6.

Finally, it can be seen that MBM showed a fermentation trend similar to SS, which could be attributed to the fact that the nature of both substrates is similar, i.e. both substrates are rich in proteins Figure 3. 3 c). Interestingly, under 35 °C, although acetic acid was the major VFA and increased up to 1,517 mg $O_2$ $L^{-1}$, it degraded gradually down to 1,022 mg $O_2$ $L^{-1}$, with a simultaneous increase of valeric acid. The shift in minor VFA to valerate production is in line with the pH change from 5.5 to values greater than 6, which could be related to the degradation of proteins and a concomitant ammonia release (1,288 mg N $L^{-1}$). Additionally, it is worth noting that the temperature increase promoted a mixed acid fermentation pattern with a predominance of acetate (i.e. 1,684 mg $O_2$ $L^{-1}$) followed by butyrate (665 mg $O_2$ $L^{-1}$).

### 3.5.1.2. Fermentation tests under alkaline pH

According to Figure 3. 4, acetic acid was the main fermentation product at alkaline conditions, reaching levels within the range 1,803–3,047 mg $O_2$ $L^{-1}$, which was greater than the acid pH. This is in agreement with previous researchers who stated that metabolic pathways set at alkaline pH most likely direct organic matter conversion towards acetate production (Zhang *et al.*, 2009, Hao and Wang, 2015, Maspolim *et al.*, 2015). Moreover, it has been suggested that alkaline conditions may reduce microbial diversity, while favouring the predominance of specific microorganisms (Ma *et al.*, 2016, Wang *et al.*, 2016). Homoacetogenic bacteria might also contribute to the increase in acetate production, which could be a relevant alternative pathway for increasing the final acetic acid yield in 10-day incubation period.

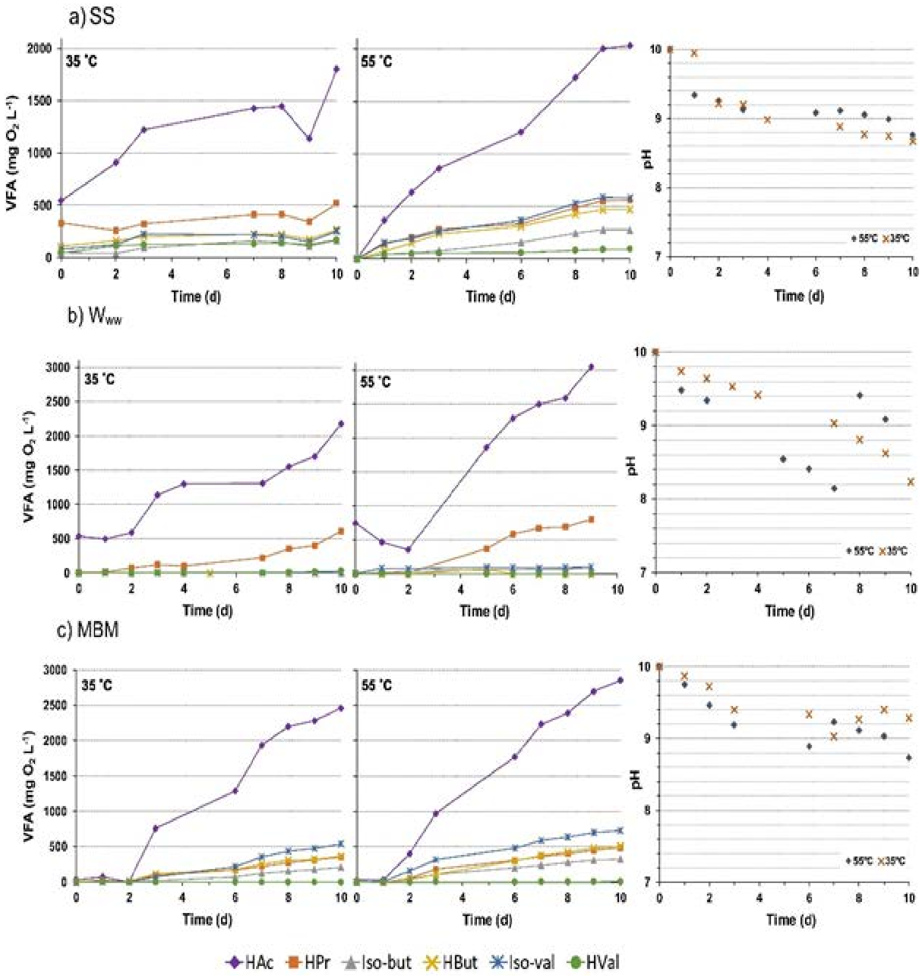


**Figure 3. 4.** Individual VFA profile and pH evolution at mesophilic and thermophilic temperature during laboratory-scale tests in alkaline conditions (pH 10): SS (a), $W_{ww}$ (b) and MBM (c).

Regarding to other VFA, propionic acid was the second most significant VFA in SS fermentation at 35 °C (i.e., 521 mg $O_2$ L$^{-1}$) and $W_{ww}$ fermentation at 35 °C

(610 mg $O_2$ $L^{-1}$) and 55 °C (i.e., 803 mg $O_2$ $L^{-1}$) (Figure 3. 4a and b). Likewise, isovalerate production was notable in SS fermentation at 55 °C, where the acid reached a final concentration almost equal to propionate (i.e., 580 mg $O_2$ $L^{-1}$) (Figure 3. 4a). Moreover, isovalerate was the second major VFA in MBM fermentation, where it increased up to 541 and 731 mg $O_2$ $L^{-1}$ under mesophilic and thermophilic temperatures, respectively (Figure 3. 4c). It is remarkable that in MBM fermentation, propionate and butyrate were equally accumulated, possibly suggesting that their metabolic pathways were activated to a close extent.

Concerning temperature, it can be observed that VFA production at 55 °C started earlier and the increase was sharper than at 35 °C (Figure 3. 4a and b). Exceptionally, the VFA production pattern for MBM fermentation was practically equal at 35 °C and 55 °C, although the final VFA level for individual acids was greater at thermophilic conditions (Figure 3. 4c). During MBM fermentation the VFA spectrum was clearly dominated by acetate, followed by isovalerate. In contrast, during SS fermentation, the temperature increase promoted a product shift in terms of minor VFA, from propionic acid at 35 °C to a mixture of isovaleric, propionic and butyric acids at 55 °C. Isovaleric accumulation reported here is in line with previous studies that reported significant amounts of isovaleric acid during SS alkaline fermentation (Xiong *et al.*, 2012). Additionally, the greater production of isovalerate may be due to the decomposition of proteinaceous content, which was favoured at the higher temperature, as stated by Liu *et al.* (2011).

The pH values dropped below 9 during SS fermentation at both temperature conditions (Figure 3. 4a), whereas the ammonium release was slightly higher compared to the acid tests, increasing up to 946 and 1,020 mg N $L^{-1}$ at 35 °C and 55 °C, respectively. Similarly, in MBM fermentation, the system seemed to be buffered enough to regulate the pH itself at both temperature conditions, and it was kept mainly above 9 (see Figure 3. 4c). This was most likely due to the ammonium release, which increased up to 1,204 mg N $L^{-1}$ in thermophilic conditions. However, the pH tended to drop faster and a manual pH adjustment (i.e. up to 9.5) was made in order to keep the methanogenesis inhibited during $W_{ww}$ fermentation at 55 °C (Figure 3. 4b). The pH trend is in agreement with the observed low ammonium values close to 650 mg N $L^{-1}$. However, the observed

VFA pattern, suggested that pH and ammonia had a negligible effect on the VFA production compared to the effect of temperature.

### 3.5.1.3. Further implications of laboratory-scale testing on individual VFA production

Table 3. 3 gathers the maximum percentage for major VFA and the total VFA yield for laboratory-scale fermentation. It can be seen that VFA yields are greater for thermophilic temperature and alkaline pH, ranging from 0.20 to 0.49 g $VFA_{total}$ $g^{-1}$ $COD_{in}$. Moreover, it is noteworthy that pH and temperature increase resulted in a similar enhancement of the VFA yield for $W_{ww}$ and MBM (45%) albeit largely different in nature. The results for SS strongly suggest that alkaline conditions and thermophilic temperature are beneficial for its acidification. Similarly, Hao and Wang (2015) found that thermophilic conditions led to a higher VFA accumulation, higher enzymatic activity and the proliferation of specific microorganisms that enhanced the hydrolysis and the acidification of sludge. Total VFA yield and acidification degree for each substrate are presented in detail elsewhere (Garcia-Aguirre et al., 2017).

Likewise, acid pH seemed to be favourable for specific VFA recovery other than acetic acid. At pH 5.5, 33% iso-valerate and 53% propionate was measured in the fermentation broth of SS, butyrate accounted for 75% of the total VFA in the thermophilic test for $W_{WW}$, and valeric acid reached 25% of the fermentation broth in the test for MBM at 35 °C. In contrast, the fermentation broth in alkaline tests for SS and $W_{WW}$ was dominated by acetic acid at 35 °C and 55 °C, ranging from 50% to 77%, which is likely due to the loss of microbial diversity. The results of this study are in line with previous studies which suggested that the dynamic behaviour of pH may influence the fermentation pathways (Wang *et al.*, 2016). Moreover, it seems that the effect of temperature on anaerobic fermentation of all streams under study was more significant when the pH was close to 5.5. A product shift from acetic acid (39%) and propionic acid (42%) to butyric acid (75%) was detected when moving from 35 °C to 55 °C in $W_{ww}$ fermentation, whereas the temperature shift revealed greater accumulation of acetic acid in 55 °C (51%) and a dominance of isovaleric acid (33%) at 35 °C for SS.

**Table 3. 3**. Distribution of the major VFA and total VFA yield in laboratory-scale fermentation tests.

| | | SS | | | | $W_{ww}$ | | | | MBM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | HAc (%) | HPr (%) | Iso-Val (%) | Yield(gVFA $_{total}$ $g^{-1}$ $COD_{in}$) | HAc (%) | HPr (%) | HBut (%) | Yield (g $VFA_{total}$ $g^{-1}$ $COD_{in}$) | HAc (%) | Iso-Val (%) | HVal (%) | Yield (g $VFA_{total}$ $g^{-1}$ $COD_{in}$) |
| 35 ºC | pH 5.5 | 2 | 53 | 33 | 0.12 | 40 | 42 | 10 | 0.26 | 37 | 9 | 25 | 0.27 |
| | pH 10 | 57 | 16 | 8 | 0.32 | 77 | 22 | 0 | 0.28 | 63 | 14 | 0 | 0.39 |
| 55 ºC | pH 5.5 | 51 | 11 | 16 | 0.2 | 16 | 9 | 75 | 0.35 | 49 | 14 | 1 | 0.34 |
| | pH 10 | 51 | 14 | 15 | 0.4 | 75 | 20 | 0 | 0.4 | 58 | 15 | 0 | 0.49 |

Therefore, individual VFA profiles might be indicative of significant variations in microbial community in response to changing environmental conditions such as the pH. In other words, variations in [$H^+$] and hydrogen concentration in the liquid phase during fermentation seem to greatly influence the thermodynamics of the system and thus the mix of biochemical reactions, as suggested by Aymerich *et al.* (2013).

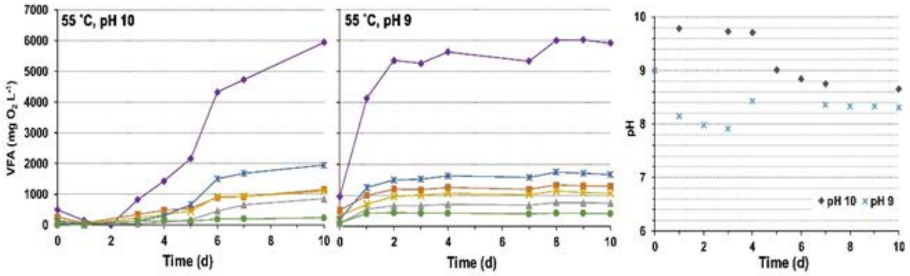
### 3.5.2. Pilot-scale fermentation tests

This section provides the results of up-scaling the fermentation of SS, $W_{ww}$ and MBM and two co-fermentation scenarios, namely SS + $W_{ww}$ and SS + MBM. pH and temperature conditions for up-scaling were selected according to the VFA production profiles discussed above. Biogas production was negligible during batch pilot-scale testing (data not shown), and the methane content remained at low values ranging between 1.7 and 16.3%.

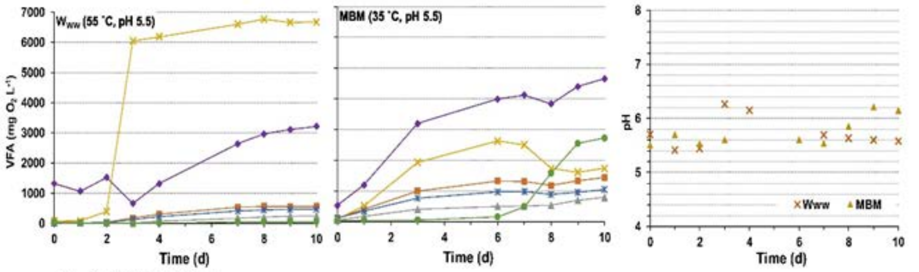
#### 3.5.2.1. Mono-fermentation tests

The results suggest that the pH exerted a small effect on the VFA spectrum in SS fermentation, which was dominated by acetic acid, followed by isovaleric acid (Figure 3. 5a). In fact, isovalerate increased up to 1,950 mg $O_2$ $L^{-1}$ (pH 10) and 1,667 mg $O_2$ $L^{-1}$ (pH 9), followed by propionate. The pH tended to decrease with time, which was especially significant in the test at pH 10, due to the high VFA accumulation. Thus, a manual adjustment was performed. The total ammonium nitrogen increased from 893 to 1,775 mg N $L^{-1}$ at pH 10 and from 1,075 to 1,954 mg N $L^{-1}$ at pH 9. In terms of kinetic behaviour in SS fermentation, pH 10 showed a lag phase that could be attributed to biomass acclimation, where only 25% of the final VFA were produced on day 4, as compared to 97% under pH 9. The VFA values indicate that reducing the initial pH to 9 enhanced the fermentation kinetics of SS and the acidification rate, although final acetic acid concentration on the 10th day was beyond 6 g $O_2$ $L^{-1}$ in both cases (Figure 3. 5a). However, the final VFA concentration increased to 11.2 g $O_2$ $L^{-1}$. This is in agreement with the results from Chen *et al.* (2017), who stated that despite the fact that pH 10 may be more efficient in achieving a higher hydrolysis, a higher acidification may be obtained at pH 9.

a) SS mono-fermentation



b) $W_{ww}$ and MBM mono-fermentation



c) Co-fermentation

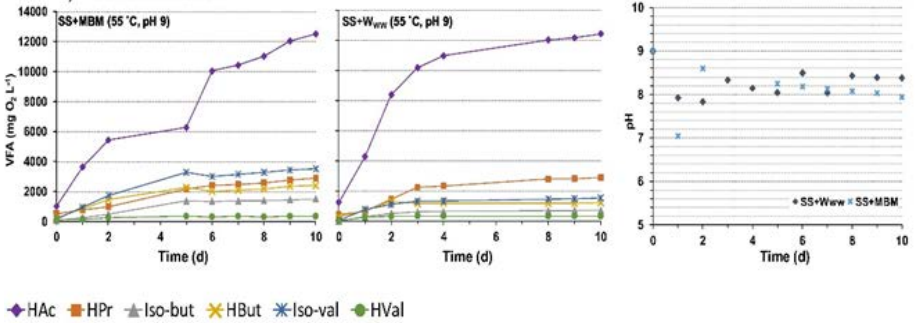


**Figure 3. 5** Individual VFA profile and pH evolution during pilot-scale fermentation tests: SS mono-fermentation at pH 10 (Test 1) and pH 9 (Test 2) (a), $W_{ww}$ (Test 3) and MBM (Test 4) mono-fermentation (b), and SS + $W_{ww}$ (Test 5) and SS + MBM (Test 6) co-fermentation (c).

Regarding $W_{ww}$ mono-fermentation (Figure 3. 5b), the fermentation was dominated by butyrate at 55 °C and acid pH, reproducing laboratory-scale results. It is noteworthy that butyric acid increased sharply moving to 6 g $O_2$ $L^{-1}$ between days 2 and 3. Afterwards, it remained relatively stable with a peak value of 6.67 g $O_2$ $L^{-1}$. The shift from acetic acid to butyric acid on day 3 might be related to the pH increase to values above 6. The kinetics of other VFA was different since their concentration remained nearly stable for the first 3–4 days, which is similar to the trends for SS fermentation under pH 10. On the one hand, acetic acid tended to increase progressively from day 4, and values up to 3.21 g $O_2$ $L^{-1}$ were reached at the end of the test. On the other hand, other minor VFA were barely detected, and they were kept below 1 g $O_2$ $L^{-1}$ at the end of the experiment.

Mono-fermentation of MBM was dominated by acetic and butyric acid, which accumulated during the first 6 days, increasing up to 3,984 and 2,623 mg $O_2$ $L^{-1}$, respectively (Figure 3. 5c). The pH remained stable, which could be partly attributed to the high ammonium release, which increased to 3,688 mg N $L^{-1}$ (Figure 3. 5b). Interestingly, a fermentation shift towards valeric acid production was detected, which reached a final concentration of 2,713 mg $O_2$ $L^{-1}$, with a noticeable reduction of butyric acid down to 1,713 mg $O_2$ $L^{-1}$ and a slight decrease in acetic acid. The results fit with the laboratory assays, where valeric acid accumulated after day 6. These results suggest that higher pH values may be favourable for valeric acid production. Concerning other VFA, propionic and isovaleric acid concentration increased to 1,433 and 1,047 mg $O_2$ $L^{-1}$.

#### 3.5.2.2. Co-fermentation tests

Concerning co-fermentation, it not only made it possible to obtain higher VFA production for the same volume of waste material, but it also showed significant accumulation of other primary fermentation products than acetate on a pilot-scale basis, i.e. propionic, butyric and isovaleric acids.

During SS + $W_{ww}$ co-fermentation, total VFA concentration increased up to 16.8 g $O_2$ $L^{-1}$ on the 4th day, and 19.1 g $O_2$ $L^{-1}$ was reached afterwards, which means a 1.72-fold increase compared to SS mono-fermentation (Figure 3. 5c). It can be seen that the major VFA was acetic acid, followed by propionic and isovaleric acids, which increased to 12.4, 2.89, and 1.54 g $O_2$ $L^{-1}$, respectively. These results suggest that co-fermentation of SS with $W_{ww}$ promoted propionic acid accumulation under 55 °C and pH values that fell within 7.8–8.5. In addition, the total VFA level by the end of the SS + $W_{ww}$ scenario accounted for 75% of the final sCOD, which might be beneficial for subsequent downstream purposes, i.e. by membrane filtration (Zacharof and Lovitt, 2013). In contrast to the $W_{ww}$ mono-fermentation, no significant accumulation of butyric acid was observed in SS + $W_{ww}$, which could be indicative of the fact that mixed SS nature was dominant over carbohydrate rich $W_{ww}$ composition in determining final VFA distribution.

Regarding SS + MBM co-fermentation, VFA production was further enhanced and values up to 23.2 g $O_2$ $L^{-1}$ where reached, which supposed a two-fold increase compared to the SS mono-fermentation (Figure 3. 5c). Acetic acid was predominant over other VFA in the fermentation broth during the whole 10-day experiment and it reached a final 12.5 g $O_2$ $L^{-1}$ concentration. Interestingly, high amounts of isovaleric acid were obtained, up to 3.5 g $O_2$ $L^{-1}$, followed by propionic acid and butyric acid, which increased to 2.89 and 2.40 g $O_2$ $L^{-1}$, respectively. The pH tended to decrease with time, especially during the initial phase, where a manual adjustment was required in order to avoid methanogenic conditions. The VFA profile during SS + MBM could be indicative of the fact that alkaline pH was beneficial for the hydrolysis and solubilizationof not only SS but also MBM, which led to further increasing the VFA concentration as compared to single SS fermentation. The concentration of total ammonium nitrogen increased to values of 4,228 mg N $L^{-1}$, which is almost twice the level in SS + $W_{ww}$. This is in accordance with the fact that MBM is a protein-rich co-substrate as opposed to carbohydrate-rich $W_{ww}$. Due to the proteinaceous nature of both SS and MBM, their co-fermentation displayed a final VFA distribution that is similar to single SS fermentation, which again was dominated by the acetate.

The results of the present study are in line with previous works that reported positive effects of co-fermentation for VFA production. According to Wu *et al.* (2016), the hydrolysis and the acidification yield of food waste and sewage sludge was enhanced during co-treatment, increasing up to 867 mg COD $g^{-1}$ VS, where the high buffering capacity of the system make it possible to maintain the pH within a considerably narrow range (i.e. 5.2–6.4). Interestingly, Yin *et al.* (2016) suggested that mixing carbohydrates, proteins and lipids may have a synergistic effect on acidogenic fermentation. Previous studies also showed that modifying carbohydrates to match the SS ratio could promote higher amounts of propionic acid (Luo *et al.*, 2014, Zhou *et al.*, 2013). Recently, Den Boer *et al.* (2016) showed promising results in co-fermentation where different microbial cultures were obtained under micro-aerobic conditions, which opens a new spectrum for further research.

With respect to VFA production kinetics in co-fermentation tests, a stepwise accumulation was observed for the SS + MBM scenario (Figure 3. 5c), with the greatest production rate within days 0–2 and days 5–10 for acetic acid and between days 0–5 for other VFA. In contrast, the greatest VFA production took place within days 0–4 in the SS + $W_{ww}$ scenario for acetic acid and for other minor VFA as well. These results might suggest that liquid co-substrates might be favourable for achieving greater VFA accumulation in a shorter period, and thus potentially reducing the retention time of the fermentation process.

### 3.5.2.3. Further implications of up-scaling on individual VFA production

Overall, the VFA profiles obtained in pilot-scale fermentation indicate that laboratory testing represents a good basis for the selection of favourable up-scaling conditions. Interestingly, the most meaningful individual trends during the mono-fermentation of SS, MBM and $W_{ww}$ were reproduced in up-scaling, even though the inoculum to substrate ratio was notably modified and the pH showed differences compared to laboratory-scale. These results suggest that pH and temperature influence the fermentation process to a greater extent than the biomass (inoculum) to substrate ratio and/or substrate concentration itself. As for temperature, it showed a more remarkable effect on the kinetics of VFA production rather than in determining the spectrum of the fermentation product in pilot-scale experiments, as observed in laboratory-scale. In this respect, temperature and kinetics should be given special consideration when thinking of further up-scaling since they seem to be crucial for adjusting the retention time.

The maximum percentage for the major VFA and the VFA yield of pilot-scale fermentation (Tests 1–6) are summarized in Table 3. 4. The distribution of VFA possibly reveals the significance of the composition of the substrate(s) in determining the VFA spectrum. On the one hand, acetic and isovaleric acids were promoted during the fermentation of proteinaceous SS and MBM, and thus they could be target acids in up-scaling. Acetic acid represented close to 55% and 40% of the fermentation broth in Tests 1/2 and Test 4, respectively, whereas isovalerate reached almost 20% of the total VFA mix in Test 2. Additionally, valeric acid was promoted in significant amounts during MBM fermentation, when the pH increased to values greater than 6, reaching a maximum concentration of 22%. On the other hand, it is remarkable that the results for fermentation of $W_{ww}$ (Test 3) suggest that individual recovery of butyric acid could be feasible in further up-scaling. To be precise, butyrate was the major VFA by comprising 60% of VFA, which is slightly lower than 75% in laboratory-scale. Co-fermentation of SS with MBM in Test 6 seemed to promote the production of VFA other than acetic acid, where 15% isovaleric acid, 13% propionic acid, and 10% butyric acid percentages could be reached.

**Table 3. 4.** Distribution of the major VFA and total VFA yield in pilot-scale fermentation tests

| | Mono-fermentation | | | | Co-fermentation | |
|---|---|---|---|---|---|---|
| | Test 1 (SS) | Test 2 (SS) | Test 3 ($W_{ww}$) | Test 4 (MBM) | Test 5 (SS + $W_{ww}$) | Test 6 (SS + MBM) |
| HAc (%) | 54 | 53 | 29 | 38 | 65 | 54 |
| HPr (%) | 12 | 10 | 5 | 12 | 15 | 13 |
| HBut (%) | 10 | 10 | 60 | 14 | 6 | 10 |
| HVal (%) | 4 | 2 | 0 | 22 | 2 | 2 |
| Iso-Val (%) | 15 | 17 | 4 | 8 | 8 | 15 |
| Yield (g $VFA_{Total}$ $g^{-1}$ $COD_{in}$) | 0.51 | 0.52 | 0.25 | 0.24 | 0.49 | 0.31 |

VFA yield has been previously expressed on a COD basis by other authors (Garcia-Aguirre *et al.*, 2017, Silva *et al.*, 2013). When comparing pilot-scale tests (Table 4), SS + MBM scenario (Test 6) showed a lower VFA yield close to 0.3 g $VFA_{Total}$/g $COD_{In}$ compared to mono-fermentation of SS in Tests 1 and 2 (0.51–0.52 g $VFA_{Total}$/g $COD_{In}$), and Test 5. The VFA yield as expressed in Table 3. 4 is useful for the comparison of different waste streams and organic mixtures. However, it is important to note that the latter might not be convenient when evaluating the outcome of up-scaling or the economic feasibility of fermentation. Regarding economic estimates for the implementation of anaerobic fermentation on a larger scale, the following figures could be meaningful for a preliminary analysis. First, the individual VFA concentration expressed in mass units (i.e. mg acid $L^{-1}$) could be considered, which ranged within 3,011–11,735 mg $L^{-1}$ for acetic acid, 371–1,917 mg $L^{-1}$ for propionic acid, 585–3,675 mg $L^{-1}$ for butyric acid, 135–822 mg $L^{-1}$ for isobutyric acid, 26–1,681 mg $L^{-1}$ for valeric acid and 227–1,731 mg $L^{-1}$ for isovaleric acid. One must bear in mind the fact that the greater the accumulation for individual or total VFA in terms of volumetric concentration, the more favourable the conditions for further downstream and the more feasible the whole recovery process. Secondly, the specific VFA outcome per mass unit of the organic stream could be useful, which highest values are listed here: 12.2 g HAc $kg^{-1}$ SS; 7.35 g HBut $kg^{-1}$ $W_{ww}$, and 1.63–1.92 g Iso-Val $kg^{-1}$ SS, 10.74 g Iso-Val $kg^{-1}$ MBM 27.9 g HVal $kg^{-1}$ MBM and 91.4 g HAc $kg^{-1}$ MBM. During co-fermentation tests, 23.5 g HAc $kg^{-1}_{mixture}$, 3.5 g Iso-val $kg^{-1}_{mixture}$, 3.8 g HPr $kg^{-1}_{mixture}$ and 2.6 g HBut $kg^{-1}_{mixture}$ were obtained. Considering the aforementioned figures and the market value of VFA (Zacharof and Lovitt, 2013), fermentation could involve an economic income within 3.6–40 € $tonne^{-1}_{waste}$, which might make VFA recovery economically feasible, as recently suggested Wang *et al.* (2016). A deep economic analysis could include not only the operating costs due to fermentation and chemical reagents for the control of pH, but it should also consider the costs associated with the transport and the storage of the organic waste and the cost of downstream processing.

## 3.6 CONCLUSIONS

In the present study it was demonstrated that laboratory-scale testing provides a good basis for the up-scaling of anaerobic fermentation and that the recovery of significant amounts of individual VFA might be feasible by manipulating temperature and pH. On the one hand, in laboratory-scale alkaline fermentation the highest VFA production corresponded to 55 °C tests. Total VFA production during SS $W_{ww}$ and MBM fermentation was 4,014 mg $O_2$ $L^{-1}$ (0.4 g $VFA_{total}$/g$COD_{in}$), 4,039 mg $O_2$ $L^{-1}$ (0.4 g $VFA_{total}$/g $COD_{in}$) and 4,921 mg $O_2$ $L^{-1}$ (0.5 g $VFA_{total}$/g $COD_{in}$), respectively. On the other hand, in batch pilot-scale fermentation, the individual trends for the VFA were reproduced. The following numbers were obtained: (i) 5.91 g $O_2$ $L^{-1}$ of acetic acid (53%) and 1.67 g $O_2$ $L^{-1}$ of iso-valeric acid (17%) during SS alkaline fermentation at 55 °C, (ii) 6.67 g $O_2$ $L^{-1}$ of butyric acid (60%) from $W_{ww}$ fermentation at acid pH and 55 °C, and (iii) 2.71 g $O_2$ $L^{-1}$ of valeric acid (22%) during MBM fermentation at 35 °C and acid pH.

In addition, the results of batch pilot-scale testing could be valuable in selecting critical parameters for further implementation of fermentation under continuous operation. The operational conditions for the waste streams under study could be pH 9 and 55 °C for SS, pH 6 and 55 °C for $W_{ww}$ and pH 6 and 35 °C for MBM. Furthermore, retention time within 5–10 days might be reasonable and an automatic system for controlling the pH would be strongly recommendable. Nonetheless, how pH is adjusted and controlled needs further study and clarification. As for co-fermentation, both organic streams ($W_{ww}$ and MBM) could be assessed as co-substrates for SS fermentation. However, it seems that liquid co-substrates might be favourable in terms of acidification kinetics, and in terms of operational feasibility since their dosing could be driven automatically without major technical impediments.

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# CHAPTER 4

# CONTINUOUS ACIDOGENIC FERMENTATION: NARROWING THE GAP BETWEEN LABORATORY TESTING AND INDUSTRIAL APPLICATION

This chapter contains the publication:

*Garcia.Aguirre, J., Esteban-Gutiérrez, M., Irizar, I., González-Mtnez de Goñi, J., Aymerich, E., 2019. Continuous acidogenic fermentation: narrowing the gap between laboratory testing and industrial application. Bioresource Technology, 282, 407-416.*

*The results were presented in an oral presentation at the IWA Anaerobic Digestion conference, AD16, in June 2019, Delft, The Netherlands*

## 4.1 GRAPHICAL ABSTRACT

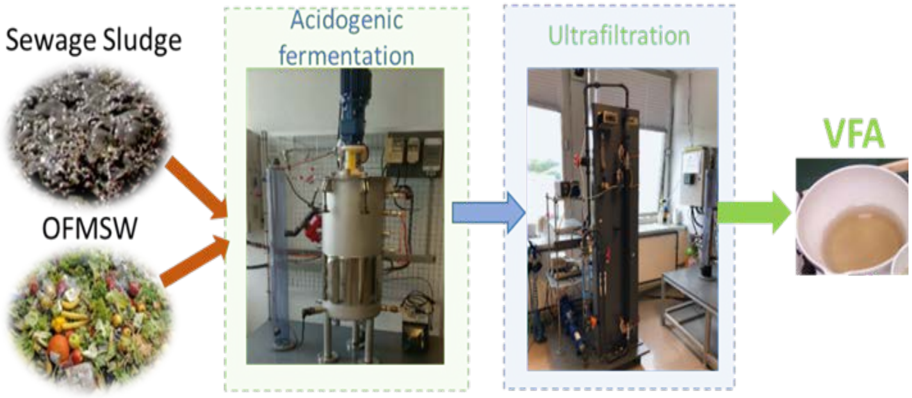

**Figure 4. 1**. Graphical abstract.

## 4.2 ABSTRACT

This study explored the potential of acidogenic fermentation of sewage sludge (SS) in an 80 L automatized pilot scale platform. A high-rate VFA production was obtained at HRT 5 d and pH 9, with a volatile fatty acid (VFA) yield of 336 mg VFA $g^{-1}$ VS and a VFA productivity of 2.15 kg VFA $m^{-3}$ $d^{-1}$. During co-fermentation of SS with OFMSW, a reversible pH shift from pH 9 to pH 6, evidenced a higher acidogenic activity which promoted the butyrate metabolic pathway, with 13.97 g COD $L^{-1}$ of butyric acid and a VFA peak 23.2 g COD $L^{-1}$. The results show the degree of flexibility of mixed culture fermentation systems, where other pH control methods other than steady control could be used to enhance the fermentation process. Ultrafiltration was a feasible technology to obtain a VFA rich permeate where 12.3 – 26.6 g COD $L^{-1}$ could be recovered.

## 4.3 INTRODUCTION

The pressure on natural resources is expected to be intensified over the next decades due to the acelerated population growth and significant increase in the living standards. Waste management rethinking will open new opportunities by closing the economic loop and promoting the resource recovery of linear take-make-dispose production systems.

Circular economy strategies highlight anaerobic fermentation as a sustainable option to produce value-added biochemicals, such as market demanded volatile fatty acids (VFA). Bio-based VFA consist of intermediate chemicals produced during the acidogenesis and acetogenesis step of conventional anaerobic digestion process, when methanogenesis is successfully inhibited (Lee *et al.*, 2014). With a fast market growth and emerging applications (e.g. production of bioplastics), vast amounts of high quality VFA from biomass fermentation needs to become commercially available in the following years.

Mixed culture VFA production from cost-effective waste carbon sources, offers significant advantages over pure fermentation processes, i.e. using sugars and engineered bacterial strains, in terms of economic feasibility and process cost (Bathia *et al.*, 2017). Waste derived VFA could feed back the industry, by providing valuable “building blocks” for its subsequent conversion into marketable products (Zacharof and Lovitt, 2013) and replace the traditional dependence with fossil fuels. Currently, the lower cost of oil-based VFA production methods and the higher yield achieved, pose an economically favourable condition for non-renewable VFA production (Atasoy *et al.*, 2018). However, the shift towards a sustainable approach is compulsory, to meet the future climate needs. The changes in the policy framework in addition to the annual VFA market growth (Global Market Insights, 2018), opens a positive prospective for bio-based VFA production.

Recent efforts have been directed towards understanding how the operational factors affect the acidification process on a laboratory-scale basis (Jiang *et al.*, 2013; Garcia-Aguirre *et al.*, 2017; Han *et al.*, 2016; Jankowska *et al.*, 2017) and influence the metabolic pathways (Zhou *et al.*, 2017). However, synergistic effects occur between different operational factors, i.e. the temperature, the pH and the composition of the waste stream. Additionally, an effective VFA production methodology should consider all the factors determining the efficiency of the process, such as the availability of the waste stream, the requirement of additional pre-treatment steps, the VFA productivity and the VFA yield of the fermentation process and the subsequent downstream to obtain concentrated or purified VFA (Bathia *et al.*, 2017).

Regarding different waste streams, sewage sludge (SS) and the organic fraction of municipal solid waste (OFMSW) are attractive substrates for VFA production. These waste streams can pose a direct threat on health and hygiene, and usually pose a handling challenge in many municipalities. Additionally, both waste streams are generated in sufficient amounts to constantly feed a full-scale fermentation system, i.e.  261 – 777 kg per capita of municipal waste were generated in 2016 and sludge production varied from 0.067 – 1.82 M tonne in the Euro area in 2015 (Eurostat, 2018).

Despite the promising results of the current state of the art, VFA fermentation is not a mature technology. There is a gap to be overcome the following years between laboratory scale VFA production and full-scale implementation. To the author's knowledge, the study of Liu *et al.* (2018) was the first work describing a full-scale operation of VFA production with the aim of biological nutrient removal in WWTP. During the study, pH 12 and 70 °C were applied as a pre-treatment, prior to fermentation, which was performed at pH 10-11 and a hydraulic retention time (HRT) of 14 d.  A recent study of Li *et al.* (2018a) suggested that waste activated sludge alkaline fermentation could be conducted at shorter HRT of 8 d, under pH 10, with NaOH (40%) and $Ca(OH)_2$ (10 %) as dosing chemicals. Interestingly, Esteban-Gutiérrez *et al.* (2018) proposed that sewage sludge alkaline fermentation could be performed at 3-5 days retention time and pH 9.  HRT is an important design parameter that will define the size of the full-scale fermenter, which determines to a great extent the initial investment cost.

However, industrial processes using purified VFA from fermentation broths are not a state of the art yet. From a process engineering point of view, robust and efficient fermentation systems are required which go a step forward laboratory tests. Effective methanogenic inhibition and proper process monitoring control pose an engineering challenge during VFA up-scaling. pH control and HRT selection can offer a means for inhibiting the growth of methanogenic bacteria, which might be a more feasible option than adding methanogenic chemical inhibitors (Lee *et al*., 2014, Vanwonterghem *et al.,* 2015). Although some pre-treatment methods like ultrasonic or thermal treatments might improve the extent of hydrolysis (Zhou *et al.*, 2014, Xu *et al.,* 2015), these should also be scalable and should not enhance the energetic and economic costs (Strazzera *et al.,* 2018). Finally, cost-effective downstream processes that can obtain high purity VFA from fermentation broths are required. Among different downstream technologies, i.e. liquid-liquid extraction with anionic solvents, adsorption with ion-exchange resins and novel membrane technologies (Sighnaia *et al*., 2013, Atasoy *et al.*, 2018), the use energy intensive methods and chemicals should be avoided. Additionally, VFA recovery requires a fist downstreaming step for the removal of the biomass from the fermentation broth (Rebecchi *et al.*, 2016).

The main objective of this work was to up-scale the VFA production process in an 80 L automatized pilot-scale platform and to obtain a high-rate continuous fermentation process with SS as model substrate. OFMSW was evaluated as a potential co-substrate during SS fermentation. The influence of HRT and pH were evaluated and the viability of ultra-filtration as a first VFA recovery step was assessed.

## 4.4 MATERIAL AND METHODS

### 4.4.1. Organic waste streams

The SS used in this study was obtained from the thickener of a municipal wastewater treatment plant (WWTP) in San Sebastian (Spain), which has a high load biological treatment unit. As shown in Table 1, this substrate contains and average chemical oxygen demand (COD) content of 60.6 g COD $L^{-1}$ and 38.4 g $L^{-1}$ of VS (Volatile Solids), which represent 76.2% of TS (Total Solids). The OFMSW was collected from a university canteen in the campus of the University of Navarra in San Sebastian, which holds the daily activity of over 1,000 people. The OFMSW was collected during subsequent days and a representative characterization was made. This substrate contains a high solid percentage of 29.1% TS and the VS account for 75% of TS (Table 4. 1).

During the co-fermentation test, the mixture containing 20% of OFMSW and 80% of SS (v.v) was characterized. Due to the addition of OFMSW, the total COD increased up to 104.1 g COD $L^{-1}$ with a VS content of 6.8% (Table 1).

During fermentation tests a mixed culture inoculum was used. This inoculum consisted of anaerobically digested sludge collected from the above mentioned WWTP. The inoculum provides a buffer capacity to the system, with 6,250 mg CaCO3 L$^{-1}$ of total alkalinity (TA) and an ammonium content of 2,646 mg N L$^{-1}$ (Table 4. 1). All waste samples and inoculum were stored at 5 ºC prior to use.

### 4.4.2. Fermentation tests

An automatized 80 L stainless steel reactor was operated during the fermentation tests (Figure 4. 2).

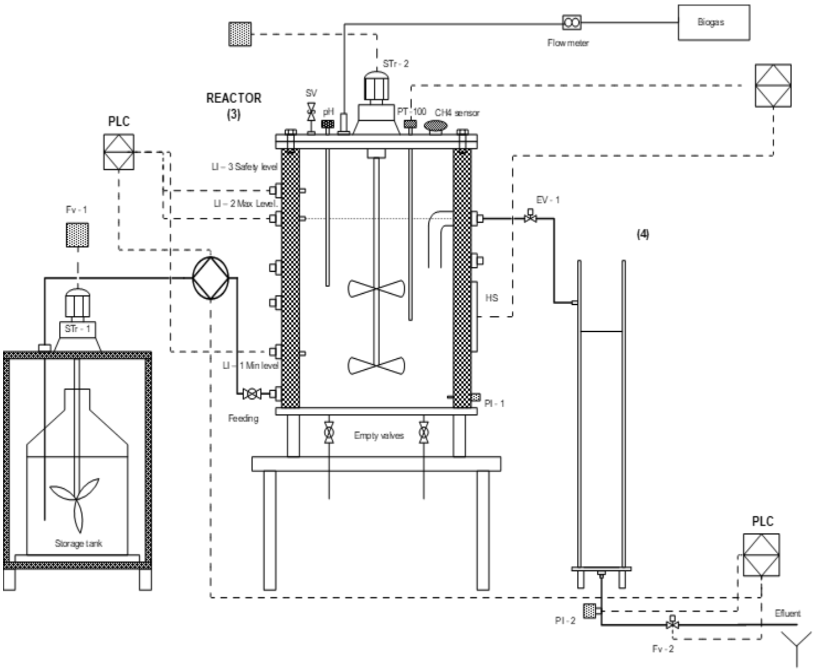

**Figure 4. 2.** 80 L pilot-scale reactor used in batch and continuous tests.

The pilot plant was equipped with a PLC and Scada software that enabled its automatization in terms of hydraulic retention time (HRT) and the organic loading rate (OLR). A system to automatically control the pH was installed so that the methanogenic activity was adequately suppressed.

**Table 4. 1**. Characteristics of the fermentation substrates.

| | **SS** | **Inoculum** | **OFMSW** | **Co-fermentation** |
|---|---|---|---|---|
| TS (%) | 5.04 ± 0.5 | 3.267 ± 0.32 | 29.25 ± 1.6 | 8.58 ± 0.71 |
| TSS (%) | 4.59 ± 0.5 | 2.85 ± 0.26 | N.A. | 7.14 ± 1.0 |
| VS (%) | 3.84 ± 0.4 | 1.89 ± 0.20 | 21. 78 ± 2.2 | 6.83 ± 0.9 |
| VSS (%) | 3.62 ± 0.3 | 1.72 ± 0.13 | N.A. | 4.63 ± 2.4 |
| pH | 6.10 ± 0.2 | 7.89 ± 0.11 | 5.1 ± 0.205 | 4.88 ± 0.7 |
| TA (mg $CaCO_3$ $L^{-1}$) | 1,282.5 ± 492 | 8,250 ± 746 | N.A. | 7,000 ± 2,500 |
| PA (mg $CaCO_3$ $L^{-1}$) | 287.5 ± 48 | 6,350 ± 545 | N.A | 500 ± 123.2 |
| TAN (mg N $L^{-1}$) | 213.5 ± 197 | 2,646 ± 331 | N.A | 1,022 ± 580.6 |
| tCOD (mg $O_2$ $L^{-1}$) | 60,585.2 ± 6148 | 26,111 ± 4,118 | 1,370* | 104,074 ± 11,056 |
| sCOD (mg $O_2$ $L^{-1}$) | 5,914.3 ± 3234 | 1,800 ± 487 | N.A | 9,172.8 ± 2,842 |

*mg $g^{-1}$ ST

NaOH 8 M and HCL 4 M were used as dosing agents to adjust the pH during fermentation tests. The pilot plant was equipped with a PT100 temperature probe and an electrical heater which enabled to keep the temperature of the process in the desired range, i.e. at mesophilic (35 °C) or thermophilic conditions (55 °C).

A mechanical mixing was employed. A biogas flow-meter was used to monitor daily biogas production while methane was quantified by a gas chromatograph.

Firstly, batch fermentation tests were performed with SS and the OFMSW (Table 4. 2). During the fermentation tests, the food to microorganism ratio was adjusted to 1:1 (v.v), and the pH was controlled manually, with intermediate adjustment that enabled to keep the fermentation pH in the desired range during 10 days of fermentation. Concretely, SS required one intermediate manual adjustment to keep the fermentation process within pH 8-9. OFMSW showed a high fermentation activity and required five intermediate adjustments in order to prevent the pH fall below 5.

Secondly, continuous fermentation process was assessed with SS as model substrate. According to Table 4. 2, SS continuous fermentation tests were performed at HRT 10 d, with steady control of pH setting the target value in pH 10 and 9. With the aim of analysing the influence of the SS retention time in the fermentation system, the HRT effect was analysed at 7.5 and 5 d (Table 4. 2). During these tests, the operation time was kept at a minimum of three times the HRT in order to achieve a steady VFA production. Herein the pH was adjusted initially to the target value, and the reactors were maintained at uncontrolled period during 5 days, followed by a period of steady pH control and continuous reactor feeding. Due to the particulate nature of OFMSW and the current reactor configuration, OFMSW evidenced technical difficulties to perform continuous fermentation tests, since blockages were continuously faced in the pumping system. According to previous studies, leaching bed reactor (LBR) configuration could be used during OFMSW fermentation (Han and Shin, 2002).

The potential of OFMSW was assessed as co-fermentation substrate, where the dilution with SS enabled the correct feeding in the automatized fermentation reactor. During co-fermentation, 20% of OFMSW and 80% was assessed during 30 days of fermentation.

**Table 4. 2**. Detailed description of the fermentation tests performed during the study.

| Operation mode | Substrate | Reactor pH | T (ºC) | HRT (d) | pH control method | F:M[1] | Feed (Kg d$^{-1}$) | Test duration (d) |
|---|---|---|---|---|---|---|---|---|
| Batch | SS | 9 | 55 | 10 | Manual adjustment | 1:1 | N.A. | 10 |
| | OFMSW | 5.5 | 35 | 10 | Manual adjustment | 1:! | N.A. | 10 |
| Continuous | SS | 10 | 55 | 10 | Steady-control | 1:1 | 5 | 30 |
| | SS | 9 | 55 | 10 | Steady-control | 1:1 | 5 | 18 |
| | SS | 9 | 55 | 7.5 | Initial adjustment (uncontrolled inoculation + steady control) | 1:1 | 6.67 | 35 |
| | SS | 9 | 55 | 5 | nitial adjustment (uncontrolled inoculation + steady control) | 1:1 | 10 | 20 |
| | SS + OFMSW | 9 | 55 | 5 | Steady-control | (0.2:0.8)):1 | 10 | 30 |

[1] Food to microorganism ratio

### 4.4.3. Ultrafiltration unit

An external filtration unit was used to get the soluble fraction from the fermentation broth of the reactor (Figure 4. 3). The unit consisted of a helicoidal pump and a ceramic cross-flow membrane. The pump was regulated with a frequency variator which allows to operate the filtration system with a cross flow velocity of approximately 100 – 500 L $h^{-1}$ and 1-5 m $s^{-1}$. Transmembrane pressure was regulated using a back pressure diaphragm valve in the outlet of the membrane. Additionally, a maximum pressure alarm device was installed in the circuit, which enabled to stop the flow when pressure exceeded 2.5 bar. A PVC tank was used to store the reactor fermentate before the ultra-filtration step. A tubular monochannel ceramic membrane of 300 kD was tested. The membrane contained a hydraulic diameter of 6 mm and 1,200 mm length, with a surface area of 220 $cm^2$. The permeate was collected for its subsequent analysis. A peristaltic pump was used to obtain a constant flux of permeate. Pressure sensors of the inlet, outlet and permeate side enabled to control the transmembrane pressure.

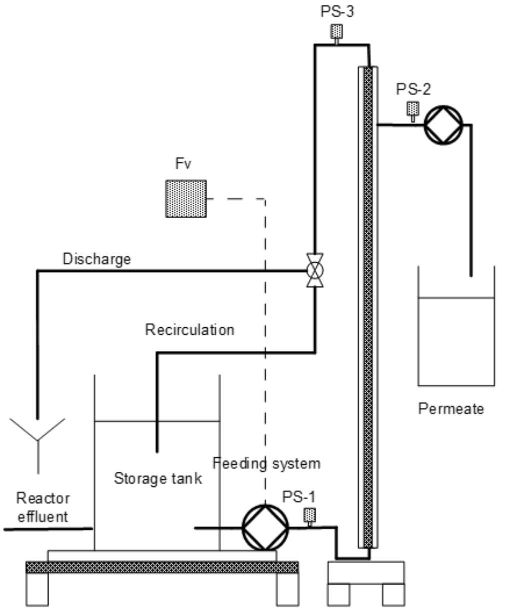

**Figure 4. 3**. Ultra-filtration module for downstream of the fermentation broth.

### 4.4.4. Analytical methods

During this study, fermentation broth samples were taken periodically and analysed. Analyses of the total fraction were performed directly over the raw samples and the soluble fraction was obtained by means of centrifugation (i.e. 12,000 rpm, 5-10 min), followed by a vacuum filtration (Whatman 1.5 µm). TS, VS, TAN (total ammonia nitrogen), tCOD (total chemical oxygen demand), sCOD (soluble chemical oxygen demand), TA and PA (partial alkalinity) were measured according to Standard Methods 21st Edition (APHA, 2005).

The VFA concentration, i.e. acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid were determined by gas chromatography, by means of an Agilent GC-6890 gas chromatograph equipped with a FID (Flame Ionization Detector) and a capillary column (DB-FFAP, 30m x 0.25 mm i.d., 0.25 µm film, Agilent J&W: ref. 122 – 3232E). Pivalic acid was used as an internal standard solution.

The biogas composition was monitored by taking samples from the fermentation reactor in order to check that the methanogenic activity was sufficiently inhibited. The biogas composition was measured by a GC-TCD HP6890 column SUPELCO 60/80 Carboxen, Ref. 10001-2390-U.

The VFA concentration was expressed in COD units, with the following conversion rates: 1.07 g $O_2$ $g^{-1}$ acetate, 1.51 g $O_2$ $g^{-1}$ propionate, 1.81 g $O_2$ $g^{-1}$ butyrate and 2.04 g $O_2$ $g^{-1}$ valerate (Eastman and Ferguson 1981). The VFA production was also expressed on a daily production basis, which was referred to as VFA productivity, by using the units, mg VFA L $d^{-1}$. Additionally, the fermentation tests were evaluated by considering the acidification degree (DA), which is considered as the readily fermentable matter present in soluble fraction of the final fermentation broth, i.e. $COD_{VFA}\ sCOD_{final}^{-1} \cdot 100$.

## 4.5 RESULTS AND DISCUSSION

### 4.5.1. Batch fermentation tests

Previous laboratory scale tests provided a good methodology for the selection of favourable conditions for the up-scaling and revealed the significance of the substrate(s) and the synergistic effects of temperature and pH, in determining the final VFA spectrum (Garcia-Aguirre *et al.,* 2017). From previous results, thermophilic conditions and alkaline pH were considered as promising conditions for SS fermentation, giving a maximum yield of 332 mg VFA $g^{-1}$ $COD_{fed}$. Contrarily, acidic conditions and 35 ºC performed best during OFMSW acidogenic fermentation, where the VFA yield raised up to 611 mg VFA $g^{-1}$ $COD_{fed}$ and the VFA accounted 94% of the final sCOD. These results were considered as baseline for subsequent up-scaling. According to Figure 4. 4 a fast hydrolysis and acidogenesis rate could be obtained during SS alkaline fermentation. The VFA spectrum was dominated by acetic acid at pH 9 and 55 ºC, followed by propionic and iso-valeric acid, with 6,000, 912 and 871 mg COD $L^{-1}$, respectively (Figure 4. 4a). The results evidenced that a short HRT of 3 days could be used for continuous VFA production process.

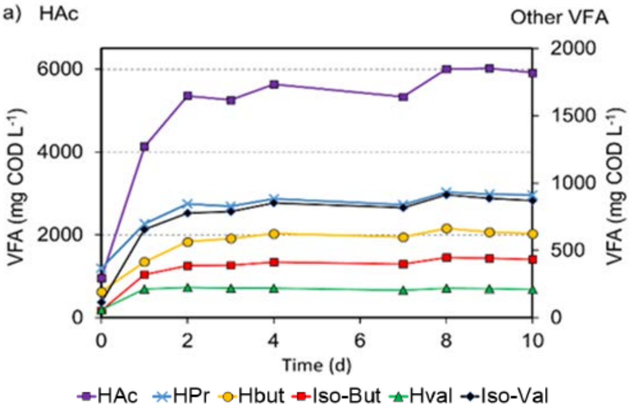

**Figure 4. 4**. VFA profile during pilot-scale fermentation test. SS and pH 9 (a)

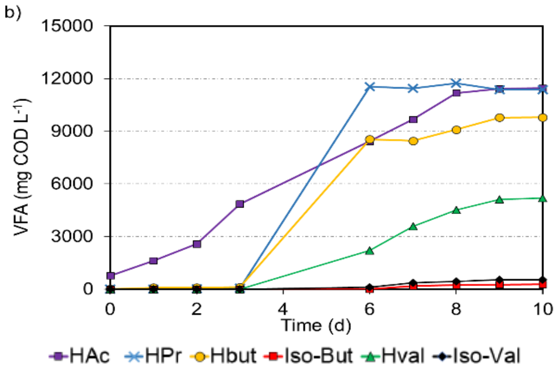

**Figure 4. 4** (Continued)**.** VFA profile during pilot-scale fermentation test. SS and pH 9 (a)VFA profile during pilot-scale fermentation test of OFMSW and pH 5.5 (b)

OFMSW is an interesting substrate regarding its VFA production profile, since a rich VFA composition can be obtained (Strazzera *et al.*, 2018). The high VFA production potential of OFMSW was confirmed, where 55.5 g COD $L^{-1}$ of total VFA was registered, which is close to the values reported with synthetic food waste mixtures (Jiang *et al.*, 2013, Yin *et al.*, 2016). The VFA production was dominated by a mixed acid type fermentation (Zhou *et al.*, 2017), and the fermentation mixture was equally dominated by acetic and propionic acid, with 11.4 and 11.3 g COD $L^{-1}$ respectively, while butyric and valeric acid reached up to 9.78 and 5.12 g COD $L^{-1}$ (Figure 4. 4b). As previously reported, the distribution of the metabolic products depends on the various factors affecting the fermentation process, such as the concentration and characteristics of the substrates, the pH of the process and the type of the bacteria present in the inoculum (Zhou *et al.*, 2017). While acetic, propionic and butyric acid may be produced from OFMSW fermentation (Strazzera *et al.*, 2018), other fermentation products like valerate are less frequent. For example, valeric acid might be the result of protein degradation (Jankowska *et al.*, 2017, Garcia-Aguirre *et al.*, 2017) which occurred after day 3, reaching 5.2 g COD $L^{-1}$.

### 4.5.2. Inoculation and start-up of the fermentation process

Continuous fermentation tests were started with 10 d HRT by feeding 5 L of fresh SS per day, based on previous batch trials. The feed was governed by the Scada software, which was set at 500 mL charges of sludge every 144 min. Thus, the performance of the fermentation could be comparable to a full continuous process.

pH is a key parameter governing the VFA production rate and the VFA yield. However, there is not a clear consensus on the influence of pH on the composition of the products (Zhou *et al.*, 2017). In line with previous work, pH 10 enhanced the hydrolysis of SS releasing high amounts of soluble COD, i.e. 33.96 g sCOD $L^{-1}$ (Jie *et al.*, 2014, Chen *et al.*, 2017). However, the soluble COD was not consumed by acidogenic bacteria into VFA, where only 11.2 mg COD $sCOD^{-1}$ where obtained. During 30 days of fermentation, the VFA remained stable with 3,673 ± 517 mg COD $L^{-1}$ with a moderate productivity of 367.3 mg COD $L^{-1}$ $d^{-1}$, evidencing that the process was far from its optimum (Figure 4. 5a). While some authors obtained maximum VFA production at pH 10 (Wu *et al.*, 2010), other studies suggested that the metabolic activities of acid-producing bacteria could be markedly inhibited under alkaline conditions (Jie *et al.*, 2014, Zhao *et al.*, 2015). At pH 10, the fermentation outcome was dominated by acetic acid and propionic acid, which accounted for 57 ± 2.7% and 22 ± 0.9%, respectively (Figure 4. 5). According to Figure 4. 5c, the biogas production remained stable, with an average value of 6.75 mL $min^{-1}$ and less than 2% of methane. These results are a good indicative that alkaline pH environment is favourable for the hydrolysis of organic matter, sCOD release, and inhibitor of methanogenic bacteria (Jie *et al.*, 2014). Yuan *et al.* (2015) also reported a low presence of methanogenic *Archaea* during waste activated sludge alkaline fermentation at pH 10.

Based on the optimal results obtained in the previous batch test, a steady pH 9 was tested with an HRT of 10 days. During this test, the VFA production tended to increase with time, from 1,235 mg COD $L^{-1}$ to a maximum of 2,880 mg COD $L^{-1}$ within 8 days (Figure 4. 5b). Chen *et al.* (2017) reported the highest VFA yield related from the abundance of *Clostridia* at pH 8.9 during the fermentation of a mixture of sewage sludge and waste activated sludge. According to Figure 4. 5d a higher biogas production and higher methane content, i.e. 3-8%, were measured during this period. Nonetheless, the subsequent reduction in the biogas and methane production, in addition to the VFA production profile, most probably indicated an inhibition or inactivation phenomenon of acidogenic bacteria.

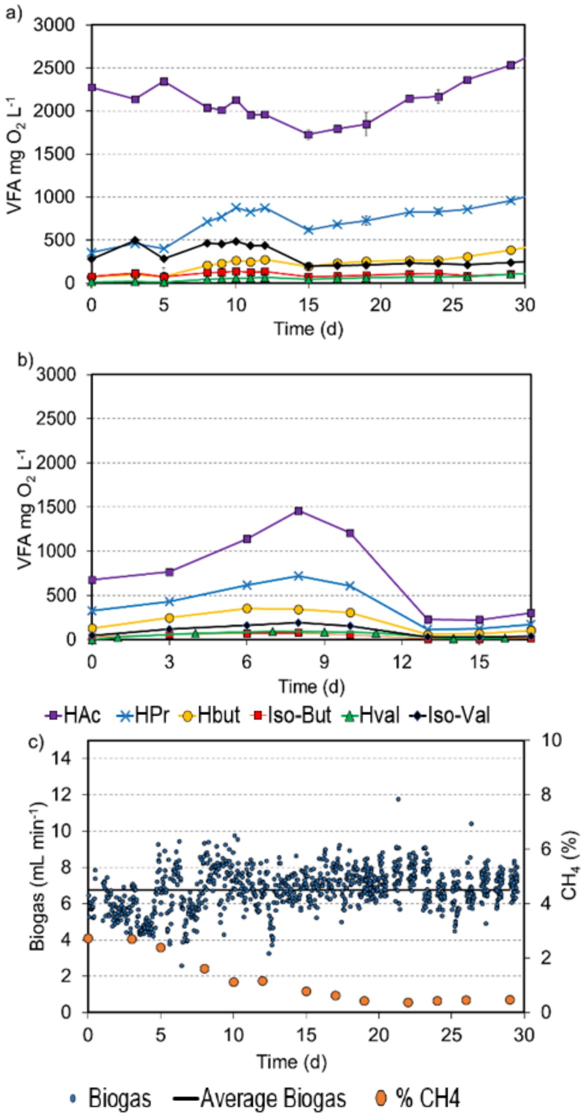

**Figure 4. 5**. VFA production during start-up of the fermentation process at pH 10 (a), pH 9 (b). Biogas and methane content in the start-up at pH 10 (c).

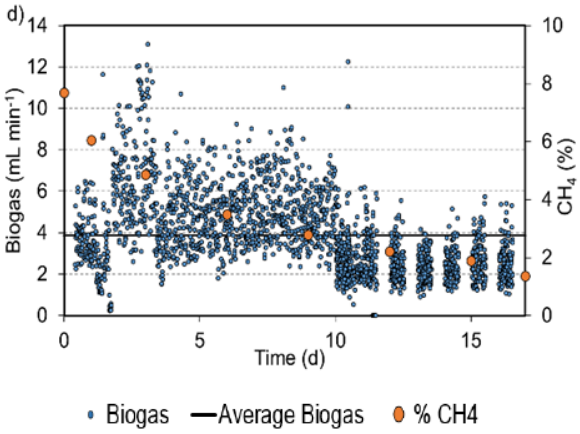

**Figure 4. 5** (Continued). Biogas and methane content at pH 9 (d)

The degree of acidification obtained on day 8, which raised to a peak value of 8.94%, was reduced down to 1.37-1.96 % in the following fermentation days. Due to the abiotic effect of NaOH dosing, the sCOD remained at high values of 32,113 ± 1,222 mg COD $L^{-1}$, accounting for 60% of the total COD and indicating that soluble proteins and carbohydrates remained unchanged.

These results suggest that the process was inhibited and that the acidogenic bacteria could not be acclimated at both pH 10 and 9 values, which contradicts the results obtained during previous batch tests and previous literature work. Many of the previous work, e.g. Jankowska *et al.* (2017), were performed by initial adjustment of pH followed by uncontrolled period where the pH monitoring shows a dynamic behaviour and a tendency to decrease when VFA accumulate. For example, during acidogenic tests performed at alkaline conditions at pH 11, the pH stabilized within 7.4-9.7 for different organic waste streams (Jankowska *et al.*, 2017). The alkaline shock induces a high solubilization, favourable for the subsequent acidogenic step. However, during continuous fermentation tests performed in this study, the pH was maintained at the target value, i.e. pH 9 or 10 and the high solubilization rate but low VFA production rates suggest that this pH control was beneficial for the hydrolysis, but detrimental for acidogenic microorganisms. Contrarily, uncontrolled pH would favour the activity of acidogenic microorganisms and the activity of key enzymes involved during acidogenic fermentation. Thus the start-up methodology and proper pH control strategy are critical factors affecting the acidogenic fermentation which was barely reported in previous work. Therefore, the inoculation strategy for the next tests consisted of an initial adjustment, followed by 5 days of acclimation without pH control, and a steady control of pH afterwards.

### 4.5.3. Influence of the HRT during SS continuous fermentation

HRT is a critical design parameter in full-scale reactors that determines the volume of the reactor to be constructed. This parameter defines the time that the biomass and the substrate will be retained in the reactor and, thus, the time period available for hydrolysis of complex organic matter and biodegradation. Regarding the anaerobic fermentation, it is well known that it requires a lower HRT to avoid the proliferation of methanogenic microorganisms (Kim *et al.*, 2013). However, the HRT should be long enough to guarantee an adequate hydrolysis and the release of large amounts of soluble substances that can be converted into VFA. The selection of the HRT is inherently linked to the complexity of the substrate and according to previous studies, this parameter can influence the VFA production and composition (Lim *et al.,* 2008; Lee *et al*., 2014). According to the results of Lim *et al.* (2008), the prolonged HRT could only induce a higher VFA production up to a certain value, were not a further increase was noticed.

During the subsequent tests, pH 9 fermentation tests were performed by setting the HRT to 7.5 and, then, to 5 to analyse the effect of this parameter on the VFA yield and the VFA spectrum. The results obtained during HRT 7.5 d, showed a stable continuous VFA production and thus, confirmed that the inoculation method was effective to improve the VFA production. The fermentation composition was dominated by acetic acid, with an average value of 4,878 ± 647 mg COD $L^{-1}$ after day 10 of fermentation (Figure 4. 6). Secondary fermentation products were propionic, iso-valeric and butyric acid, which accounted for 1,440 ± 241 mg COD $L^{-1}$, 1,436 ± 178 mg COD $L^{-1}$ and 1,338 ± 167 mg COD $L^{-1}$, respectively during the steady fermentation period. The composition of the fermentation broth was similar to the composition reported during batch fermentation tests at pH 9, suggesting that the operation mode had a negligible effect in the VFA distribution.

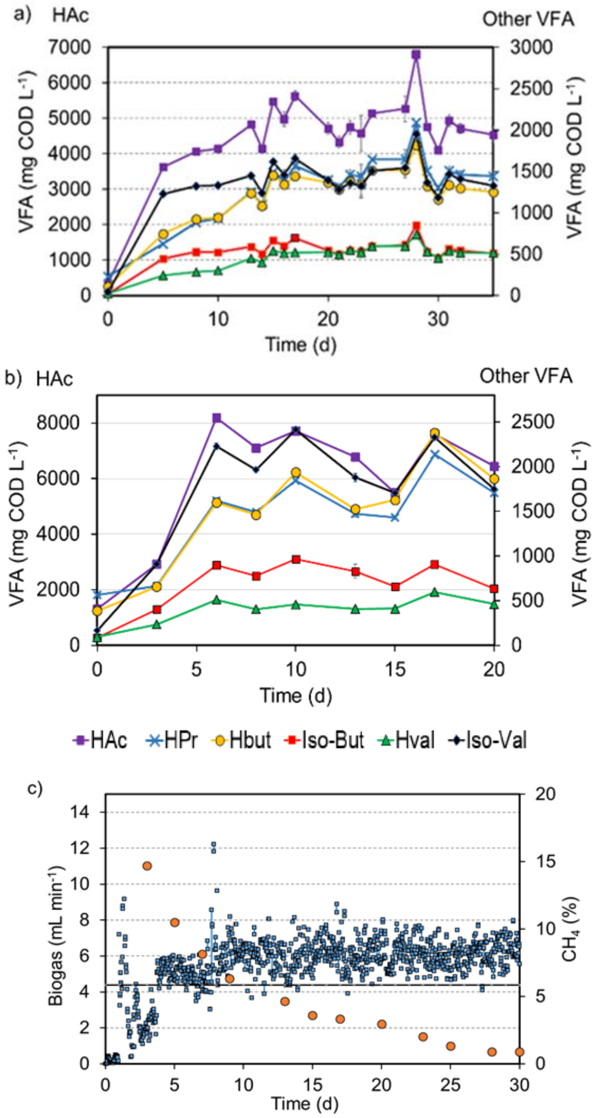

**Figure 4. 6**. VFA distribution profile at HRT 7.5 (a) and HRT (5), biogas and methane content at HRT 7.5 (c)

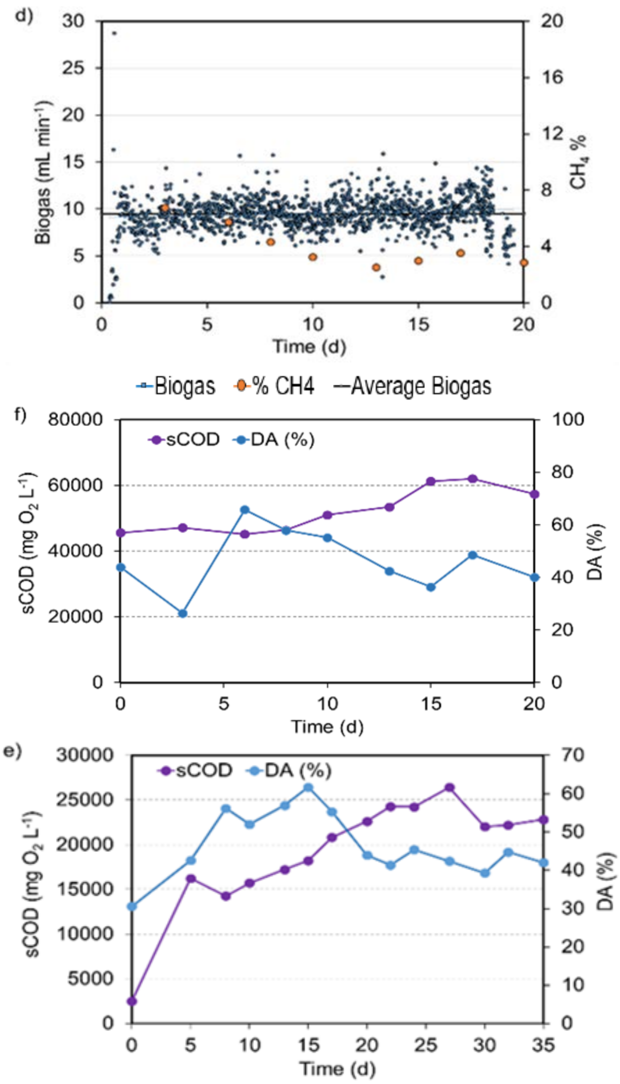

**Figure 4. 6** (Continued). Biogas and methane content at HRT 7.5 (c) and HRT 5 (d), and sCOD and the DA over time at HRT 7.5 (e) and HRT 5 (f).

This test produced an overall VFA concentration of 10,067 mg COD L$^{-1}$, with a VFA productivity of 1,343 mg COD L d$^{-1}$. The degree of acidification ranged within 48.4 ± 7.5% and the sCOD remained within 23,172 ± 1,734 mg COD L d$^{-1}$ after two HRT (Figure 4. 6 e). The average biogas production was 5.84 ± 2.8 mL min$^{-1}$ and the methane production was successfully inhibited from initial 20% to final values within the range 0.52-1.33% (Figure 4. 6c). The ammonium release provided buffer capacity to the fermentation system with an average TAN value of 1,604 mg N $L^{-1}$ which enabled the acidogenic bacteria stand high VFA concentration (Dahiya *et al.*, 2019).

Figure 4. 6b shows that VFA concentration was improved at HRT of 5 days, which had an average value of 13,799 ± 1,667 mg COD $L^{-1}$. This could be attributed to the fact that the OLR was increased from 4.32 kg SV $m^{-3}$ $d^{-1}$ to 8.19 kg SV $m^{-3}$ $d^{-1}$ at an HRT of 5 days. Additionally, the higher biogas production, i.e. 9.48 mL $min^{-1}$, evidenced a higher activity of acidogenic microorganisms (Figure 4. 6 d). Regarding the VFA profile achieved, 5 d HRT was enough to assure the hydrolysis of the organic matter where the sCOD increased up to 32 g COD $L^{-1}$. During this period the degree of acidification ranged within 49.5 ± 10 mg VFA $sCOD^{-1}$ (Table 4.3) and the ammonium nitrogen release increased to an average concentration of 2,269 ± 221 mg N $L^{-1}$, enhancing the buffer capacity of the system.

Additionally, it was seen that the decrease in the HRT to 5 days had a noticeable effect in the distribution of secondary fermentation products. Iso-valeric reached an average concentration of 2,038 ± 238 mg COD $L^{-1}$ followed by butyric and propionic acid with 1,770 ± 318 mg COD $L^{-1}$ and 1,671 ± 251 mg COD $L^{-1}$, respectively. The results suggest that secondary fermentation products, i.e. iso-valeric acid, could be promoted by reducing the HRT of the process.

**Table 4.3**. Summary table of the main values obtained during pilot-scale fermentation.

| Process conditions | | | | | Fermentation outcome | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Subs. | pH | HRT (d) | OLR (kg SV $m^{-3}$ $d^{-1}$) | Averg. VFA (g $O_2$ $L^{-1}$) | Averg. VFA distribution (%) | Fermentation yield (mg VFA $g^{-1}$ SV) | Acidification degree (VFA $sCOD^{-1}$) | Productivity (kg VFA $m^{-3}$ $d^{-1}$) |
| Batch | SS | 9 | 10 | N.A | 8.96 | 66: 10.2: 9.7 (HAc, HPr, Iso-Val) | 339 | 49.5 | 0.9 |
| | OFMSW | 5.5 | 10 | N.A | 55.5 | 16:19:14 (HAc, HPr,HBut) | 438.3 | 64.4 | 5.55 |
| Cont. | SS | 10 | 10 | 3.69 | 3.69 ± 0.53 | 57:22:8.2 (HAc, HPr, Iso-Val) | 100.0 ± 14.4 | 10.9 ± 1.6 | 0.37 ± 0.05 |
| | SS | 9 | 10 | 4.3 | 1.53 ± 0.66 | 49:25:13 (HAc, HPr, Hbut) | 35.6 ± 15.3 | 4.8 ± 2.0 | 0.15 ± 0.07 |
| | SS | 9 | 5 | 8.19 | 13.80 ± 1.7 | 51:15:13 (HAc, Iso-Val, HBut) | 336.8 ± 40.7 | 49.5 ± 10.6 | 2.15 ± 1.25 |
| | SS | 9 | 7.5 | 4.32 | 10.07 ± 1.4 | 48:14:14 (Hac: HPr: Iso-Val) | 305.6 ± 48.1 | 41.8 ± 6.6 | 1.34 ± 0.18 |
| | SS + OFMSW | 9 | 5 | 13.66 | 14.25 ± 4.7 | 57:23:8 (HAc, Hbut, Iso-Val) | 288.3 ± 48.5* | 30.7 ± 10.1 | 2.99 ± 0.97 |

*Average value after day 17. Maximum yield of 351 mg VFA $g^{-1}$ SV

### 4.5.4. Co-fermentation of SS and OFMSW

During this study the effect of co-fermenting 20 % of OFMSW with SS was analysed (*v.v*). The operational parameters were decided according to the high VFA productivity obtained during SS continuous fermentation tests, i.e. at HRT 5 d and pH 9, under thermophilic temperature. The co-fermentation was performed by adding the OFMSW during the SS test performed with an HRT of 7.5 d.

According to Figure 4. 7a, an initial two-fold increase in the acetic acid concentration was observed, which remained stable at 7,695 ± 561 mg COD $L^{-1}$ indicating a positive effect of OFMSW addition. Previous work suggested a synergistic effect of microorganisms when mixing glucose, peptone and glycerol during batch acidogenic fermentation tests (Yin *et al.*, 2016). Interestingly, Li *et al.* (2018b) reported benefits on VFA production by mixing SS and OFMSW. The authors concluded that the increase of the food waste proportion in the SS mixture would reduce the microbial diversity, and that the food waste would favour the growth of dominant species such as *Veillonella*, *Clostridium*, *Bacteroidales and Lactobacillales.*, which are responsible for the higher VFA and hydrogen production. A similar conclusion was provided by Wu *et al.* (2016), who stated that the co-fermentation scenario of OFMSW and excess sludge increased the relative abundance of hydrolysis and acidification of bacteria, such as *Clostridium,* which were related to accelerating the hydrolysis of polysaccharides and generating organic acids. During the test, a maximum VFA peak of 14,242 mg COD $L^{-1}$ was registered on day 3, which decreased moderately to 11,096 ± 791 mg COD $L^{-1}$ for the following 14 days (Figure 4. 7a). Despite the stable VFA production, the alkaline conditions released high amounts of sCOD, likely resulting from the hydrolysis of OFMSW, and this seemed to trigger a reduction of the degree of acidification, from a maximum value of 38.4% to 17.2% on day 17. Despite the high release of sCOD, the fermentation system operated at a low VFA yield of 160 ± 9.7 mg VFA $g^{-1}$ SV which evidenced that the fermentation system was not reaching its optimal acidogenic potential. As previously reported, this could be related to the lower microbial richness and the hindered metabolism of acidogenic microorganisms at alkaline conditions (Ma *et al.,* 2016).

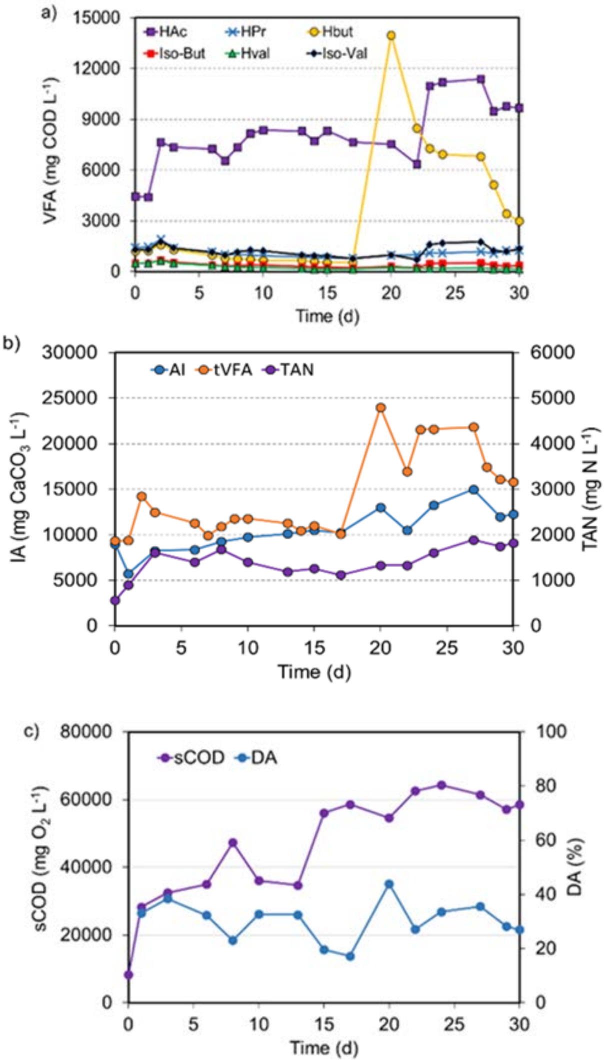

**Figure 4. 7.** VFA distribution profile during co-fermentation (a), intermediate alkalinity, total VFA and TAN (b), evolution of sCOD and DA (c)

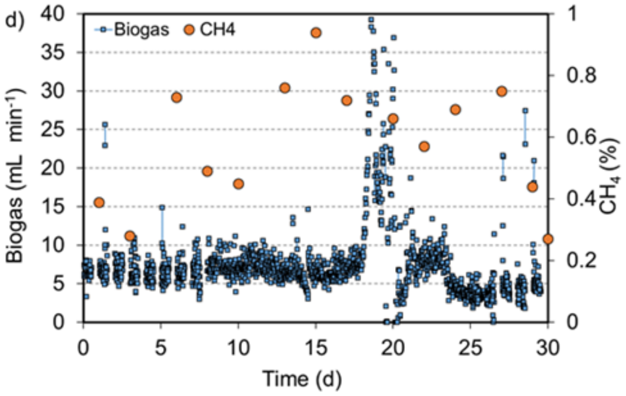

**Figure 4. 7** (Continued). Biogas composition and methane content (d)

Despite the high release of sCOD, the fermentation system operated at a low VFA yield of 160 ± 9.7 mg VFA g$^{-1}$ SV which evidenced that the fermentation system was not reaching its optimal acidogenic potential. As previously reported, this could be related to the lower microbial richness and the hindered metabolism of acidogenic microorganisms at alkaline conditions (Ma *et al.*, 2016).

With the aim of improving the acidification performance, and considering good results obtained during OFMSW fermentation in batch mode, the pH control was stopped during 48h, between day 17 and 19. Without a steady control, pH shows a dynamic behaviour in acidogenic fermentation systems. pH also plays a major role in the enrichment of acidogenic microorganism and influencing the main fermentation routes of acidogenic bacteria (Feng *et al.*, 2018). According to Liu *et al.* (2012), a higher abundance of acidogenic microorganisms were identified during neutral pH compared to acidic pH 3, and alkaline pH 12 environments. In this study, during the uncontrolled pH period, the pH of the fermentation system dropped progressively down to 6. In line with the results of Liu *et al.* (2012), the high biogas peak registered of 43 mL biogas min$^{-1}$ (Figure 4. 7 d) evidences a high microbial activity during this phase, where the proliferation and activity of acidogenic bacteria was enhanced and resulted in a higher VFA production. The acidogenic environment enabled to maintain the methane content at low values, within 0.66 – 0.72% (Figure 4. 7 d) showing a correct inhibition of methanogenic microorganism. Interestingly, the acidic environment, promoted a metabolic shift towards butyric acid production, most probably due to the contribution of

OFMSW, which reached a peak value of 13,970 mg COD $L^{-1}$. As previously reported, butyric acid is an expected fermentation product resulting from mixed culture fermentation of OFMSW. For example, butyric acid production was also promoted during the fermentation of food waste under pH 7 and thermophilic conditions, with 13 g $L^{-1}$ of butyric acid at 6 d HRT (Stein *et al.*, 2017) resulting from the decomposition of carbohydrates. According to Figure 4. 7 b, the ammonium level and intermediate alkalinity in the reactor show that the reactor remained stable during this phase.

Remarkably, the fermentation conditions were reversible, since returning the pH at the set point of 9, enhanced the acetogenic route, and reduced the butyrate metabolic pathway. This is in line with Dahiya *et al.* (2015) and Garcia-Aguirre *et al.* (2017) who reported higher presence of acetic acid during alkaline fermentation of OFMSW. With regards to the overall outcome, the pH disruption, enhanced the degree of acidification, which boosted to a peak value of 43.9% and remained at higher values afterwards, within 32.6 ± 6.6 %. Overall, the pH shift, exerted a positive effect in the fermentation system, where the VFA production was enhanced from an average value of 11,071 ± 1,340 mg COD $L^{-1}$ to 19,408 ± 3,170 mg COD $L^{-1}$ and a peak value of 23,984 mg COD $L^{-1}$ was reached at pH 6, with a maximum VFA yield of 351 mg VFA $g^{-1}$ SV (Table 4.3).

These are valuable results which highlight the level of flexibility of mixed culture anaerobic fermentation systems. Another study performed by Wu *et al.*, (2016), reported the dynamic behaviour of anaerobic fermentation and product shift within the pH range 4 – 5.5. During the fermentation of fruit and vegetable waste, pH 4 promoted the production of ethanol while pH 5.5 shifted the metabolic routes towards butyric acid, showing the influence of pH in the metabolic routes promoted. Interestingly, recent studies have focused on pH control strategy to enhance the VFA production process. For example, the study of Zhao *et al.* (2018) proposed a stepwise pH control for waste activated sludge alkaline fermentation. According to the authors, pH 11 was optimal to enhance the hydrolysis of complex organic matter, while pH 9 significantly enhanced the activity of acidogenic bacteria. This was refuted by a recent study performed by Wang *et al.* (2019). Herein, the authors highlighted that strong alkaline conditions at the initial fermentation stage (pH 11 during 3 days) facilitated the hydrolysis and the selection of more clustered bacterial populations, i.e. *Anaerobrancaceae,* capable of protein conversion into VFA under alkaline conditions. Reducing the pH in the second stage, promoted a niche for diverse acidogenic bacteria (such as *Acinetobacter sp.*, *Proteiniborus sp.*, *Proteiniclasticum sp.*, etc.). Additionally, the authors raised a critical question on the efforts that have been directed in previous work in controlling the fermentation pH at constantly high levels, throughout the entire fermentation process which can result in counteracting effects of acidogenic process and hinder the VFA production.

In line with recent work, the results of this study suggest that setting an optimal pH strategy is a key aspect to be optimized in acidogenic fermentation processes, and to maximize synergism for enhanced VFA production. Regarding the degree of flexibility of the mixed culture fermentation system, a dynamic pH control strategy should be further studied in future work, in order to direct the fermentation products towards target chemicals, i.e. butyric acid, which is a valuable bioproduct, with a market share of 2,000 – 2,500 USD tonne$^{-1}$ (Zacharof and Lovitt, 2013).

### 4.5.5. Ultra-filtration of the fermentation broth

Downstreaming of VFA can be considered a bottle-neck of mixed culture anaerobic fermentation, which is more complicated when high purity individual acids want to be recovered. During this study, a pilot-scale ultrafiltration unit was tested to recover the soluble VFA rich permeate and with the above-mentioned fermentation broths.

As shown in Table 4. 4, an up-concentration effect was noticed during the ultra-filtration tests both for SS fermentation and for co-fermentation of SS and OFMSW. The final total VFA concentration obtained from the HRT 5 fermentation broth contained 19.7 g COD L$^{-1}$, with the predominance of acetic acid, followed by iso-valeric and propionic acid. The amount of VFA recovered from SS fermentation contained lower amounts of iso-valeric and propionic acids when fermentation was performed at HRT 7.5 test, and 11.8% of butyric acid was obtained. Due to the particulate nature of OFMSW, the fermentation broth of the co-fermentation caused difficulties for the ultra-filtration unit operation. Similarly, Longo *et al.* (2015) reported clogging phenomena related to the fibrous materials present in the sewage sludge. However, a VFA concentrated permeate could be obtained, with 26.6 g COD L$^{-1}$. Acetic acid and butyric acid were the major fermentation products when OFMSW was subjected to ultra-filtration, which accounted for 45.7 and 41.6%, respectively.

The ultrafiltration unit provided a solid free and VFA rich permeate, which could be easily manipulated in subsequent downstream steps. According to these results, ultrafiltration technology could be up-scaled, and commercially available membranes could successfully be used for downstream of the fermentation broth. Additionally, coupling the membrane filtration unit to a CSTR fermentation reactor could offer a means for controlling the SRT (Solids Retention Time) and the HRT in an anaerobic membrane fermentation system (AnMBR-FS).

**Table 4. 4.** VFA recovery in the ultra-filtration unit.

| Fermentation substrate | pH | HRT (d) | VFA (g COD $L^{-1}$) permeate | % HAc | % Iso-Val | % HPro | % Hbut |
|---|---|---|---|---|---|---|---|
| SS | 9 | 5 | 19.75 | 47.3 | 23.5 | 21.2 | 5.3 |
|  | 9 | 7.5 | 12.35 | 47.9 | 13.9 | 14.9 | 11.8 |
| OFMSW + SS | 9 | 5 | 26.63 | 45.7 | 5.1 | 4.7 | 41.6 |

According to the study of Ataei and Vasheghani-Farahani (2008), removing the VFA from the fermentation broth, will benefit the process by decreasing the toxic effect of high VFA concentration. This novel configuration may add complexity to the process automatization and control, but it could most likely lead to a significant improvement of the VFA production and recovery process.

### 4.5.6. Batch Vs Continuous configuration

As for the feeding strategy in fermentation processes, it is not clear whether batch or continuous fermentation mode of operation might be beneficial for VFA production. The continuous fermentation process implies an engineering challenge due to the pH control, charge automation, and to the process monitoring and control requirements. Contrarily, batch fermentation could offer the advantage that it is easier to perform and control, but the major drawback raises from the time consumption and the complexity of periodic cleaning and filling up of the reactor.

According to Table 4.3, batch fermentation tests allowed for reaching a VFA yield of 339 mg VFA $g^{-1}$ SV during SS alkaline fermentation. During SS continuous fermentation tests, the VFA yield was notably increased under no-pH control inoculation, and 305 – 336 mg VFA $g^{-1}$ SV production was achieved. The degree of acidification achieved during continuous SS fermentation tests was similar to the SS batch test and ranged within 42 – 49%. Thus, the low differences observed in this study possibly suggest that continuous SS fermentation is a feasible approach for full-scale implementation.

Regarding the OFSMW, a high VFA yield and productivity were obtained during batch mode i.e. 438 mg VFA $g^{-1}$ SV and 5.55 kg VFA $m^{-3}$ $d^{-1}$ (Table 4.3). However, the complex nature of the substrate did not allow to perform continuous fermentation tests of OFMSW with the current reactor configuration. Alternatively, OFMSW was used as a co-substrate during the fermentation of SS. As previously reported, co-fermentation scenario constitutes a positive approach likely due to the nutrient balance that is stablished between SS and OFMSW which balances the C/N ratio (Zhou *et al.*, 2018). Interestingly, co-fermentation of OFMSW with SS enabled boosted the VFA productivity, where an average value of 2.99 kg VFA $m^{-3}$ $d^{-1}$ was obtained, with a maximum VFA yield of 351 mg VFA $g^{-1}$ SV (Table 4.3). In a recent study, Li *et al.* (2018b) reported the highest VFA yield of 281 mg $g^{-1}$ VS during co-fermentation of SS and 80% food waste (on TS basis), where the pH was kept within 7.2-7.6. Due to the effect of OFMSW, co-fermentation not only enabled to reach a high VFA concentration and a high VFA yield, which was especially significant in the last phase, but it also enabled to reach an interesting product shift from acetic to butyric acid.

## 4.6 CONCLUSIONS

This study showed the feasibility of pilot-scale batch and continuous acidogenic fermentation tests, performed with SS as model substrate. According to the results, the pH showed a marked effect in the VFA production yield and final product distribution, which raised up to 336 mg VFA $g^{-1}$ VS, with the predominance of acetic acid, iso-valeric and butyric acid, at pH 9 and HRT 5 d. The VFA productivity could be enhanced by co-fermenting OFMSW and SS, up to 2.99 kg VFA $m^{-3}$ $d^{-1}$. Optimal pH control was identified as a key aspect in ensuring the process stability and a steady VFA yield. The results highlight an interesting degree of flexibility of anaerobic fermentation systems where pH shift could enrich the bacterial community and direct the metabolic routes towards valuable bioproducts, such as butyric acid, with 13.97 g COD $L^{-1}$. Ultrafiltration technology was a feasible downstream alternative that enabled to obtain up to 26.6 g COD $L^{-1}$ VFA rich permeate.

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# CHAPTER 5

# FORWARD OSMOSIS TECHNOLOGY FOR DOWNSTRAM RECOVERING OF VALUABLE ACIDOGENIC FERMENTATION BIOPRODUCTS

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*The results were presented at Sustain Conference 2018, Technical University of Denmark (DTU), 28-29 November 2018.*

## 5.1 GRAPHICAL ABSTRACT

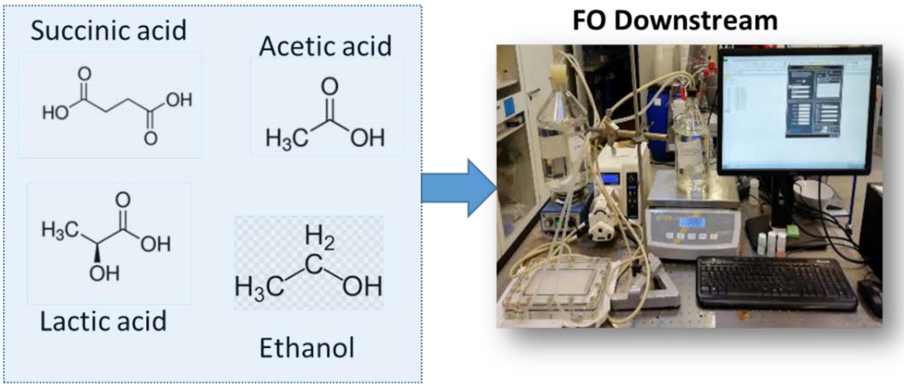

**Figure 5. 1**. Graphical abstract.

## 5.2 ABSTRACT

Anaerobic fermentation processes require competitive and cost-effective downstream technologies to recover the bioproducts from the fermentation broth. This study examined Forward Osmosis (FO) as a novel downstream solution to recover fermentation bioproducts. Binary model mixtures consisting of 20 g $L^{-1}$ of acetic acid (HAc), succinic acid (HSuc), lactic acid (HLac) and ethanol (EtOH), respectively, were tested as feed solution, at pH 3 and pH 7. Two membranes, namely, flat sheet cellulose triacetate and thin film composite hollow fibre were evaluated. The results obtained showed promising potential of FO technology in recovery of HSuc and HLac. Best results were obtained with hollow fibre thin film composite membranes at pH 3; with water removals > 84%. HSuc could be upgraded in a saturated solution of 71.3 g $L^{-1}$ and HLac reached a maximum concentration peak of 65 g $L^{-1}$. Additionally, pH 7, resulted in rejection rates higher than >99%, of the acids, by both types of membranes. In contrast, EtOH showed a different behaviour compared to pH dependent acids and a limited potential of FO upgrading.

## 5.3 INTRODUCTION

Anaerobic fermentation gives an opportunity to produce valuable biomolecules of industrial importance from organic waste in line with the bioeconomy approach. Among these biomolecules, succinic acid (HSuc), lactic acid (HLac) ethanol (EtOH) and acetic acid (HAc) have been highlighted as some of the most important chemicals from biomass. These bio-products are expected to contribute in mitigation of adverse climate effects and dependence on fossil fuels by substituting their petrochemical equivalents. HSuc can be produced from the sugars present on second and third generation biomasses (Marinho *et al.*, 2016, Luthfi *et al.*, 2017). HSuc was reported to be on the top 12 platform chemicals that can be produced from renewable resources (U.S. Department of Energy, 2004). This chemical can be polymerized with 1, 4 butanediol to produce polybutylene, a bioplastic that is expected to have a high economic growth in the following years (Sreedevi *et al.*, 2014). HLac is one of the most important organic acids for the biotechnology industry and it is precursor of products such as, acrylic acid, pyruvic acid, biosolvents and esters (Ryu *et al.*, 2008). Additionally, lactic acid can be used to produce polylactic acid, a biodegradable and biocompatible aliphatic polyester (Vandenberghe *et al.*, 2018). EtOH can be synthesized from sugar present in a variety of waste streams such as household food waste and agroindustrial waste (Beyene *et al.* 2018) and it is widely used as a biofuel but also as feedstock for the chemical industry. Acetic acid (HAc) a major intermediate can be produced from any feedstock during anaerobic digestion. As an example, HAc production was reported as the main product of sewage sludge alkaline fermentation (Liu *et al.*, 2018, Garcia-Aguirre *et al.*, 2019), which has shown potential application as intermediate chemical for the synthesis of vinyl acetate monomer, which is polymerized as polyvinyl acetate for extensive use in paints and adhesives (Pal and Nayak, 2016).

During anaerobic fermentation processes, usually the target chemicals are produced along production of other bioproducts, such as EtOH and HAc (Wu *et al*, 2017), or mixture of volatile fatty acids (VFA) resulting from mixed culture anaerobic fermentation (Garcia-Aguirre *et al.*, 2017). Additionally, fermentation processes performed by specific microbes, can be combined to produce the target chemicals, i.e. HSuc and EtOH (Kuglarz *et al.*, 2018). Overall, the product(s) concentrations are in the range of i.e. 0.19 - 30 g $L^{-1}$ (Lee *et al.*, 2014) for mix culture anaerobic fermentation. A lot of attention has been paid to improve the bioproduct titer such as using engineered bacterial strains. For example, HSuc concentrations titers of 146 g $L^{-1}$ were reported from glucose with genetically engineered R(Δ*ldh*A-pCRA717) (Jiang *et al.* 2017).

Currently, a major bottleneck of acidogenic fermentation processes is the final bioproduct recovery step (Atasoy *et al.*, 2018) constituting a significant cost of the overall process, and is largely dependent on the achieve titers in the fermentation broth. This bottleneck makes the fuels and chemicals produced from biomass less economically competitive compared to their petrochemical equivalents (Katafatakis *et al.*, 2017). Thus, it is of utmost importance to develop solutions to eliminate water from the fermentation mixture and make downstream solutions of biomass-derived chemicals competitive and cost-effective.

Forward Osmosis (FO) can be used as a novel application to extract water from fermentation broths. FO uses the natural pressure gradient that is stablished between two solutions with a different saline concentration. This driving force induces a water flux through a semi-permeable membrane, from the side of the low salinity (i.e. high solute) concentration or feed solution, to the side of high saline (low solute) concentration, or draw solution (Cath *et al.*, 2006). As a result, the feed solution is up-concentrated because water is moving to the more concentrated side. The main advantages of FO technology raise from the low energy requirement, low hydraulic pressure and the lower fouling propensity of the FO membranes compared to other membrane-based technologies, i.e. reverse osmosis or nanofiltration (Phuntsho *et al.*, 2013). Additionally, the water removal from fermentation broths will result in cost-effective distillation downstream, which consume 25 - 120 kWh $m^{-3}$ treated and hinder the current economic feasibility (Cho *et al.*, 2012). FO is an emerging technology that has shown potential application for desalination (Thabit *et al.*, 2019), wastewater treatment (Coday *et al.*, 2014) and food processing (*Petrotos and Lazarides* 2001). The application of FO as a downstream option in anaerobic fermentation processes is a novel approach that may lead to potential benefits and new market opportunities.

Regarding the few works available in literature, Jung *et al.* (2015) performed a study assessing cellulose triacetate asymmetric semipermeable membrane for the concentration of VFA and other bioproducts. The process was studied by altering the temperature, the pH, the concentration of draw solution and the type of draw solutes, providing valuable insight into the separation mechanisms of FO technology. Recently, the study of Law and Mohammad (2018) reported the high influence of pH in the rejection mechanisms of HSuc ions on cellulose triacetate based FO membrane, which could increase to optimal values, with a 100% rejection at higher pH than the $pK_{a2}$ value. Blandin *et al.,* (2019) reported optimal results for the upgrading of VFA using seawater and desalination brine draw solution using commercially available polyamide thin film composite membranes. Nonetheless, low VFA concentrations of 60 – 80 mg $L^{-1}$ were treated with aims of optimizing the microbial desalination cell.

Therefore, the aim of the current study was to provide a deeper insight and understanding regarding use of FO membranes as a technological solution for the downstream and upgrading of fermentation target chemicals (i.e. HAc, HSuc, HLac and EtOH). To accomplish this aim, a systematic study on the influence of pH on the water flux and transport mechanisms for the target chemicals was performed. Additionally, two different types of FO membranes (i.e. flat sheet cellulose triacetate based and thin film composite hollow fibre) were evaluated.

## 5.4 MATERIAL AND METHODS

### 5.4.1. Feed and draw solutions

For the FO tests, four chemicals were tested as feed solutions, which included HAc, HSuc, HLac and EtOH. The properties of the chemicals including the molecular weight, $pK_a$ value, hydrophobicity (log D) and Osmotic Pressure ($\Pi$) at pH 3 and pH 7 are detailed in Table 5. 1. The chemicals used during FO tests were analytical grade (Sigma Aldrich) with 99% purity. The feed solutions were prepared by dilution of the chemical with distilled water, up to a concentration of 20 g $L^{-1}$. Sodium chloride (Sigma Aldrich) was used as draw solution and the amount of salt was adjusted close to 1 M to keep the osmotic pressure difference constant at $\Delta P$ 43.5 bar in all FO tests. The osmotic pressure was calculated by using the Eq. 1. According to previous work, NaCl offers advantages due to its high solubility, non-toxicity and due to the fact that it is easy to up concentrate for reuse (Achili *et al.*, 2010, Chun *et al.*, 2017).

**Table 5. 1**. Properties of the analysed chemicals

| Compound | Acetic acid | Lactic acid | Succinic Acid | Ethanol |
|---|---|---|---|---|
| Formula | $C_2H_4O_2$ | $C_3H_6O_3$ | $C_4H_6O_4$ | $C_2H_5OH$ |
| Molecular weight (g mol$^{-1}$) | 60.05 | 90.08 | 118.09 | 46.07 |
| Pk$_a$ | 4.76 | 3.86 | 4.21, 5.63 | 15.9 |
| Log D (pH 3)* | -0.24 | -0.54 | -0.51 | -0.16 |
| Log D (pH 7)* | -2.65 | -3.52 | -4.92 | -0.16 |
| Π pH 3 (bar) | 8.1 | 4.8 | 4.5 | 12.0 |
| Π at pH 7 (bar) | 14.4 | 7.8 | 10.4 | 10.9 |

**Values were estimated by using the ChemAxon Marvin Sketch 17.27 software*

### 5.4.2. FO membranes

Flat sheet (FlS) membranes consisted of asymmetric cellulose triacetate (CTA) membranes provided by FTSH$_2$O Fluid Technology Solutions (Albany, OR, USA). These membranes have been widely used in FO tests (Ansari *et al.*, 2017, Jung *et al.*, 2015). The CTA active layer is supported with an embedded polyester screen mesh and has a thickness less than 50 µm and shows a hydrophilic nature, which contributes to reduce the internal concentration polarization (ICP) (McCutcheon and Elimeleh 2008, Xie *et al.*, 2018). As previously reported, the pore size of the membrane is 0.74 nm. During this study, the size of the membrane was adjusted to give a surface area of 0.014 m$^2$ and to fit the membrane chamber.

**Table 5. 2.** Summary table of the main characteristics of the FO membranes

| | Flat sheet | Thin film composite hollow fibre |
|---|---|---|
| Active layer | Cellulose triacetate | Aquaporin protein channels |
| Surface area | 0.014 m$^2$ | 0.3 m$^2$ |
| Water flux | N.A. | >7 L m$^{-2}$ h$^{-1}$ |
| Reverse salt flux | N.A. | < 2.5 g m$^{-2}$ h$^{-1}$ |

Additionally, thin film hollow fibre (TFHF) membranes (Aquaporin A/S, Copenhagen, Denmark) were tested. The hollow fibre membranes had a membrane surface area of 0.3 m$^2$ and were with biomimetic protein channels incorporated into proteoliposomes layer supported by a microporous structure (Zhao *et al.*, 2012). The aquaporin proteins are water selective and can operate with turnover rates up to 10$^9$ water molecules per second (Benga, 2009, Madsen *et al.*, 2015), which translates into a fast permeability. According to the manufactures, these membranes show a water flux, > 7 L m$^{-2}$ h$^{-1}$ with 1 M NaCl and a reverse salt flux of < 2.5 g m$^{-2}$ h$^{-1}$, against DI water and 300 mL min$^{-1}$ flow rate.

### 5.4.3. FO experimental procedure

Bench scale FO tests consisted in a counter-current configuration similar to previous work (Engelhardt *et al*., 2018, Lanbrecht and Sheldon, 2019). The orientation of the membrane was settled in FO mode, thus the feed solution facing the active side of the membrane. Feed solution and draw solution reservoirs were adjusted to 2 and 4 L respectively. The cross-flow rate was settled with a variable-speed peristaltic pump (Longer BT100, 2 pump heads YZ1515X) and a constant flow rate of 200 mL min$^{-1}$ was maintained. The draw solution was placed over a magnetic stirrer and the feed solution was placed on a digital scale (Kern Balance 572, + software balance connection 4), where the weight change was monitored over time and used to determine the water flux. Due to the lower surface area of flat sheet membranes, flat sheet FO tests were performed during 24 h, and thin film composite FO membrane tests were performed during 60 min. The pH of the feed was adjusted at the beginning of the tests either at 3 or 7, with NaOH or

HCl 4 M, respectively. An osmometer (Genotec Osmomat 030 Cryoscopic) was used to determine the osmotic pressure of feed solution and draw solution. The experiments were carried out at room temperature and all the air bubbles were eliminated at the beginning of the tests. A conductivity meter was used to qualitatively assess the increase of salt concentration in the feed caused by the reverse salt flux.

After feed solution and draw solution preparation, 20 mL samples were taken for conductivity and osmotic pressure measurement. Prior to FO tests, distilled water was run continuously through the membrane in order to clean the membrane chamber and remove any pollutants present in the system. During the FO tests with the flat sheet, the change of weight in the feed solution reservoir was recorded every 5 min. Due to higher water flux for the FO tests with the hollow fibre the change in weight was recorded every 10 seconds. Feed and draw solution tubing were emptied at the end of the tests and final samples were taken for subsequent measurement of conductivity, osmotic pressure and composition.

### 5.4.4. Analytical methods

The composition of HAc, HSuc, HLac and EtOH mixtures was analysed by HPLC (Ultimate 3000, Thermo Scientific) with a refractive index detection, equipped with a resin based sulfonated divinyl benzene-styrene–hydrogen column (Aminex HPX-87H300 x 7.8 mm, BIO-RAD), under isocratic conditions and sulphuric acid (0.005 M) as eluent. The detection of the chemicals is achieved by refractive index detection using a RefractoMax 521 (ThermoScientific) operated at 35 degrees. The flow in the HPLC was kept constant at 0.6 mL min$^{-1}$, the column compartment at 50 ºC. Due to the clean composition of the mixtures, no pre-treatment of samples was needed. 10 µL of sample were injected and dilutions were made to operate in the HPLC range of 1 – 10 g L$^{-1}$.

### 5.4.5. Calculations

The osmotic pressure of feed solution and draw solution was calculated based on the Van't Hoff equation:

$$\Pi = iMRT \qquad \text{(Eq. 1)}$$

Where *i* is the Van't Hoff factor, M the molar concentration of the solution, R is the ideal gas constant and *T* the temperature. The osmotic pressure values were used to adjust the NaCl concentration and provide a $\Delta P$ of 43.5 bar. The water flux, $J_w$ (L $m^{-2}$ $h^{-1}$) was quantified by measuring the amount of water transported from the feed to the draw solution over time, by using the following equation (Schneider *et al.*, 2019):

$$J_w = \frac{\Delta V_f}{\rho A \Delta t} \qquad \text{(Eq. 2)}$$

Where, $\Delta V_f$ is the change in volume (g) of feed solution; ρ is the density of water (g $L^{-1}$), *A* is the surface side ($m^2$) of the membrane and $\Delta t$ (h) accounts for the difference in time between measurements. An important aspect to assess is the up-concentration effect of feed solution as a result of water transportation through the membrane from the feed to the draw solution side. Herein, the indicators proposed by Blandin *et al.*, (2019) were employed, i.e. the water concentration factor (WCF) and the solute concentration factor (SCF):

$$\mathrm{WCF} = \frac{V_{\mathrm{feed,\,0}}}{V_{\mathrm{feed,\,f}}} \qquad \text{(Eq. 3)}$$

$$\mathrm{SCF} = \frac{C_{\mathrm{solute,\,0}}}{C_{\mathrm{solute,\,f}}} \qquad \text{(Eq. 4)}$$

$V_{\mathrm{feed\ 0}}$ and $V_{\mathrm{feed\ f}}$ (L) represent the initial and final volume of the feed solution, respectively. Similarly, $C_{\mathrm{solute\ 0}}$ (g $L^{-1}$) and $C_{\mathrm{solute\ f}}$ (g $L^{-1}$) represent the concentration of the solute at the beginning and at the end of the FO test, respectively. Additionally, the rejection rate of the solutes was calculated based on the expression of Engelhardt *et al.*, (2018):

$$\text{Rejection} = 1 - \frac{V_{ds} \cdot C_{ds}}{V_w \cdot \frac{C_{f0} + C_{fe}}{2}} \cdot 100 \qquad \text{(Eq. 5)}$$

Where $V_{ds}$ (L) and $C_{ds}$ (g $L^{-1}$) represent the final volume of the draw solution and the concentration of solute in the draw solution, $V_w$ (L) is the volume of water transported through the membrane, $C_{f0}$ (g $L^{-1}$) and $C_{fe}$ (g $L^{-1}$) represent the concentration of solute in the feed solution and the beginning and at the end of the test.

## 5.5 RESULTS AND DISCUSSION

### 5.5.1. Water flux, reverse salt flux and water removal

FO technology enabled to remove high amounts of water from the feed solution to the draw solution side due to the osmotic pressure difference that is generated between both solutions, which results in a water flux across the semipermeable membrane. According to Table 5. 3, for the same initial osmotic pressure difference ($\Delta\Pi$ = 43.5 bar), the type of membrane, i.e. flat sheet or thin film composite hollow fibre; the properties of the solute (e.g. the molecular weight, $pK_a$) and the pH of the mixture, had a marked effect in the water flux.

#### 5.5.1.1. Water flux

Overall, higher water fluxes were obtained with thin film composite hollow fibre membranes at the beginning of the tests (11.6 - 15.6 L $m^{-2}$ $h^{-1}$), compared to the water fluxes with flat sheet membranes (6 - 7.22 L $m^{-2}$ $h^{-1}$) **(**Figure 5. 2). As observed the dilution effect in the draw solution, reduces the osmotic pressure difference overtime resulting in a decrease in the water flux (Figure 5. 2 a-d). The dilution effect can be avoided by increasing (Law and Mohammad, 2018) the concentration of the draw solution; or by keeping the concentration of draw solution constant (Abousina and Nghiem, 2014); or using different draw solutes (Achili *et al.*, 2010, Ansari *et al.*, 2015).

The feed solution pH had a marked effect in the water flux obtained, which is in agreement with previous work performed by Law and Mohammad (2018).

For the organic acids (HAc, HSuc and HLac), the highest water flux was observed at pH = 3 – which is well below the acids pKa´s (see Table 5. 1) – at which mainly the unionised (free) form of the acids (R-COOH) is present.

**Table 5. 3**. Water flux and water removal during FO tests

| | | Water flux (L $m^{-2}$ $h^{-1}$) | | Water removal (%) | |
|---|---|---|---|---|---|
| | | FlS | TFHF | FlS | TFHF |
| HAc | pH 3 | 5.72 ± 0.88 | 6.31 ± 2.97 | 94.46 | 95.03 |
| | pH 7 | 2.89 ± 1.22 | 4.43 ± 3.46 | 49.68 | 67.9 |
| HSuc | pH 3 | 4.70 ± 1.57 | 5.71 ± 2.55 | 66.98 | 83.99 |
| | pH 7 | 4.35 ± 1.41 | 4.87 ± 4.27 | 66.84 | 68.9 |
| HLac | pH 3 | 5.39 ± 1.12 | 6.12 ± 4.32 | 84.55 | 84.33 |
| | pH 7 | 3.98 ± 1.27 | 5.64 ± 4.80 | 69.35 | 78.15 |
| EtOH | pH 3 | 1.85 ± 0.45 | 6.19 ± 3.96 | 31.76 | 96.84 |
| | pH 7 | 6.12 ± 1.87 | 6.48 ±4.71 | 98.89 | 98.5 |

At pH 3, the water flux was influenced by the characteristics of the chemicals, which resulted in higher fluxes for lower molecular weight solutes, i.e. HAc > HLac > HSuc (Table 5. 3). Contrarily, at pH 7, the anionic nature of the solutes reduced the water flux. This can be related to the interaction of the chemicals with the membrane and the ICP phenomenon. The $Ac^{-}$, $Lac^{-}$ and $Suc^{-2}$ contribute to the ICP, which occurs at the porous layer of the membrane, reducing the osmotic pressure potential and the subsequent water flux. As previously reported, ICP is a complex phenomenon affected by the characteristics of the membrane, i.e. thickness, and porosity, which occurs with all types of feed solutions including deionized water (Achili *et al.*, 2010) and it is more severe when the active layer faces the feed solution.

Chun *et al.* (2017) reported that the ICP effect was enhanced due to the adjective water flux and direct diffusion that brings the solutes that enter and exit the membrane into the surface layer, which builds-up solutes within the porous layer. The lower water flux obtained at pH 7, might be related to the ICP effect.

EtOH showed a different behaviour compared to pH dependent solutes, suggesting that the water flux is also dependent on the specific characteristics of the solute (Jung *et al.*, 2015) and its interaction with the surface layer of the membrane. This was especially significant for flat sheet membranes, at the same initial osmotic pressure difference ($\Delta\Pi$ = 43.5 bar), EtOH provided different water fluxes of 2 L m$^{-2}$ h$^{-1}$ for flat sheet at pH 3, and 6.1-6.8 L m$^{-2}$ h$^{-1}$ at pH 7. The pH of the medium and complementary anions related to pH adjustment might be responsible for the reported differences.

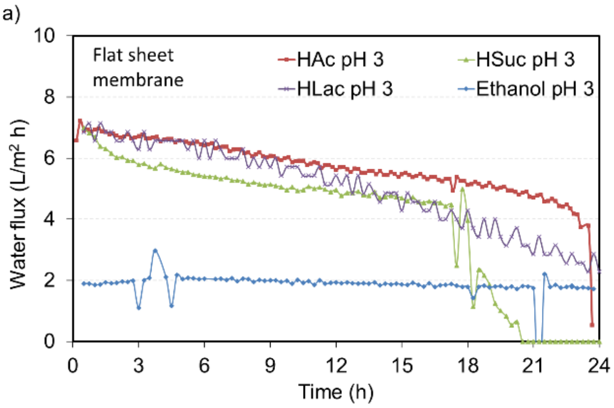

**Figure 5. 2.** Water flux of flat sheet membrane tests at pH 3 (a)

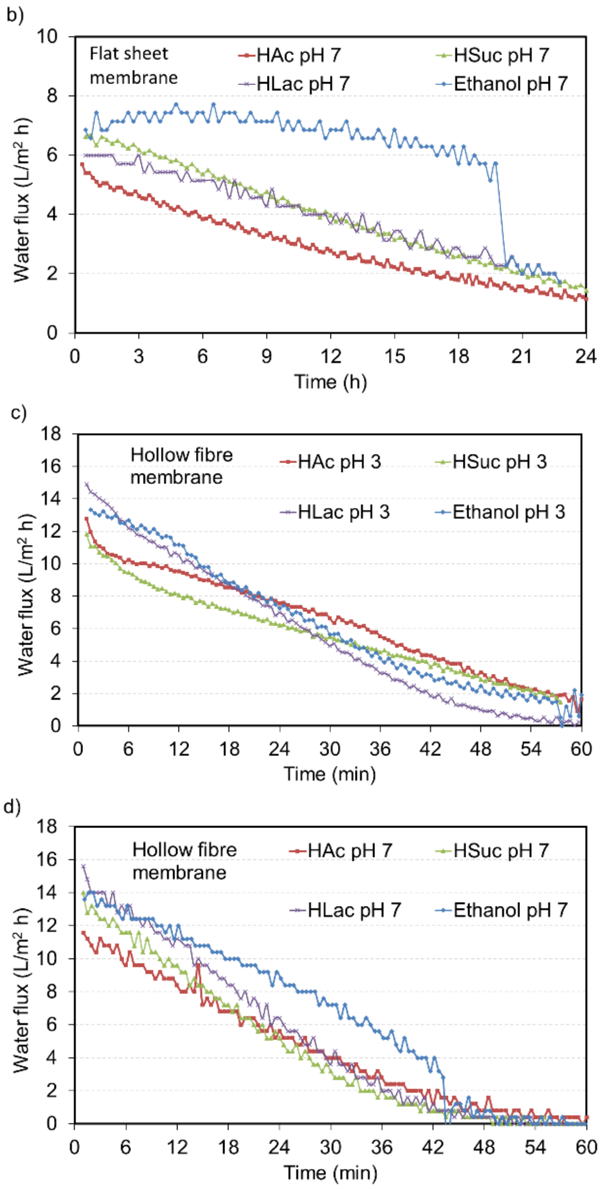

**Figure 5. 2** (Continued). Water flux of flat sheet membrane tests at pH 7 (b). Water flux of hollow fibre FO tests at pH 3 (c) and pH 7 (d).

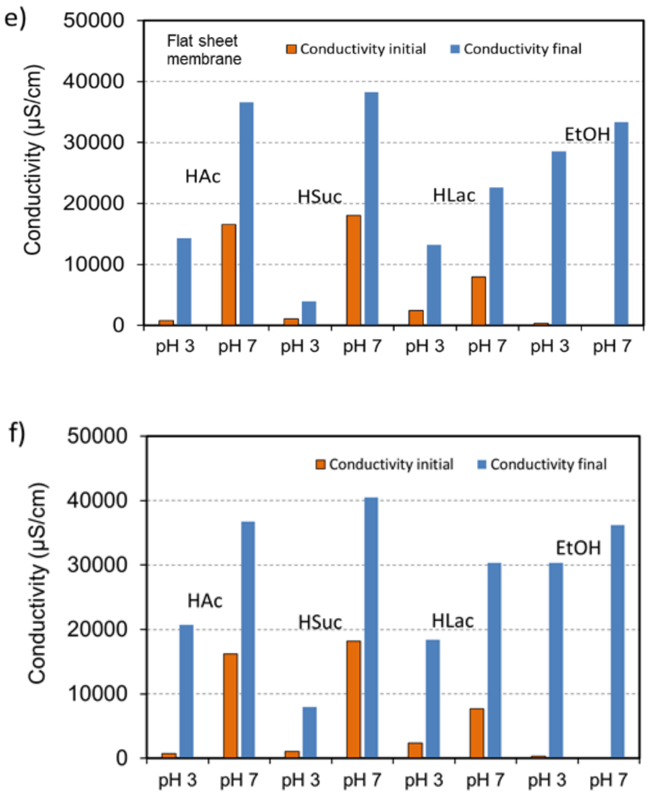

**Figure 5. 2** (Continued). Conductivity of flat sheet (e), conductivity hollow fibre tests (f).

#### 5.5.1.2. Reverse salt flux

Besides water flux from feed to draw solution, salts of the draw solution might also diffuse back to the feed side, creating a reserve salt flux (Hancock and Cath, 2009), contributing to the ICP effect and as a consequence reducing the water flux. As shown in Figure 5. 2 e-f, the reverse salt diffusion could be qualitatively assessed by the increase in the conductivity of the feed solution at the end of the FO tests. The reverse salt diffusion is the result of the high concentration difference between draw solution and feed solution and it was correlated with the diffusion coefficient of the draw solute (Achili *et al.*, 2010).

During these tests, and due to the low anionic size of NaCl and higher diffusion coefficient, significant reverse salt flux occurred (Ansari *et al.*, 2015). The reverse salt flux was moderately higher during thin film hollow fibre membrane tests and pH 7, where the final conductivity increased up to 30 - 40 mS $cm^{-1}$. Augustine *et al.* (2017) also reported a higher reverse salt flux of thin film hollow fibre membranes, compared to the flat sheet CTA membranes during winery wastewater treatment. However, despite the contribution to the ICP effect, the high specific area of the membrane and hollow fibre enabled to obtain highest water flux.

### 5.5.1.3. Water removal

In line with the water flux, optimal water removals were obtained at pH = 3 tests. Both flat sheet and hollow fibre membranes enabled high water removals, of 95% and 84% of water from the HAc and HLac mixtures. However, flat sheet membranes required a significant higher amount of time, 24 hours compared to the 60 minutes of hollow fibre membranes. The high water removals of HAc and HLac mixtures resulted in final volumes of 100 and 320 mL respectively. Similarly, 84% of water was removed from the HSuc mixture with the hollow fibre membrane (Figure 5. 3a).

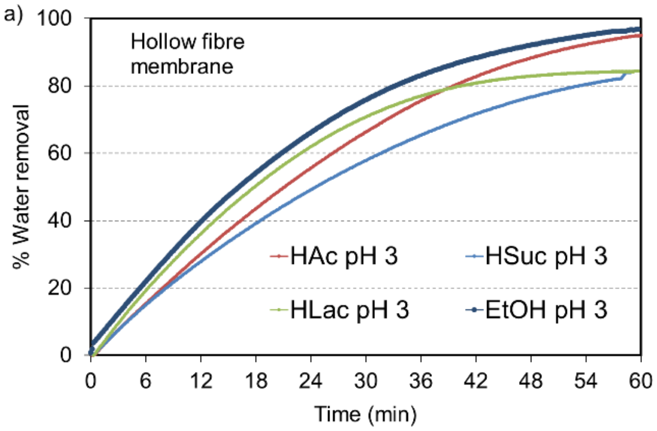

**Figure 5. 3.** Percentage of water removal during hollow fibre FO tests at pH 3 (a)

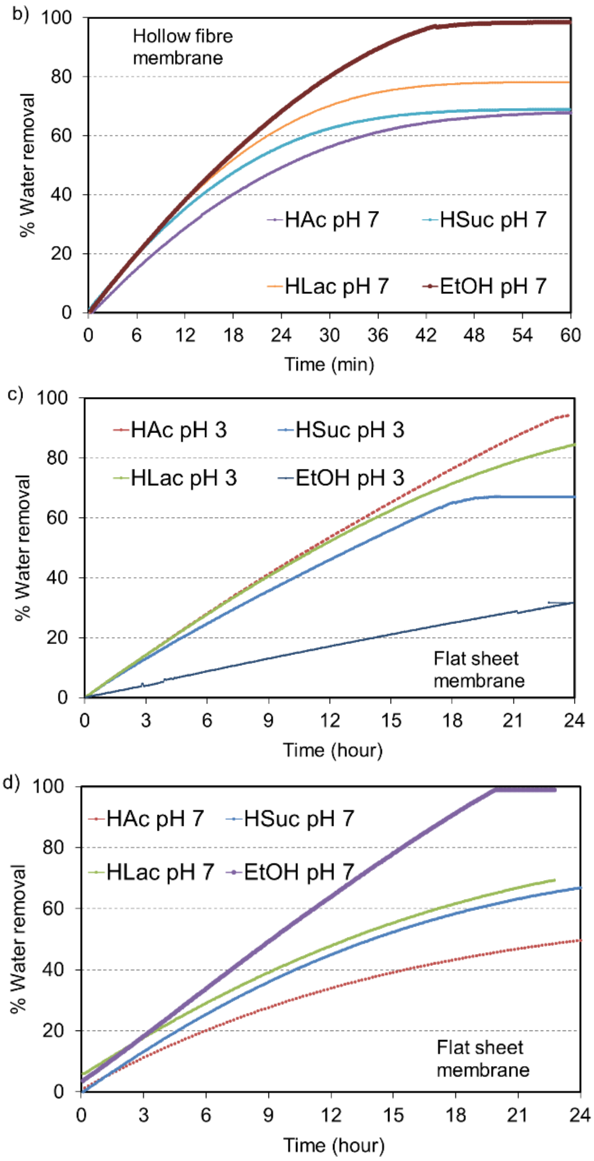

**Figure 5. 3**. (Continued). Percentage of water removal during hollow fibre FO tests at pH 7 (b). Water removal during flat sheet tests at pH 3 (c) and pH 7 (d).

In contrast, the lower water flux at pH 7 resulted in lower water removals from the mixtures. The amount of water removed during flat sheet ranged within 50 - 69% and 67 – 78% during hollow fibre membranes, which resulted in final volumes of 610 – 1,010 mL and 440 – 660 mL respectively.

EtOH mixtures showed a marked behaviour, enabling the highest water removals for both membrane configurations at pH 7, which resulted in final volumes < 30 mL. Remarkably, the lower water flux obtained at pH 3 with the flat sheet membranes, enabled after 24 hours only 32% of water removal, indicating that FO technology was not suitable for EtOH upgrading with CTA membranes at acidic conditions.

### 5.5.2. Rejection rate of membranes

Despite the high water fluxes, the performance of FO technology needs to be assessed by evaluating the rejection rate of the membranes, which enable to retain the target chemical in the feed solution. According to the results, the rejection of chemicals was influenced by pH and it was optimal at pH 7, since the ionised form of the target acids is repulsed by the negative charge of the surface layer, keeping them in the feed solution, ending to their up-concentration.

#### 5.5.2.1. Rejection of CTA-FO membranes

During acidic tests with flat sheet membranes, the undissociated forms of HAc, HSuc and HLac molecules could penetrate the active layer of flat sheet membranes. A 0.997 $R^2$ suggests that steric dependent rejection was the separation mechanism for HAc, HSuc and HLac (Figure 5. 4a). It is obvious that the chemical rejection was associated with the pore size of the membrane (Engelhart *et al.*, 2018). The results indicated that larger molecules, i.e. HLac, were better rejected than small size molecules. Herein, HAc showed the lowest rejection of 1.5%. Recently, Blandin *et al.* (2019), came to a similar conclusion, reporting better rejection rates for higher molecular weight VFA, i.e. butyric acid > HAc. Additionally, the authors highlighted the complexity of separation mechanisms, since the geometry of the chemical also influenced the rejection mechanism, for example, n-butyric acid was better rejected than iso-butyric acid. This may also explain the higher rejection of EtOH at pH 3 than HAc, which might be related to the specific interaction of EtOH with the surface material of CTA membrane.

In contrast, at pH 7, the amount of solute measured in the draw solution was negligible for all pH dependent chemicals indicating that $Ac^-$, $Lac^-$ and $Suc^{-2}$, anions could not penetrate the active layer of CTA membrane. The 100%

evidences the strength of electrostatic repulsion of the anionic charges of the solutes with the negatively charged surface of CTA membranes (Jung *et al.*, 2015, Law and Mohammad, 2018). Abousina and Nghiem (2014), reported an increasing rejection of HAc alongside the pH increase. Additionally, the same authors suggested that the intricate relationship between the ionic strength of the solutes, the type of membrane surface and charge, and the solution pH, were the factors governing the separation mechanism. A recent work of Law and Mohammad (2018) indicated a higher rejection of succinate of cellulose triacetate FO membranes at higher pH, which was independent of the concentration of anions present in the feed solution. At pH values higher than the $pKa_2$ a strong succinate rejection was observed by the larger size hydrated succinate anions dominating the feed solution. Additionally, the divalent succinate anions also contributed to the stronger electrostatic repulsion mechanism.

According to previous work, the hydrophobicity (Log D) of the chemicals also contributed to the enhancement of the rejection rate at higher pH (Engelhart *et al.*, 2018). However, the tested chemicals are hydrophilic, which tend to dissolve in water and as shown in Figure 5. 4c and d, there was not a clear correlation between the hydrophilicity and the rejection rate of the membranes.

Surprisingly, the results suggest that the pH of the feed solution also influenced the rejection behaviour of EtOH during flat sheet FO tests. At pH 3, a 7.8% was reported, which increased up to 36.4 % at pH 7. EtOH is a pH independent solute, and thus, it is hypothesized that there was a compensation effect of additional anions present in the medium, i.e. $OH^-$, which result from the NaOH dosing during pH adjustment at pH 7. The presence of negative anions contributed to the electrostatic repulsion at the surface material of the membrane, and likely affected the EtOH rejection.

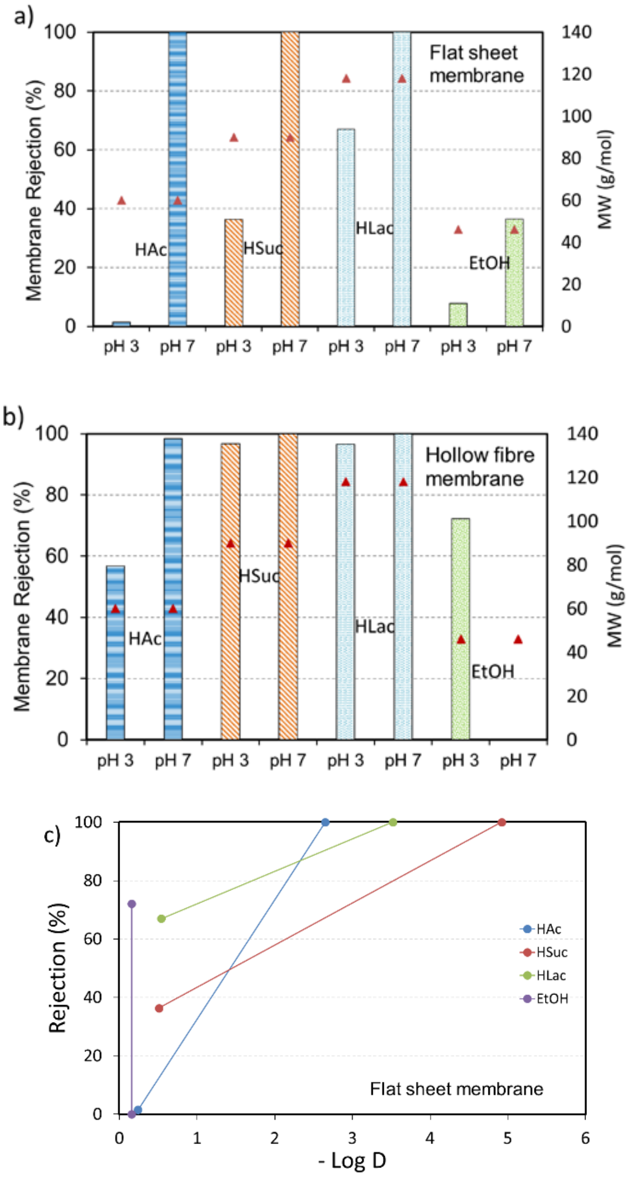

**Figure 5. 4**. Membrane rejection rate of FlS (a) and TFHF tests (b). Rejection rate in function of the hydrophobicity of the solute for FlS (c).

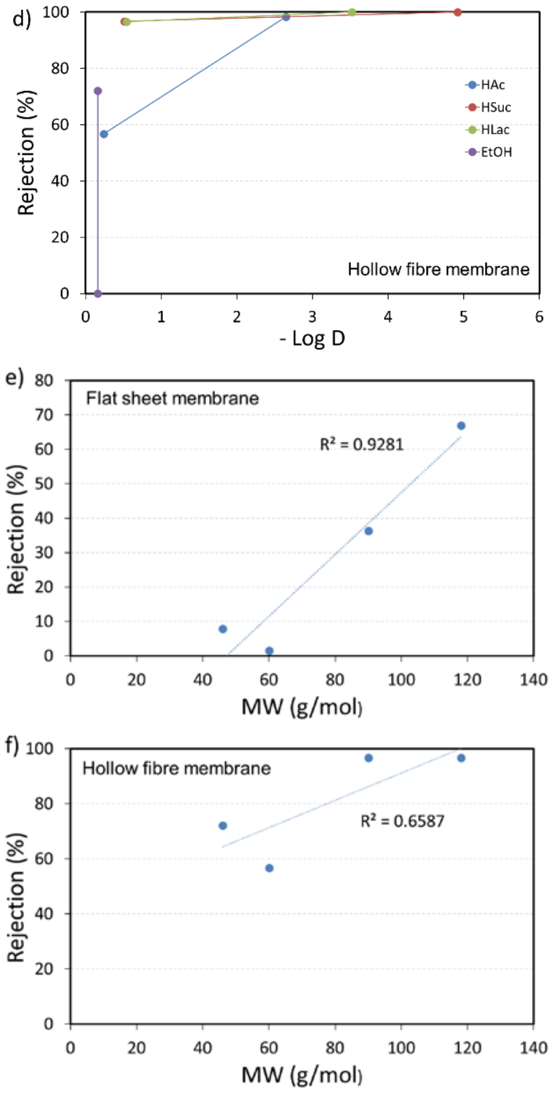

**Figure 5. 4** (Continued). Rejection rate in function of the hydrophobicity of the solute for TFHF (d). Rejection rate at pH 3, in function of the molecular weight for FlS (e) and TFHF (f).

### 5.5.2.2. Rejection of thin film composite hollow fibre membranes

During thin film hollow fibre tests, the rejection values at acidic conditions were higher, and $Ac^-$, $Suc^{-2}$ and $Lac^-$ rejections accounted for 56.7, 96.7 and 96.6 %, respectively. At acidic pH, lower molecular weight HAc and EtOH rejection was higher compared to flat sheet membrane tests, but the molecules could still pass the active layer of the thin film hollow fibre membranes and be transported to the draw solution. $R^2$ 0.65 suggests a lower influence of molecular weight in the diffusion of molecules, although molecular sieving had a significant impact in the diffusion through the membrane. According to Xie *et al.*, (2018) non-ionic contaminants transport mechanism was dominated by the molecular size and steric hindrance on the hollow fibre membranes. However, in their study, only high molecular weight molecules were tested, such as dioxan, erythritol and xylose (88.1 - 150 g $mol^{-1}$).

Similar to previous CTA FO tests, the rejection at pH 7, accounted for 98.3 % for HAc, and 100% for succinic and lactic acids. The results highlight the efficiency of the protein channels in selectively only permitting the transport of water through the membrane and influencing the negative charge on the active layer of the membrane and rejecting organic solutes at pH 7. Contrarily to previous flat sheet membranes, there was not EtOH rejection at pH 7, which means that the hollow fibre were not able of retaining the solute and EtOH could pass through the selective layer of the membrane.

Overall, the results show a clear influence of membrane material composition, the molecular weight of the chemicals, the pore size of the membrane, and electrostatic repulsion in the rejection mechanisms. Additionally, Engelhart *et al.* (2018) also proposed that structural differences between flat sheet and hollow fibre membranes may influence the rejection characteristics, which might have also help explain the differences observed in this study. Regarding the influence of pH in the rejection mechanisms, neutral pH was optimal to obtain a 100% rejection rate for pH dependent chemicals.

### 5.5.3. Upgrading of the effluents

Regarding the final concentrations of the mixtures (Figure 5. 5 a-b), FO technology showed a high potential for up-concentrate the target chemicals. From the initial concentration of 20 g $L^{-1}$ of chemical that could be expected from anaerobic fermentation processes, the final concentration could be increased up to 33 – 71 g $L^{-1}$ and the up-concentration potential was directly linked to the rejection of the membranes.

Regarding the results of flat sheet membrane tests, maximum concentrations were obtained at pH 7 for HSuc, followed by HLac and HAc, with 52.6 g $L^{-1}$, 36.3 g $L^{-1}$ and 33.4 g $L^{-1}$. This resulted in a Solute Concentration Factor (SCF) of 2.65, 2.77 and 1.81, respectively. These results are in line with the values reported by Ruprakobkit *et al.* (2016) where the authors reported a good agreement between experimental data and the developed model to assess the concentration of carboxylic acids. Specifically, 1.65 and 2.5 concentration factors were reported for acetic and lactic acid by using thin film composite membranes during 30 h tests. Additionally, Jung *et al.*, stated that VFA mixtures could be up concentrated from 35 g $L^{-1}$ to 65 g $L^{-1}$, with a SCF of 1.87.

The up-concentration effect of hollow fibre membranes were optimal at pH 3 and enhanced the results of previous flat sheet membranes. Very high concentrations of target chemicals were obtained after FO treatment where HAc mixture reached a peak value of 51 g $L^{-1}$, and HLac was concentrated to maximum value of 65 g $L^{-1}$. The results were noteworthy during HSuc test at pH 3. Herein, the effluent reached the saturation point and succinate salt crystals were obtained in the concentrated effluent (Li *et al.*, 2010) which contained a HSuc concentration of 71 g $L^{-1}$. The results of hollow fibre membranes at pH 7 were slightly lower than acidic values, although high up-concentration effects of 48 – 66 g $L^{-1}$ for HAc, HSuc and HLac were possible. These are high solute concentrations, and significantly higher than values achieved from acidogenic fermentation processes (Lee *et al.*, 2014).

Additionally, the best results of EtOH mixture upgrading were obtained with hollow fibre membranes, which reached maximum concentration of 34 g $L^{-1}$ at pH 3. Overall, the SCF during hollow fibre membrane tests were significantly higher than previous flat sheet test, ranging within 1.64 - 5.34. The high SCF are indicative of the potential of FO membranes for the target application.

### 5.5.4. Evaluation of the FO technology

The performance of FO technology needs to be assessed by considering all the above mentioned factors. According to Table 5. 4, promising results were obtained regarding the application of FO technology for upgrading fermentation effluents, especially for HSuc and HLac.

Table 5. 4. Summary table of optimal conditions of FO tests.

| | **Flat sheet** | | | | **Hollow fibre membrane** | | | |
|---|---|---|---|---|---|---|---|---|
| | Hac | HSuc | HLac | EtOH | Hac | HSuc | Hlac | EtOH |
| WCF | 1.99 | 2.65 | 6.47 | 28.57 | 20.13 | 6.25 | 6.38 | 29.9 |
| SCF | 1.81 | 3.02 | 2.56 | 0.8 | 2.66 | 3.49 | 5.34 | 1.64 |
| Optimal pH | 7 | 7 | 7 | 3 | 3 | 3 | 3 | 3 |
| Rejection (%) | 100 | 100 | 100 | 7.8 | 56.7 | 96.7 | 96.6 | 72.1 |
| Titer (g $L^{-1}$) | 33.4 | 52.6 | 36.3 | 21.2 | 51 | 71.3 | 64.9 | 33.9 |

#### 5.5.4.1. Chemical upgrading of flat sheet membranes

According to the results, optimal values were obtained for HLac, HSuc and HAc at pH 7. Best results were obtained for HLac, with a water and solute concentration factors (WCF and SCF) of 6.47 and 2.56, with a final volume of 309 mL. The WCF and SCF represented, 2.65 and 3.02 respectively, for HSuc, with a final volume of 663 mL and the SCF factor obtained was higher than the values reported by Jung *et al.* (2015) of 1.58. However, during their study, the initial concentration was 105 g $L^{-1}$ of HSuc and 5 M NaCl was used as draw solution. The WCF and SCF attained for HAc were lower, with 1.99 and 1.81, which resulted in a final volume of 1006 mL. Despite the high WCF of 18 for HAc at pH 3, the low rejection rate of the membranes indicated a poor performance. It should also be noted that significant amounts of HAc were lost during the tests by evaporation, i.e. 7.7 – 9.0%, since at pH 3, the unionised HAc can evaporate.

Flat sheet membranes demonstrated poor performance for EtOH concentration. For example, despite the highest water flux obtained at pH 7, i.e. WCF 28.57, the SCF was only 0.8 indicated a product lost and poor performance of FO technology. According to the mass balance, 42.5% of EtOH was lost by evaporation.

Overall, flat sheet membranes and FO technology enabled to upgrade the HLac and HSuc mixtures, where best results were obtained at pH 7. Contrarily, lower performances were obtained for HAc, and EtOH.

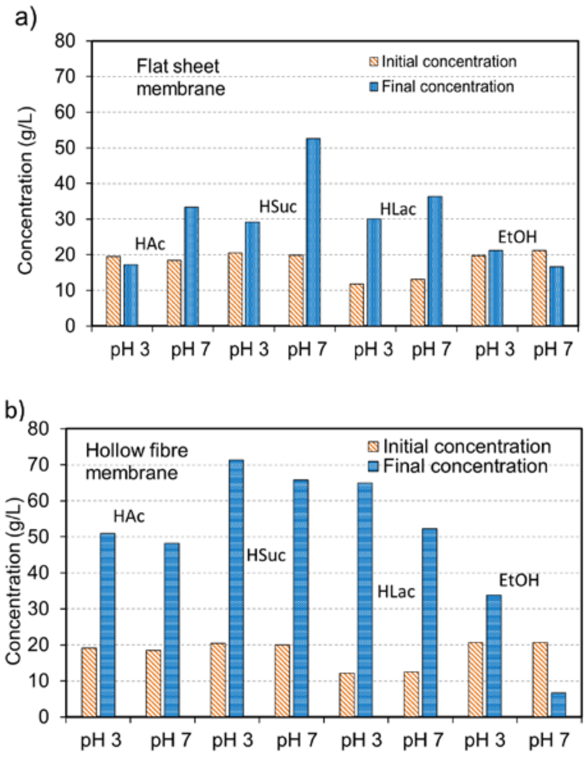

**Figure 5. 5.** Initial and final concentration of the FO tests during flat sheet FO tests (a) and TFHF tests (b).

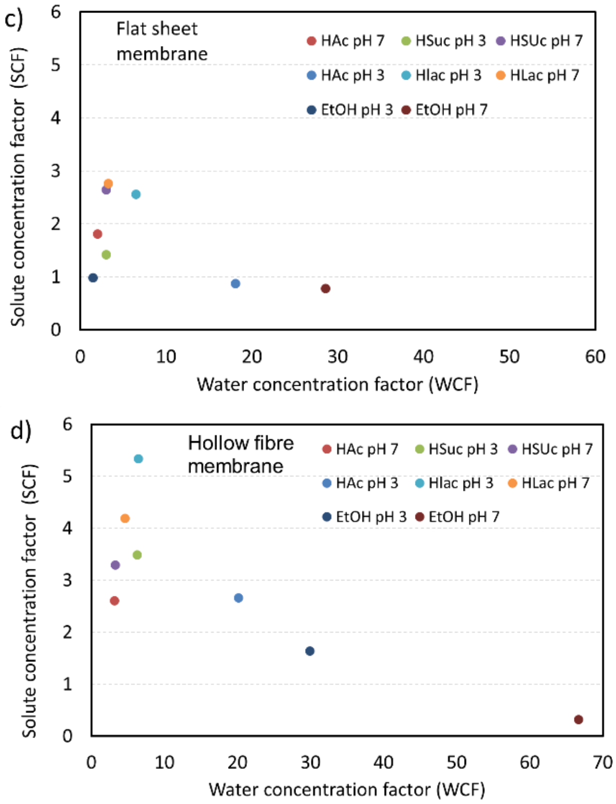

**Figure 5. 5** (Continued). Solute concentration factor in function of the WCF for FlS membrane (c) and for TFHF membrane (d)

#### 5.5.4.2. Chemical upgrading of thin film composite hollow fibre membranes

The results of hollow fibre were optimal compared to flat sheet tests and interestingly, enhanced performances were obtained for HSuc and HLac at pH 3 (Figure 5. 5d). The results of HSuc were noteworthy, were the WCF and SCF increased up to 6.25 and 3.49 respectively. Herein, a highly concentrated and saturated solution of 320 mL was recovered. The WCF and SCF increased up to 6.38 and 5.34 for HLac, were 313 mL of a high concentrated solution were obtained. Additionally, promising results were obtained at pH 7, with slightly lower SCF of 3.29 – 4.19, but which enhanced the values of previous flat sheet tests. Regarding the results of HAc and EtOH, high WCF were obtained for both solutes which increasing up to 20.1 and 29.9 WCP at pH 3. However, the amount of mass lost by evaporation reached 16 and 20% respectively. Thus, under the conditions tested in this study, EtOH and HAc were not adequate to be up concentrated by means of FO technology.

## 5.6 CONCLUSIONS

The results obtained showed promising potential of FO technology as a first step in the downstream of HSuc and HLac. The up-concentration of the target chemicals by using FO membranes, could change the feasibility of subsequent chemical purification, as for example by distillation, and become FO technology a core downstream solution to recover valuable bioproducts from fermentation mixtures. Thin film composite hollow fibre membranes enabled the highest water fluxes and highest up-concentration effect, where HSuc could be recovered in a saturated solution of 71.3 g $L^{-1}$ of solute and remaining succinate salt crystals. In addition HLac could be upgraded up to 65 g $L^{-1}$ with optimal WCF and SCF. The pH was a key factor influencing the separation mechanism, the behaviour of solutes in contact with the active layer of the membranes and determining the water flux. At pH 3, size exclusion was the main separation mechanism while chemical speciation and electrostatic repulsion were dominant at pH 7 for pH dependent solutes. In contrast, lower performances were obtained for EtOH upgrading and significant evaporation was obtained during HAc processing. Further studies should be conducted in order to specifically assess the effect of draw solute in the water flux and reverse salt flux, and to optimize the FO upgrading process.

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# CHAPTER 6

# RECOVERY OF SUCCINIC ACID, LACTIC ACID AND ETHANOL FROM FERMENTATION BROTHS BY FORWARD OSMOSIS MEMBRANES

The content of this Chapter has been submitted to Environmental Science and Technology:

*Garcia-Aguirre, J., Alvarado-Morales, M., Fotidis, I.A., Angelidaki, I. 2018. Recovery of succinic acid, lactic acid and ethanol from fermentation broths by forward osmosis membranes. Environmental Science and Technology.*

## 6.1 GRAPHICAL ABSTRACT

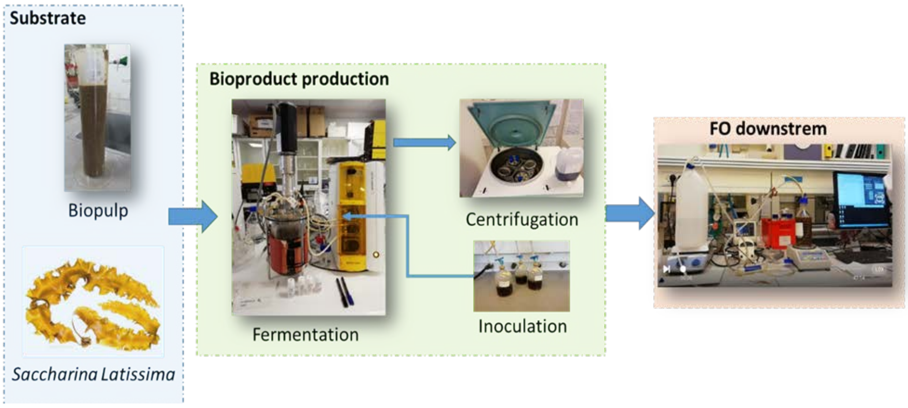

**Figure 6. 1**. Graphical abstract.

## 6.2 ABSTRACT

In this study, the potential of Forward Osmosis (FO) technology for the recovery of biofermentation products, namely, succinic acid, lactic acid and ethanol was tested. First, fermentation tests were produced. Lactic acid and ethanol were obtained from municipal organic waste or biopulp, and third generation biomass, i.e. Saccharina Latissima macroalgae was used for succinic acid production. Herein, bioproduct concentration titers of 14.98 g $L^{-1}$ of lactic acid, 19.11 g $L^{-1}$ of ethanol and 38.8 g $L^{-1}$ of succinic acid were obtained. Second, the fermentation broths were centrifuged and concentrated using thin film composite hollow fibre FO membranes, with different concentrations of NaCl, i.e 1.5 and 5 M, as draw solution. The results evidenced the potential of FO technology to achieve solute concentration factors of 4 – 5.5 and water concentration factors of 3.6 – 6.75. Best results were obtained with succinic acid fermentation broth, which resulted in a final bioproduct titer of 186.7 g $L^{-1}$ and 85% of water removal from the mixture. Remarkable enhancement of lactic acid concentration was obtained, with 57.6 g $L^{-1}$, while significant mass was evaporated during the downstream of ethanol. The findings of this study, highlight the potential of a novel application of FO membranes, in the downstream of fermentation products.

## 6.3 INTRODUCTION

In a petroleum based economic model, the need of oil supply and the market fluctuations produce continuous instability and geopolitical issues (IAGS, 2019). Currently, oil based refineries are essential to produce the daily consumer goods and fuels. However, a transition towards a sustainable society is compulsory to meet the Sustainable Development Goals and avoid the climate change. In the mid-term future, biorefineries will constitute an attractive platform to develop a bio-based economy, since these are analogous to oil based refineries, where a number of integrated processes are used with biomass as feedstock to produce energy, biomaterials and a variety of chemicals, reducing the dependence on fossil fuels (Clark and Deswarte, 2015).

Among different bioproducts, lactic acid and succinic acid were envisioned as key platform chemicals (Datta and Henry, 2006, U.S. Deparment of Energy, 2004). As shown in Table 6. 1, these chemicals are used in a wide range of applications and their market growth prospects are promising. In addition, these biochemicals can be produced with energy efficient anaerobic fermentation processes (Gunnarsson *et al.*, 2014, Jiang *et al.*, 2017) from different sources of organic waste that are rich in carbohydrates but avoiding the use of edible crops. Currently, 80 – 90% of lactic acid is produced through biological fermentation using edible crops (Valdes de Oliveira *et al.*, 2018). In addition, the production of succinic acid relies on maleic anhydride, which is a derivative of oil.

One of the most studied bacterial strain for sustainable succinic acid production is *Actinobacillus succinogens*, which can produce mainly succinic acid from glucose (Sun *et al.*, 2014). Marinho *et al.* (2016) obtained a succinic acid titer of 36.8 g $L^{-1}$ with carbohydrate rich 3rd generation macroalgae. Additionally, among different strains, *Lactobacillus delbrueckii was reported to* produce mainly the isomer L-lactic acid, which is desirable for PLA production and other commercial applications (Singhvi *et al.*, 2018). This bacterial strain can produce significant amounts of ethanol during fermentation of carbohdyrates (Zhang *et al.*, 2016), which is a valuable biofuel (Table 6. 1).

During fermentation, process inhibition can occur due to the bioproduct accumulation. Besides, regarding the bioproduct recovery from fermentation broths, this can account for 50% of the overall process cost (Komesu *et al.*, 2015, Law *et al.*, 2019) and thus, bioproduct concentrations ranging within 120 – 150 g $L^{-1}$ are required to make the biofermentation processes cost-effective and competitive. Currently, there is an existing gap between the production capacity and the current bioproduct production, related to the up-scaling and subsequent separation step (Dusselier *et al.*, 2013).

**Table 6. 1.** Applications and market share of lactic acid, succinic acid and ethanol.

| Chemical | Applications | Market share | References |
|---|---|---|---|
| **Lactic acid** | • Food additive (Acidulant, preservative, emulgator)<br>• Biopolymer production (PLA). Chemical intermediates (acrylic acid, propylene glycol, 2,3 pentanedione, 1, 2 –propanodiol acetaldehyde, pyruvic acid, lactide etc.).<br>• Esters (alkyl lactates) | • Demand: 1960 kt (2025)<br>• Market size: USD 8.77 billion | Varadarajan *et al.*, 1999<br>Dusselier *et al.*, 2013<br>Alves de Oliveira *et al.*, 2018 |
| **Succinic acid** | • Solvents (1-4 butanediol, tetrahydrofuran, 2-purrolidone, adipic acid, gamma-butyrolactone.<br>• Linear aliphatic esters<br>• Biodegradable polymers (polybutylene) | • Market size: USD 183 million<br>• Growth rate: 6.8% | Sreevi *et al.*, 2014<br>Jiang *et al.*, 2017 |
| **Ethanol** | • Transport fuel<br>• Power generation fuel<br>• Fuel for fuel cells<br>• Feedstock in the chemical industry | • Market size: USD 77.74 billion (2018)<br>• Growth rate: 5.6% | Reports and Data, 2019 |

Research community is making efforts in studying recovery technologies of biofermentation products. For example, precipitation methods with calcium salts were used with succinic acid broths (Li *et al.*, 2016). However, the chemical consumption ($Ca(OH)_2$/$CaCO_3$) in addition to the bypdroducts generated are undesirable. Similarly, high sulphuric consumption and product impurity were related to lactic acid precipitation (Komesu *et al.*, 2017) where a non-soluble waste sodium gypsium cake is formed. Li *et al.* (2010) proposed direct crystallization for succinic acid upgrading. Despite the secondary fermentation products present in the fermentation mixture, i.e. lactic acid, acetic acid and formic acid, a succinic acid yield of 70% with a purity of 90% was obtained. Distillation constitutes another traditional technology, with the inconvenience of the high energy demand mainly associated with the high water content of the mixtures (Danner *et al.*, 2000). Besides, solvent extraction has been studied to upgrade the fermentation mixtures where high distribution coefficients are required. Herein, high molecular weight amines were identified as promising extractants (Vayda *et al.*, 2005). However, the main inconvenience of solvent extraction is that it requires high specific area for separation, and an additional stripping unit to recover the solvent, which results in higher process cost.

In the last years, novel advance purification solutions have been tested, which include different membrane technologies, such as pervaporation, nanofiltration and electrodyalisis or the combination of the latter (Delgado *et al.*, 2010, Sosa *et al.*, 2016, Ma *et al.*, 2019). Recently, Mai *et al.* (2018) combined microfiltration, nanofiltration and pervaporation-assisted esterification for the production of high purity D-lactic acid from starch. During the pervaporation, ethanol was used to produce ethyl lactate, with a water removal of 95%, the authors reported a lactic acid conversion yield of 0.93. The process was completed by distillation and hydrolysis with deionized water and a final polymerization into poly (D-lactide). Interestingly, Ma *et al.* (2019) used bipolar membrane electrodyalisis to recover lactic acid from stillage, with bioproduct recovery yields of 71.2%. Nanofiltration was also employed at laboratory scale by Oonkhanond *et al.* (2017) to recover lactic acid from s sugarcane bagasse fermentation, where a selectivity of 82.5% was reported. Similarly, Sosa *et al.* (2016), combined a three step membrane process including, electrodyalisis, nanofiltration and Donnan dialysis, to recover succinic acid from synthetic fermentation mixtures based on carob pod. Herein, rejections > 90% succinate were obtained, although technical difficulties associated to membrane fouling and reduced fluxes were reported. In line with other studies, Nguyen *et al.* (2017) proposed a multi-stage process that consisted of microfiltration and nanofiltration-assisted crystallization to recover high purity succinic acid (i.e. 99.18%).

Despite the promising achievements in the downstrem field, the proposed solution needs to be environmentally friendly, avoiding high amounts of chemicals and energy consumption, and to gather the potential characteristics to be feasible on a commercial level.

Regarding further downstream and to increase the fermentation product titer, water removal from fermentation mixtures might be highly beneficial. With this aim, Forward Osmosis (FO) technology poses significant advantages over other membrane-based technologies, i.e. nanofiltration or reverse osmosis, due to the low membrane fouling and low energy requirement (Phuntsho *et al.*, 2013). FO uses the natural water flux that is stablished due to the osmotic pressure gradient that is generated between the feed solution and draw solution, which contain different saline concentrations (Achili *et al.*, 2010, Law and Mohammad, 2017). The water flux in FO results, dilutes the draw solution and results in an up-concentration effect in the feed side. The application of FO technology with fermentation mixtures, might lead to up-concentrate the target chemicals which is valuable for subsequent downstream (Jung *et al.*, 2015). The application of FO membranes for fermentation broth downstream is a novel approach that has been barely explored in previous work. Recently Garcia-Aguirre *et al.,* (2019) highlighted the potential of FO technology to up-concentrate synthetic binary mixtures containing succinic acid and lactic acids. Interestingly, a novel work by Law *et al.* (2019) obtained promising results regarding the succinic acid upgrading with FO assisted crystallization process. The authors used cellulose triacetate flat sheet membranes and reported solute concentration factors of 3.9 with a succinic acid titer of 111.16 g $L^{-1}$. The crystallization led to a purity and yield of succinate crystals of 90.52% and 67.09% respectively. Nonetheless, the application of FO with real fermentation broths needs further study and clarification, for example by evaluating different types of membranes, or testing additional draw solutes than NaCl (Cai and Hu, 2016).

In this study, for the first time thin film composite hollow fibre FO membranes were evaluated to upgrade real fermentation broths containing target bioproducts, namely, succinic acid, lactic acid and ethanol. Fermentation tests were performed to obtain the real broths that were used as feed solution in FO tests. A straightforward downstream process was employed, where the broths were centrifuged to remove the cell mass, but not further pretreatments were performed prior to FO. Different concentrations of NaCl, i.e. 1.5 M and 5 M, were used as draw solution. During the FO tests, process parameters were evaluated, such as the water flux, the rejection rate of the membranes, the water and the solute concentration factor.

## 6.4 MATERIAL AND METHODS

### 6.4.1. Fermentation substrates

S*accharina Latissima* macroalga biomass was used for succinic acid production, which consists of an abundant renewable resource with a high carbohydrate content as Table 6. 2 ilustrates. The substrate was collected from a cultivation area granted at Hjarnø Havbrug A/S.

**Table 6. 2.** Composition of the substrates used for this study.

| | ***Saccharina Latissima Hydrolyzate*** | **Biopulp hydrolyzate** | **Diluted Biopulp (1:1)** |
|---|---|---|---|
| Glucose (g $L^{-1}$) | 28.23 | 13.56 | 1.52 |
| Xylose (g $L^{-1}$) | 0.0 | 20.24 | 0.0 |
| Mannitol (g $L^{-1}$) | 11.996 | N.A. | 0.0 |
| Arabinose (g $L^{-1}$) | 0.0 | N.A. | 0.0 |
| HSuc (g $L^{-1}$) | 0.426 | 0.0 | 0.23 |
| HLac (g $L^{-1}$) | 1.998 | 11.07 | 8.09 |
| HFor (g $L^{-1}$) | 0.0 | 0.292 | 0.0 |
| HAc (g $L^{-1}$) | 0.0 | 0.0 | 1.52 |
| EtOH (g $L^{-1}$) | 0.0 | 0.0 | 3.51 |

Ethanol and lactic acid were obtained from biopulp. This substrate represents the source separated organic fraction of municipal solid waste (collected from supermarket and food residues from restaurants and canteens), that undergoes a pretreatment at Gemidan Ecogy A/S.

The pretreatment performed consists of the waste shredding to obtain and homogeneous biopulp. Subsequently, the biopulp is utilized for bioenergy production in an anaerobic digestion process.

In the fermentation tests, the biopulp was diluted 1:1 with deionized water (Table 6. 2) and subsequently fermented to obtain ethanol. All substrates were stored at -18 ºC prior to use.

All additional chemicals, e.g NaOH or HCl for pH adjustment were analytical grade, purchased from Sigma Aldrich ApS (Brondy, Denmark).

## 6.4.2. Fermentation broths

### 6.4.2.1. Fermentation test set-up

Batch fermentation tests were performed in a 3-L BIOSTAT Aplus bioreactor, connected to a digital controller and a computer with control panel from Sartorius (Figure 6. 2 a, c). The bioreactor was equipped with EASYFERM plus K8 200 pH probe (Hamilton Bonaduz AG, Switzerland) and temperature probes from Sartorius. The substrate and inoculum solution were added to the bioreactor and a warming jacket was connected and set to 37 ºC.

**Figure 6. 2**. 3-L fermentation reactor (a). Inoculation of *Lactobacillus delbrueckii* (b), monitoring and control system (c).

Samples were collected at periodic intervals to analyze the fermentation composition. The fermentation broths were centrifuged in a Hereaus Multifuge

X3R centrifuge for 15 min at 4,500 rpm to separate the cells and solids, from the liquid fraction.

#### 6.4.2.2. Succinic acid broth

The succinic acid broth was obtained from Marinho *et al.* (2016). The authors performed batch fermentation tests with enzymatically hydrolized *Saccharina Latissima* macroalgae. Herein, the pH was adjusted to 4.8 and temperature increased up to 70 ºC during 15 min. Cellulase, β-glucosidase and Alginate lyase were used as enzymes and incubated during 48h, at 50 ºC and 150 rpm. After centrifugation, the liquid fraction was employed during fermentation tests, with the strain of *Actinobacillus Succinogens* 130 (DSM 22257). All technical specifications can be found elsewhere, at Marinho *et al.* (2016).

#### 6.4.2.3. Lactic acid broth

An enzymatic hydrolysis was performed with biopulp, to release the sugars available in the substrate for lactic acid fermentation. Preliminary screening tests were performed in 50 mL falcon tubes to find the most appropriate substrate and enzyme loading which released the highest sugar content. The enzymatic hydrolysis was up-scaled in the 3-L reactor, with a substrate loading of 20% (w/v) and an enzyme loading of 10 U g DM$^{-1}$ β-glucosidase and 20 U g DM$^{-1}$ cellullase C2730. Herein, the temperature was adjusted to 50 ºC. The biopulp hydrolysate was centrifuged to separate the solids from the liquid fraction.

The fermentation was performed with the strain of *Lactobacillus delbrueckii* (DSM 20074) obtained from DSMZ (German Collection of Microorganisms and Cell Cultures). The microbes were grown at 37 ºC for 24h in 20 mL flasks at a concentration of 10% (v/v) and 90% (v.v) of sterilized MRS medium. For the inoculation medium growth, the *Lactobacilli* culture was transferred to 100 mL serum bottles under the same operational conditions as the first generation. The medium was used as inoculum for the fermentation tests (Figure 6. 2b).

The fermentation was performed during 27h in the abovementioned 3-L fermenter. Herein, biopulp hydrolysate and inoculum were mixed with 90% and 10% (*v.v.*) in a total volume of 2L. Samples were collected every 2 – 10 h for HPLC analysis. During the fermentation, the pH was adjusted to 6.2 and maintained constant throughout the fermentation process.

#### 6.4.2.4. Ethanol broth

Diluted Biopulp with deionized water 1:1 (*v.v*) was used as substrate for ethanol production. The substrate to inoculum ratio was adjusted with the same quantities expressed in the former lactic acid fermentation, with a 9:1 (*v.v*) substrate to inoculum ratio, and a total volume of 2 L. Herein, *L. delbrueckii* (DSM 200774) bacterial strain was used as inoculum and the fermentation time was adjusted to 14h. Again, pH 6.2 was maintained during the fermentation test.

### 6.4.3. FO tests with the fermentation broths

FO tests were performed with the liquid fraction of the abovementioned fermentation broths. The membranes used consisted of thin film composite hollow fibre membranes obtained from Aquaporin A/S (Copenhagen, Denmark) which contain a surface area of 0.3 $m^2$ and a water flux > 7 L $m^{-2}$ $h^{-1}$, when 1 M NaCl is used as draw solution and DI water as feed solution.

During FO tests, the feed solution reservoir of 1L contained the liquid fraction of the fermentation broth, while the draw solution reservoir of 4L contained different concentrations of NaCl, i.e. 1.5 M and 5M (Nayak *et al.*, 2010, Law *et al.*, 2019).

**Table 6. 3.** Conditions of FO tests

| Substrate | NaCl concentration | ΔP initial (bar) | pH of feed solution | ΔP final (bar) | Time (min) |
|---|---|---|---|---|---|
| Succinic acid broth | 1 M | 43.2 | 7.50 | 0 | 45 min |
| | 5 M | 155.5 | | 75.06 | 30 min |
| Lactic acid broth | 1 M | 45.3 | 5.90 | 0 | 45 min |
| | 5 M | 148.2 | | 11.50 | 30 min |
| Ethanol broth | 1 M | N.A[1] | 7.10 | N.A[1] | 45 min |
| | 5 M | N.A[1] | | N.A[1] | 30 min |

[1]: Crystallization errors of the sample prevented any possible measurement in the osmometer.

NaCl was employed as draw solution, since this solute is inexpensive, nontoxic, produces a high osmotic pressure and it is easy to be regenerated. Indeed, the high NaCl concentrations enabled to increase the osmotic pressure difference between substrates and to enable a high water flux.

The FO consisted in a counter-current configuration similar to previous studies (Engelhart *et al.*, 2018). The feed solution was faced against the active side of the membrane and the cross flow rate was adjusted with a variable-speed peristaltic pump (Longer BT100, 2 pump heads YZ1515X) at a constant flow rate of 200 mL min$^{-1}$. The draw solution was placed over a magnetic stirrer for constant mixing and a digital scale (Kern Balance 572, + software balance connection 4) recorded the feed solution weight change. The osmotic pressure difference between the feed and draw solution was measured by an osmometer (Genotec Osmomat 030 Cryoscopic). All FO tests were performed at room temperature. The reverse salt flux was qualitatively assessed by measuring the conductivity change of the feed solution and draw solution at the beginning and at the end of the FO test.

### 6.4.4. Analytical methods

The composition of the fermentation broths and FO outcome was analyzed by HPLC (Ultimate 3000, Thermo Scientific) which contains a refractive index detection, equipped with a resin based sulfonated divinyl benzene-styrene-hydrogen column (Aminex HPX – 87H300 x 7.8 mm, BIO-RAD), under isocratic conditions and $H_2SO_4$ acid (0.005 M) eluent. The detection of the chemicals (sugars, VFA and ethanol) is achieved by refractive index detection using a RefractoMax 521 (ThermoScientific) operated at 35 degrees. The flow in the HPLC was kept constant at 0.6 mL min$^{-1}$, the oven temperature at 63.5 ºC.

### 6.4.5. Calculations

The Van’t Hoff equation was used to the osmotic pressure calculation:

$$\Pi = iMRT \tag{1}$$

Where is the Van’t Hoff factor, M (mol $L^{-1}$) is the molar concentration of the solution, R (atm L $mol^{-1}$ $K^{-1}$) is the ideal gas constant and T (K) the temperature.

The water concentration was measured using the following equation (Law *et al.*, 2019).

$$J_w = \frac{\Delta V}{\rho\ A \Delta t} \qquad (2)$$

Where ΔV represents the weight change in the feed solution (g), ρ is density of water (g $L^{-1}$), A is the surface area of the membrane ($m^2$) and Δt (h) represents the difference in time between measurements.

In order to assess the overall performance of FO technology, the indicators of Blandin *et al.* (2019) were used, i.e. the solute concentration factor and the water concentration factor.

$$SCF = \frac{V_0}{V_f} \qquad (3)$$

$$WCF = \frac{C_0}{C_f} \qquad (4)$$

Herein, $V_0$ and $V_f$ represent (L) represent the initial and final volume of the feed solution and $C_0$ and $C_f$ (g $L^{-1}$) represent the concentration of the target chemicals at the beginning and at the end of the FO tests.

The rejection rate of the membrane was calculated based on the expression of Engeldhart *et al.* (2018):

$$\text{Rejection} = 1 - \frac{V_{ds}\ C_{ds}}{V_w\ \frac{C_{f0}+C_{fe}}{2}} \qquad (5)$$

Where $V_{ds}$ (L) is the final volume of the draw solution, $C_{ds}$ (g $L^{-1}$) is the final concentration of the target chemical in the draw solution. $V_w$ (L) represents the volume of water transported through the membrane and $C_{f0}$ and $C_{fe}$ are the concentration of the target chemicals in the feed solution at the beginning and the end of the FO tests.

## 6.5 RESULTS AND DISCUSSION

### 6.5.1. Fermentation broths

The fermentation of the hydrolyzed *Saccharina Lattisima* reached a succinic acid titer of 38.8 g $L^{-1}$ (Figure 6. 3a) and low amounts of secondary fermentation products (Marinho *et al.*, 2016), suggesting that macroalga were attractive feedstock for fermentation process in a biorefinery.

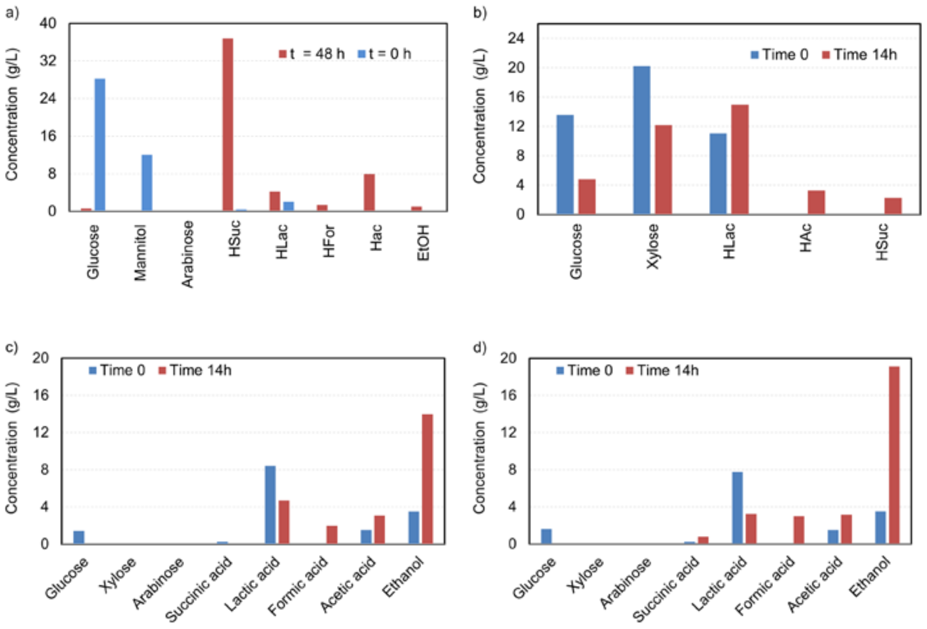

**Figure 6. 3.** Succinic acid fermentation (a), lactic acid fermentation (b), ethanol fermentation (c, d).

Lactic acid from biopulp hydrolyzate, reached a bioproduct titer of 14.98 g $L^{-1}$ (Figure 6. 3b). The presence of glucose and xylose after 27h of fermentation suggests that the acidogenic potential was not fully exploited. The fermentation profile evidences that there was a slow biodegradation profile during the first 21h, with a slight decrease of glucose and xylose consumption, suggesting a lack of acclimation of *L. delbrueckii* which is hypothesized that might be related to constant pH adjustment. According to the acidogenic profile, a higher fermentation time might have increased the lactic acid production. Additionally, 2.25 g $L^{-1}$ of succinic acid and 3.27 g $L^{-1}$ of acetic acid were obtained. Nonetheless, regarding the aim of the current study, the composition of the fermentation broth was considered interesting to be evaluated by the FO technology.

Diluted raw biopulp fermentation enabled to obtain ethanol concentration titers of 13.94 and 19.11 g $L^{-1}$ (Figure 6. 3 c, d). It should be highlighted that metabolic routes of *L. debrueckii* enable to produce ethanol as main fermentation product from mixed sugar fermentation, which occurs through pyruvate and acetyl-coA production (Zhang *et al.*, 2016).

The liquid fraction of the fermentation broths was subjected to FO evaluation, in order to remove water from the mixtures, and assess the feasibility of obtaining product titers within the commercial range, i.e. 120 – 150 g $L^{-1}$.

## 6.5.2. Forward osmosis tests with the fermentation broths

### 6.5.2.1. FO water flux and water removal

The osmotic pressure difference between the draw solution and feed solution promotes a natural water flux trough the membrane, without additional pressure requirements, from the feed solution side (low osmotic pressure) to the draw solution side.

As shown in Figure 6. 4b, 5 M NaCl draw solution promoted the highest water flux, from 12.52 L $m^{-2}$ $h^{-1}$ with the lactic acid broth to 22.06 L $m^{-2}$ $h^{-1}$ with the ethanol broth at the beginning of the tests. These values were are higher than the water flux range obtained by Law *et al.* (2019) with cellulose triacetate based flat sheet membranes. Due to the dilution effect in the feed solution and the net osmotic pressure loss, the water flux tended to decline with time (Law *et al.*, 2019) which resulted in a negligible flux after 30 min of FO tests were for example, the osmotic pressure difference between the feed and draw solution was negligible with NaCl 1.5 M. Due to the high specific area of the membranes, the FO tests could be concluded in a reduced time, compared to the > 12 h required with flat sheet membranes (Garcia-Aguirre *et al.*, 2019, Law *et al.*, 2019).

The highest water flux of the ethanol broth might be related to the specific iteration of the chemicals present in the broth, with the active layer of the membrane. A preliminary study with binary synthetic mixtures suggested that the water flux of thin film hollow fibre membranes was related with the molecular size of the chemicals and their speciation in ionic or free acid form (Garcia-Aguirre *et al.*, 2019). Concretely, the ethanol mixture contained the lower molecular weight chemicals, i.e. HFor, HAc and EtOH, and low amounts of HLAc and HSUc, which could induce the highest water flux.

**Table 6. 4.** Properties of the chemicals present in the fermentation broth (PubChem, 2019).

| **Compound** | **HFor** | **EtOH** | **HAc** | **HLac** | **HSuc** | **Xylose** | **Glucose** |
|---|---|---|---|---|---|---|---|
| Formula | $CH_2O_2$ | $C_2H_5OH$ | $C_2H_4O_2$ | $C_3H_6O_3$ | $C_4H_6O_4$ | $C_5H_{10}O_5$ | $C_6H_{12}O_6$ |
| Molecular weight (g mol$^{-1}$) | 46.02 | 46.07 | 60.05 | 90.08 | 118.09 | 150.13 | 180.16 |
| p$K_a$ | 4.60 | 15.9 | 4.76 | 3.86 | 4.21, 5.63 | 12.14 | N.A. |

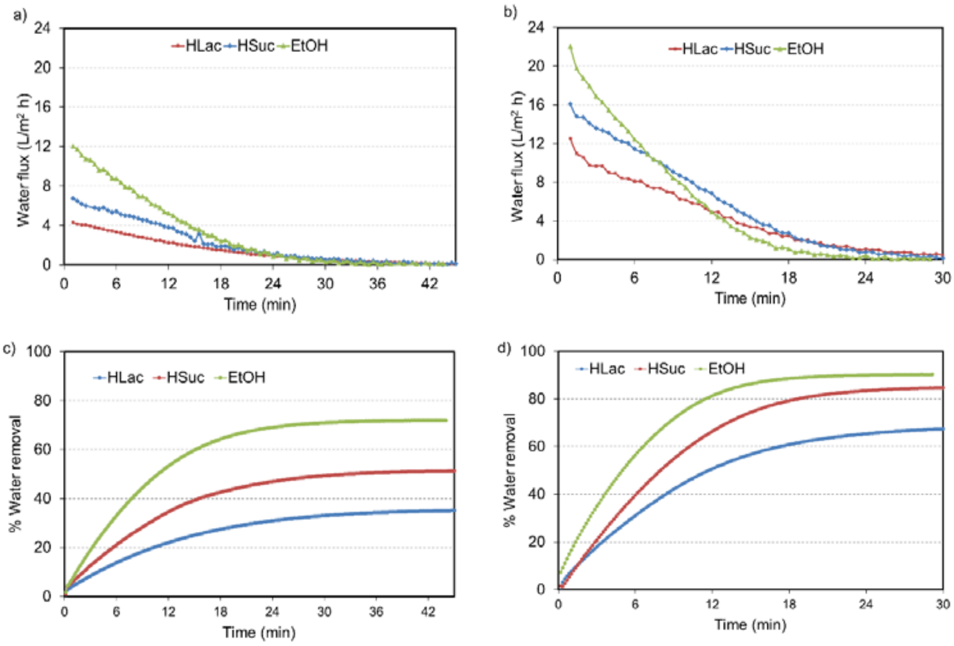

**Figure 6. 4**. Water flux of the fermentation broths with NaCl 1.5M (a) and NaCl 5M (b).

Lactic acid broth showed the lowest water flux, which accounted for 4.26 ± 3.4, during the first 30 min and NaCl 5 M as draw solution. Herein, the fermentation broth contained high amounts of lactic acid, glucose and xylose which are the highest molecular weight chemicals of the evaluated broths. Since size exclusion with thin film composite hollow fibres is a significant factor affecting the water flux it is hypothesized that the higher molecular weight solutes hindered the water flux. This is in line with the results of Engelhart *et al.*, (2018) who reported different water fluxes for different types of organic compounds, namely, 2,4 D, Bisphenol A and methyl paraben. In addition, this broth contained high amounts of impurities, i.e. coloring organics, that were adsorbed in the membrane and which might have contributed lo lower the water flux (Kim *et al.*, 2014). The remaining sugars can be removed from the liquid fraction by adsorption materials, such as activated carbon as reported by Law *et al.* (2019).

The water removal obtained with NaCl 1.5 M, ranged within 35 – 72%, and as expected, it was enhanced by increasing the osmotic pressure difference between the feed and draw solution (Law *et al.*, 2019). According to Table 6. 3 increasing the NaCl concentration to 5M enabled to reach very high osmotic pressure differences between the feed and draw solution, for example a 155.5 bar pressure difference was reached with the succinic acid broth, which resulted in a high water removal from the fermentation broth. The final volumes of the ethanol and the succinic acid mixture were 97.10 and 149.78 mL respectively, where a water removal of 85% and 90.3% was obtained. The lowest water removal of 67.4% was produced with the lactic acid broth.

Overall, the high water flux and high water removals obtained with thin film composite FO membranes and high draw solute concentrations suggested a positive scenario, which needs to be validated with the rejection rate of the membranes.

#### 6.5.2.2. Rejection rate, up-concentration and reverse salt flux

The composition analysis of the draw solutions after individual FO tests showed positive results of FO membranes, with a 100% rejection of the target chemicals.

There was not succinic acid detection in the succinic acid broth, for both draw solution concentrations. Similarly, lactic acid and ethanol concentrations were absent in the fermentation mixtures for both draw solute concentrations. This is an indicative factor of the high selectivity of the thin film composite hollow fibre membranes in enabling high water fluxes but rejecting the rest of organic compounds.

NaCl 1.5 M enabled to achieve target chemical titers of 26.8 g L$^{-1}$ of lactic acid, 34.6 g L$^{-1}$ of ethanol and 63.1 g L$^{-1}$ of succinic acid (Figure 6. 5, a,c,e). However, this concentration was significantly increased by increasing the osmotic pressure differenc, with 5 M NaCl concentrations.

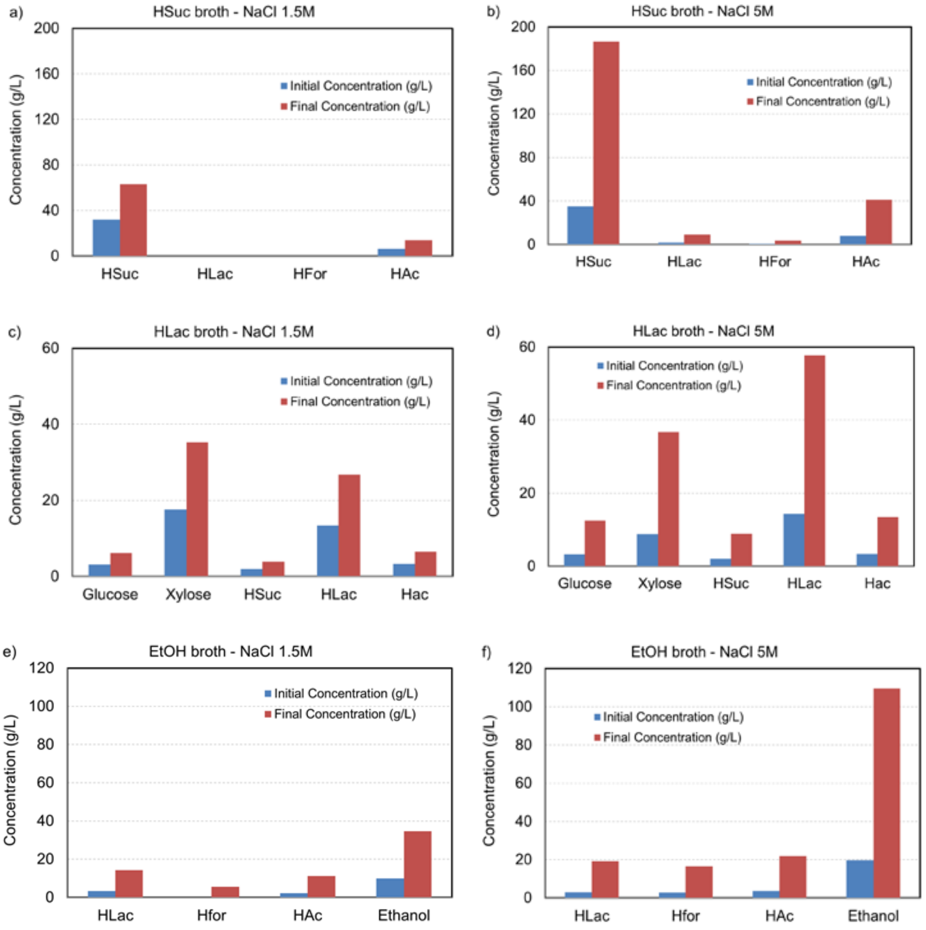

**Figure 6. 5.** Up-concentration of the succinic acid broth (a, b), of the lactic acid broth (c, d) and the ethanol broth (e, f).

Concretely, lactic acid could be concentrated up to 57.7 g L$^{-1}$ (Figure 6. 5d). A concentration titer of 109.6 g L$^{-1}$ was achieved with ethanol and succinic acid was up-concentrated up to 186.8 g $L^{-1}$ (Figure 6. 5 b, f). Surprisingly, a high ethanol rejection was observed, which contradicts the results of previous binary synthetic mixtures (Garcia-Aguirre *et al.*, 2019) where a low rejection rate of thin film composite membranes was reported. Thus, it is hypothesized that a compensation effect of additional anions influenced in the rejection mechanisms, and contributed to retain ethanol in the feed side. At pH 7.1 all the anionic form of the chemicals is favored beyond their $pK_a$ (Table 6. 4), and thus electrostatic repulsions occur with the negative charged active layer of thin film hollow fibre membranes (Law and Mohammad, 2018).

In addition, FO technology showed a high potential to up-concentrate other secondary fermentation products. Acetic acid could be upgraded up to 41.1 g $L^{-1}$ in the succinic acid broth and formic acid titer of 16.4 g $L^{-1}$ was obtained during the ethanol broth up-concentration and 5 M NaCl as draw solution. These are additional valuable chemicals from the carboxylic platform with potential applications and market opportunities (Liu *et al.*, 2015, Garcia-Aguirre *et al.*, 2019). Besides, the sugar content present in the soluble fraction could be upgraded with FO technology. For example, xylose could be upgraded > 3.8 times, up to 36.7 g $L^{-1}$.

According to the conductivity measurements of the samples at the beginning and the end of FO tests, a reverse salt flux occurred for all samples and NaCl 1.5 M. Herein, the feed mixtures increased the conductivity content 1.2, 1.26 and 2.0 times during HLac, HSuc and EtOH broths, respectively (Figure 6. 6a). In contrast, increasing the NaCl concentration did not translate into a higher reverse salt flux. Specifically, during lactic acid FO tests, the reverse salt flux was negligible and only a 1% increase in the salt content was noticed during HSuc test. Contrarily, the ethanol broth showed the highest reverse salt flux, achieving a 57% increase and reaching 49.3 mS $cm^{-1}$ (Figure 6. 6b).

The reverse salt flux competes with the direct water flux (Achili *et al.*, 2010). Thus, one could think that the higher water flux contributes to hinder the reverse salt flux. Surprisingly, the results show a lower reverse salt flux in the lactic acid mixture. It is suggested that the specific iteration of the high charged ions of the lactic acid broth, i.e. lactate, exerted a strong repulsion with $Cl^-$ anions, reducing the reverse salt flux. In addition, it was hypothesized that chemical adsportion in the membrane, might help hinder the reverse salt flux (Law *et al.*, 2019). In line with this, the ethanol broth promoted the highest water flux, which might have contributed to a reduced reverse salt flux. However, the results suggest that the weakest and lower molecular weight ions, such as formate and acetate, exerted a weaker repulsion with NaCl, contributing to the highest reverse salt flux.

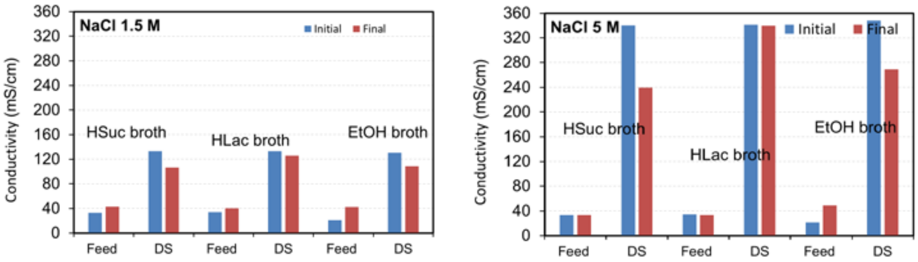

**Figure 6. 6**. Conductivity measurements with NaCl 1.5 M (a) and NaCl 5M (b).

### 6.5.3. Remarks of FO downstream

FO is an emerging technology that has been used for desalination and wastewater reclamation (Awad *et al.*, 2019), but which has been barely explored as fermentation product downstream. Overall, the results of this study evidence the high potential of FO to remove water from real fermentation broths. This might constitute a significant step in the subsequent downstream, contributing to obtain product concentration titers which fall in the commercial range and enhancing the limitation of anaerobic fermentation processes.

Assessing all the parameters together, the highest water concentration factor (i.e. highest water removal) and highest solute concentration factor are desirable. Overall, increasing the NaCl concentration, enabled to increase the solute concentration factor up to 4 – 5.5 and a water concentration factor of 3.6 – 6.75 was obtained. These values are in line with the values reported by Law *et al.* (2019), where a succinic acid concentration factor of 3.9 was reported with flat sheet cellulose triacetate membranes. However, during their study, the authors performed a pretreatment with activated carbon to remove residual compounds and clarify the fermentation mixture. In this study, best results were obtained for succinic acid upgrading with a straightforward FO processing of the fermentation mixtures and with thin film hollow fibre membranes. Concretely, a water concentration factor of 6.88 and a solute concentration factor of 5.33 were obtained, which resulted in a bioproduct titer of 186 g $L^{-1}$. The high concentrated succinic acid mixture can be further purified by removing volatile fatty acids, i.e. HFor and HAc, by vacuum distillation and by direct crystallization (Luque *et al.*, 2009, Law *et al.*, 2019). As shown in Figure 6. 6b, decreasing the pH of the mixture down to 3, and cooling the mixture during 12 h at 5 ºC, enabled to obtain succinate salt precipitate.

It is important to emphasize that high fermentation yields and product concentration titers are desirable. For example, during lactic acid up concentration, the initial 14.9 g $L^{-1}$ concentration was remarkably enhanced, with a solute concentration factor of 4, and a product titer of 57.6 g $L^{-1}$. However, this concentration might not be enough for commercial purposes. Contrarily, and based on the results of FO tests, at least a solute concentration of 4 could be expected with FO membranes, where for example an initial product concentration of 40 g $L^{-1}$ might lead to > 160 g $L^{-1}$. Current fermentation yields and product titer greatly depend on the bacterial strains employed and the fermentation process variables. For example, Srivastava *et al.* (2014) reported lactic acid concentration of 84.5 g $L^{-1}$ during the fermentation of cane molasses, with *L. delbrueckii* strain, during 12 h fermentation. Remarkably, engineered bacterial strains have enhanced succinic acid production yield from glucose, beyond 0.75 g/g and succinic acid concentration ranging within 40 – 107 g $L^{-1}$. Nonetheless, it is important to adapt current fermentation processes to real organic waste streams and to make current processes, technically feasible, cost-effective and scalable. For example, Lam *et al.* (2014) reported a positive economic scenario for succinic acid production from bakery waste in a pilot scale facility.

Another important factor is the mass lost during the downstream process. Mass balance calculation indicate that significant amounts of ethanol were lost during FO downstream, i.e. 17% with NaCl 5 M. This was also noted in the preliminary tests performed with synthetic binary mixtures (Garcia-Aguirre *et al.*, 2019), where 42.5% was lost. In contrast, the mass lost during the upgrading of lactic acid was negligible and <4% was lost during succinic acid upgrading. Thus, this is an important aspect that should be considered during ethanol FO downstream, wehre additional alternatives to avoid solute evaporation should be considered.

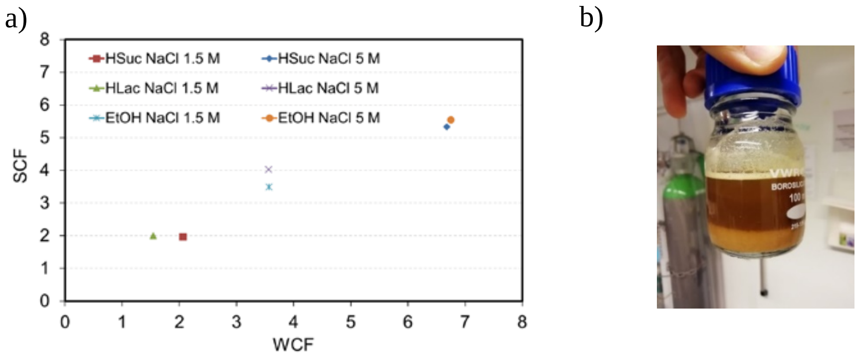

**Figure 6. 7.** Solute concentration factor and water concentration factor of the target solutes (a), crystallization of succinate salt particles (b).

In this study, best results were obtained with NaCl 5M, which resulted feasible at laboratory scale, but which might include a high product consumption at pilot/demonstration scale, making the FO downstream unfeasible. Many draw solution have been evaluated for FO application, which include different salts and organic compounds (Ansari *et al.*, 2015, Kim *et al.*, 2019) Herein, other process configurations might enable to reduce the osmotic pressure requirement, for example by continuous supply of the draw solution and minimizing the dilution effect. Interestingly, Blandin *et al.* (2019) evaluated seawater and desalination brine for volatile fatty acid up-grading, where high solute concentration factors were reported. It is noteworthy that the desalination brine constitutes a major managing challenge at desalination facilities, associated to high discharge cost (Jones *et al.*, 2019). With a high saline content and with an estimated production of 142 M $m^3$ $d^{-1}$, this waste stream constitutes a potential resource that should be tested as draw solution, and which may help incrase the sustainability of current desalination facilities. Hereby, further studies are required regarding the application of different draw solutes in fermentation and FO downstream, where the influence of draw solute in the water flux, reverse salt flux and byproduct concentration need to be assessed.

## 6.6 CONCLUSIONS

For the first time, this study explored the potential of thin film composite hollow fibre FO membranes to up concentrate target chemicals from real fermentation processes. FO was applied to the liquid fraction of the fermentation broths, which contained succinic acid, lactic acid and ethanol, at room temperature and keeping the liquid pH, without additional pretreatment steps. The target chemicals could be significantly upgraded with a solute concentration factor of 4 -5.5, where high water removals were obtained, and 5 M NaCl draw solution. The results evidenced the high potential of a novel application of FO membranes, which has been barely explored in previous work, with >99% target solute rejection rates and secondary chemical upgrading, where a low fowling propensity of the membranes was noted. Overall, optimal results were obtained for succinic acid upgrading, were a product titer of 186.7 g $L^{-1}$ was obtained, with a water removal of 85%, which falls within the commercial range, making the fermentation and subsequent downstream a promising and robust approach. In addition, the potential for lactic acid upgrading was remarkable, although it is highlighted the need of optimized fermentation processes, which enhance the bioproduct yield and titer. Additionally, secondary fermentation product concentrations could be significantly increased, i.e. HAc and HFor. Although ethanol concentration could be significantly enhanced, mass balance calculations indicate that a significant amount was lost by evaporation. In addition, further work is required to elucidate additional draw solutes, to deeply understand the separation mechanisms of FO and to optimize the downstream process.

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## CHAPTER 7

# CONCLUSIONS AND FUTURE RESEARCH LINES

## 7.1 CONCLUSIONS

This thesis explored acidogenic fermentation as a promising solution that could be used for the valorisation of organic waste. VFA and additional bioproduct production from organic waste could contribute as a novel solution to reduce the environmental impact and to change the perception of waste towards a valuable resource, which could be used in future biorefineries.

Controlled anaerobic fermentation processes were performed at laboratory scale to produce valuable industrial bioproducts from different waste streams of urban and agro-industrial origin. The fermentation process was up-scaled in an automatized pilot-scale platform where batch, and continuous fermentation tests were performed. During the final step, pure culture fermentation processes were evaluated to obtain other value-added fermented bioproducts such as succinic acid, lactic acid and ethanol.

Additionally, regarding the current need for sustainable and cost-effective separation technologies to recover the fermented bioproducts, this thesis gained deep insights into advance membrane separation technologies, by exploring ultra-filtration and forward osmosis technology.

**The first contribution of this thesis consisted of assessing the acidogenic potential of different organic waste streams**. These included, different wastewater, i.e. winery wastewater, crude glycerol, slaughterhouse wastewater, paper mill wastewater, solid waste, i.e. the OFMWS, meat and bone meal, and sewage sludge from WWTP. The most significant aspects of this study are listed below:

- Mixed culture fermentation was successful in producing different types of acidogenic fermentation products.
- The process pH and temperature had a clear effect in the acidogenic fermentation process, determining the VFA production yield and the extent of acidification of the treated organic waste streams.
- The highest VFA concentration and the highest VFA/sCOD ratio was obtained during the OFMSW fermentation, with 8,320 mg COD $L^{-1}$ and 94%, at alkaline pH and mesophilic temperature, suggesting that OFMSW is a potential feedstock for the VFA platform.
- The parameters evaluated in combination with the composition of the waste stream had a combined effect on the metabolic routes and determining the product spectrum.
- The results suggested that the VFA production can be directed towards desired bioproducts, i.e. valeric acid or butyric acid, through the waste stream selection and process parameter control. For example, valeric acid can be promoted by selecting proteinaceous waste stream, while butyric can be obtained from carbohydrate-rich waste streams.

**The second contribution of this thesis was to up-scale the fermentation tests in a pilot-scale system using mixed culture anaerobic inoculum**. With this aim, SS, $W_{ww}$ and MBM were selected from previous tests as substrates of interest. The results from pilot-scale tests were compared with previous laboratory-scale tests. Additionally, co-fermentation tests were evaluated.

Laboratory-scale acidogenic potential tests provided a robust methodology for subsequent up-scaling. At pilot-scale the individual trends of VFA could be reproduced compared to previous laboratory scale tests, where SS led to acetic acid and iso-valeric acid as main fermentation products, butyric acid could be produced from $W_{ww}$ at acidic conditions and thermophilic temperature and significant amounts of valeric acid were obtained from MBM at acidic conditions and mesophilic temperature.

- The results of batch tests indicated that RT of 5 – 10 days might be reasonable during fermentation and automatic control of the pH would be recommendable.
- 6,670 mg COD $L^{-1}$ of butyric acid were obtained with Www at acidic pH and thermophilic temperature.
- MBM led to significant amounts of valeric acid, with 2,700 mg COD $L^{-1}$ at mesophilic conditions and acidic pH.
- Regarding co-fermentation, a liquid substrate (i.e. $W_{ww}$) was beneficial in terms of the acidification kinetics and the operational feasibility, since their dosing could be driven automatically without major technical impediments.
- Co-fermentation scenario enabled synergistic effects between substrates and enhanced the acidification yield 1.7 – 2 fold.

From an industrial point of view, continuous acidogenic fermentation processes are desirable which enable to obtain the target chemicals in continuous mode of operation. **The third main contribution of this thesis consisted of showing the feasibility of reaching a stable continuous acidogenic fermentation process at pilot-scale**. With this aim, SS was selected as model substrate, and the potential of OFMSW was evaluated as co-substrate. Additionally, ultrafiltration technology was used as a preliminary step before further downstream. This study led to interesting findings which are listed below:

- The results showed the feasibility of continuous acidogenic fermentation processes, at pilot-scale, which enable obtain a controlled continuous VFA production with similar results of previous batch tests.
- Optimal pH control was identified as a critical aspect in ensuring the process stability and a steady VFA yield.
- Sewage sludge alkaline fermentation performed best results at pH 9 and HRT 5 days, enabling a high-rate VFA production, with a VFA yield of 336 mg VFA $g^{-1}$ VS and a VFA productivity of 2.15 kg VFA $m^{-3}$ $d^{-1}$.
- Co-fermentation of SS and OFMSW enabled to boost the VFA productivity evidencing synergistic effects between both substrates.

- The results showed an interesting degree of flexibility of anaerobic fermentation systems where the pH shift could enrich the bacterial community and direct the metabolic routes towards valuable products, such as butyric acid where 13.97 g COD $L^{-1}$ were obtained, with a VFA peak of 23.2 g COD $L^{-1}$.
- Ultrafiltration technology enabled to recover a high concentrated VFA permeate, with VFA concentrations up to 26.6 g COD $L^{-1}$, which can be used for further downstream.

The fermented effluents need a further downstream to recover the fermentation products. This was identified as a major bottleneck of current anaerobic fermentation processes, which makes the fermentation processes uncompetitive compared to oil-based processes. Thus**, the fourth contribution of this thesis was to gain insights into a novel application of an advance membrane separation technology, namely forward osmosis**, to obtain high concentrated fermented solutions.

The application of forward osmosis technology for concentrating the fermentation products obtained in controlled acidogenic fermentation processes is a novel approach that was barely explored in previous work. The results obtained were promising, suggesting that FO technology could be used to recover the fermented bioproducts, becoming a core technology in future biorefineries, rising the industrial interest and opening new market opportunities.

During the FO tests, synthetic mixtures of four valuable chemicals were evaluated, namely acetic acid, succinic acid, lactic acid and ethanol. HSuc, HLac and EtOH were included (beyond VFA), since these are value-added industrial bioproducts, that are widely used in different applications, such as biofuels, bioplastics and chemical industry. The main finding of this part of the thesis are listed below:

- FO technology showed a high potential to remove water from the synthetic mixtures containing acetic acid, succinic acid, lactic acid, ethanol (20 g $L^{-1}$), enabling to obtain highly concentrated mixtures (except for ethanol mixtures).
- The pH of the mixtures was a key factor influencing the separation mechanism, the behaviour of solutes in contact with the active layer of the membrane and determining the water flux across the membrane.

- At acidic conditions (pH 3) size exclusion was the main separation mechanism, while chemical speciation and electrostatic repulsion were dominant for pH-dependent chemicals, i.e. HAc, HSuc and HLac, at pH 7.
- Interestingly, > 99% rejection rate of solutes was obtained at pH 7 for all the acids tested.
- Thin film composite hollow fibre membranes enabled to obtain high solute concentration factors, with high water removals (e.g. > 84%) in a reduced period, reaching concentrations up to 71.3 g $L^{-1}$ of solute.
- FO showed the best results for succinic acid concentration, at pH 3, where the saturation point was reached, and part the of solute remained crystalised.
- High water concentration factors and solute concentration factors were also obtained for lactic acid with 6.38 and 5.34, respectively.
- Ethanol showed a different behaviour compared to pH dependent acids, with a significant evaporation of the solute (e.g. 42%), and showing a limited potential for FO upgrading.
- Regarding the results, FO could become a key technology to concentrate the fermentation products prior to the recovery and purification step.

Regarding the promising results with synthetic binary mixtures, **the fifth main contribution of this thesis was to validate the application of FO technology with real fermentation broths**, which contained lactic acid, succinic acid and ethanol as main target chemicals.

- The fermentation with enzymatically hydrolized Saccharina Latissima macroalgae and Actinobacillus Succinogens bacterial strain enabled to obtain a succinic acid titer of 39 g $L^{-1}$.
- Lactobacillus delbrueckii bacterial strain enabled to obtain a lactic acid titer of 14.9 g $L^{-1}$ from enzymatically hydrolized biopulp (OFMSW).
- Interestingly, the same bacterial strain enabled to reach significant amounts of ethanol as main fermentation product and biopulp as substrate without further pretreatment, with 13 – 19 g $L^{-1}$ of ethanol.
- For the first time, the potential of thin film composite hollow fibre FO membranes was explored with real fermentation broths.
- The FO test results showed that the target chemicals could be significantly upgraded with a solute concentration factor of 4 – 5.5, where high water removals were obtained.

- Incrasing the NaCl concentration up to 5M, resulted in higher water fluxes and higher solute concentration factors. Interestingly, the reverse salt flux, was not proportional to the increase in the salt content.
- >99% of solute rejection rates were obtained for target chemical upgrading and for secondary fermentation products, such as acetic acid and formic acid. In addition, a low fouling propensity of the membranes was observed.
- Optimal results were obtained for succinic acid upgrading, with a bioproduct titer of 186.7 g $L^{-1}$ and 85% of water removal from the mixture.
- The potential of lactic acid upgrading was remarkable, although optimized fermentation processes are required to increase the bioproduct titer.
- Ethanol concentration could be significantly enhanced, due to the compensatory effect of additional anions, but significant amounts of the solute were lost through evaporation, i.e. 17% during the test with NaCl 5M.
- Further work with FO membranes is necessary to elucidate the separation mechanism and to assess the performance of different draw solution and additional process variables, such as the composition of the membranes, the water flux and orientation of the membranes.

## 7.2 FUTURE RESEARCH LINES

Mixed culture fermentation processes represent a complex group of biochemical reactions that are affected by the many factors that govern the acidogenic fermentation process, such as the process temperature, the pH, the configuration of the process and the nature of the substrate among others.

This thesis opened a new research line at the Water and Waste group of Ceit-IK4 and set the basic principles to understand the VFA platform and mixed culture fermentations. From preliminary laboratory-scale tests to continuous tests performed at pilot-scale, this thesis gained insights into the process chemistry and fermentation outcome and additionally, promising efforts were performed in the downstream field in collaboration with the Technical University of Denmark, by using forward osmosis membranes.

The process chemistry understanding throughout the experimental period led to different observations that could be further studied in future research lines constituting new ideas for future master thesis or PhD topics.

Among different observations, the pH was identified as a key aspect governing the metabolic routes of mixed culture fermentation systems and influencing the fermented product distribution and the fermentation yield. However, **the control of pH needs further study and clarificaiton**. This aspect has been neglected in previous literature studies and only recent works of 2019 suggested the possibility of other pH control strategies other than steady control. Thus, pH control needs further clarification in order to achieve optimal control strategies:

- During this thesis, first pH control was identified as a major aspect governing the up-scaling of continuous fermentation tests, where a steady control resulted detrimental to the anaerobic microorganism. Optimal start-up of the fermentation process should be clarified at pilot-scale by the proposal of different pH control strategies and with their experimental validation.
- During continuous fermentation tests, the constant control of pH enabled to reach a stable VFA production process, such as with SS at HRT 5d and pH 9. However, during co-fermentation of SS and OFMSW when the pH was reduced to 6, the VFA yield and the metabolites richness was enhanced by for example promoting the butyric acid metabolic pathway from the degradation of carbohydrate-rich OFMSW. Thus, one could think on a stepwise pH control or subsequent periods of controlled and uncontrolled pH, as novel pH control strategies.

**Different sources of inoculum should be tested during mixed culture anaerobic fermentation systems**. There is a lack of studies regarding this aspect which determines the microorganisms that are present in the biomass that is introduced in the fermentation system.

- During these tests, inoculum coming from a mesophilic anaerobic digestion system of a WWTP was evaluated. Other types of inoculum could include AD inoculum from thermophilic systems, or different sources of inoculum from AD plants treating other types of feedstock, such as OFMSW or other types of agro-industrial waste.
- Different sources of inoculum coming from the fermentation industry should be considered such as the brewery or wine industry.
- Laboratory-scale studies could be performed by assessing the performance of different types of inoculum at different substrates to inoculum ratios.

**Bioaugmentation strategies should be considered and studied to improve the acidogenic fermentation yield**. Previous experience at anaerobic digestion processes indicated that bioaugmentation could improve ammonium inhibition phenomenon or acidification phenomenon, and partially recover the anaerobic digestion process. Bioaugmentation can enhance the yield of specific microorganisms that work for particular aims. This solution includes different approaches such as pre-adapted culture addition, a pre-adapted consorptium addition, genetically engineered bacteria addition or biodegradation relevant genes addition in a vector. There is only one study of 2018 reporting the benefits of the application of bioaugmentation in acidogenic fermentation, which enabled to increase the butyric and caproic acid concentrations. This opens promising possibilities for further research, which could be perfoermed by inoculating specific bacterial strains that might be added to the anaerobic fermentation process to assess the process enhancement and final product titer.

Beyond mixed culture fermentation systems, **the applicability of pure cultures with organic waste streams could lead to enhanced results and process optimization,** specially considering the final product titer and fermentation yield. Despite the lower economic efficiency of pure cultures, and the associated technical difficulties of up-scaling (e.g. the nedd for sterilization), pure cultures might enable to gain further insights of process chemistry and boost the fermentation yield. The applicability and the technical difficulties associated with up-scaling should be assessed and the potential benefits of using pure-cultures further evaluated. In line with the exploration of pure culture anaerobic fermentation, **the production of additional value-added chemicals should be considered**, such as lactic acid or surccinic acid. These are promising platform chemicals that can be obtained from additional carbon sources, such as third generation biomass. Interestingly, both chemicals have shown promising potential in the bioplastics field and the applicability to synthesize subsequent products. Thus, the production of these chemicals should be further studied and optimized both at laboratory and pilot-scale.

**Co-fermentation** enabled to increase the fermentation yield when substrates of different composition, i.e. carbohydrates and proteins, were mixed, such as SS and the OFMSW. These synergistic effects should be studied from a metabolic point of view, by analysing the specific type of bacteria involved in the fermentation process and the significant changes that are produced due to the combination of both substrates.

- Different co-fermentation scenarios need to be studied, with different substrates ratios with the aim of optimising the fermentation processes.
- The detailed characterization of bacteria involved in the fermentation process should be performed, which might help understand the biochemistry of the process and give further process insights of mixed culture anaerobic fermentation.
- The effect of co-substrate addition in the fermentation yield and product distribution should be further evaluated.

During this thesis, ultrafiltration tests were performed at pilot scale. **Coupling the ultrafiltration membrane to the anaerobic fermentation system** in continuous mode of operation may lead to the process optimisation, by decoupling the solid retention time and the hydraulic retention time of the process. This was identified as a critical aspect that has not been tested in previous work.

- Long-term fermentation tests should be performed by assessing the influence of the HRT and SRT in the fermentation yield, product distribution and the technical challenges involved during the process.
- Optimization of the fermentation system might by selecting the appropiate HRT/SRT and assessing the effect on the acidogenic fermentation permeate composition.

**Mathematical modelling** of acidogenic fermentation processes is a complex approach that was attempted in a few literature studies. Mathematical models need to show a significant degree of accuracy while minimizing the complexity of the process. Anaerobic fermentation can be modelled by simple empirical models which focus on the conversion of biomass into cell biomass through the use of yield coefficients. However, this type of model does not consider all the level of detail of metabolites and bioproducts involved during the anaerobic fermentation process. On the other hand, more complexity is added with metabolic models, which focus on individual metabolites as they are consumed and converted into the cell structure. These are obtained from pure substrate degradation, such as glucose, and the level of detail in intermediate metabolites and final products distribution is well known. An intermediate approach could constitute the application of Lumped pathway models which used simplified metabolic models between the substrate consumption and biomass generation. This approach could be applied for example with the ADM1 model. The ADM1 is based on kinetic equations with a stoichiometric matrix approach for mass balance fulfilment. The ADM1 has been widely used for anaerobic digestion modelling. The integration of metabolic routes to ADM1 could be performed to add further details of the acidogenic routes of the substrates and the main product metabolites involved during the fermentation process. Since the details of the metabolic routes may add a high degree of complexity and hinder the potential application of the model, a compromise between the level of detail and efficient model development needs to be considered. Likewise, inhibition related to the free form of the chemicals should be considered. The developed model might be calibrated and validated with experimental data, from which, batch laboratory-scale experiments constitute a robust tool.

Regarding the downstream technology, **further FO studies should be performed** to optimise the membrane separation technology.

- Different draw solutions chemicals other than NaCl should be studied, i.e. $CaCl_2$, $MgCl_2$ or NaAc, by analysing the water flux and reverse salt flux and evaluating the solute concentration factor and water concentration factor.
- Different solute concentrations of draw solution should be used at the laboratory scale, to optimise the up-concentration effect and product distribution. An interesting substrate with high osmotic pressure potential is the desalination brine, which constitutes a challenging waste in desalination plants. Interestingly, the application of desalination brine for downstream processes might increase the sustainability of current desalination processes.
- Different membrane materials should be studied or synthesised. During this thesis, the thin film composite hollow fibre membranes performed better results than cellulose triacetate based flat sheet membranes. This could open new collaboration opportunities with material research groups to synthesise new polymeric materials for the active layer of FO membranes.
- The FO process should be further optimized regarding the flow rate of feed solution and draw solution, process configuration design and the temperature

of the FO tests. As previously reported, the orientation of the membranes can induce changes in the results obtained.

- FO technology should be up-scaled and coupled to the ultrafiltration membrane. In other words, an integrated solution of fermentation – separation – downstream needs to be validated at pilot-scale.
- FO technology needs to be studied with additional feed solutions, such as other major VFA, i.e. propionic acid, butyric acid, valeric acid, formic acid or caproic acid which constitute value-added bioproducts with potential market applications.

Regarding the downstream of target chemical, FO showed the potential to remove high amounts of water and to achieve high bioproduct titers. This could become a core technology in the downstream field and shorten the distance between real application of fermented bioproducts and academic research However, **a final purification step is required to recover these bioproducts**. The current state of the art is developing and studying new solutions for the purification of target chemicals. This opens many possibilities and opportunities for future research lines. For example:

- Crystallisation could be performed to crystallise the fermentation products, such as succinic acid, and to recover the bioproducts in the form of salts.
- Vacuum distillation could be used to separate the mixtures of the high concentrated fermentation effluents that are generated after FO treatment, where for example VFA could be easily evaporated.
- Pervaporation technology could be studied which showed potential results in preliminary studies to recover target VFA and fermentation products.

Finally, **life cycle assessment (LCA) and life cycle cost assessment (LCCA)** analysis should be performed to assess the environmental and economic feasibility of mixed and pure culture fermentation technology and downstream solutions. These tools are necessary to assess the whole pitcture of the integrated fermentation and downstream process in the the context of biorefineries.

From waste generation and processing, regarding the anaerobic fermentation process, and considering the impact of the downstream solution, the technological solution proposed should be assessed from an integrated approach.

In addition, the LCCA might contribute to assess the cost-effectiveness of the technology and subsequent market opportunities of the bioproducts generated from the fermentation of organic waste.

# APPENDIX A

# PUBLICATIONS GENERATED

The scientific publications derived from the present thesis are listed below:

## INTERNATIONAL JOURNALS

### Published papers

Garcia-Aguirre, J., Esteban-Gutiérrez, M., Irizar, J., González-Mtnez de Goñi, J., Aymerich, E. 2019. Continuous acidogenic fermentation: narrowing the gap between laboratory testing and industrial application. Bioresour. Technol. 282. 407-416.

Esteban-Gutiérrez, M., Garcia-Aguirre, J., Irizar, I., Aymerich, E. 2018. From sewage sludge and agri-food waste to VFA: individual acid production protential and up-scaling. Waste Manage. 77: 203-212.

Garcia-Aguirre, J., Aymerich, E., González-Mtnez de Goñi, J., Esteban-Gutiérrez, M. 2017. Selective VFA production of organic waste streams: assessing temperature and pH influence. Bioresour. Technol. 244: 1081-1088.

### Submitted papers

Garcia-Aguirre, J., Alvarado Morales, I. Fotidis, Schneider, C., Angelidaki, I., 2019. Forward Osmosis technology for downstream recovering of valuable acidogenic fermentation bioproducts. Water Research.

### Papers in preparation

Garcia-Aguirre, J., Alvarado Morales, M., Fotidis, I., Angelidaki, I. 2019. Recovery of succinic acid, lactic acid and ethanol from real fermentation broths by Forward Osmosis membranes. Environmental Science and Technology.

## INTERNATIONAL CONFERENCE PROCEEDINGS

**Conferences related to VFA production and downstream**:

Garcia-Aguirre, J., Esteban-Gutiérrez, M., Aymerich, E. 2016. Assessing the potential of industrial waste streams for volatile fatty acid (VFA) production. **First International Conference on Bioenergy and Climate Change**, June 2016, Soria, Spain.

Garcia-Aguirre, J., Aymerich, E., Esteban-Gutiérrez, M. 2017. From WAS to VFA scaling-up the process and comparison with other organic waste streams. **The IWA specialist conference on sludge management (SludgeTech)**. July 2017, London UK.

Garcia-Aguirre, J., Fotidis, I., Alvarado-Morales, M., Schneider, C., Angelidaki, I. 2018. Recovery of Acetic, succinic and lactic acid through Forward Osmosis: a novel downstreaming approach. **Sustain conference 2018**. Technical University of Denmark (DTU), November 2018, Denmark.

Garcia-Aguirre, J., Esteban-Gutiérrez, M., González-Mtnez de Goñi, J., Irizar, I., Aymerich, E. 2019. From laboratory to continuous pilot scale VFA production: the challenge of up-scaling and process engineering. **IWA World Conference on Anaerobic digestion AD16**, Delft University of Technology, 23-27 June 2019, The Netherlands.

**Conferences related to anaerobic digestion and wastewater treatment**

J. Garcia-Aguirre, M. Esteban-Gutiérrez, L. Pastor, J.E. Sánchez-Ramirez, S. Doñate, R. Romaguera, C. Lardín, C.E. Mino. 2019. Dry anaerobic digestion of Sewage Sludge in small and medium WWTP. **IWA World Conference on Anaerobic Digestion AD16**. Delft Unviersity of Technology, 23 – 27 June 2019, The Netherlands.

J. Garcia-Aguirre, M. Esteban-Gutiérrez, Y. Tejido-Núñez, J. Luis García-Heras, E. Aymerich, 2016. SARASWATI, supporting consolidation, replication and up-scaling of sustainable wastewater treatment and reuse technologies for India. **I Sustainable Engineering Forum**. 28-29 September 2016. Granada (Spain).

L. Pastor, J.E. Sánchez-Ramírez, S. Doñate, E. Aymerich, M. Esteban-Gutiérrez, J. Garcia-Aguirre, R. Romaguera, C. Lardín, E. Mino. Life-ANADRY: start-up for thermophilic dry anaerobic digestion of Sewage Sludge in small and medium wastewater treatment plant (WWTP). **SMICE 2018 (IWA)**. 23-25 May 2016, Rome.

## NATIONAL CONFERENCE PROCEEDINGS

Garcia-Aguirre, J., Esteban-Gutiérrez, M., Aymerich, E. 2016. Valoración del potencial acidogénico de diferentes residuos agro-industriales, **Jornada de la Red Española de Compostaje (REC)**, Sevilla, 16 – 18 noviembre de 2016.

Aymerich, E., Garcia-Aguirre, J., Esteban-Gutiérrez, M. 2016. Nuevas vías para la valorización de residuos orgánicos y aguas industriales: obtención de Biocompuestos: AGV. **Red Novedar**. Madrid, 3 de noviembre de 2016.

Garcia-Aguirre, J., Aymerich, E., Esteban-Gutiérrez, M. 2017. De lodo a AGV, escalado del proceso y comparación con otras corrientes residuales. **VII jornada sobre gestión y tratamiento de lodos**. Barcelona, 22 de noviembre de 2017.

Garcia-Aguirre, J., Esteban-Gutiérrez, M., Aymerich, 2019. Aplicación de osmosis directa para recuperar bioproductos de alto valor añadido obtenidos por fermentación anaerobia. **VII jornada sobre biorreactores de membrana**. Barcelona, mayo de 2019.

## PROJECTS

### DIGESTAIR - A novel anaerobic digester solution in air transport for on-board safe and efficient waste management

- Funding organism: European Union, Clean Sky 2 Programme
- Partners: Ceit-IK4, CITD, Fraunhofer
- Period: April 2019 – April 2021.

### Life-ANADRY- Dry anaerobic digestion as an alternative management & treatment solution for sewage sludge

- Funding organism: European Union, LIFE Programme
- Partners: Ceit-IK4, DAM, INDEREN, ESAMUR, EMWIS-SEMIDE
- Period: September 2015 – February 2019.

### SARASWATI-Supporting consolidation, replication and up-scaling of sustainable wastewater treatment and reuse technologies for India

- Funding organism: European Commission (FP7- Collaborative Project)
- Partners: Ceit-IK4, BOKU, BRGM, CENTA, University of Exeter, A3I, SIMBIENTE, HYDROK UK LTD
- Period: September 2012 – August 2016.

### PROAZETIK - Producción de acético a partir de RSU (Residuos Sólidos Urbanos): avanzando hacia una economía circular

- Funding organism: Diputación Foral de Gipuzkoa
- Partners: Ceit-IK4
- Date: January 2018 – October 2018.

**PRODIGEST- Valorization of pulp and paper mill sludge through anaerobic co-digestion with WWTP sludge**

- Funding organism: IHOBE – Basque Government
- Partners: Ceit-IK4, CONAQUA, Añarbe, Iberpapel.
- Date: September 2014 – June 2015