Universidad de Valladolid

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PROGRAMA DE DOCTORADO EN INGENIERÍA QUÍMICA Y AMBIENTAL

TESIS DOCTORAL:

New insights into anaerobic digestion: hybridization, bioproducts and microparticles

Presentada por **Alfonso García Álvaro** para optar al grado de
Doctor por la Universidad de Valladolid

Dirigida por:
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A mi familia

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Resumen

El proceso de digestión anaerobia se ha estudiado desde hace años resultando en una gran cantidad de aplicaciones. Las investigaciones dentro de esta tecnología se han orientado hacia la mejora con diferentes enfoques científicos: el estudio más fundamental del proceso, la cinética de las etapas, el uso final de los productos y subproductos obtenidos, o los pretratamientos para la optimización del proceso, entre otros.

Se trata de un proceso biológico que de manera controlada puede suponer una gran oportunidad para la generación de energía renovable y su integración dentro del *mix* energético de los países. Su principal producto es el biogás que tiene un alto poder calorífico y se usa como una fuente de bioenergía que sustituye a los combustibles fósiles, los cuales actualmente siguen suponiendo más del 75% del consumo energético global. Una mayor implementación de la producción de biogás supondría un ahorro en emisiones de gases de efecto invernadero y la mitigación del uso de combustibles fósiles derivado del crecimiento de la población, que a su vez implica un aumento de la generación de residuos orgánicos. El desarrollo de las tecnologías de digestión anaerobia puede tener un efecto doble, ya que con la producción del biogás se dejan de emitir gases de efecto invernadero a la atmósfera al tratarse de una fermentación anaeróbica controlada de residuos orgánicos, y en su consumo se evita el uso de otros combustibles más dañinos para el medio ambiente. Estas emisiones captadas por la digestión anaerobia controlada tienen un potencial enorme cuando trabajamos con sustratos o residuos orgánicos agrícolas (de origen antropogénico) ya que, de manera espontánea, suponen actualmente el 40 % de las emisiones de metano a la atmósfera siendo este gas uno de los más dañinos con el medio ambiente por su fuerte efecto en el aumento de las temperaturas, que es aproximadamente 28 veces mayor que el dióxido de carbono.

Es, por tanto, imprescindible centrar parte de los esfuerzos en mitigar este efecto con una mejor gestión de esta actividad agrícola dentro del sector primario con un doble enfoque para convertir al sector (i) mejorando su rentabilidad, por la gestión de los subproductos generados, así como (ii) atenuando su efecto como fuente de gases de efecto invernadero y, en suma, transformarlo en un proceso mucho más sostenible.

En este contexto, los desechos ganaderos como el purín de cerdo y otros animales, que además llevan asociados otros problemas ambientales por contaminación de suelos y malos olores, son sustratos adecuados y ampliamente utilizados para la producción de biogás. Los subproductos de las plantaciones de cereales básicos como el trigo, la cebada o el centeno, por su abundancia y amplia disponibilidad geográfica se presentan como sustratos clave en esta transformación. Finalmente, la industria de productos agrícolas, que normalmente demanda energía, también tiene una oportunidad de transformación hacia la sostenibilidad incluyendo procesos de digestión anaerobia con la gestión de los subproductos de bajo o nulo valor añadido. En este sentido, la industria vitivinícola por su extensa distribución y la gran cantidad de subproductos asociados a su actividad puede contribuir notablemente a la generación de bioenergía en el sector primario. Dentro de esta industria las lías de vino son uno de los sustratos con más necesidades de gestión por su alto contenido de materia orgánica y su compleja composición química que puede tener efectos nocivos en el medioambiente.

No obstante, la transformación biológica dentro del proceso de digestión anaerobia va a ser diferente para cada tipo de sustrato por su diferente composición química y propiedades físicas. Es, por tanto, imprescindible estudiar a fondo los diferentes subproductos disponibles para su gestión a partir de la fermentación anaeróbica con el objeto de maximizar su transformación. De este modo, el desarrollo tecnológico del proceso de digestión anaerobia aplicado al sector agrícola es básico para la implementación de la producción de biogás y una operación más eficiente de las instalaciones, permitiendo que sea, más respetuoso con el medio ambiente y rentable para el usuario final.

El objetivo de esta tesis es el estudio a escala de laboratorio de tecnologías incipientes en el proceso de la digestión anaerobia aplicada a sustratos orgánicos procedentes de la actividad agrícola. Estos estudios se acompañan de un análisis de proyección para la integración a escala real a partir de simulaciones con datos experimentales. Específicamente, se busca optimizar la digestión anaerobia mediante el control y seguimiento de los parámetros operativos gracias al (i) estudio microbiológico del medio, (ii) la generación de subproductos de alto valor durante el proceso, (iii) la eficiencia del uso de micropartículas para acelerar la transformación biológica, (iv) el uso de pretratamientos en residuos lignocelulósicos y (v) la viabilidad de combinar la digestión anaerobia con otras fuentes de energía renovable.

Para ello, en las dos primeras secciones se presenta el estado del arte del proceso de digestión anaerobia y sus nuevas perspectivas (**Capítulo 1**) así como los objetivos y desarrollo de la tesis (**Capítulo 2**). Los posteriores cinco capítulos (3-7) son las líneas de trabajo específicas que preceden a una última sección de conclusiones y líneas futuras de trabajo (**Capítulo 8**).

En el **Capítulo 3**, se propuso una aplicación de tecnología híbrida para la generación de biogás y biometano en granjas aisladas a partir de la digestión anaerobia del purín de cerdo generado en la actividad ganadera y la instalación de paneles solares híbridos con capacidad de producir simultáneamente calor y electricidad para proporcionar energía térmica para la fermentación anaeróbica (entre 35 y 55 °C) y energía eléctrica/térmica para los consumos del proceso y de la transformación del biogás en biometano (upgrading).

Para ello, se estudiaron cinco localizaciones diferentes a nivel mundial en zonas de alta actividad porcina, evaluando el balance energético y la generación final de energía en forma de biometano en función de las condiciones climáticas de cada localización comprobando que: (i) la configuración híbrida planteada garantiza un mayor rendimiento energético y acceso a bioenergía en zonas aisladas, (ii) las altas temperaturas y, en particular, la disponibilidad de luz solar, favorecen este sistema híbrido en términos de eficiencia energética y conversión a biometano, con valores máximos en zonas tropicales, hasta el 83,3% del potencial, y mínimos en climas templados fríos, menos del 40 % en promedio anual y (iii) los climas templados permiten una producción intermitente de biometano con una marcada estacionalidad que limita la operación de upgrading a los meses cálidos. Se realizó un análisis económico considerando los costes de capital y operativos del sistema híbrido propuesto con el objeto de compararlo con otras energías renovables en términos de "Coste nivelado de la energía" (LCOE), que es un indicador normalizado del coste promedio neto de generación de energía durante el ciclo de vida del sistema, obteniendo un valor de entre 0,042 y 0,055 USD/kWh en función de la localización, resultando notablemente competitivo en comparación con otras tecnologías del sector energético.

En el **Capítulo 4**, se evaluó la producción simultánea de biogás y carboxilatos o ácidos grasos volátiles (AGVs) por fermentación anaerobia como subproducto intermedio del proceso. Para ello, se estudiaron las condiciones operativas óptimas de la fermentación acidogénica cuyos principales productos son los AGVs -ácidos acético (C2), propiónico (C3), butírico (C4) y valérico (C5)- utilizando como sustrato agrícola paja de cultivos de cereal (trigo, cebada y centeno), con diferentes condiciones de pretratamiento mecánico, a partir de un experimento por lotes. Las transformaciones bioquímicas se modelizaron mediante las ecuaciones y formulaciones validadas aplicadas para la producción de biogás (ADM1), y se calcularon balances de masas para evaluar la bioconversión de diferentes sustratos.

La modelización de las fases de digestión anaerobia ayudó a comprender el proceso y a establecer una estrategia para maximizar la producción de AGVs. El uso de un tiempo hidráulico de residencia (THR) de entre 9 y 12 días permitió alcanzar una concentración total de AGVs por encima 4000 mg/L paralelamente a una producción de biogás en torno a 300 mL CH_4/g SV. La generación simultánea de AGVs y biogás permitiría suplir el consumo térmico para la operación del digestor en la integración del proceso dentro de una planta de biorrefinería. Esta configuración propuesta maximiza la bioconversión de biomasa en bioproductos y garantiza las necesidades energéticas del proceso.

La paja de cebada presentó un índice de conversión por encima del 60 % de la DQO inicial, siendo un 30 y un 35% superior al de la paja de centeno y trigo, respectivamente, probablemente debido a que su menor contenido en lignocelulosa favorece su biodegradabilidad. El pretratamiento mecánico, por otra parte, permitió aumentar, para los tres sustratos, tanto el rendimiento de la bioconversión de la paja en biogás y carboxilatos como la cinética del proceso de digestión anaerobia (hasta un 40%).

En el **Capítulo 5**, se evaluó el efecto de la adición de micropartículas en el rendimiento del proceso de digestión anaerobia de las lías del vino, subproducto de la industria vitivinícola. Para ello se pusieron en marcha reactores anaerobios discontinuos en los que se estudió el potencial de producción metanogénico con la agregación de micropartículas de base férrica (magnetita) y carbonosa (grafito), para determinar su efecto en la mejora del potencial de producción de biometano (BMP). Al mismo tiempo, se monitorizaron otros parámetros clave del proceso, como la composición del biogás, el pH y la concentración de AGVs y compuestos fenólicos.

La adición de micropartículas de magnetita dio lugar a un aumento sustancial del 39,1% en las tasas de producción de biometano, mientras que el uso de micropartículas de grafito produjo un aumento del 35,6%. No obstante, durante el proceso de digestión anaerobia se detectaron factores inhibidores, como un elevado contenido de AGVs y bajos valores de pH, que no se evitaron con la presencia de las partículas. Además, el contenido en el biogás de ácido sulfhídrico (H_2S) mostró un rápido aumento a lo largo del proceso de digestión anaerobia lo que supuso otro factor inhibitorio del proceso.

En el **Capítulo 6**, se pusieron en marcha dos reactores anaerobios paralelos alimentados con purín de cerdo trabajando en condiciones semicontinuas durante 150 días monitorizando los parámetros fisicoquímicos relevantes en el proceso de digestión anaerobia trabajando a un bajo THR con el objeto de identificar posibles inhibiciones y analizar las poblaciones microbianas en cada escenario, a partir de las abundancias relativas basadas en el ARNr 16s.

La producción de biometano registró una fuerte inhibición debida a las altas concentraciones de amoniaco y AGVs que se superó tras más de 70 días de marcha. Estas tendencias indican que las condiciones del reactor cambian con el tiempo y subrayan la importancia de una cuidadosa monitorización y control de los parámetros del sistema (químicos y biológicos) para optimizar la producción de biogás en aplicaciones de digestión anaerobia. Además, el análisis microbiológico reveló que los taxones de bacterias y arqueas identificadas en el purín de cerdo muestran resiliencia durante el proceso de digestión anaerobia, lo que sugiere que la población microbiana que se encuentra en este sustrato podría ser suficiente para la rápida puesta en marcha de la digestión anaerobia sin necesidad de añadir fuentes externas de microorganismos como lodos anaerobios.

En el **Capítulo 7**, se evaluó el efecto de la aplicación de un pretratamiento mecánico y térmico (explosión de vapor) en la paja de trigo como sustrato para la mejora cinética de su transformación biológica en biometano. Para ello, se establecieron experimentos por lotes dando seguimiento a la producción de biogás y a otros parámetros críticos del proceso. También se llevó a cabo una evaluación energética de todo el proceso para determinar su viabilidad a escala real comprobando que la etapa de hidrólisis térmica optimizaba el balance energético del proceso hasta un 15 % por descomposición de la hemicelulosa de la paja de trigo que hace que se aumente la biodegradabilidad del sustrato.

Los resultados obtenidos en esta tesis confirman el potencial del proceso de digestión anaerobia en la gestión de los residuos y subproductos agrícolas como una tecnología

eficiente, limpia y sostenible que favorece la economía circular. La necesaria transformación del sector pasa por este tipo de alternativas que además deben resultar rentables para su inversión. El desarrollo tecnológico del proceso se hace necesario para mejorar el rendimiento del proceso e incentivar su uso. En este desarrollo se puede mejorar sustancialmente el rendimiento mediante: (i) la hibridación de digestores anaerobios con energía solar, en el caso de sustratos procedentes de la ganadería, (ii) la conversión de sustratos agrícolas abundantes en bioproductos (AGVs), (iii) la adición de micropartículas en sistemas destinados a sustratos recalcitrantes de difícil digestión, (iv) la caracterización de poblaciones microbianas en procesos susceptibles de sufrir inhibiciones y (v) la aplicación de pretratamiento térmico para mejorar la biodegradabilidad de sustratos de composición lignocelulósica.

Abstract

The anaerobic digestion process has been studied for years, leading to numerous applications. Research in this technology has been focused on improvement through various scientific approaches: the most fundamental study of the process, the kinetics of the stages, the final use of the products and by-products obtained as well as pretreatments for process optimization, among other aspects.

This is a biological process which, in controlled conditions, can represent a great opportunity for the generation of renewable energy and its integration into the country’s energy mix. Its primary product is biogas, which has a high calorific value and is used as a source of bioenergy with the capacity to be a direct substitute for fossil fuels, which currently still account for more than 75% of global energy consumption. Greater implementation would mean savings in greenhouse gas emissions and mitigation of the use of fossil fuels due to global population growth, which leads to a constant increase in energy consumption and the generation of organic waste. Its implementation would also result in a double effect. From production, where greenhouse gas emissions to the atmosphere are avoided through controlled anaerobic fermentation, to its final consumption, which avoids the use of other more environmentally harmful fuels. The emissions captured through controlled anaerobic digestion have a significant potential, particularly when dealing with agricultural organic substrates from the anthropogenic activity since, these emissions account for 40% of methane released into the atmosphere spontaneously. Methane is one of the most environmentally damaging gases due to its strong effect on temperature increase, approximately 28 times greater than carbon dioxide.

Therefore, it is imperative to focus efforts on mitigating this effect through a better management of this agricultural activity within the primary sector. This involves a double approach of (i) enhancing the sector's profitability through the management of the by-product generated and (ii) mitigating its impact as a source of greenhouse gas emissions, ultimately transforming it into a much more sustainable process.

In this context, livestock waste such as pig slurry, which are also associated with environmental issues due to soil contamination and unpleasant odours, are suitable and widely substrates for the biogas production. By-products from basic cereal crops like wheat, barley, or rye, due to their abundance and high geographical availability, emerge as key substrates in this transformation. Finally, the agricultural products industry, that normally requires energy in its activity, also has an opportunity for transformation towards sustainability by including anaerobic digestion processes with the management of low or no added-value by-products. In this sense, the wine industry, due to its extensive distribution and the significant quantity of by-products associated with its activities, can contribute significantly to bioenergy generation in the primary sector. Within this industry, wine lees are one of the substrates with the greatest management needs due to their high organic matter content and complex chemical composition, which can have harmful effects on the environment.

The biological transformation in process of anaerobic digestion varies for each type of substrate due to differences in chemical composition and physical properties. In such a way that is essential to study the different available by-products for their management through anaerobic fermentation to maximise the biotransformation. In this way, technological development in the anaerobic digestion process applied to the agricultural sector is fundamental for a biogas production implementation and a more efficient operation of the installations. This not only ensures environmental improvement but ultimately makes it profitable for the end user.

The aim of this thesis is to investigate, at a laboratory scale, the emerging technologies in the anaerobic digestion process using organic substrates from agricultural activities. Additionally, the study includes their integration on a larger, real scale through a simulation projection based on experimental data. Specifically, the aim is to enhance the anaerobic digestion process by controlling and monitoring operating parameters through (i) microbiological analysis of the medium, (ii) the generation of high-value by-products during the process, (iii) the efficient use of microparticles addition to accelerate the biological transformation, (iv) the application of pretreatments on lignocellulosic wastes, and (v) the feasibility of integrating anaerobic digestion with other renewable energy sources.

For this purpose, the first two sections provide an overview of the state of the art in the anaerobic digestion process and its new perspectives (**Chapter 1**), as well as the objectives and development of the thesis (**Chapter 2**). The following five chapters (3-7) represent the specific lines of work, leading to a final section of conclusions and future lines of work (**Chapter 8**).

In **Chapter 3**, a hybrid technology application was proposed for biogas and biomethane generation on isolated farms. This involved anaerobic digestion of pig slurry from livestock activities and the installation of hybrid solar panels capable of simultaneously producing heat and electricity. The generated heat serves the anaerobic fermentation process (maintained between 35 and 55 °C), while the electricity/heat powers the overall process and the upgrading of biogas into biomethane.

To achieve this, five different locations worldwide with a high pig farming activity were studied, evaluating the energy balance and the final energy generation in the form of biomethane based on the climatic conditions of each location. The findings confirmed that: (i) the proposed hybrid configuration ensures higher energy efficiency and access to bioenergy in isolated areas, (ii) high temperatures, especially sunlight availability, favour this hybrid system in terms of energy efficiency and biomethane conversion, reaching maximum values in tropical zones, up to 83.3% of the potential, and minimums in cold temperate climates, averaging less than 40% annually, and (iii) temperate climates allow intermittent biomethane production with a marked seasonality that limits upgrading operation to warm months. An economic analysis was carried out considering the capital and operational costs of the proposed hybrid system in order to compare it with other renewable energies in terms of the "Levelized Cost of Energy" (LCOE), which is a standardized indicator of the average net cost of energy generation over the system's lifecycle. The obtained value ranged between 0.042 and 0.055 USD/kWh depending on the location, proving to be significantly competitive compared to other technologies in the energy sector.

In **Chapter 4**, a simultaneous production of biogas and carboxylates or volatile fatty acids (VFAs) as an intermediate by-product of the anaerobic fermentation process was evaluated. Optimal operational conditions for acidogenic fermentation were studied, focusing on the production of VFAs - acetic acid (C2), propionic acid (C3), butyric acid (C4), and valeric acid (C5). The agricultural substrate used in this investigation was cereal straw (from wheat, barley, and rye crops), with a different mechanical pretreatment conditions, in a batch experiment setup. Biochemical transformations were modelled using validated equations and formulations applied for biogas production (ADM1). Mass balances were calculated to evaluate the bioconversion of different substrates.

The modelling of anaerobic digestion phases facilitated an understanding of the process and to establish a strategy to maximize the production of Volatile Fatty Acids (VFAs). Employing a hydraulic retention time (HRT) between 9 and 12 days led to achieving a total VFA concentration exceeding 4000 mg/L, concurrently with a biogas production of around 300 mL CH_4/g VS. The simultaneous generation of VFA and biogas would allow to supply the thermal consumption for the digester operation in the integration of the process within a biorefinery plant. This proposed configuration aims to optimize the biomass bioconversion into bioproducts while ensuring the energy requirements of the process.

Barley straw showed a conversion rate above 60% of the initial chemical oxygen demand (COD), which was 30% and 35% higher than that of rye and wheat straw, respectively. This difference is likely attributed to its lower lignocellulose content, promoting higher biodegradability. On the other hand, mechanical pretreatment increased both the conversion efficiency of straw into biogas and carboxylates and the kinetics of the anaerobic digestion process for all three substrates, achieving an improvement of up to 40%.

In **Chapter 5**, the impact of adding microparticles on the performance of the anaerobic digestion process of wine lees, a by-product of the wine industry, was evaluated. Discontinuous anaerobic reactors were employed to investigate the methanogenic production potential with the addition of microparticles, specifically iron-based (magnetite-Fe_3O_4) and carbon-based (graphite) to determine their effect on improving the biochemical methane potential (BMP). Simultaneously, other key process parameters, such as biogas composition, pH, and concentrations of VFAs and phenolic compounds, were monitored.

The addition of Fe_3O_4 microparticles resulted in a substantial increase of 39.1% in biomethane production rates, while the use of graphite microparticles led to a 35.6% increase. However, inhibitory factors were identified during the anaerobic digestion process, such as a high concentration of VFAs and low pH values, which were not mitigated by the presence of the particles. Additionally, the hydrogen sulphide (H_2S) content in the biogas showed a rapid increase throughout the anaerobic digestion process, serving as another inhibitory factor.

In **Chapter 6**, two parallel anaerobic reactors were initiated, fed with pig slurry, and operated under semi-continuous conditions for 150 days, monitoring the relevant physicochemical parameters in the anaerobic digestion process, operating at a low hydraulic retention time (HRT). The objective was to identify potential inhibitions and analyse microbial populations in each stage based on relative abundances derived from 16S rRNA.

The production of biomethane experienced a significant inhibition due to high concentrations of ammonia and VFAs, a challenge that was overcome after more than 70 days of operation. These trends highlight that reactor conditions evolve over time and underscore the importance of careful monitoring and control of system parameters (chemical and biological) to optimize biogas production in anaerobic digestion applications. Additionally, the microbiological analysis revealed that the intrinsic bacteria and archaea taxon's present in pig slurry exhibit resilience and could be very significative during the anaerobic digestion process. This suggests that the microbial population inherent to this substrate might be sufficient for the rapid initiation of anaerobic digestion without the need to introduce external sources of microorganisms, such as anaerobic sludge.

In **Chapter 7**, the impact of implementing mechanical (milling) and thermal pretreatments (steam explosion) on wheat straw as a substrate was evaluated to enhance the kinetic aspects of its biological transformation into biomethane. Batch experiments were set up to monitor biogas production and other critical process parameters finding the highest biomethane production with a steam explosion conditions of 170 ˚ and 7 bars for 15 minutes.

An energy assessment of the whole process was also carried out to determine its feasibility at a real scale, proving that the thermal hydrolysis stage optimized the energy balance of the process by up to 15% through the decomposition of wheat straw hemicellulose, enhancing the substrate's biodegradability.

The results obtained in this thesis confirm the potential of the anaerobic digestion process in the management of agricultural wastes and other by-products as an efficient, clean, and sustainable technology that favours the circular economy. The necessary transformation of the sector relies on such alternatives, which must also prove profitable for investment.

Technological advancements in the process are essential to enhance its performance and encourage its widespread implementation.

In this development, anaerobic digestion performance can be substantially enhanced through: (i) the hybridization of anaerobic digesters with solar energy, particularly for substrates from livestock, (ii) the conversion of abundant agricultural substrates into bioproducts (AGVs), (iii) the addition of microparticles in systems designed for recalcitrant substrates with challenging digestibility, (iv) the characterization of microbial populations in processes susceptible to inhibitions, and (v) the application of thermal pretreatment to improve the biodegradability of lignocellulosic substrates.

List of publications

The following publications are presented as part of the current thesis. All papers were published in international journals indexed in Journal Citation Reports.

Manuscript I. Alfonso García Álvaro, César Ruiz Palomar, Daphne Hermosilla Redondo, Raúl Muñoz Torre, Ignacio de Godos Crespo (2023). Hybridization of anaerobic digestion with solar energy: A solution for isolated livestock farms. *Energy Conversion and Management: X*, 100488.

https://doi.org/10.1016/j.ecmx.2023.100488

Manuscript II. Alfonso García Álvaro, César Ruiz Palomar, Daphne Hermosilla Redondo, Raúl Muñoz Torre, Ignacio de Godos Crespo (2023). Simultaneous production of biogas and volatile fatty acids through anaerobic digestion using cereal straw as substrate. *Environmental Technology & Innovation*, 103215.

https://doi.org/10.1016/J.ETI.2023.103215

Manuscript III. Alfonso García Álvaro, César Ruiz Palomar, Daphne Hermosilla Redondo, Raúl Muñoz Torre, Ignacio de Godos Crespo (2023). Improving the anaerobic digestion process of wine lees by the addition of microparticles. *Water*, *16*(1). https://doi.org/10.3390/w16010101

Manuscript IV. Alfonso García Álvaro, César Ruiz Palomar, Edgardo I. Valenzuela, Daphne Hermosilla Redondo, Raúl Muñoz Torre, Ignacio de Godos Crespo (2023). Simultaneous production of biogas and volatile fatty acids through anaerobic digestion using cereal straw as substrate. *Journal of Water Process Engineering*. (In review)

Manuscript V. Alfonso García Álvaro, César Ruiz Palomar, Israel Díaz Villalobos, Daphne Hermosilla Redondo, Raúl Muñoz Torre, Ignacio de Godos Crespo (2023). Improving biogas yield from wheat straw with thermal pretreatment. Energy integration study. *Energies*. (In review)

Contribution to the papers included in the thesis

Manuscript I. In this research, I was responsible for designing the methodology for the techno-economic evaluation of the proposed hybrid system of anaerobic digestion and solar energy. I collected all the necessary data to simulate the system and processed the data, conducting the calculations to obtain the energy balances. Some of the experimental data used came from the experiments conducted by MSc Eng. César Ruiz Palomar. Finally, I made the result analysis and the writing of the original manuscript. The review and editing of the manuscript were carried out by Dr. Ignacio de Godos, Dr. Daphne Hermosilla, and Dr. Raúl Muñoz.

Manuscript II. In this work, I was responsible for the experimental design based on the original idea proposed by Dr. Ignacio de Godos regarding the production of carboxylates from AD. I carried out the set-up of the batch experiment and the analytical measurements in coordination with MSc Eng. César Ruiz Palomar. I processed the data and evaluated the results in the manuscript writing, which was ultimately supervised by Dr. Ignacio de Godos, Dr. Daphne Hermosilla, and Dr. Raúl Muñoz.

Manuscript III. In this work, I was responsible for the experimental design in coordination with the Universidad Politécnica de Madrid -UPM- (Dr. Daphne Hermosilla and Dr. Antonio Gascó). I carried out the setup and analytical measurements of the experiment with the collaboration of MSc Eng. César Ruiz Palomar. Once the laboratory stage was completed, I processed the data, conducted mass balance calculations, evaluated the results, and the manuscript writing under the supervision of Dr. Ignacio de Godos, Dr. Daphne Hermosilla, and Dr. Raúl Muñoz.

Manuscript IV. In this work, I was responsible for the design, setup, and operation of the experimental setup in collaboration with MSc Eng. César Ruiz Palomar under the supervision of Dr. Ignacio de Godos. I conducted mass balance calculations, evaluated the results, and drafted the manuscript under the supervision of Dr. Ignacio de Godos, Dr. Daphne Hermosilla, and Dr. Raúl Muñoz. Dr. Edgardo Valenzuela was responsible for the characterization of microbial populations, where I contributed to the analysis and discussion of the data.

Manuscript V. In this work, I was responsible for the experimental design. I carried out the preliminary pre-treatments with the assistance of Dr. Israel Díaz. I conducted the setup of the batch experiment and the analytical measurements with the collaboration of MSc Eng. César Ruiz Palomar. I processed the data and performed the energy balance. Finally, I completed the manuscript writing under the supervision of Dr. Ignacio de Godos, Dr. Daphne Hermosilla, and Dr. Raúl Muñoz.

Chapter 1

Introduction

1.1. Current global situation

The continuous growth of the global population has supposed a significant challenge in sustainability. This phenomenon has increased the pressure on natural resources and intensified the demand for resources as food or water, and energy. Population growth and the rise in energy consumption are directly correlated (**Fig. 1**). Fossil fuels, including coal, natural gas, and oil, currently account for 78.9% of the origin of consumed energy (**Fig. 1, a**). Despite the prominent use of fossil fuels, renewable energy sources are growing rapidly and in recent years they have registered an increasing share of generation, surpassing 20,000 TWh and supplying 12.6% of global energy and 29.9% of electricity generation [1].

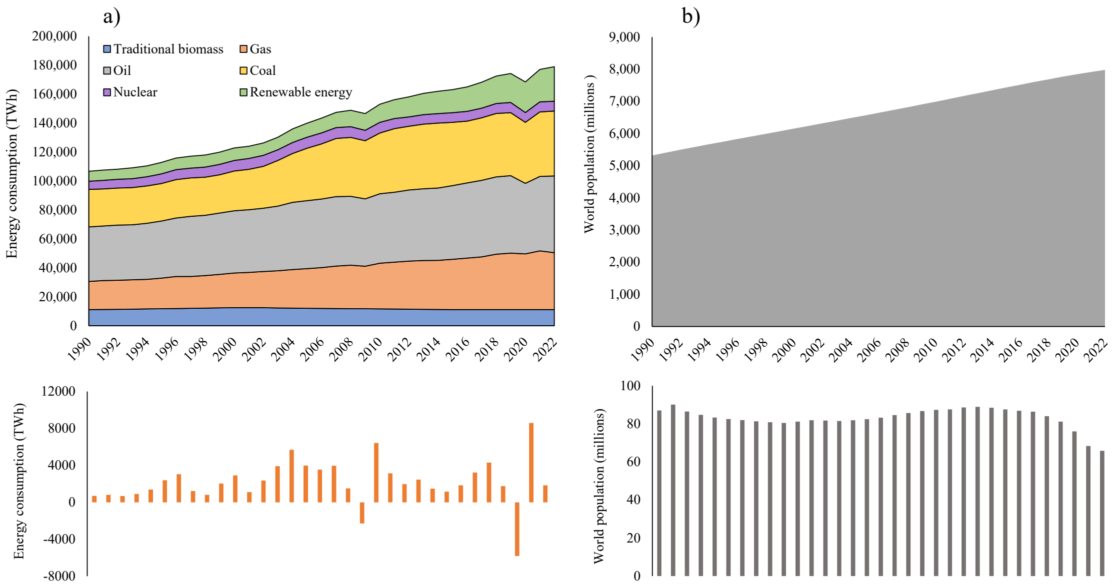


Fig. 1. a) Global primary energy consumption by source and **b)** World population evolution and their annual change, 1990-2022; [2].

This high dependence on fossil resources to meet global energy demand creates an unsustainable framework. The continuous extraction and burning of fossil fuels, besides contributing to greenhouse gas emissions (GHG), has led to the accelerated depletion of these non-renewable resources. In this sense, the term defined as peak oil, referring to the point in time when global oil production reaches its maximum level and starts to decline irreversibly generating energy insecurity, has been already surpassed (**Fig. 2, a**). This situation underscores the urgent need to a transition towards alternative energy sources.

In parallel, the increased consumption of natural resources has also had a huge impact on waste generation (**Fig. 2, b**). The production of substantial amounts of solid waste, coupled with inefficient waste management practices, contributes to soil and water degradation, among other impacts. The composition of waste generated globally averages in 40 % of organic fraction, in case of urban activities and more than 90 % in rural activities. That means that most part of the waste produced could be easily transformed into energy or commodities [3,4].

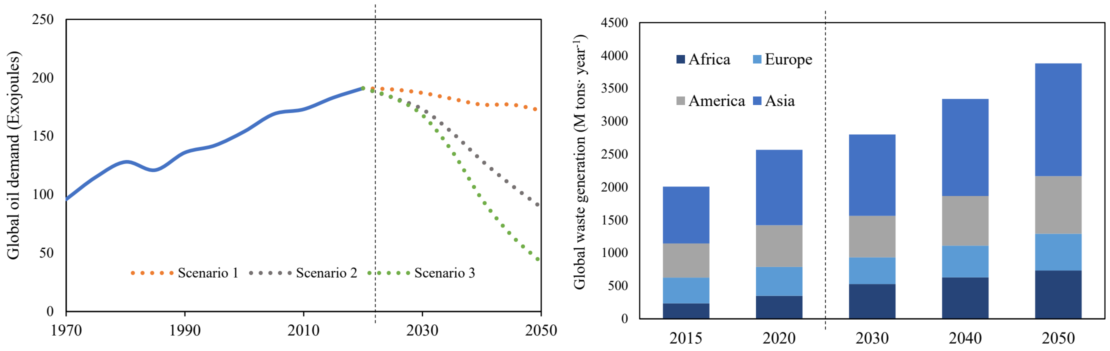


Fig. 2: a) Global oil demand in exajoules with three scenarios projected between 1970-2050. [5,6] **b)** Waste generation worldwide and the projection 2015-2050. [7].

This rise in resource depletion and waste generation is significantly impacting on greenhouse gases (GHG) emissions, and contributing to the global warning (**Fig. 3, a)**. In this manner, emmissions have reached values close to 40 Gt CO_2 equivalents, mainly pushed by industrial activity, transportation, and energy generation. [8]. This global warning is perceptible with indicators as rising temperatures, melting ice caps, droughts and more frequent extreme weather events. These dangerous consequences have motivated international efforts to address the GHG emissions mitigation: including the key agreements such as the Paris Agreement, where nations commit to limiting global temperature increases or The Kyoto Protocol, outlined emission reduction targets between others [9,10].

In addition to CO_2 emissions, the impact of other emissions that contribute to global warming, such as methane (CH_4), nitrogen oxides (NO_x), or hydrofluorocarbons (HFCs), among others, must also be considered. Collectively, these emissions contribute to climate change by trapping heat in the atmosphere, intensifying the greenhouse effect [11].

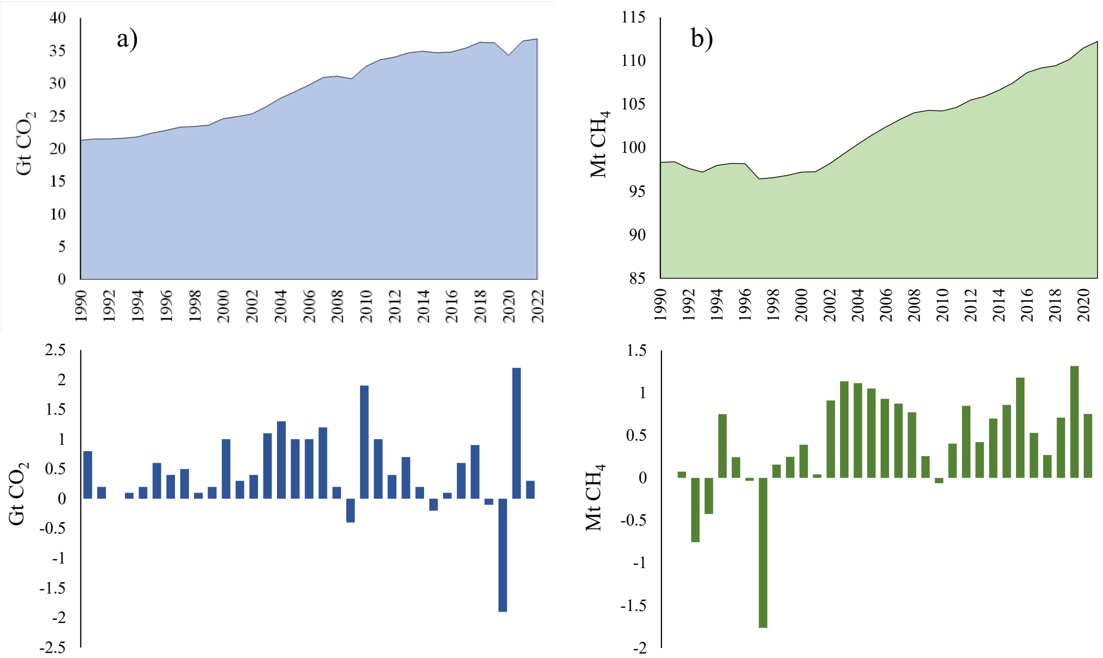


Fig. 3: a) Global CO_2 and **b)** CH_4 emissions and their annual change (Gt and Mt), 1990-2022 [8,12]

Methane emissions into the atmosphere may account for approximately 30% of the increase in global temperatures since the Industrial Revolution. This effect is attributed to two factors: (i) the duration it remains in the atmosphere and (ii) its capacity to absorb energy. Methane has a much shorter atmospheric lifetime than CO_2, around 12 years compared to centuries, but it absorbs significantly more energy while present in the atmosphere, resulting into 28 times the impact of CO_2 [13].

The origin of methane emissions includes approximately 35% from natural sources and 65% from human activities (**Fig. 4, b**). The most significant anthropogenic source is agriculture, accounting for about a quarter of total emissions, closely followed by the energy sector including emissions from coal, oil, natural gas, and biofuels [14]. Agricultural methane emissions come from livestock production, crop cultivation, landfills, and wastewater management. The largest contributor to the CH_4 emissions globally is livestock farming, primarily due to the ruminant activity of animals that emit methane as a by-product of the microbial activity in their digestive systems [15,16]. The primary factor contributing to the rise in emissions is the continual growth of the total number of livestock, once again linked to global population expansion (**Fig. 4, a**), even though in recent years the use of more modern technologies and optimization of diets has changed the linearity between the number of heads and the emission, with a clear reduction of methane release [17].

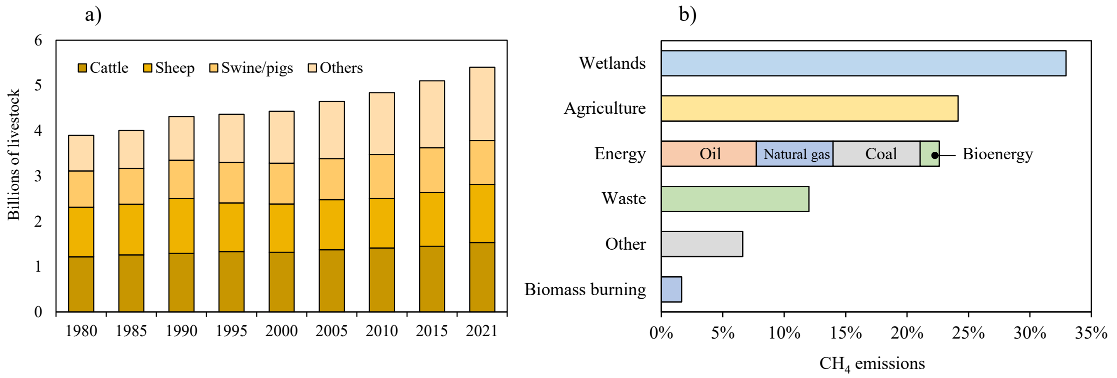


Fig. 4. a) Number of livestock in the world, 1980-2021 [18]; **b)** Sources of methane emissions [14]

An important contribution to methane emissions includes the management of liquid manure, including storage of sludge in lagoons, ponds or tanks, where the organic matter is spontaneously transformed into methane, which is responsible for 10-15% of total anthropogenic methane emissions [19]. Another important source are agricultural plantations, where waterlogged crops such as rice in flooded fields represent a significant source of methane emissions. Likewise, the management of by-products such as straw from staple crops like wheat, rice, and corn, with a huge worldwide production (**Fig. 5**), must also be considered since their natural decomposition releases GHG emissions, also contributing to soil and water pollution. Furthermore, the common practice of burning the straw as a disposal method has an even more damaging effect on air quality.

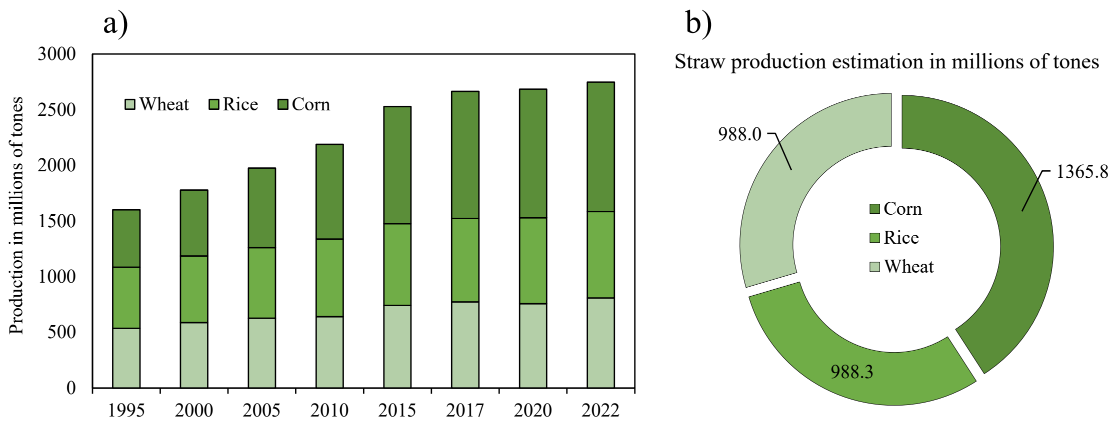


Fig. 5. a) Basic cereals production in the world, 1995-2022 and **b)** straw production estimation in millions of tons [18,20]

A third source of GHG emission is the agro-industrial waste management based in landfill deposition., becoming a significant methane production source [21]. That is the case of by-products derived from wine industry, that is globally widespread and especially important in Spain. (**Fig. 6**). During the process, by-products like grape pomace or wine lees are generated, and their organic composition represents an environmental challenge that requires a proper management.

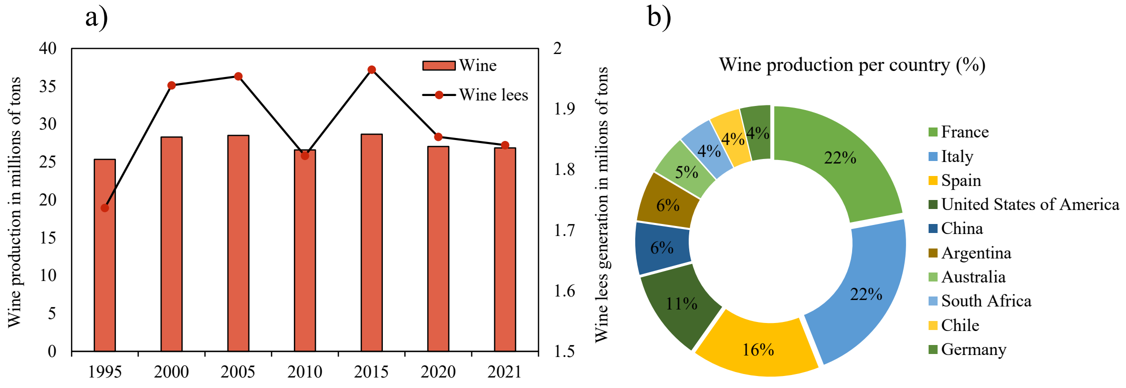


Fig. 6. a) Wine production and wine lees generation in the world in millions of tons, 1995-2021 and **b)** Wine production per country [18]

In all cases, these uncontrolled agricultural CH_4 emissions can considerable reduced by improving the management of their sources through two strategies (i) minimize such emissions and (ii) capture and convert these emissions into available energy, considering that methane gas has a high energy content. A significant portion of the proposed solutions to decrease CH_4 emissions into the atmosphere involves implementing the best available techniques aimed at enhancing the environmental efficiency of processes. This includes reducing pollutant emissions and resource consumption under economically and technically viable conditions. In the livestock sector, the strategies include applying nutritional techniques, enhancing housing design and management, implementing improvements during manure or slurry storage and optimizing water and energy usage [22]. In agricultural plantations, techniques such as periodic drainage and aeration can be applied. On one hand, these methods oxidize existing soil CH_4, and on the other hand, they inhibit the additional production of CH_4 in the soil [23].

In this context, anaerobic digestion emerges as an alternative for managing agricultural by-products. Through a controlled biological process, it does not only minimize CH_4

emissions but also valorises it for a final energy use. This approach has a double impact on the emissions balance of agricultural activities, potentially transforming a GHG emissions source into an emission sink [24] (**Fig. 7**). In addition to the environmental positive impact of this management, there is also an economic revenue due to the added value of the by-products generated in the process. Both biogas as an energy source and digestate as a fertilizing source contribute to this economic effect [25].

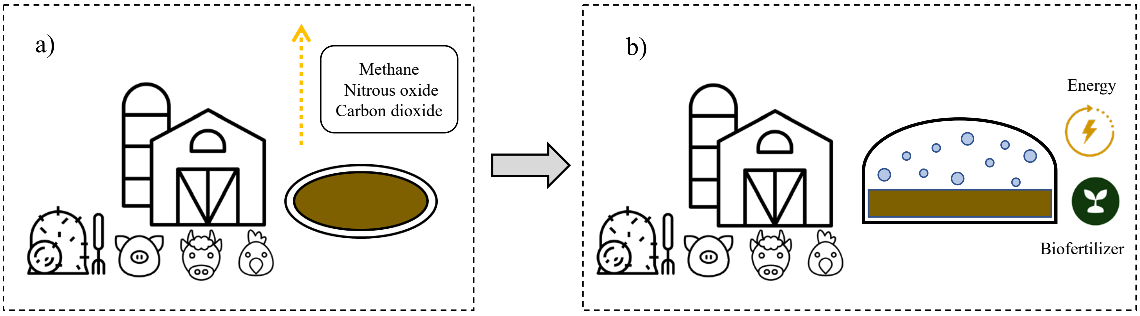


Fig. 7. Livestock farm **(a)** transformed using anaerobic digester **(b).**

Further improvement is needed to enhance biogas production performance, incorporating initiatives such as (i) microbiological characterization for process stability, (ii) pre-treatment in substrates with higher biodegradation difficulty, or (iii) using additives to improve biotransformation rates. Additionally, enabling the technology implementation in areas with organic substrate availability, even in energetically isolated regions through (iv) the hybridization with other energy sources to optimise the energy balance. Furthermore, agricultural wastes can be transformed by simple fermentation into (v) valuable bioproducts (such short chain organic acids) useful in the chemical industry. Finally, (vi) the process validation is needed for different organic substrates, paying special attention in those of agricultural origin due to their greater availability.

The development of these lines of work will make the anaerobic digestion process a more robust, efficient, and ultimately, a more profitable environmental technology for waste management.

1.2. Anaerobic digestion process

1.2.1. Background

Anaerobic digestion (AD) is a biological process where the organic matter is transformed from complex compounds to simpler compounds by microbiological activity in the absence of oxygen. The growing interest in this spontaneous process in recent decades has different perspectives, with the main ones being the reduction of organic load of substrates with a high organic content, the production of a biogas with a high energy content, or the obtaining of intermediate by-products from the process that can be applied in various industries.

In the research field, new lines of work are also being continuously explored, focused on gaining a deeper understanding of all process variables with the final objective of improve the biogas production ensuring the operation and stability. This includes studying the physicochemical parameters of operation, the substrates used in the process and their biodegradability, as well as the microbiological characterization of organic matter transformers.

AD, as a technology that is increasingly integrated into the research development field was identified and used centuries ago, with significant scientific and technological contributions over the years, as depicted in **Fig. 8**. Historical documents suggest that the Sumerians, 5,000 years ago, employed anaerobic cleaning of waste and wastewater. 2,000 years ago, the Roman scholar Pliny described bright lights appearing under the surface of swamps [26].

The fundamental biochemical principles of AD began to be clearly understood only a few decades ago. The process has been studied as a proven scientific matter since the 1930s. Even before that, some renowned scientists, including Alessandro Volta (Italy) at the end of 18th century, Michael Faraday (United Kingdom), and Louis Pasteur (France) in the 19th century, discovered fundamental facts about biogas and its generation process. The chemical structure of methane was formulated through the works of John Dalton (United Kingdom), Henry Cavendish (United Kingdom), Humphrey Davy (United Kingdom), and Amedeo Avogadro (Italy) [27]. The first documented and designed anaerobic digester was commissioned in Mumbai (India), in 1859. Another significant milestone in the development of AD was the construction of the first industrial-scale biogas plant in Exeter (United Kingdom) in 1895. This facility treated solid and liquid waste to produce methane, which was then used to illuminate public places in the city. These events marked the beginning of the practical application of large-scale anaerobic digestion for the generation of biogas with energy purposes.

Following research in the 19th and 20th centuries focused on the characterization of the microorganisms involved in the process, where microbiologists such as Sergei Winogradsky (Ukraine), Martinus Beijerinck (Netherlands), Ralph Stoner Wolfe (United States of America), Arthur Mckenzie Buswell (United States of America), and Horace Albert Barker (Untied States of America). Their contributions played a crucial role in clarifying the anaerobic digestion process [28].

Concerning the practical application of the process in the first half of the 20th century, reactor designs were achieved, and two-stage reactors were implemented, among other milestones such as the sale of biogas in Germany in 1923. In the second half of the 20th century, encouraged by the oil crisis of the 1970s, many biogas plants were established, with Germany notably leading the way, a fact that still continues today. Industrial new technologies were also developed that are still in use nowadays, such as UASB (Upflow Anaerobic Sludge Blanket) reactors, immobilised digesters, which are efficient systems that retain microorganisms on a solid support, the annamox process (ANaerobic AMMonium OXidation) to eliminate ammonium content, among others, and also a new line of biogas purification or up-grading that allowed greater added value to the biogas produced. In the 21st century, the development of biogas and biomethane plants remains to grow in number each year.

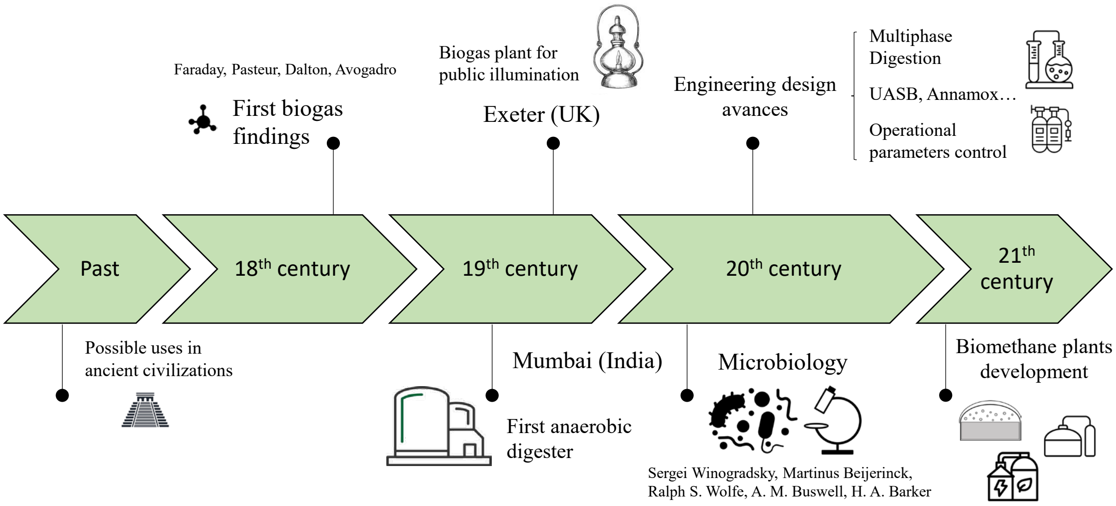


Fig. 8. Anaerobic digestion application and advances evolution.

A novel implement for diagnosing the main research trends in the field of AD is through a bibliometric study that allows the identification of research trends and gaps in this field. Scientific collections from Web of Science (WOS) and Scopus were examined using an “advanced search” on their platforms with the equation code provided in *Eq. 1*. Over 130,000 publications were registered from 1980 to the present (search end date: 20/11/2023). The collected data were exported and processed using the bibliometric software VOSviewer to creates a keyword network, thus identifying research trends in AD following the methodology described by Ampese et al., (2022) [29].

$$\text{Topic} = \text{“Anaerobic” and “Digestion”}\ ;\ \text{Keyword} = \text{“Anaerobic” and “Digestion} \qquad (1)$$

Fig. 9 illustrates the evolution of publications over the years 1980 and 2023, demonstrating a significant increase from the year 2000 to the present. The total number of publications has surpassed 10,000 annually since 2020. This exponential growth in publications related to anaerobic digestion reflects the growing interest of researchers in the subject and a global trend toward the use of renewable fuels. Authors like Yaoyang & Boeing (2013) emphasize this trend due to the strong interest in bioenergy and biofuels generation from waste [30].

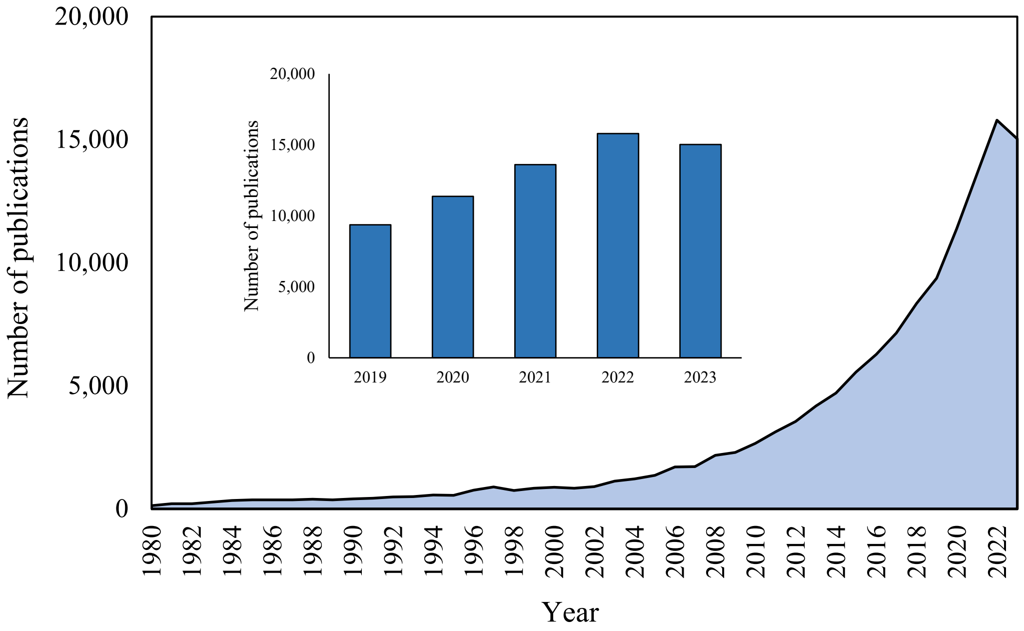


Fig. 9. Evolution of the number of publications over the years (1980–2023).

In **Fig. 10, a**, different research lines related to anaerobic digestion can be observed through the co-occurrence analysis of the clustering of the top 50 most frequent keywords in the described bibliometric search. This analysis is based on a selection of the 2,500 most relevant documents determined by the associated citation numbers. This tool can be used to identify critical points and research frontiers [31]. The most frequent research themes, with a larger size in the image, include biogas and methane, microbial community, wastewater, sewage sludge, and waste management. This result is an indicative of the interest in utilizing the AD process for energy recovery from waste, as well as a tendency to obtain a greater knowledge on the bioprocess and the microbiology features together with the operational optimization. On the other hand, it has been identified as a research frontier the heavy focus on wastewater and urban waste as substrates, while other substrates are receiving less attention in the scientific research.

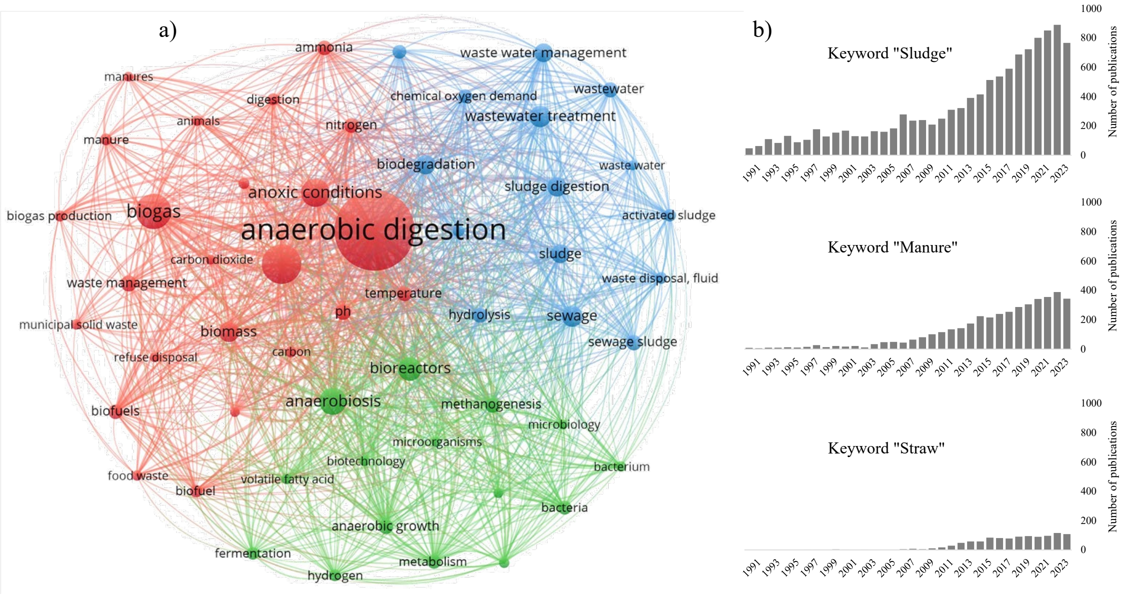


Fig. 10. a) Bibliometric mapping using a Cluster of the 50 most frequent keywords in the 2,500 most cited research publications related with "anaerobic digestion". **b)** Number of publications with the keyword "Anaerobic digestion" and "sludge", "manure" or "straw" since 1990.

1.2.2. Biochemical reactions

The biological process of AD is divided into four stages where organic matter is degraded from more complex to simpler molecules. Each of these stages is carried out by specific microorganisms that will be responsible for the kinetics of this transformation and as a whole for the final production of biogas whose main components are CH_4 (≈ 60 %) and CO_2 (≈ 40 %). The general equation for biogas formation can be seen in *Eq. 2* [32].

$$C_cH_hO_oN_nS_s + y\,H_2O \rightarrow x\,CH_4 + n\,NH_3 + s\,H_2S + \,(c-x)CO_2 \qquad (2)$$

where: $x = \frac{1}{8} \cdot (4c + h - 20 - 3n - 2s)$ / $y = \frac{1}{4} \cdot (4c - h - 20 + 3n + 3s)$

Specifically, these products can be divided into carbohydrates, fats and proteins according to *Eq. 3-5*:

Carbohydrates: $C_6H_{12}O_6 \rightarrow 3CH_4 + 3CO_2$ (3)

Fats: $C_{12}H_{24}O_6 + 3H_2O \rightarrow 7.5CH_4 + 4.5CO_2$ (4)

Proteins: $C_{13}H_{25}O_7N_3S + 6H_2O \rightarrow 6.5CH_4 + 3NH_3 + H_2S + 6.5CO_2$ (5)

These well-known and characterized stages, as established in various studies, are distinguished by the different activities of microorganisms in each of the four phases, as illustrated in **Fig. 11**. The first phase involves hydrolysis, where complex organic substances such as carbohydrates, lipids, and proteins, included polymers, fatty acids, and amino acids respectively, are broken down through the secretion of exoenzymes by anaerobic hydrolytic bacteria. [33].

In the second stage known as acidogenesis, the monomers generated are absorbed by acidogenic bacteria to transform them into short-chain fatty acids (C1 to C5 molecules), also called volatile fatty acids (VFAs), including acetic acid, propionic acid, butyric acid, and valeric acid. In this stage, alcohols, CO_2, H_2, and H_2O are also formed. In the third stage of acetogenesis, sometimes grouped together with the acidogenesis stage, fatty acids are oxidized into acetic acid (C2), also generating H_2 in the process through the activity of acetogenic bacteria, also producing H_2. The formation of acetate is thermodynamically possible only with a very low partial pressure of hydrogen, and therefore, these acetogenic bacteria must live in symbiosis with methane-producing microorganisms that consume this H_2. There is another pathway called homoacetogenesis where the CO_2 is reduced with H_2 to produce acetic acid ($2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$) [34].

In the last stage of the process, methanogenesis, CO_2, H_2, acetic acid, and methyl groups are transformed into CH_4 by the activity of archaea through three different metabolic pathways: (i) the hydrogenotrophic pathway from H_2 and CO_2, (ii) the acetoclastic pathway from the oxidation of acetic acid and CO_2 and finally, through (iii) the pathway of methyl groups which are oxidised to form CH_4, CO_2, and other compounds (see Eq. 6-16) [35]. There are several methane-forming reactions with different energy yields. Most of the methane generated comes from the acetotrophic pathway (≈ 70 %), while the remaining 30 % of the methane comes from the hydrogenotrophic and methylotrophic pathways.

Hydrogenotrophic pathway:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad (6)$$

$$4HCOOH_{Formate} \rightarrow CH_4 + 3CO_2 + 2H_2O \qquad (7)$$

$$4CH_3OH_{Methanol} \rightarrow 3CH_4 + CO_2 + 2H_2O \qquad (8)$$

Acetoclastic pathway:

$$CH_3OH_{Acetate} \rightarrow CH_4 + CO_2 \qquad (9)$$

Methylotrophic pathway:

$$4(CH_3)_3N_{Trimethylamine} + 6H_2O \rightarrow 9CH_4 + 3CO_2 + 4NH_3 \qquad (10)$$

$$2(CH_3)_2NH_{Dymethyl\ sulfate} + 2H_2O \rightarrow 3CH_4 + CO_2 + 2NH_3 \qquad (11)$$

$$4(CH_3)NH_{2\,Methylamine} + 2H_2O \rightarrow 3CH_4 + CO_2 + 4NH_3 \qquad (12)$$

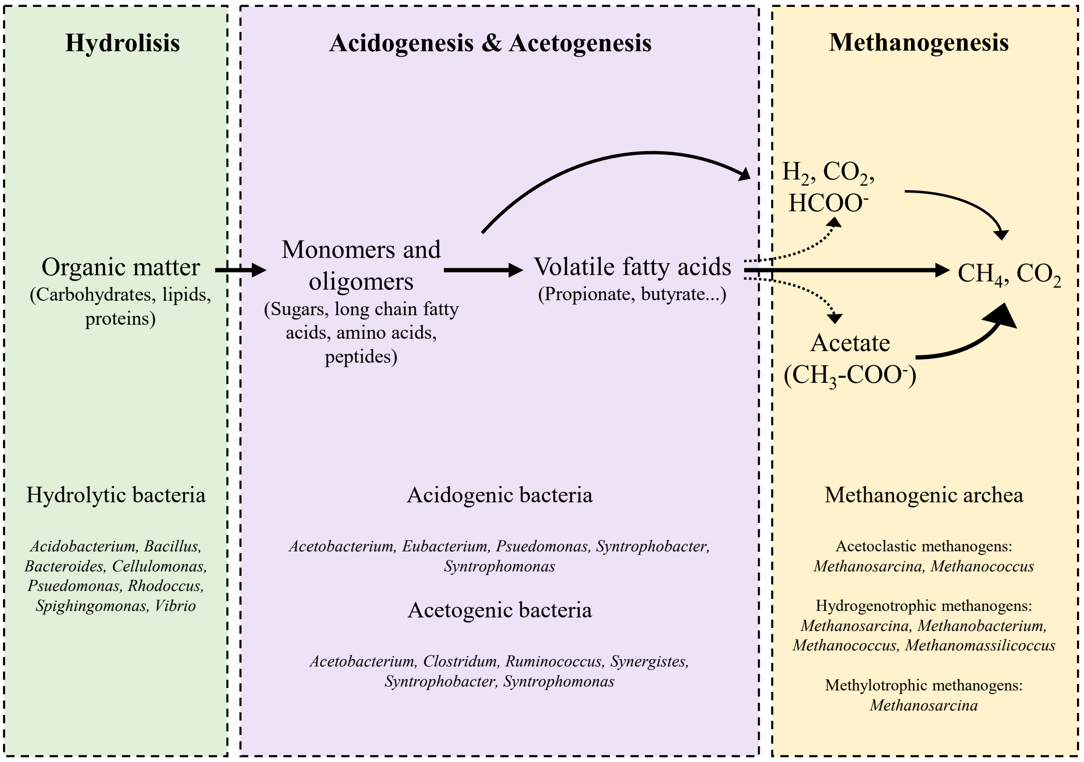


Fig. 11. The microorganism and pathways involved in the anaerobic digestion of organic substrates. Adapted from [36].

1.2.3. Microorganisms participating in the AD process

Each stage exhibits its own kinetic depending on the substrate composition and the environmental conditions where microorganisms interact. Generally, the hydrolytic phase can be limited by substrates with a high a complex structure, as lignocellulose, making the biodegradation slower. Meanwhile, carbohydrate hydrolysis occurs in a few hours, and protein and lipid hydrolysis take a few days [37]. Under certain circumstances, the balance in microbiological activity between the acetogenic and methanogenic phases may take longer due to hydrogen consumption in other metabolic pathways, ultimately leading to excessive acidification (VFAs accumulation), making these stages the limiting factors.

As mentioned, there are two major groups of microorganisms involved in the AD process: bacteria, primarily responsible for breaking down carbonaceous compounds into simple intermediate products, and archaea, capable of converting them into CH_4 and CO_2. The species of microorganisms are highly diverse. The most common genera are presented in **Table 1**.

Currently, there are more than 150 identified species of microorganisms in anaerobic digestion [38]. Focusing on bacteria, 58 species across 18 genera are involved in the first and second stages of degradation, and an additional 81 species and 4 orders are involved in the third and fourth stages, with concentrations ranging between 10^8 y 10^9 fermentative bacteria per mL [39]. Species of the genus *Clostridium, Ruminococcus, Eubacterium* y *Bacteroide* are the most common [36]. The activity of these bacteria involves the decomposition of polymers, fatty acids, and amino acids through the secretion of lipases, proteases, cellulases, pectinases, and amylases. Pentoses and hexoses are metabolized through the Embden-Meyerhof-Parnas (EMP) pathway, producing pyruvate and NADH as intermediaries for their subsequent transformation into mainly acetate, propionate, or lactate. **Table 1** provides a list of bacterial genera that participate in each stage.

Hydrolytic products are fermented into VFAs by acidogenic microorganisms during acidogenesis, where CO_2, H_2, ammonia, and sulfide are also produced. Acetogens include both hydrolytic and fermentative bacteria. *Firmicutes, Bacteroidetes, Chloroflexi, Proteobacteria*, and *Atribacteria* are the main phyla containing many acidogenic species reported in AD bioreactors. Acidogenesis is generally rapid and can lead to the accumulation of volatile fatty acids (VFA) and a significant pH drop [40].

Acetate, formate, H_2, and CO_2 resulting from acidogenesis can be directly utilized by methanogens for biogas production. However, other acidogenesis products, including propionate, butyrate, isobutyrate, valerate, and isovalerate, cannot be used for methanogenesis. It is necessary to further degrade and transform them through syntrophic acetogenesis. During this process, the previous hydrolytic and acidogenic products are further degraded/oxidized into acetate, H_2, and CO_2.

The methanogenic archaea, forming part of the archaea domain, appear in the final stage of the anaerobic decomposition process. These are unicellular microorganisms with unusual shapes that have the ability to convert organic matter into CH_4 and CO_2 through three different metabolic pathways [41]: Acetoclastic, where acetate is the precursor substrate; hydrogenotrophic, utilizing H_2 and CO_2; and methylotrophic, using methyl groups such as methanol, methylamines, and methyl sulfides. The archaea participating in the methanogenesis stage are classified into 7 orders identified in **Table 1** based on their metabolic pathway.

In recent years, new strains of hydrolytic/acidogenic bacteria have been characterized, including *Clostridium bornimense, Herbinix hemicellulosilytica, Herbinix luporum, Herbivorax saccincola, Proteiniphilum saccharofermentans, Petrimonas mucosa, Fermentimonas caenicola,* y *Proteiniborus indolifex*, thanks to improved techniques in anaerobic microbial cultivation and biomolecular techniques. Additionally, new species of methanogenic archaea, such as *Methanobacterium aggregans* y *Methanosarcina flavescens* have been reported [42].

Understanding the transforming microorganisms in the different stages of anaerobic digestion is fundamental to advance the technological development of the process. Similarly, selecting and maintaining an appropriate anaerobic inoculum is essential to initiate the digestion processes of complex substrates. However, in the case of livestock wastewater substrates, little attention has been given to the microbiota of manure, which is already adapted to the chemical substrate conditions and can play a decisive role in the bioprocess (**CHAPTER 6**).

Table 1. Anaerobic digestion process main microorganisms divided in Bacteria (genus) and Archaea (order).

Bacteria			Archaea	
Genus		**Stage**	**Order**	**Pathway**
Acetobakterium	*Eubacterium*	All the process	*Methanobacteriales*	Hydrogenotrophic / Methilotrophic
Lactobacillus	*Sporobacterium*	Hydrolysis		
Propioni-bacterium	*Megasphaera*		*Methanococcales*	Acetoclastic
Sphingomomas	*Bifidobacterium*		*Methanomicrobiales*	Hydrogenotrophic
Clostridium	*Cloacimomas*	Hydrolysis & Acidogenesis	*Methanosarcinales*	Acetoclastic
Ruminococcus	*Cytophaga*		*Methanomassiliicoccales*	Hydrogenotrophic
Acetivibrio	*Flavobacterium*		*Methanocellales*	Hydrogenotrophic
Thermotogae	*Bacteroides*		*Methanopyrales*	Hydrogenotrophic
Proteiniphilum	*Terrisporobacter*			
Anaerobacter	*Tissierella*			
Cloacibacillus	*Turicibacter*			
Bifidobacterium	*Syntrophus*	Acetogenesis		
Anaerolineaceae	*Syntrophomonas*			
Cloacibacillus	*Desulfovibrio*			
Syntrophobacter	*Aminobacterium*			
Pelotomaculum	*Acidaminococus*			

For a success biological activity by the microbiological communities and consequently, a proper anaerobic digestion of organic substrates, there are several parameters that will determine the living conditions and ultimately define the dominant microorganism populations in the environment. These parameters can often be controlled to maximize biodegradation, biomethane production, or even the accumulation of other intermediate by-products such as volatile fatty acids.

An important factor to consider is that, in general, the environmental requirements of fermentative bacteria involved in the firsts stages of anaerobic digestion differ from the requirements of methane-producing archaea and it can be complex to optimize all process stages. In this regard, two-stage technology offers a solution by optimizing hydrolysis, acidogenesis, and acetogenesis in a first reactor, and methanogenesis in a second reactor under different working conditions [43,44].

1.2.4. Process parameters and substrates

Anaerobic decomposition as a biological process is highly complex, involving the interaction of numerous biotic and abiotic elements. Its development depends on various environmental and operational factors [45,46]. The main parameters affecting the kinetics of anaerobic digestion as a whole are outlined in **Fig. 12.**

It has been identified that the most crucial elements influencing the anaerobic digestion process are temperature, pH, the presence of toxic or inhibitory substances, and the nature of the raw material (composition, nutrients) [47]. Another important factor, given that it is a biological process, is the persistence or continuity in the environmental conditions, as microorganisms, especially methanogenic archaea, are highly sensitive to unfavourable and/or fluctuating conditions for their survival in the medium.

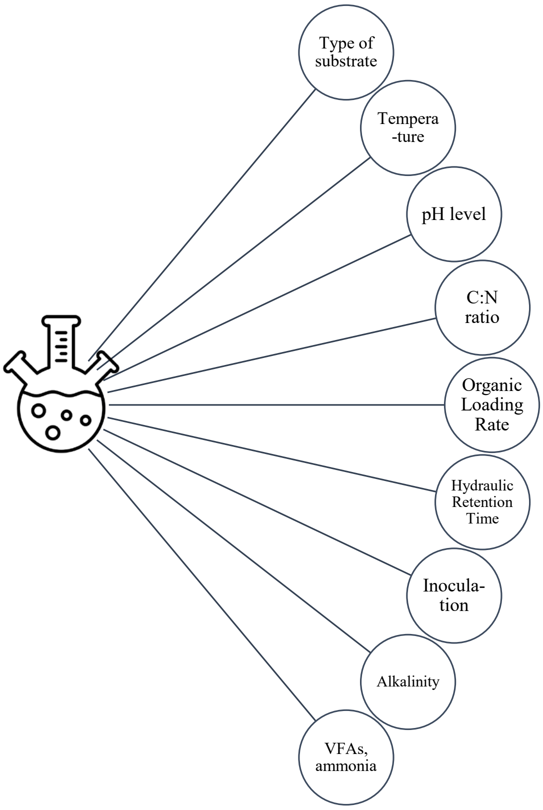


Fig 12. Parameters influencing anaerobic digestion.

The type or nature of substrate is one of the operational parameters, which, besides being dependent on geographical location, is crucial as its chemical composition and physical properties will define the metabolic pathways and the rate of anaerobic degradation, potentially leading to process limitation or inhibition. AD can be carried out using different types of substrates, which can be categorized based on their origin: (i) urban, such as sewage sludge and municipal solid waste [48]; (ii) industrial, including those from processing industries, slaughterhouses or paper mills and (iii) agricultural, composed of manure and lignocellulosic biomass principally. While most of the published research papers deals with sewage sludge or urban organic waste [49], there has not been the same emphasis on the by-products of primary sector activities, which, as mentioned in Section 2, are one of the main sources of anthropogenic CH_4 emissions.

Therefore, managing these by-products, particularly livestock manure, agricultural wastewater, and agricultural residues, possess both a challenge and an opportunity for the sector due to its high availability as can be seen in **Fig. 13** with two representative indicators. **Fig. 13, a** show the rapid worldwide economic development of the main agriculture production since 1995 derived from the rise of the number of livestock heads, crops and vegetables plantations. On the other hand, **Fig. 13, b** shows the waste origin used in biogas and biomethane plants in Europe, where two thirds come from the agricultural sector showing its high importance and interest.

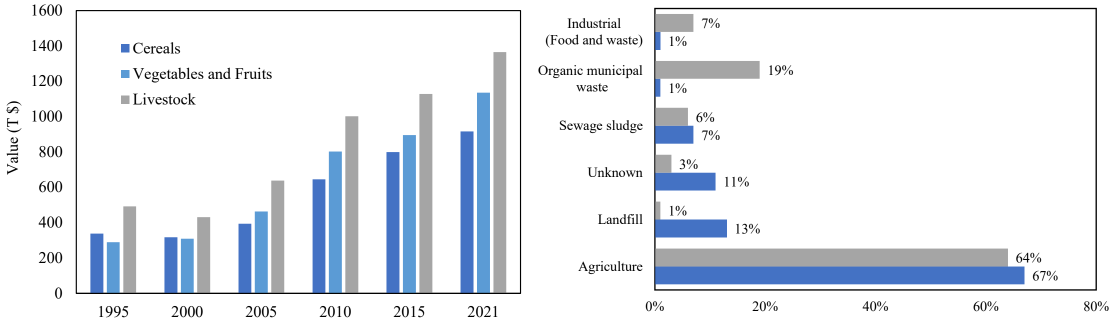


Fig. 13. a) Economic value of the main agriculture production between 1995-2021 [18]; **b)** Substrates used in the anaerobic digestion of biogas (blue colour) and biomethane plants (grey colour) [50,51].

This management proposed involves mitigating the significant environmental impact of CH_4 emissions and, simultaneously, capturing these emissions through anaerobic digestion for the generation of useful energy or other value-added by-products. The optimal environmental and operational conditions for the anaerobic digestion of these agricultural by-products are analysed in **Table 2**, distinguishing between livestock manure, agricultural straw and industrial agricultural waste.

Table 2. Operational conditions for the livestock manure, agricultural straw and industrial agricultural waste anaerobic digestion [52–63].

	Livestock manure	**Agricultural straw**	**Industrial agricultural waste***
Parameter	Average conditions		
Total solids (%)	2-30	60-95	40-21
Volatile solids (% of TS)	70-85	80-95	70-85
C:N ratio	5-30	80-150	25-115
Temperature (˚C)	35-55	35-55	35-55
pH value	7.5-8.5	7-8	6.5-8
Hydraulic retention time (d)	20-50	25-70	21-45
Biogas yield ($m^3 \cdot kg\ VS^{-1}$)	0.2-0.6	0.1-0.9	0.1-0.4
CH_4 content (%)	55-80	45-60	49-67
Potential problems	NH_4^+, Inhibition, scum layers	Poor degradation of lignocellulose, sediments	Phenolic compounds inhibition

* Due to the great heterogeneity of the substrates, wine-making industry waste has been selected including grape pomace, grape stalk and wine lees.

The differences observed in the table are primarily attributed to the composition of each by-product. Livestock manure as a substrate with high moisture content, exhibits lower volatile solids and a much lower carbon-to-nitrogen ratio (<10 most of the times) due to its high nitrogen content. This factor often leads to ammonia toxicity [64]. In contrast, agricultural residues are generally drier and predominantly with a high content of lignocellulose in composition, resulting in slower degradation, which often requires a prior stage of pretreatment for the hydrolytic phase. To address these operational difficulties of these substrates, co-digestion is frequently employed for various purposes: (i) balancing nutrient content, (ii) mitigating the negative effects of inhibitors, (iii) increasing the system's buffering capacity, (iv) improving methane production capacity, and finally (v) enhancing the economic profitability of the process [65].

One of the most common operational strategies used with agricultural residues, involves the implementation of pretreatments. The primary purpose is the acceleration of the AD process by enhancing the biodegradability of substrates through different technologies outlined in **Fig. 14.**

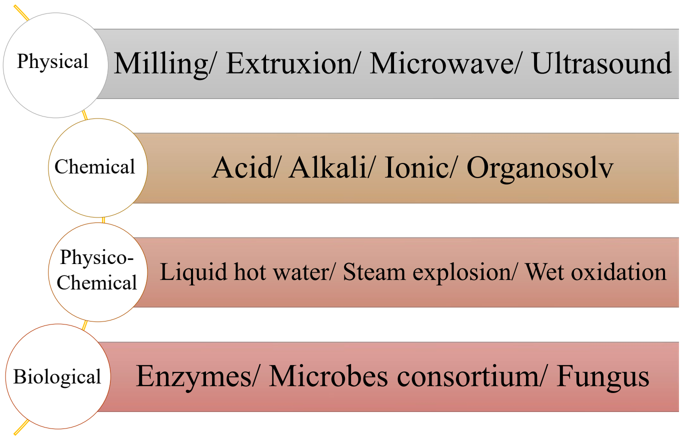


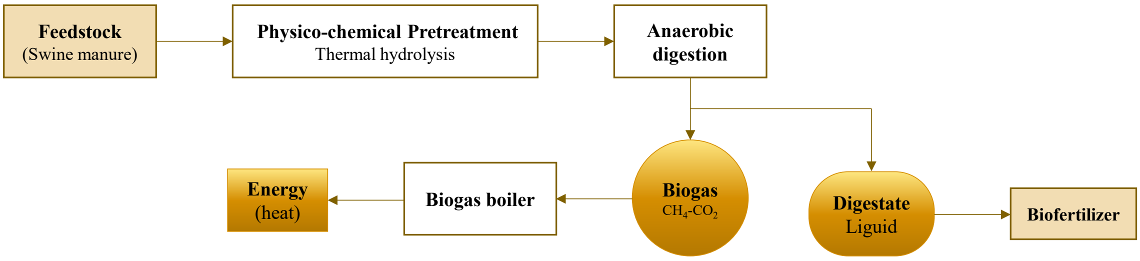
Fig. 14. Pretreatment more used in the anaerobic digestion process.

The nature and composition of the substrate, once again, will determine the type of pretreatment to be used before anaerobic digestion. Lignocellulosic biomass is highly resistant to degradation and requires one or various pretreatments to enhance its susceptibility to enzymes and transformative microorganisms, (i) increasing the accessible surface area, (ii) decrystallizing the cellulose, and (iii) solubilizing the hemicellulose and lignin [66]. Wastewater from the livestock sector achieves better efficiency through thermal pretreatments, especially in the case of manure from ruminant animals such as cows. **Table 3** provides a summary of studies realized on the pretreatment technologies used with agricultural substrates.

Table 3. Agricultural feedstock pretreatment strategies for improved anaerobic digestion

Pretreatment Feedstock	Physical	Chemical	Physico-Chemical	Biological
Productive livestock manure	Milling [67,68]	Alkali [69,70]	Thermal [71,72] Steam explosión [73,74]	
Animal waste			Pasteurisation [75]	
Agricultural residues- crops	Milling [76] Extrusion [81]	Alkali/ Acid [77]	Liquid hot water [78,79] Steam explosion [82,83]	Enzymes [80] Fungal [84]

Most of the studies found explore new laboratory-scale pretreatment methods under various conditions and scenarios to identify potential effects on biogas production rates, the kinetics of each stage, etc. However, the integration of these proposals into real scale applications is not always considered, including the application of energy and/or economic balances. This integration would allow the replication to full-scale plants (**CHAPTER 7**). Similarly, this integration should include flow diagrams that identify each stage at an industrial level. For agricultural by-product management, two examples with the key stages are depicted in **Fig. 15** considering a biogas plant feed with swine manure and cereal straw as the substrates:



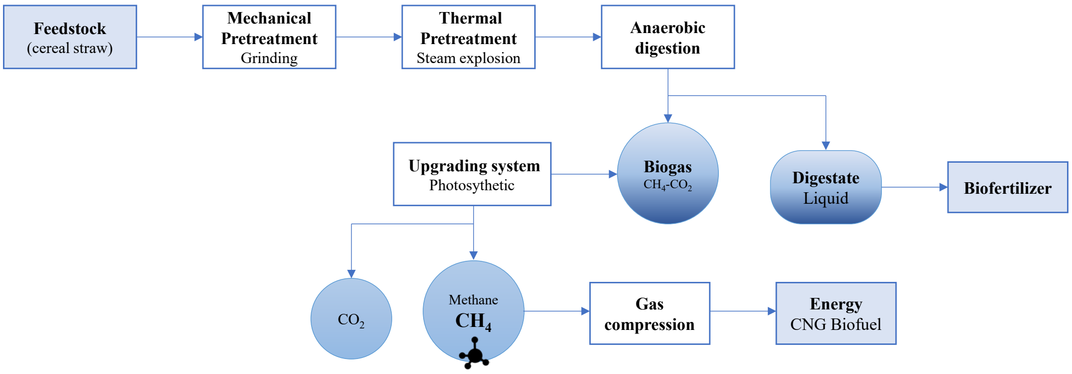


Fig. 15. Conceptual Process Flow Diagram for the anaerobic digestion of **a)** swine manure and **b)** cereal straw as substrates

1.2.5. Biogas and biomethane use

Biogas represents, the most useful by-product resulting from anaerobic digestion. The combined microbiological activity of bacteria and archaea allows the transformation of biodegradable organic by-products from the agricultural sector, such as livestock manure, crop residues, or other solid wastes with varied compositions, into a homogeneous gaseous mixture primarily composed of methane and carbon dioxide. It also contains minor proportions of hydrogen sulphide, carbon monoxide, hydrogen, oxygen, nitrogen, siloxanes, ammonia, volatile organic compounds (VOCs), and halocarbons [85]. The proportion of each component and the overall composition of the generated biogas is, therefore, influenced by the nature of the substrate and the technology employed in its decomposition. The specific properties of a standard biogas are shown in **Table 4**.

Table 4. General characteristics of a conventional biogas generated by the biodegradation of organic matter under anaerobic conditions. Adapted from [27]

Composition	55 – 70% methane (CH_4) 30 – 45% carbon dioxide (CO_2) Traces of other gases
Energy content	6.0 – 6.5 kWh m^{-3}
Fuel equivalent	0.60 – 0.65 L oil m^{-3} biogas
Explosion limits	6 – 12 % biogas in air
Ignition temperature	650 – 750 ˚C
Critical pressure	75 – 89 bar
Critical temperature	−82.5 ˚C

Depending on the final use, it could be necessary to enhance the quality of the biogas through a process known as upgrading. This upgrading stage is achieved not only to maximize the energetic performance but also to eliminate components that may cause technical or operational issues. **Table 5** provides a summary of the effect of each component on biogas.

Table 5. Biogas components and impurities and their effects. Adapted from [27]

Component	Content (%)	Effect
CH_4	45-75	- Gives the calorific value
CO_2	25-50	- Lowers the calorific value - Increases the anti-knock properties of engines - Causes corrosion if the gas is wet.
H_2S	0-1	- Corrosive effect in equipment and piping systems - SO_2 emissions after burners - Spoils catalysts
NH_3	0-0.1	- NO_x emissions after use - Increases the anti-knock properties of engines
Water vapour	1-0.5	- Condensate's damage instruments and plants. - Risk of freezing of piping systems and nozzles
N_2	0-10	- Lowers the calorific value. - Increases the anti-knock properties of engines
Siloxanes	0-0.005	- Act like an abrasive and damages engines

There are different uses for raw biogas or refined gas, as can be seen in **Fig. 16**. When employed to generate electricity and heat through combustion engines or turbines, the removal of hydrogen sulfide and siloxanes is necessary. Purifying biogas into biomethane for use as vehicle fuel or injection into the grid involves eliminating most contaminants present in biogas (CO_2, N_2, O_2, H_2, CO, H_2S, NH_3, siloxanes, halocarbons, and VOCs).

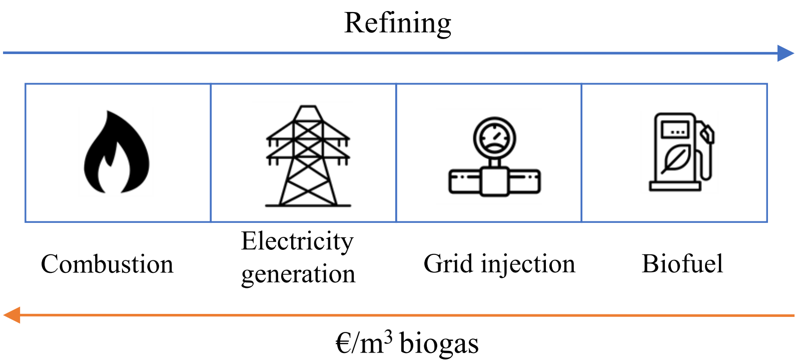


Fig. 16. Biogas applications depending on its quality in terms of composition.

Various technologies are employed to enhance biogas, including absorption methods such as water scrubbing, amine scrubbing, and physical scrubbing, as well as adsorption techniques like pressure swing adsorption. Additionally, membrane separation and cryogenic separation are utilized [86,87]. These improvement technologies are applicable in both small-scale applications, such as farms and landfills, and large-scale industrial environments. However, they often involve significant costs in terms of both investment and operation. Consequently, conventional upgrading systems are not always economically viable on a small scale. Nevertheless, emerging technologies like photosynthetic upgrading may provide a new alternative, proving cost-effective in smaller biomethane production scales, as demonstrated in pilot projects like the LIFE SMART Agromobility [88] or EU Horizon 2020 Urbiofin [89–91].

Depending on the biogas/biomethane use, there are regulations related with the quality of biomethane with defined quality parameters. These standards differ among countries and regions; however, the criteria address methane purity, impurities concentration and the presence of contaminants. In Spain, there is a standard regulation derived from the European Union with minimum quality standards and specifications for injecting biomethane into the natural gas network (UNE-EN 16723-1) and for vehicular biomethane in transport sector (UNE-EN 16723-2).

1.2.5.1 Biogas and biomethane. Current global situation

The scenario for the development of technologies related to biogas has experienced a significant change due to geopolitical conflicts in this decade, leading to a price increase resulting from excessive dependence on external energy suppliers. This new context of energy insecurity has accelerated the development and adoption of renewable energies, with a more focused emphasis on renewable gases such as biogas and biomethane, which are becoming essential components of the energy mix [50]. This strategic direction has a double perspective: on one hand, reducing dependence, and on the other, substantially contributing to an integrated net-zero energy system, including energy and agroecological transitions within the circular economy context. The global trend in biogas production is emerging, doubling its production in the last 10 years (**Fig. 17**). Currently, Europe leads both biogas production and the implementation of anaerobic digesters worldwide, followed by China and the United States. The European Union, led by Germany, has made a significant contribution to biogas production, generating over 200 TWh in 2022, representing approximately 5% of natural gas consumption but with projections to cover between 35% and 62% by 2050 [50].

The reduction in the costs of solar and wind energy is affecting tax incentives for electricity generation from biogas, motivating research into alternative uses such as the production of biomethane for vehicular use, bioplastics, and other higher-value-added products. In the same way, in Europe, biomethane plants have experienced a significant growth, reaching 1323 by the end of 2023. In Spain, the number of biomethane plants has increased from 1 in 2018 to 8 in 2023, currently producing more than 400 GWh from various sources, with an estimated biomethane production potential of $14 \cdot 10^6$ Nm^3 from different organic materials.

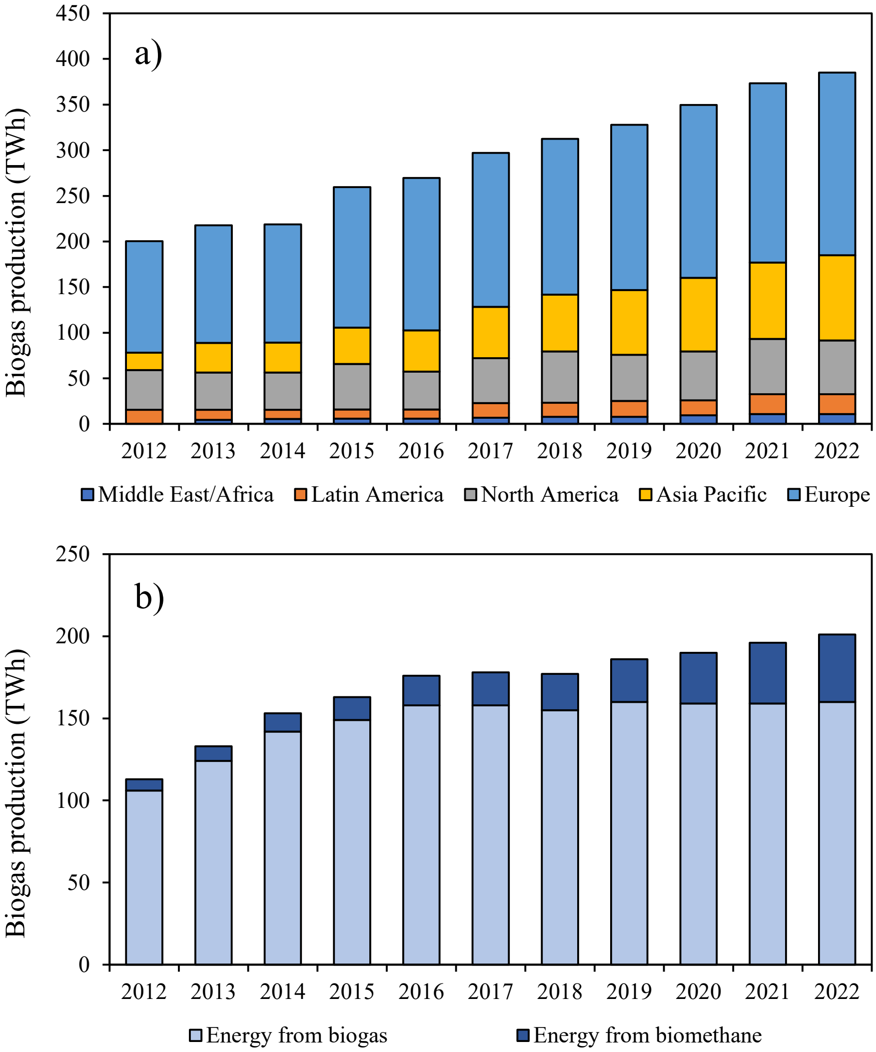


Fig. 17. a) Global and **b)** European biogas production in 2012 and its trend to 2022; [50,92]

1.3. New tendencies in anaerobic digestion: Integration, valorisation, and potential

Energy valorisation is the most common application of the biogas generated in anaerobic digestion. In recent decades, research has been carried out on the control of operational parameters to optimize the process. A significant share of this research has focused on a (ii) better understanding of the transforming microorganisms, (ii) the use of various substrates simultaneously through co-digestion, (iii) the application of pretreatments, or (iv) enhancing the final biogas quality through upgrading technologies. However, novel approaches of optimizing process performance are also being explored from alternative perspectives.

1.3.1. Energy hybridization

Simultaneous utilization of other renewable energies together with anaerobic digestion is an innovative line of work that can enhance the overall technological development of the process. This idea stems from the energy requirements of the process, which, as described, is highly dependent on operational parameters such as temperature that involves energy consumption in the form of heat. Likewise, there are additional electric power requirements for the process, including substrate feeding pumps, system agitation, or biogas upgrading if applicable. In **Table 6** can be seen the main energy inputs of a biogas plant [93]. In this context, the availability of renewable energy basis resource, such as solar irradiation, wind and biomass availability, and the difficulty of energy storage make this proposal potentially more efficient.

From the AD perspective, the goal of the hybridization is to cover this need, which can represent over 30% of the generated biogas, and maximize the production of biogas/biomethane for another energy purpose. This proposal can be highly effective, especially in isolated systems where there is an abundant availability of organic residues from agricultural sources and no access to the grid and the energy demand satisfied through the consumption of fossil fuels. Such scenarios, common in rural areas or intensive livestock farms, represent an opportunity to transform waste management with a double environmental and economic impact by reducing (i) greenhouse gas emissions associated with the activity by-products and (ii) the consumption of fossil fuels.

Table 6. Rough estimate of specific energy consumption in a biogas plant related to the energy produced in the plant [94–97].

Energy inputs for a biomethane pant operation (%)		
	Electricity demand	**Heat demand**
Small-scale plant. <500 kW_{el} installed capacity		
Heating of the substrate	-	6.3-8.5
Heat losses	-	4.0-5.1
Agitation and pumping	1.3-1.7	
Up-grading system*	10.0-20.0	
Other components	4.0-5.0	
Large-scale plant. >500 kW_{el} installed capacity		
Heating of the substrate		4.6-6.3
Heat losses		2.9-3.8
Agitation and pumping	3.0-4.0	
Up-grading system*	10.0-20.0	
Other components	3.5-4.4	

*Up-grading energy consumption depends strongly on the technology used.

In these isolated scenarios, solar energy has been the most explored for hybridization with AD due to its general availability and well-developed technological advancements, enabling easy and cost-effective implementation with reported positive outcomes [98,99]. Wind energy, traditionally used for electricity generation, exhibits suboptimal efficiency when employed for heat production. Additionally, standard wind turbines would be oversized, with an average power output of 2-3 MW. Mini wind energy systems could be a viable alternative; however, they still face challenges related to cost, performance, and reliability, despite recent improvements in their design and turbine efficiency [100]. Another hybridization proposal that has been studied involves the combination of AD with Compressed Air Energy Storage (CAES). In this approach, energy is stored in the form of compressed air, and upon its use, heat is generated and can be utilized in anaerobic digestion [101].

Fig. 18 shows an example of hybridization technology in a rural area with agricultural residues as feedstock for an anaerobic digester that would produce biogas por the heat needs in the farms around and for its own necessities (the average temperature of work is 35 ˚C). On the other hand, solar panels would generate the electricity to cover the farm and digester demands. Also, there is a digestate production from the digester with a fertilizer use on the crop fields around.

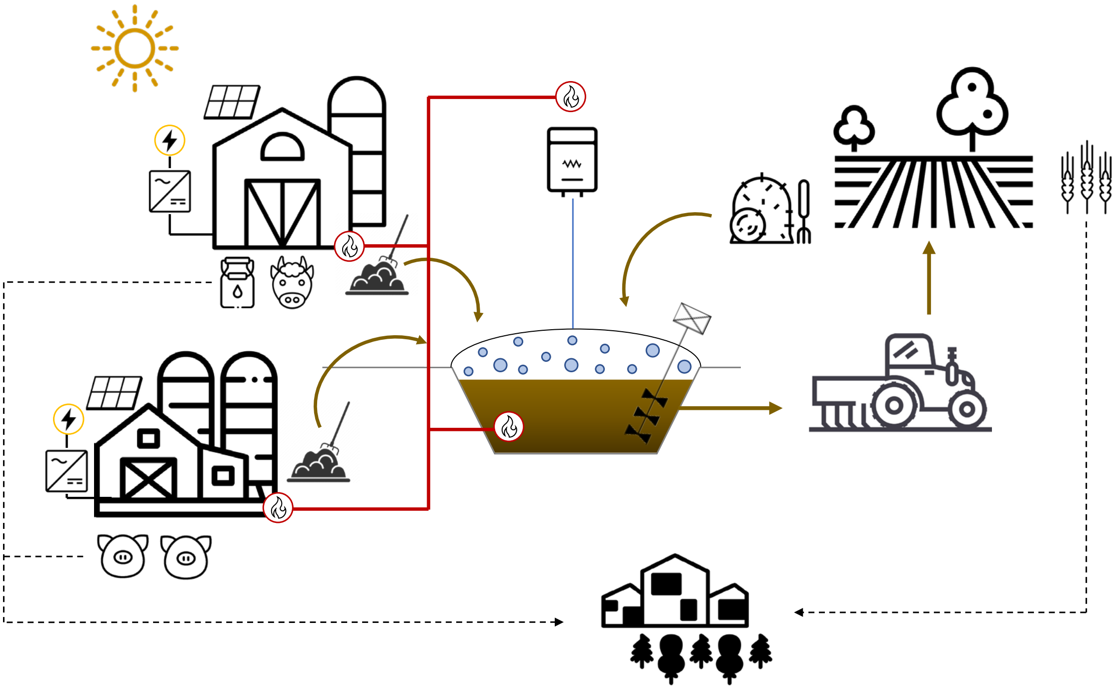


Fig. 18. Isolated farms with energy supply based on renewable energies: Anaerobic digestion and solar panels.

Another promising scenario for the use of AD is in agricultural cooperatives, food industrial zones, or urban areas where integration with other energy sources within the framework of a microgrid or district heating network can be effective and cost-efficient (**Fig. 19**). All these locations are a sources of organic waste generation, and with a well-established system for separating organic components - which is mandatory at the European level (Directive (EU) 2018/850 of the European Parliament) - they could be easily managed within a short distance [102]. The produced biogas/biomethane would become another output of the system, with a significant advantage over other technologies as its production is continuous over time, and its use is not linked to immediate production due to the ease of storage.

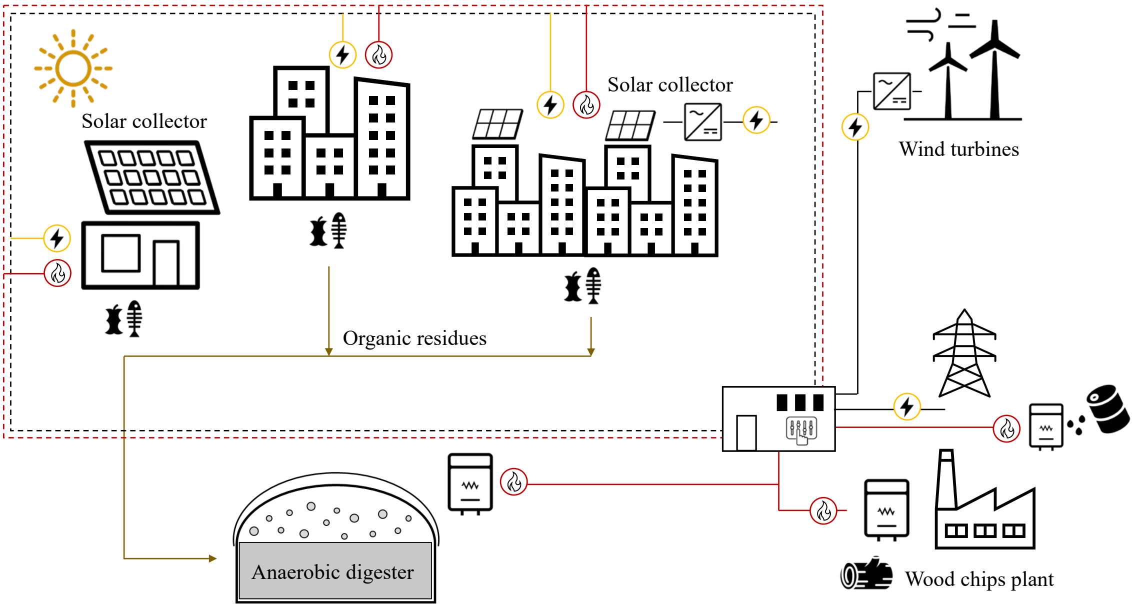


Fig. 19. District electricity and heating proposal based on renewable energies: Anaerobic digestion, solar collectors, wind turbines and a wood chips plant.

Hybridization AD with other renewable energy sources has not been thoroughly studied yet, although, it presents a significant opportunity for implementation in biogas plants. Research under different environmental conditions and with different substrates is essential to understand the energy and economic balances of its application. which can motivate the real-scale implementation (**CHAPTER 3**).

1.3.2. Volatile fatty acids production

In addition to the energy valorisation of the anaerobic digestion process, technologies for obtaining other high value bioproducts from the process have been developed in recent years in the industry [103].

A recent trend involves the accumulation of VFAs, intermediate compounds of the process, through operational strategies that inhibit the methanogenesis stage. The idea of recovering organic carbon in the form of VFAs (acetic acid (C2), propionic acid (C3), butyric acid (C4), and valeric acid (C5)) becomes highly valuable, as these can serve as building blocks in other industrial processes such as the synthesis of bioplastics, advanced biofuels, or the formation of medium-chain fatty acids [104,105] (**Fig. 18**). The market interest in VFAs increases with the higher number of carbon atoms they contain (**Fig. 20**).

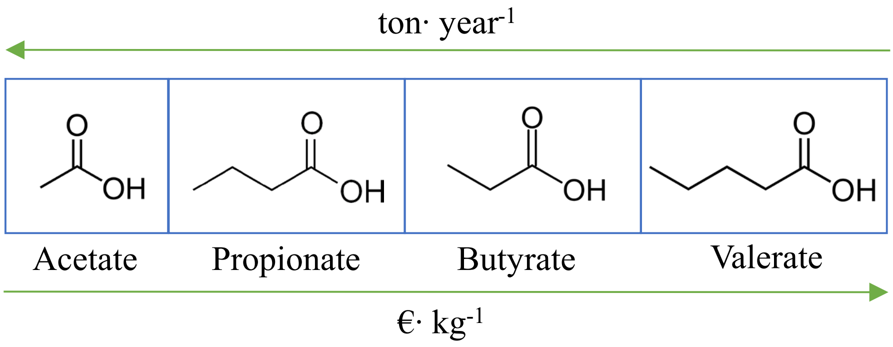


Fig. 20. Volatile fatty acids obtained through the anaerobic digestion process and its global production and market value.

Recent studies are validating operational strategies for the controlled accumulation of specific VFAs thanks to the modelling of metabolic pathways, controlling operational parameters such as pH, HRT, or substrate composition [106]. Current advancements are only at an experimental level in the laboratory conditions and with very specific substrates such as cellulose carbohydrates, xylan, glucose, and xylose, or proteins like casein and gelatine. However, these new lines of research promote new opportunities for valorising VFAs from the process, as the obtention of VFAs with a specific and control concentration. This is significant because anaerobic fermentation is typically characterized by producing a non-selective mixture of VFAs due to its complex biological nature with various interactions.

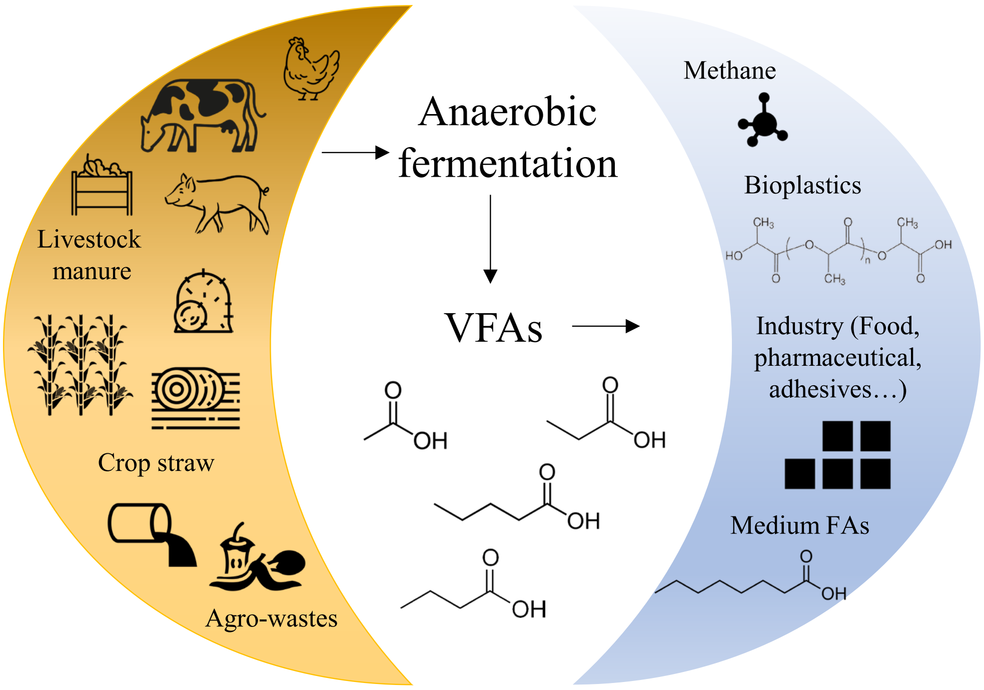


Fig. 21. VFAs production and potential uses. Adapted from [107]

Two limitations have been identified in the production of VFAs, (i) the difficulty of increasing the selective concentration due to the lack of sufficiently developed and specific technologies. The most common methods are based on ionic liquids, membranes, and electrolysis. Additionally, (ii) the integration of the process and its energy balance for the implementation of anaerobic digesters without energy valorisation can incur in significant operational costs (**CHAPTER 4**).

1.3.3. Addition of particles in anaerobic digestion process.

The new trends in the technological development of AD in this century align with the goal of enhancing biogas production rates and its quality. In this context, a novel work line involves the addition of small-sized particles (micrometric or nanometric) to serve as chemical activators due to their characteristics of small size, varied morphologies, high reactivity, chemical stability and a high surface area/volume ratio make them particularly effective for this purpose [108,109].

The effect of these particles, still in the early stages of study, primarily at the laboratory level, has different causes: (i) a significant increase in active sites for microorganisms due to the expanded support surface available [110]. The average size of bacteria can vary considerably but is normally within the range of 0.4 to 4 µm in length, and methanogenic archaea range between 0.1 and 15 µm [111] while particle sizes can reach the nanometric scale; (ii) the enhancement of direct electron transfer due to the particles' ability to disrupt cell membranes (**Fig. 22**). Regarding the substrate, there may be (iii) an acceleration of the initial hydrolysis through abrasion effect, promoting the substrate decomposition, leading to higher biogas production, and reducing the latency phase [112].

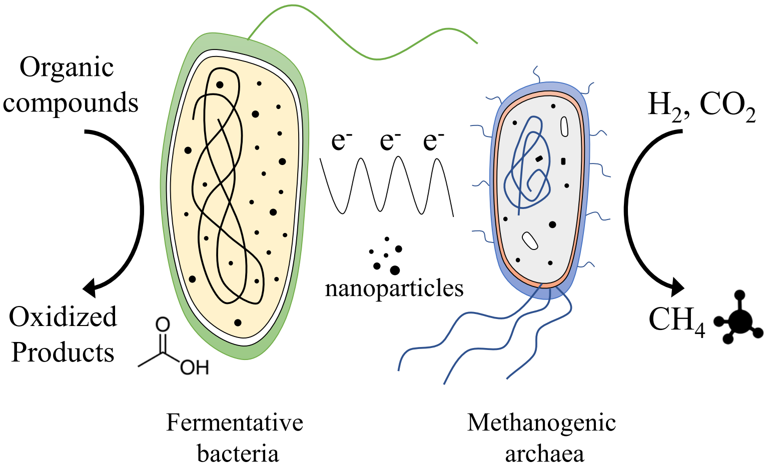


Fig. 22. Electron transfer mechanism between fermenting bacteria and methanogen.

Optimisation of particle composition is currently being explored, as well as optimal concentrations in their use depending also on the organic substrate. A summary of the main particle types and working conditions experienced with a positive impact is presented in **table 7**. However, the addition of micro or/and nanoparticles to a biodigester can have both positive and negative impacts on biogas generation. Iron particles and their compounds and carbon-based materials are the most frequently reported to have a positive effect [113] and there is a need for comparative AD performance improvement experiments.

Iron oxide-based nanoparticles, such as magnetite or maghemite, stand out for their high chemical stability and magnetic properties, making them appreciated in anaerobic digestion. These oxides play a crucial role in promoting direct electron transfer between microorganisms, facilitating the conversion of CO_2 to methane [114]. Magnetite (Fe_3O_4) particles enhance hydrogenotrophic methanogenesis by generating electrons during iron corrosion, increasing hydrogen production, and consequently, methane production. Additionally, Fe_3O_4 also contribute to breaking down volatile solids in the substrate, improving hydrolysis. However, an excessive accumulation of iron can be toxic to microorganisms and inhibit the process [115]. Similarly, carbon-based micro-nanoparticles, such as graphite or biochar, have demonstrated significant improvements in methane production due to their key properties, including high conductivity and a large specific surface area, promoting chemical reactivity and microbial growth [116]. Their ability to assist direct electron transfer makes them comparable to metallic particles, with the added advantage of being more cost-effective.

Table 7. Biogas production effect from different types of substrate and particles in AD.

Agricultural substrate	Type of nanoparticles	Highest reported concentrations	Effect on AD process (CH_4 production)	Reference
		Livestock manure		
Poultry litter	Fe_3O_4	100 mg· L^{-1}	↑ 45 %	[117]
Chicken litter	Fe_3O_4	20 mg· L^{-1}	↑ 74 %	[118]
Piggery waste	Biochar	0.0 g· g dry $matter^{-1}$	↑ 7 %	[119]
Cattle dung slurry	Fe_3O_4	20 mg· L^{-1}	↑ 115 %	[120]
Swine manure	Fe_3O_4	75 mmol	↑ 48 %	[121]
Cattle manure	Fe_2O_3	100 mg· L^{-1}	↑ 19 %	[122]
Pig manure and wheat straw	Fe_3O_4	54 mg· g TS^{-1}	↑ 57 %	[123]

		Agricultural residues		
Wheat straw	Fe_3O_4	100 mg· L^{-1}	↑ 51 %	[124]
Canola straw and banana plant wastes	Fe_3O_4	0.162 mg· g TS^{-1}	↑ 65 %	[125]
sweet sorghum	Biochar	15 g· L^{-1}	↑ 25 %	[126]
Rice straw	Fe_3O_4	12 mg· g VS^{-1}	↑ 129 %	[127]
Wine lees	Biochar	10 g· L^{-1}	↑ 18 %	[128]
Corn straw	Iron Biochar	15 g· L^{-1}	↑ 30%	[129]
Swine manure	Iron Biochar	2 mg· g VS^{-1}	↑ 86%	[130]
Fruitwoods	Biochar	50 mg· g VS^{-1}	↑ 54%	[131]

A drawback identified in the use of particles in anaerobic digestion is the lack of an exhaustive study on their potential environmental impact. Their size typically falls below 100 nanometers, making them susceptible to be dispersed in the environment, with associated high impacts dangerous to ecosystems, water and soil quality and different biological processes [132,133]. Additionally, these particles can interact with living organisms, including plants, animals, and microorganisms, potentially causing toxic or disruptive effects on their normal functioning.

Another relevant aspect is the production cost of the particles, as the studies presented are at the laboratory scale. When scaling up to an industrial plant, these costs can impact the overall economics of biogas production. Consequently, this factor must be considered to assess the economic viability of the process [134]. To conclude there is a high necessity in this AD field to study the comparation of the different particles effect in the kinetics pathways and methane production rates, to clarify its result basis and other associated effects (**CHAPTER 5**).

1.4. References

[1] REN21, Renewables 2023 Global Status Report Collection, 2023.

[2] Hannah Ritchie and Pablo Rosado, Energy Mix, Our World in Data. (2020).

[3] I. Papamichael, I. Voukkali, M. Stylianou, A.A. Zorpas, R. Baidya, S.K. Ghosh, Chapter 12 - Concept of waste-to-energy strategies, in: M. Jeguirim, A.A. Zorpas (Eds.), Advances in Biofuels Production, Optimization and Applications, Elsevier, 2024: pp. 241–267. https://doi.org/https://doi.org/10.1016/B978-0-323-95076-3.00002-8.

[4] S.K. Ghosh, U. V Parlikar, A.A. Zorpas, I. Papamichael, Chapter 15 - Sustainable supply chain for waste-to-energy facilities, in: M. Jeguirim, A.A. Zorpas (Eds.), Advances in Biofuels Production, Optimization and Applications, Elsevier, 2024: pp. 297–313. https://doi.org/https://doi.org/10.1016/B978-0-323-95076-3.00015-6.

[5] IEA. Intenation Energy Agency, International Energy Agency forecasts for 2020, 2020.

[6] D.M.-C. Chen, B.L. Bodirsky, T. Krueger, A. Mishra, A. Popp, The world's growing municipal solid waste: trends and impacts, Environmental Research Letters. 15 (2020) 074021. https://doi.org/10.1088/1748-9326/ab8659.

[7] D.M.-C. Chen, B.L. Bodirsky, T. Krueger, A. Mishra, A. Popp, The world's growing municipal solid waste: trends and impacts, Environmental Research Letters. 15 (2020) 074021. https://doi.org/10.1088/1748-9326/ab8659.

[8] IEA. Internation Energy Agency, CO2 Emissions in 2022, 2023.

[9] R. Najarzadeh, H. Dargahi, L. Agheli, K.B. Khameneh, Kyoto Protocol and global value chains: Trade effects of an international environmental policy, Environ Dev. 40 (2021) 100659. https://doi.org/https://doi.org/10.1016/j.envdev.2021.100659.

[10] Y. Wei, Y. Li, M. Wu, Y. Li, The decomposition of total-factor CO2 emission efficiency of 97 contracting countries in Paris Agreement, Energy Econ. 78 (2019) 365–378. https://doi.org/https://doi.org/10.1016/j.eneco.2018.11.028.

[11] M.E. Malerba, D.A. Friess, M. Peacock, A. Grinham, P. Taillardat, J.A. Rosentreter, J. Webb, N. Iram, A.N. Al-Haj, P.I. Macreadie, Methane and nitrous oxide emissions complicate the climate benefits of teal and blue carbon wetlands, One Earth. 5 (2022) 1336–1341. https://doi.org/https://doi.org/10.1016/j.oneear.2022.11.003.

[12] European Environment Agency, Annual European Union greenhouse gas inventory 1990–2020 and inventory. Report 2022, 2022.

[13] K.A. Mar, C. Unger, L. Walderdorff, T. Butler, Beyond CO2 equivalence: The impacts of methane on climate, ecosystems, and health, Environ Sci Policy. 134 (2022) 127–136. https://doi.org/https://doi.org/10.1016/j.envsci.2022.03.027.

[14] M. Saunois, A.R. Stavert, B. Poulter, P. Bousquet, J.G. Canadell, R.B. Jackson, P.A. Raymond, E.J. Dlugokencky, S. Houweling, P.K. Patra, P. Ciais, V.K. Arora, D. Bastviken, P. Bergamaschi, D.R. Blake, G. Brailsford, L. Bruhwiler, K.M. Carlson, M. Carrol, S. Castaldi, N. Chandra, C. Crevoisier, P.M. Crill, K. Covey, C.L. Curry, G. Etiope, C. Frankenberg, N. Gedney, M.I. Hegglin, L. Höglund-Isaksson, G. Hugelius, M. Ishizawa, A. Ito, G. Janssens-Maenhout, K.M. Jensen, F. Joos, T. Kleinen, P.B. Krummel, R.L. Langenfelds, G.G. Laruelle, L. Liu, T. Machida, S. Maksyutov, K.C. McDonald, J. McNorton, P.A. Miller, J.R. Melton, I. Morino, J. Müller, F. Murguia-Flores, V. Naik, Y. Niwa, S. Noce, S. O'Doherty, R.J. Parker, C. Peng, S. Peng, G.P. Peters, C. Prigent, R. Prinn, M. Ramonet, P. Regnier, W.J. Riley, J.A. Rosentreter, A. Segers, I.J. Simpson, H. Shi, S.J. Smith, L.P. Steele, B.F. Thornton, H. Tian, Y. Tohjima, F.N. Tubiello, A. Tsuruta, N. Viovy, A. Voulgarakis, T.S. Weber, M. van Weele, G.R. van der Werf, R.F. Weiss, D. Worthy, D. Wunch, Y. Yin, Y. Yoshida, W. Zhang, Z. Zhang, Y. Zhao, B. Zheng, Q. Zhu, Q. Zhu, Q. Zhuang, The Global Methane Budget 2000–2017, Earth Syst Sci Data. 12 (2020) 1561–1623. https://doi.org/10.5194/essd-12-1561-2020.

[15] M.S. Johnson, E. Matthews, J. Du, V. Genovese, D. Bastviken, Methane Emission From Global Lakes: New Spatiotemporal Data and Observation-Driven Modeling of Methane Dynamics Indicates Lower Emissions, J Geophys Res Biogeosci. 127 (2022) e2022JG006793. https://doi.org/https://doi.org/10.1029/2022JG006793.

[16] F.N. Tubiello, Greenhouse Gas Emissions Due to Agriculture, in: P. Ferranti, E.M. Berry, J.R. Anderson (Eds.), Encyclopedia of Food Security and Sustainability, Elsevier, Oxford, 2019: pp. 196–205. https://doi.org/https://doi.org/10.1016/B978-0-08-100596-5.21996-3.

[17] J. Chang, S. Peng, P. Ciais, M. Saunois, S.R.S. Dangal, M. Herrero, P. Havlík, H. Tian, P. Bousquet, Revisiting enteric methane emissions from domestic ruminants and their δ13CCH4 source signature, Nat Commun. 10 (2019) 3420. https://doi.org/10.1038/s41467-019-11066-3.

[18] Food and Agriculture Organization of the United Nations, Crop prospects and food situation, FAO. (2021).

[19] S. Kirschke, P. Bousquet, P. Ciais, M. Saunois, J.G. Canadell, E.J. Dlugokencky, P. Bergamaschi, D. Bergmann, D.R. Blake, L. Bruhwiler, P. Cameron-Smith, S. Castaldi, F. Chevallier, L. Feng, A. Fraser, M. Heimann, E.L. Hodson, S. Houweling, B. Josse, P.J.

Fraser, P.B. Krummel, J.-F. Lamarque, R.L. Langenfelds, C. Le Quéré, V. Naik, S. O'Doherty, P.I. Palmer, I. Pison, D. Plummer, B. Poulter, R.G. Prinn, M. Rigby, B. Ringeval, M. Santini, M. Schmidt, D.T. Shindell, I.J. Simpson, R. Spahni, L.P. Steele, S.A. Strode, K. Sudo, S. Szopa, G.R. van der Werf, A. Voulgarakis, M. van Weele, R.F. Weiss, J.E. Williams, G. Zeng, Three decades of global methane sources and sinks, Nat Geosci. 6 (2013) 813–823. https://doi.org/10.1038/ngeo1955.

[20] Ruiz C, Wolff M, Claret M, Annual crop stubble and forest residues, Institute of Agricultural Research. Quilamapu Regional Research Center. (2015).

[21] S.A. Thorneloe, M.A. Barlaz, R. Peer, L.C. Huff, L. Davis, J. Mangino, Waste Management, in: M.A.K. Khalil (Ed.), Atmospheric Methane: Its Role in the Global Environment, Springer Berlin Heidelberg, Berlin, Heidelberg, 2000: pp. 234–262. https://doi.org/10.1007/978-3-662-04145-1_13.

[22] M. para la T.E. y el R.D. MITECO, Listado de mejores técnicas disponibles para la reducción de emisiones en ganado, 2023.

[23] R. Matthews, R. Wassmann, Modelling the impacts of climate change and methane emission reductions on rice production: a review, European Journal of Agronomy. 19 (2003) 573–598. https://doi.org/https://doi.org/10.1016/S1161-0301(03)00005-4.

[24] L. Sun, Y. Wang, N. Guan, L. Li, Methane Activation and Utilization: Current Status and Future Challenges, Energy Technology. 8 (2020) 1900826. https://doi.org/https://doi.org/10.1002/ente.201900826.

[25] S. Chozhavendhan, G. Karthigadevi, B. Bharathiraja, R. Praveen Kumar, L.D. Abo, S. Venkatesa Prabhu, R. Balachandar, M. Jayakumar, Current and prognostic overview on the strategic exploitation of anaerobic digestion and digestate: A review, Environ Res. 216 (2023) 114526. https://doi.org/https://doi.org/10.1016/j.envres.2022.114526.

[26] K. Obileke, N. Nwokolo, G. Makaka, P. Mukumba, H. Onyeaka, Anaerobic digestion: Technology for biogas production as a source of renewable energy—A review, Energy & Environment. 32 (2020) 191–225. https://doi.org/10.1177/0958305X20923117.

[27] Dieter Deublein & Angelika Steinhauser, Biogas from Waste and Renewable Resources, Focus on Catalysts. 2011 (2011) 8. https://doi.org/10.1016/S1351-4180(11)70392-0.

[28] M. Wainwright, An Alternative View of the Early History of Microbiology, in: Adv Appl Microbiol, Academic Press, 2003: pp. 333–355. https://doi.org/https://doi.org/10.1016/S0065-2164(03)01013-X.

[29] L.C. Ampese, W.G. Sganzerla, H. Di Domenico Ziero, A. Mudhoo, G. Martins, T. Forster-Carneiro, Research progress, trends, and updates on anaerobic digestion technology: A bibliometric analysis, J Clean Prod. 331 (2022) 130004. https://doi.org/https://doi.org/10.1016/j.jclepro.2021.130004.

[30] X. Yaoyang, W.J. Boeing, Mapping biofuel field: A bibliometric evaluation of research output, Renewable and Sustainable Energy Reviews. 28 (2013) 82–91. https://doi.org/10.1016/J.RSER.2013.07.027.

[31] N. Ye, T.-B. Kueh, L. Hou, Y. Liu, H. Yu, A bibliometric analysis of corporate social responsibility in sustainable development, J Clean Prod. 272 (2020) 122679. https://doi.org/https://doi.org/10.1016/j.jclepro.2020.122679.

[32] S. Juntupally, S. Begum, V. Arelli, G.R. Anupoju, Chapter 8 - Microbial coculture to enhance biogas production, in: A. Yousuf, L. Melville (Eds.), Biogas to Biomethane, Woodhead Publishing, 2024: pp. 181–194. https://doi.org/https://doi.org/10.1016/B978-0-443-18479-6.00011-9.

[33] A. Schnürer, Biogas Production: Microbiology and Technology, in: R. Hatti-Kaul, G. Mamo, B. Mattiasson (Eds.), Anaerobes in Biotechnology, Springer International Publishing, Cham, 2016: pp. 195–234. https://doi.org/10.1007/10_2016_5.

[34] R. Ye, Q. Jin, B. Bohannan, J.K. Keller, S.D. Bridgham, Homoacetogenesis: A potentially underappreciated carbon pathway in peatlands, Soil Biol Biochem. 68 (2014) 385–391. https://doi.org/https://doi.org/10.1016/j.soilbio.2013.10.020.

[35] B. de S. Moraes, R. Palacios-Bereche, G. Martins, S.A. Nebra, L.T. Fuess, A.J. Silva, W. da S. Clementino, S.V. Bajay, P.C. Manduca, R.A. Lamparelli, M.D. Berni, Chapter 7 - Biogas production: Technologies and applications, in: F.I. Gómez Castro, C. Gutiérrez-Antonio (Eds.), Biofuels and Biorefining, Elsevier, 2022: pp. 215–282. https://doi.org/https://doi.org/10.1016/B978-0-12-824116-5.00009-X.

[36] V. Arelli, N.K. Mamindlapelli, S. Begum, S. Juntupally, G.R. Anupoju, Chapter 2 - Recent advances of biogas production, in: A. Yousuf, L. Melville (Eds.), Biogas to Biomethane, Woodhead Publishing, 2024: pp. 41–66. https://doi.org/https://doi.org/10.1016/B978-0-443-18479-6.00006-5.

[37] Q. Niu, L. Song, J. Li, Chapter 4 - Conversion of manure to bioenergy and biochemicals via anaerobic digestion, in: A. Pandey, Y.W. Tong, L. Zhang, J. Zhang (Eds.), Biomass, Biofuels, Biochemicals, Elsevier, 2022: pp. 69–90. https://doi.org/https://doi.org/10.1016/B978-0-323-90633-3.00011-0.

[38] C. Söhngen, A. Podstawka, B. Bunk, D. Gleim, A. Vetcininova, L.C. Reimer, C. Ebeling, C. Pendarovski, J. Overmann, BacDive – The Bacterial Diversity Metadatabase in 2016, Nucleic Acids Res. 44 (2016) D581–D585. https://doi.org/10.1093/nar/gkv983.

[39] M.A. Bruns, 5 - Bacteria and archaea, in: T.J. Gentry, J.J. Fuhrmann, D.A. Zuberer (Eds.), Principles and Applications of Soil Microbiology (Third Edition), Elsevier, 2021: pp. 111–148. https://doi.org/https://doi.org/10.1016/B978-0-12-820202-9.00005-8.

[40] J.W. Lim, T. Park, Y.W. Tong, Z. Yu, Chapter One - The microbiome driving anaerobic digestion and microbial analysis, in: Y. Li, S.K. Khanal (Eds.), Advances in Bioenergy, Elsevier, 2020: pp. 1–61. https://doi.org/https://doi.org/10.1016/bs.aibe.2020.04.001.

[41] I. Anderson, L.E. Ulrich, B. Lupa, D. Susanti, I. Porat, S.D. Hooper, A. Lykidis, M. Sieprawska-Lupa, L. Dharmarajan, E. Goltsman, A. Lapidus, E. Saunders, C. Han, M. Land, S. Lucas, B. Mukhopadhyay, W.B. Whitman, C. Woese, J. Bristow, N. Kyrpides, Genomic Characterization of Methanomicrobiales Reveals Three Classes of Methanogens, PLoS One. 4 (2009) e5797-. https://doi.org/10.1371/journal.pone.0005797.

[42] J. Hassa, I. Maus, S. Off, A. Pühler, P. Scherer, M. Klocke, A. Schlüter, Metagenome, metatranscriptome, and metaproteome approaches unraveled compositions and functional relationships of microbial communities residing in biogas plants, Appl Microbiol Biotechnol. 102 (2018) 5045–5063. https://doi.org/10.1007/s00253-018-8976-7.

[43] E. Holl, J. Steinbrenner, W. Merkle, J. Krümpel, S. Lansing, U. Baier, H. Oechsner, A. Lemmer, Two-stage anaerobic digestion: State of technology and perspective roles in future energy systems, Bioresour Technol. 360 (2022) 127633. https://doi.org/https://doi.org/10.1016/j.biortech.2022.127633.

[44] D. Yellezuome, X. Zhu, X. Liu, X. Liu, R. Liu, Z. Wang, Y. Li, C. Sun, M. Hemida Abd-Alla, A.-H.M. Rasmey, Integration of two-stage anaerobic digestion process with in situ biogas upgrading, Bioresour Technol. 369 (2023) 128475. https://doi.org/https://doi.org/10.1016/j.biortech.2022.128475.

[45] Z. Wang, Y. Hu, S. Wang, G. Wu, X. Zhan, A critical review on dry anaerobic digestion of organic waste: Characteristics, operational conditions, and improvement strategies, Renewable and Sustainable Energy Reviews. 176 (2023) 113208. https://doi.org/https://doi.org/10.1016/j.rser.2023.113208.

[46] S. Panigrahi, B.K. Dubey, A critical review on operating parameters and strategies to improve the biogas yield from anaerobic digestion of organic fraction of municipal solid

waste, Renew Energy. 143 (2019) 779–797. https://doi.org/https://doi.org/10.1016/j.renene.2019.05.040.

[47] I.A. Vasiliadou, K. Gioulounta, K. Stamatelatou, Chapter 9 - Production of biogas via anaerobic digestion, in: R. Luque, C.S.K. Lin, K. Wilson, C. Du (Eds.), Handbook of Biofuels Production (Third Edition), Woodhead Publishing, 2023: pp. 253–311. https://doi.org/https://doi.org/10.1016/B978-0-323-91193-1.00010-X.

[48] N. Annamalai, S. Elayaraja, P. Oleskowicz-Popiel, D. Zhu, C. Rodkhum, Chapter 13 - Enhanced anaerobic digestion: Recent advancements and future prospective, in: V.K. Gupta, M. Tuohy, P. Ramteke, Q. Nguyen, R. Bhat (Eds.), Valorization of Biomass to Bioproducts, Elsevier, 2023: pp. 241–255. https://doi.org/https://doi.org/10.1016/B978-0-12-822888-3.00002-5.

[49] A.M. Abdelrahman, H. Ozgun, R.K. Dereli, O. Isik, O.Y. Ozcan, J.B. van Lier, I. Ozturk, M.E. Ersahin, Anaerobic membrane bioreactors for sludge digestion: Current status and future perspectives, Crit Rev Environ Sci Technol. 51 (2021) 2119–2157. https://doi.org/10.1080/10643389.2020.1780879.

[50] European Biogas Association, EBA Statistical report 2023, 2023.

[51] IEA. International Energy Agency, Outlook for biogas and biomethane, 2020.

[52] M. Rowan, G.C. Umenweke, E.I. Epelle, I.C. Afolabi, P.U. Okoye, B. Gunes, J.A. Okolie, Anaerobic co-digestion of food waste and agricultural residues: An overview of feedstock properties and the impact of biochar addition, Digital Chemical Engineering. 4 (2022) 100046. https://doi.org/https://doi.org/10.1016/j.dche.2022.100046.

[53] A. Kushwaha, V. Mishra, V. Gupta, S. Goswami, P.K. Gupta, L.K. Singh, C.B. Gupt, K. Rakshit, L. Goswami, Chapter 5 - Anaerobic digestion as a sustainable biorefinery concept for waste to energy conversion, in: C.M. Hussain, S. Singh, L. Goswami (Eds.), Waste-to-Energy Approaches Towards Zero Waste, Elsevier, 2022: pp. 129–163. https://doi.org/https://doi.org/10.1016/B978-0-323-85387-3.00008-2.

[54] C. He, T. Luo, H. Yuan, F. Shen, Chapter 3 - Conversion of agricultural wastes to bioenergy and biochemicals via anaerobic digestion, in: A. Pandey, Y.W. Tong, L. Zhang, J. Zhang (Eds.), Biomass, Biofuels, Biochemicals, Elsevier, 2022: pp. 45–68. https://doi.org/https://doi.org/10.1016/B978-0-323-90633-3.00007-9.

[55] Y. Li, Y. Han, Y. Zhang, W. Luo, G. Li, Anaerobic digestion of different agricultural wastes: A techno-economic assessment, Bioresour Technol. 315 (2020) 123836. https://doi.org/https://doi.org/10.1016/j.biortech.2020.123836.

[56] A. Upadhyay, R. Singh, R. Kumar, N. Verma, P. Talwar, P. Ahire, V. Vivekanand, Chapter 3 - Progress in anaerobic digestion of organic wastes to biomethane, in: S. Nanda, A.K. Dalai (Eds.), Biomass to Bioenergy, Woodhead Publishing, 2024: pp. 49–72. https://doi.org/https://doi.org/10.1016/B978-0-443-15377-8.00019-9.

[57] N. Haffiez, T.H. Chung, B.S. Zakaria, M. Shahidi, S. Mezbahuddin, F.I. Hai, B.R. Dhar, A critical review of process parameters influencing the fate of antibiotic resistance genes in the anaerobic digestion of organic waste, Bioresour Technol. 354 (2022) 127189. https://doi.org/https://doi.org/10.1016/j.biortech.2022.127189.

[58] S.-Y. Pan, C.-Y. Tsai, C.-W. Liu, S.-W. Wang, H. Kim, C. Fan, Anaerobic co-digestion of agricultural wastes toward circular bioeconomy, IScience. 24 (2021) 102704. https://doi.org/https://doi.org/10.1016/j.isci.2021.102704.

[59] B. Hashemi, S. Sarker, J.J. Lamb, K.M. Lien, Yield improvements in anaerobic digestion of lignocellulosic feedstocks, J Clean Prod. 288 (2021) 125447. https://doi.org/https://doi.org/10.1016/j.jclepro.2020.125447.

[60] I. Rocamora, S.T. Wagland, R. Villa, E.W. Simpson, O. Fernández, Y. Bajón-Fernández, Dry anaerobic digestion of organic waste: A review of operational parameters and their impact on process performance, Bioresour Technol. 299 (2020) 122681. https://doi.org/https://doi.org/10.1016/j.biortech.2019.122681.

[61] J.H. El Achkar, T. Lendormi, Z. Hobaika, D. Salameh, N. Louka, R.G. Maroun, J.-L. Lanoisellé, Anaerobic digestion of nine varieties of grape pomace: Correlation between biochemical composition and methane production, Biomass Bioenergy. 107 (2017) 335–344. https://doi.org/https://doi.org/10.1016/j.biombioe.2017.10.030.

[62] B. Tamelová, J. Malaťák, J. Velebil, A. Gendek, M. Aniszewska, Energy Utilization of Torrefied Residue from Wine Production, Materials. 14 (2021). https://doi.org/10.3390/ma14071610.

[63] A.C. Deiana, M.G. Gimenez, S. Rómoli, M.F. Sardella, K. Sapag, Batch and Column Studies for the Removal of Lead from Aqueous Solutions Using Activated Carbons from Viticultural Industry Wastes, Adsorption Science & Technology. 32 (2014) 181–195. https://doi.org/10.1260/0263-6174.32.2-3.181.

[64] J. Ning, M. Zhou, X. Pan, C. Li, N. Lv, T. Wang, G. Cai, R. Wang, J. Li, G. Zhu, Simultaneous biogas and biogas slurry production from co-digestion of pig manure and corn straw: Performance optimization and microbial community shift, Bioresour Technol. 282 (2019) 37–47. https://doi.org/https://doi.org/10.1016/j.biortech.2019.02.122.

[65] W. Li, Chapter 11 - Anaerobic digestion via codigestion strategies for production of bioenergy, in: A. Pandey, Y.W. Tong, L. Zhang, J. Zhang (Eds.), Biomass, Biofuels, Biochemicals, Elsevier, 2022: pp. 233–252. https://doi.org/https://doi.org/10.1016/B978-0-323-90633-3.00016-X.

[66] Y. Zheng, J. Zhao, F. Xu, Y. Li, Pretreatment of lignocellulosic biomass for enhanced biogas production, Prog Energy Combust Sci. 42 (2014) 35–53. https://doi.org/https://doi.org/10.1016/j.pecs.2014.01.001.

[67] M. Usman Khan, B. Kiaer Ahring, Improving the biogas yield of manure: Effect of pretreatment on anaerobic digestion of the recalcitrant fraction of manure, Bioresour Technol. 321 (2021) 124427. https://doi.org/https://doi.org/10.1016/j.biortech.2020.124427.

[68] M. Mönch-Tegeder, A. Lemmer, H. Oechsner, Enhancement of methane production with horse manure supplement and pretreatment in a full-scale biogas process, Energy. 73 (2014) 523–530. https://doi.org/https://doi.org/10.1016/j.energy.2014.06.051.

[69] R. Affes, J. Palatsi, X. Flotats, H. Carrère, J.P. Steyer, A. Battimelli, Saponification pretreatment and solids recirculation as a new anaerobic process for the treatment of slaughterhouse waste, Bioresour Technol. 131 (2013) 460–467. https://doi.org/https://doi.org/10.1016/j.biortech.2012.12.187.

[70] P. Tsapekos, P.G. Kougias, A. Frison, R. Raga, I. Angelidaki, Improving methane production from digested manure biofibers by mechanical and thermal alkaline pretreatment, Bioresour Technol. 216 (2016) 545–552. https://doi.org/https://doi.org/10.1016/j.biortech.2016.05.117.

[71] R. Biswas, B.K. Ahring, H. Uellendahl, Improving biogas yields using an innovative concept for conversion of the fiber fraction of manure, Water Science and Technology. 66 (2012) 1751–1758. https://doi.org/10.2166/wst.2012.298.

[72] M. Vanegas, F. Romani, M. Jiménez, Pilot-Scale Anaerobic Digestion of Pig Manure with Thermal Pretreatment: Stability Monitoring to Improve the Potential for Obtaining Methane, Processes. 10 (2022). https://doi.org/10.3390/pr10081602.

[73] E. Bruni, A.P. Jensen, I. Angelidaki, Steam treatment of digested biofibers for increasing biogas production, Bioresour Technol. 101 (2010) 7668–7671. https://doi.org/https://doi.org/10.1016/j.biortech.2010.04.064.

[74] B.K. Ahring, R. Biswas, A. Ahamed, P.J. Teller, H. Uellendahl, Making lignin accessible for anaerobic digestion by wet-explosion pretreatment, Bioresour Technol. 175 (2015) 182–188. https://doi.org/https://doi.org/10.1016/j.biortech.2014.10.082.

[75] J. Grim, P. Malmros, A. Schnürer, Å. Nordberg, Comparison of pasteurization and integrated thermophilic sanitation at a full-scale biogas plant – Heat demand and biogas production, Energy. 79 (2015) 419–427. https://doi.org/https://doi.org/10.1016/j.energy.2014.11.028.

[76] P.P. Dell'Omo, V.A. Spena, Mechanical pretreatment of lignocellulosic biomass to improve biogas production: Comparison of results for giant reed and wheat straw, Energy. 203 (2020) 117798. https://doi.org/https://doi.org/10.1016/j.energy.2020.117798.

[77] G. Mancini, S. Papirio, P.N.L. Lens, G. Esposito, Increased biogas production from wheat straw by chemical pretreatments, Renew Energy. 119 (2018) 608–614. https://doi.org/10.1016/J.RENENE.2017.12.045.

[78] L.C. Ferreira, A. Donoso-Bravo, P.J. Nilsen, F. Fdz-Polanco, S.I. Pérez-Elvira, Influence of thermal pretreatment on the biochemical methane potential of wheat straw, Bioresour Technol. 143 (2013) 251–257. https://doi.org/https://doi.org/10.1016/j.biortech.2013.05.065.

[79] G. Shang, C. Zhang, F. Wang, L. Qiu, X. Guo, F. Xu, Liquid hot water pretreatment to enhance the anaerobic digestion of wheat straw—effects of temperature and retention time, Environmental Science and Pollution Research. 26 (2019) 29424–29434. https://doi.org/10.1007/s11356-019-06111-z.

[80] M. Schroyen, H. Vervaeren, S.W.H. Van Hulle, K. Raes, Impact of enzymatic pretreatment on corn stover degradation and biogas production, Bioresour Technol. 173 (2014) 59–66. https://doi.org/10.1016/j.biortech.2014.09.030.

[81] M. Hjorth, K. Gränitz, A.P.S. Adamsen, H.B. Møller, Extrusion as a pretreatment to increase biogas production, Bioresour Technol. 102 (2011) 4989–4994. https://doi.org/10.1016/j.biortech.2010.11.128.

[82] S. Akizuki, H. Suzuki, M. Fujiwara, T. Toda, Impacts of steam explosion pretreatment on semi-continuous anaerobic digestion of lignin-rich submerged macrophyte, J Clean Prod. 385 (2023) 135377. https://doi.org/https://doi.org/10.1016/j.jclepro.2022.135377.

[83] F. Theuretzbacher, J. Lizasoain, C. Lefever, M.K. Saylor, R. Enguidanos, N. Weran, A. Gronauer, A. Bauer, Steam explosion pretreatment of wheat straw to improve methane yields: Investigation of the degradation kinetics of structural compounds during anaerobic

digestion, Bioresour Technol. 179 (2015) 299–305. https://doi.org/10.1016/J.BIORTECH.2014.12.008.

[84] M. Yadav, V. Vivekanand, Combined fungal and bacterial pretreatment of wheat and pearl millet straw for biogas production – A study from batch to continuous stirred tank reactors, Bioresour Technol. 321 (2021) 124523. https://doi.org/https://doi.org/10.1016/j.biortech.2020.124523.

[85] M.R. Atelge, A.E. Atabani, J.R. Banu, D. Krisa, M. Kaya, C. Eskicioglu, G. Kumar, C. Lee, Y.Ş. Yildiz, S. Unalan, R. Mohanasundaram, F. Duman, A critical review of pretreatment technologies to enhance anaerobic digestion and energy recovery, Fuel. 270 (2020) 117494. https://doi.org/https://doi.org/10.1016/j.fuel.2020.117494.

[86] H. Karne, U. Mahajan, U. Ketkar, A. Kohade, P. Khadilkar, A. Mishra, A review on biogas upgradation systems, Mater Today Proc. 72 (2023) 775–786. https://doi.org/https://doi.org/10.1016/j.matpr.2022.09.015.

[87] Q. Sun, H. Li, J. Yan, L. Liu, Z. Yu, X. Yu, Selection of appropriate biogas upgrading technology-a review of biogas cleaning, upgrading and utilisation, Renewable and Sustainable Energy Reviews. 51 (2015) 521–532. https://doi.org/https://doi.org/10.1016/j.rser.2015.06.029.

[88] C. Reparaz, L. Sánchez-Martín, I. de Godos, P. Mora, B. Llamas, A Distributed Biogas Production Model and Its Use in the Livestock Sector. Case Study: Castile and León, Applied Sciences. 11 (2021). https://doi.org/10.3390/app11125326.

[89] M. del R. Rodero, R. Ángeles, O. García-Depraect, R. Lebrero, R. Muñoz, Chapter 5 - Recent advances on photosynthetic biogas upgrading to biomethane, in: A. Yousuf, L. Melville (Eds.), Biogas to Biomethane, Woodhead Publishing, 2024: pp. 117–140. https://doi.org/https://doi.org/10.1016/B978-0-443-18479-6.00010-7.

[90] R. Ángeles, E. Arnaiz, J. Gutiérrez, C.A. Sepúlveda-Muñoz, O. Fernández-Ramos, R. Muñoz, R. Lebrero, Optimization of photosynthetic biogas upgrading in closed photobioreactors combined with algal biomass production, Journal of Water Process Engineering. 38 (2020) 101554. https://doi.org/https://doi.org/10.1016/j.jwpe.2020.101554.

[91] L. Méndez, D. García, E. Perez, S. Blanco, R. Muñoz, Photosynthetic upgrading of biogas from anaerobic digestion of mixed sludge in an outdoors algal-bacterial photobioreactor at pilot scale, Journal of Water Process Engineering. 48 (2022) 102891. https://doi.org/10.1016/J.JWPE.2022.102891.

[92] M. Raboni, P. Viotti, A. Capodaglio, A comprehensive analysis of the current and future role of biofuels for transport in the European Union (EU), Ambiente e Agua - An Interdisciplinary Journal of Applied Science. 10 (2015). https://doi.org/10.4136/ambi-agua.1492.

[93] M. Pöschl, S. Ward, P. Owende, Evaluation of energy efficiency of various biogas production and utilization pathways, Appl Energy. 87 (2010) 3305–3321. https://doi.org/https://doi.org/10.1016/j.apenergy.2010.05.011.

[94] M.U. Khan, J.T.E. Lee, M.A. Bashir, P.D. Dissanayake, Y.S. Ok, Y.W. Tong, M.A. Shariati, S. Wu, B.K. Ahring, Current status of biogas upgrading for direct biomethane use: A review, Renewable and Sustainable Energy Reviews. 149 (2021) 111343. https://doi.org/https://doi.org/10.1016/j.rser.2021.111343.

[95] M. Poeschl, S. Ward, P. Owende, Prospects for expanded utilization of biogas in Germany, Renewable and Sustainable Energy Reviews. 14 (2010) 1782–1797. https://doi.org/https://doi.org/10.1016/j.rser.2010.04.010.

[96] K. Timonen, T. Sinkko, S. Luostarinen, E. Tampio, K. Joensuu, LCA of anaerobic digestion: Emission allocation for energy and digestate, J Clean Prod. 235 (2019) 1567–1579. https://doi.org/https://doi.org/10.1016/j.jclepro.2019.06.085.

[97] Biogas from Waste and Renewable Resources, Parts of Biogas Plants, in: Biogas from Waste and Renewable Resources, 2008: pp. 199–220. https://doi.org/https://doi.org/10.1002/9783527621705.ch1e.

[98] X. Su, X. Shao, Y. Geng, S. Tian, Y. Huang, Optimization of feedstock and insulating strategies to enhance biogas production of solar-assisted biodigester system, Renew Energy. 197 (2022) 59–68. https://doi.org/https://doi.org/10.1016/j.renene.2022.07.090.

[99] L. Lombardi, B. Mendecka, S. Fabrizi, Solar integrated anaerobic digester: Energy savings and economics, Energies (Basel). 13 (2020). https://doi.org/10.3390/en13174292.

[100] K. Calautit, C. Johnstone, State-of-the-art review of micro to small-scale wind energy harvesting technologies for building integration, Energy Conversion and Management: X. 20 (2023) 100457. https://doi.org/https://doi.org/10.1016/j.ecmx.2023.100457.

[101] B. Llamas, M.F. Ortega, G. Barthelemy, I. de Godos, F.G. Acién, Development of an efficient and sustainable energy storage system by hybridization of compressed air and biogas technologies (BIO-CAES), Energy Convers Manag. 210 (2020). https://doi.org/10.1016/j.enconman.2020.112695.

[102] C. Walla, W. Schneeberger, The optimal size for biogas plants, Biomass Bioenergy. 32 (2008) 551–557. https://doi.org/https://doi.org/10.1016/j.biombioe.2007.11.009.

[103] A. Regueira, R. Bevilacqua, J.M. Lema, M. Carballa, M. Mauricio-Iglesias, A metabolic model for targeted volatile fatty acids production by cofermentation of carbohydrates and proteins, Bioresour Technol. 298 (2020) 122535. https://doi.org/https://doi.org/10.1016/j.biortech.2019.122535.

[104] J.A. Magdalena, C. González-Fernández, Archaea inhibition: Strategies for the enhancement of volatile fatty acids production from microalgae, Waste Management. 102 (2020) 222–230. https://doi.org/10.1016/j.wasman.2019.10.044.

[105] G. Strazzera, F. Battista, B. Tonanzi, S. Rossetti, D. Bolzonella, Optimization of short chain volatile fatty acids production from household food waste for biorefinery applications, Environ Technol Innov. 23 (2021) 101562. https://doi.org/https://doi.org/10.1016/j.eti.2021.101562.

[106] A. Regueira, R. Turunen, K.S. Vuoristo, M. Carballa, J.M. Lema, J. Uusitalo, M. Mauricio-Iglesias, Model-aided targeted volatile fatty acid production from food waste using a defined co-culture microbial community, Science of The Total Environment. 857 (2023) 159521. https://doi.org/https://doi.org/10.1016/j.scitotenv.2022.159521.

[107] A. Regueira, R. Bevilacqua, M. Mauricio-Iglesias, M. Carballa, J.M. Lema, Kinetic and stoichiometric model for the computer-aided design of protein fermentation into volatile fatty acids, Chemical Engineering Journal. 406 (2021) 126835. https://doi.org/https://doi.org/10.1016/j.cej.2020.126835.

[108] M. François, K.-S. Lin, N. Rachmadona, K.S. Khoo, Advancement of nanotechnologies in biogas production and contaminant removal: A review, Fuel. 340 (2023) 127470. https://doi.org/https://doi.org/10.1016/j.fuel.2023.127470.

[109] C.M. Ajay, S. Mohan, P. Dinesha, M.A. Rosen, Review of impact of nanoparticle additives on anaerobic digestion and methane generation, Fuel. 277 (2020) 118234. https://doi.org/https://doi.org/10.1016/j.fuel.2020.118234.

[110] M. Dehhaghi, M. Tabatabaei, M. Aghbashlo, H. Kazemi Shariat Panahi, A.-S. Nizami, A state-of-the-art review on the application of nanomaterials for enhancing biogas production, J Environ Manage. 251 (2019) 109597. https://doi.org/https://doi.org/10.1016/j.jenvman.2019.109597.

[111] Y. Cai, M. Zhu, X. Meng, J.L. Zhou, H. Zhang, X. Shen, The role of biochar on alleviating ammonia toxicity in anaerobic digestion of nitrogen-rich wastes: A review, Bioresour

Technol. 351 (2022) 126924. https://doi.org/https://doi.org/10.1016/j.biortech.2022.126924.

[112] J. Zhang, Z. Wang, T. Lu, J. Liu, Y. Wang, P. Shen, Y. Wei, Response and mechanisms of the performance and fate of antibiotic resistance genes to nano-magnetite during anaerobic digestion of swine manure, J Hazard Mater. 366 (2019) 192–201. https://doi.org/https://doi.org/10.1016/j.jhazmat.2018.11.106.

[113] A. Hassanein, A. Naresh Kumar, S. Lansing, Impact of electro-conductive nanoparticles additives on anaerobic digestion performance - A review, Bioresour Technol. 342 (2021) 126023. https://doi.org/https://doi.org/10.1016/j.biortech.2021.126023.

[114] G. Kassab, D. Khater, F. Odeh, K. Shatanawi, M. Halalsheh, M. Arafah, J.B. van Lier, Impact of Nanoscale Magnetite and Zero Valent Iron on the Batch-Wise Anaerobic Co-Digestion of Food Waste and Waste-Activated Sludge, Water (Basel). 12 (2020). https://doi.org/10.3390/w12051283.

[115] E. Casals, R. Barrena, A. García, E. González, L. Delgado, M. Busquets-Fité, X. Font, J. Arbiol, P. Glatzel, K. Kvashnina, A. Sánchez, V. Puntes, Programmed Iron Oxide Nanoparticles Disintegration in Anaerobic Digesters Boosts Biogas Production, Small. 10 (2014) 2801–2808. https://doi.org/https://doi.org/10.1002/smll.201303703.

[116] İ.A. Başar, C. Eskicioglu, N.A. Perendeci, Biochar and wood ash amended anaerobic digestion of hydrothermally pretreated lignocellulosic biomass for biorefinery applications, Waste Management. 154 (2022) 350–360. https://doi.org/https://doi.org/10.1016/j.wasman.2022.10.014.

[117] A. Hassanein, S. Lansing, R. Tikekar, Impact of metal nanoparticles on biogas production from poultry litter, Bioresour Technol. 275 (2019) 200–206. https://doi.org/https://doi.org/10.1016/j.biortech.2018.12.048.

[118] G.S. Aguilar-Moreno, E. Navarro-Cerón, A. Velázquez-Hernández, G. Hernández-Eugenio, M.Á. Aguilar-Méndez, T. Espinosa-Solares, Enhancing methane yield of chicken litter in anaerobic digestion using magnetite nanoparticles, Renew Energy. 147 (2020) 204–213. https://doi.org/https://doi.org/10.1016/j.renene.2019.08.111.

[119] C. Herrmann, E. Sánchez, M. Schultze, R. Borja, Comparative effect of biochar and activated carbon addition on the mesophilic anaerobic digestion of piggery waste in batch mode, Journal of Environmental Science and Health, Part A. 56 (2021) 946–952. https://doi.org/10.1080/10934529.2021.1944833.

[120] E. Abdelsalam, M. Samer, Y.A. Attia, M.A. Abdel-Hadi, H.E. Hassan, Y. Badr, Comparison of nanoparticles effects on biogas and methane production from anaerobic digestion of cattle dung slurry, Renew Energy. 87 (2016) 592–598. https://doi.org/https://doi.org/10.1016/j.renene.2015.10.053.

[121] J. Zhang, Z. Wang, T. Lu, J. Liu, Y. Wang, P. Shen, Y. Wei, Response and mechanisms of the performance and fate of antibiotic resistance genes to nano-magnetite during anaerobic digestion of swine manure, J Hazard Mater. 366 (2019) 192–201. https://doi.org/https://doi.org/10.1016/j.jhazmat.2018.11.106.

[122] M. Farghali, M. Mayumi, K. Syo, A. Satoshi, Y. Seiichi, S. Takashima, H. Ono, Y. AP, T. Yamashiro, M.M. Ahmed, S. Kotb, M. Iwasaki, I. Ihara, K. Umetsu, Potential of biogas production from manure of dairy cattle fed on natural soil supplement rich in iron under batch and semi-continuous anaerobic digestion, Bioresour Technol. 309 (2020) 123298. https://doi.org/https://doi.org/10.1016/j.biortech.2020.123298.

[123] Y. Wang, G. Ren, T. Zhang, S. Zou, C. Mao, X. Wang, Effect of magnetite powder on anaerobic co-digestion of pig manure and wheat straw, Waste Management. 66 (2017) 46–52. https://doi.org/https://doi.org/10.1016/j.wasman.2017.04.031.

[124] Y. Liu, Q. Xiao, X. Ye, C. Wang, Z. Jia, J. Du, X. Kong, Y. Xi, Effect of different charged Fe3O4 nanoparticles on methane production for anaerobic digestion of wheat straw, J Clean Prod. 328 (2021) 129655. https://doi.org/10.1016/J.JCLEPRO.2021.129655.

[125] A.A. Noonari, R.B. Mahar, A.R. Sahito, K.M. Brohi, Anaerobic co-digestion of canola straw and banana plant wastes with buffalo dung: Effect of Fe3O4 nanoparticles on methane yield, Renew Energy. 133 (2019) 1046–1054. https://doi.org/https://doi.org/10.1016/j.renene.2018.10.113.

[126] H. Ma, Y. Hu, T. Kobayashi, K.-Q. Xu, The role of rice husk biochar addition in anaerobic digestion for sweet sorghum under high loading condition, Biotechnology Reports. 27 (2020) e00515. https://doi.org/https://doi.org/10.1016/j.btre.2020.e00515.

[127] M.J. Khalid, Zeshan, A. Waqas, I. Nawaz, Synergistic effect of alkaline pretreatment and magnetite nanoparticle application on biogas production from rice straw, Bioresour Technol. 275 (2019) 288–296. https://doi.org/https://doi.org/10.1016/j.biortech.2018.12.051.

[128] C.B. Arenas Sevillano, M. Chiappero, X. Gomez, S. Fiore, E.J. Martínez, Improving the anaerobic digestion of wine-industry liquid wastes: Treatment by electro-oxidation and

use of biochar as an additive, Energies (Basel). 13 (2020). https://doi.org/10.3390/en13225971.

[129] Z. Ning, B. Xu, W. Zhong, C. Liu, X. Qin, W. Feng, L. Zhu, Preparation of phosphoric acid modified antibiotic mycelial residues biochar: Loading of nano zero-valent iron and promotion on biogas production, Bioresour Technol. 348 (2022) 126801. https://doi.org/https://doi.org/10.1016/j.biortech.2022.126801.

[130] Y. Deng, J. Xia, R. Zhao, J. Xu, X. Liu, Iron-coated biochar alleviates acid accumulation and improves methane production under ammonium enrichment conditions, Science of The Total Environment. 809 (2022) 151154. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.151154.

[131] J. Cai, P. He, Y. Wang, L. Shao, F. Lü, Effects and optimization of the use of biochar in anaerobic digestion of food wastes, Waste Management & Research. 34 (2016) 409–416. https://doi.org/10.1177/0734242X16634196.

[132] M.A. Bhat, K. Gedik, E.O. Gaga, Chapter 23 - Environmental impacts of nanoparticles: pros, cons, and future prospects, in: M. Ozturk, A. Roy, R.A. Bhat, F. Vardar-Sukan, F.M. Policarpo Tonelli (Eds.), Synthesis of Bionanomaterials for Biomedical Applications, Elsevier, 2023: pp. 493–528. https://doi.org/https://doi.org/10.1016/B978-0-323-91195-5.00002-7.

[133] R. Singh, Chapter 7 - Toxic risk assessment of engineered nanoparticles used in ink formulations, in: R.K. Gupta, T.A. Nguyen (Eds.), Smart Multifunctional Nano-Inks, Elsevier, 2023: pp. 159–194. https://doi.org/https://doi.org/10.1016/B978-0-323-91145-0.00023-2.

[134] P. Jadhav, Z. Bin Khalid, A.W. Zularisam, S. Krishnan, M. Nasrullah, The role of iron-based nanoparticles (Fe-NPs) on methanogenesis in anaerobic digestion (AD) performance, Environ Res. 204 (2022) 112043. https://doi.org/https://doi.org/10.1016/j.envres.2021.112043.

Chapter 2

Aims and scope

1.1. Justification

Enhancing the efficiency of anaerobic digestion is a key factor for its implementation in the field of organic waste and by-product management.

The generation of organic waste is becoming increasingly large due to the rise in the world's population. This surge in population is correlated with increases in resource and energy consumption, predominantly sourced from fossil fuels, which are finite. This consumption has led to an uncontrolled release of greenhouse gas emissions, with a direct impact on the global warming, which has made necessary the development of global measures through international agreements, as well as, the development of new energy production alternatives, including renewable energies such as solar, wind, and biomass, alongside an improved waste management strategy.

In recent years, anaerobic digestion has emerged as an environmentally friendly and cost-effective technology. This process transforms organic waste into energy in the form of biogas. The biogas produced consists mainly of CH_4 and CO_2, effectively capturing potentially harmful greenhouse gases. It also provides energy valorisation while simultaneously avoiding the consumption of fossil resources, which would otherwise generate CO_2 emissions through combustion.

Nevertheless, this process, reliant on the biological activity of transforming microorganisms, can provoke difficulties, leading to operational challenges with low biotransformation yields and finally a reduced biogas production. Thus, technological development is necessary to overcome these barriers, making anaerobic digestion a more robust and attractive alternative for waste management and energy recovery. Particularly, the agricultural sector, with its expanding activity and substantial organic waste production, stands as a strategic area for the implementation of anaerobic digesters.

1.2. Main objectives

The overall objective of this thesis was the study and development of innovative technologies that enhance the anaerobic digestion process for energy production and the generation of other value-added intermediate by-products. A multidisciplinary approach was employed, focusing on agricultural by-products with an extensive availability worldwide, giving significant potential for transformation and valorisation. The specific objectives to achieve this overarching goal included:

1. Evaluate the hybridization of anaerobic digestion with other renewable energy sources.
2. Control the production of high-value by-products in the anaerobic digestion process.
3. Enhance the biogas production efficiency in the anaerobic digestion process by monitoring operational parameters.
4. Conduct microbiological studies and characterization to identify stable conditions in the process.
5. Projection of the implementation of the process on a real-scale level.

These objectives were addressed through experimental basis at a laboratory level, adopting a real-scale integration forecast approach to ensure the replicability of the knowledge generated.

1.3. Development of the thesis

To achieve the objectives, various experiments were conducted. The first objective was addressed in **Chapters 3** where a simulation was carried out based on experimental data, exploring the integration of a hybrid solar installation into a biomethane plant on a pig farm in an energy isolated scenario. Different locations were studied to observe the climatic effect on the proposed system, and an economic analysis was performed.

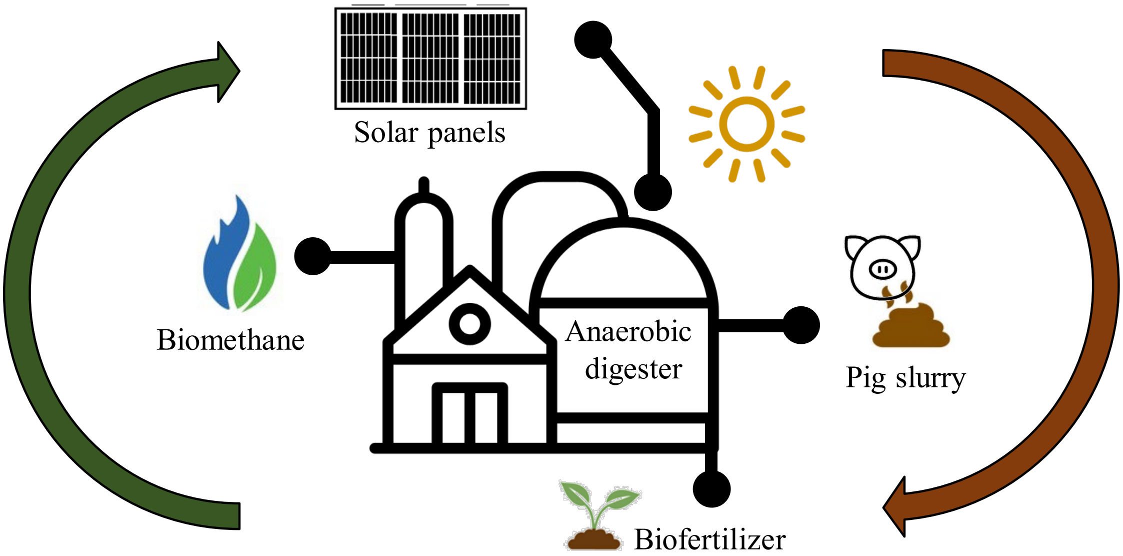
The second objective was developed in **Chapter 4**, where the kinetics of each stage were studied with the implementation of batch reactors fed with straw from different cereals (wheat, barley and rye) to optimize the production of volatile fatty acids as a valuable intermediate by-product in the industry, serving as a building-block precursors for other products.

The third objective was addressed in **Chapters 5, 6, and 7**. It involved the operation of batch reactors fed with wine lees with the addition of iron-bases and carbon-based microparticles (**Chapter 5**). Using the same methodology, reactors fed with wheat straw as a substrate were performed, subject to different thermal (steam explosion) pre-treatment conditions (**Chapter 7**). Additionally, two semi-continuous reactors were operated with pig slurry until reaching stable optimal conditions for biomethane production (**Chapter 6)**. This study also included a microbiological analysis, providing insights into the biological interactions of bacterial and archaeal species, aligning with the fourth objective of the thesis.

The last objective of the real-scale projection is overarching in all the works developed in the thesis, across **Chapters 3, 4, 6, and 7**.

Chapter 3

Hybridization of anaerobic digestion with solar energy: a solution for isolated livestock farms



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Hybridization of anaerobic digestion with solar energy: a solution for isolated livestock farms

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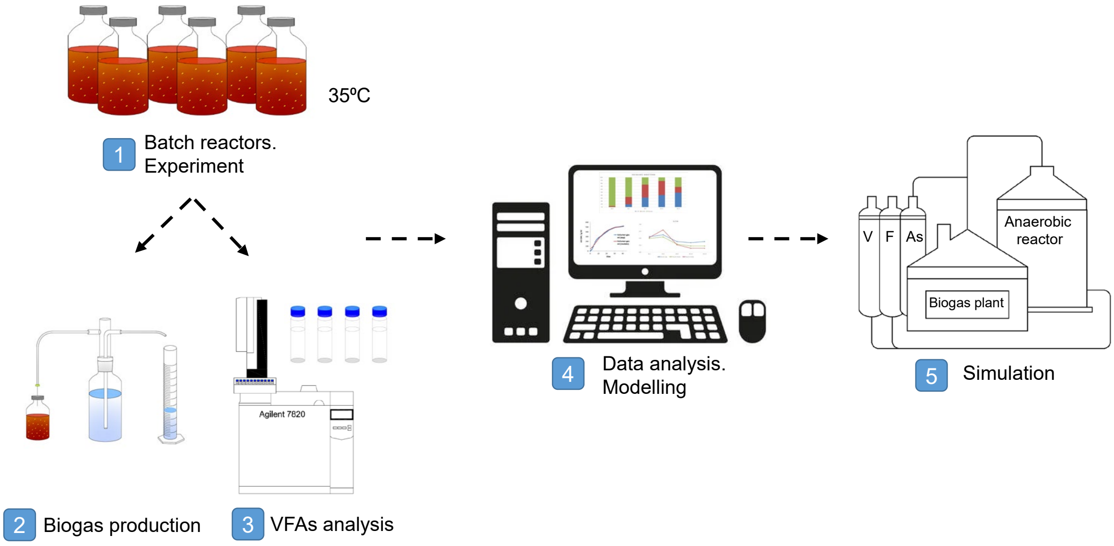
Abstract: Intensive farming causes an important amount of greenhouse gases emissions. This situation can be significantly reduced by the implementation of renewable technologies and transforming farms from being energy consumers to become energy providers. In case of livestock production, biogas and solar energy reduce greenhouse gas emissions and the energy demand of the installations. However, the implementation of these technologies requires solutions adapted to the circumstances of the facilities, such as connectivity to the energy grids. In this work, a biogas/biomethane production system, energetically covered with hybrid solar panels is proposed as a solution for isolated areas where biodegradable substrates (manure) are abundant. In this manner, the electrical and thermal necessities of the digester are supplied by solar panels, reducing the biogas self-consumption and the energy inputs from the electrical grid. Hybrid solar panels also provide sufficient energy for operation of an upgrading system to obtain biomethane of fuel vehicle quality, increasing the energy self-sufficiency of the agricultural activities. This solution has been simulated in five different climatic regions corresponding to areas of intense pig farming activity. The results demonstrate the sustainable bioenergy production in isolated farms with limited connection to the energy grid and organic matter availability. Furthermore, the economic study showed that the proposed technology is competitive compared to other technologies in the energy sector.

Chapter 4

Simultaneous production of biogas and volatile fatty acids through anaerobic digestion using cereal straw as substrate



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Simultaneous production of biogas and volatile fatty acids through anaerobic digestion using cereal straw as substrate

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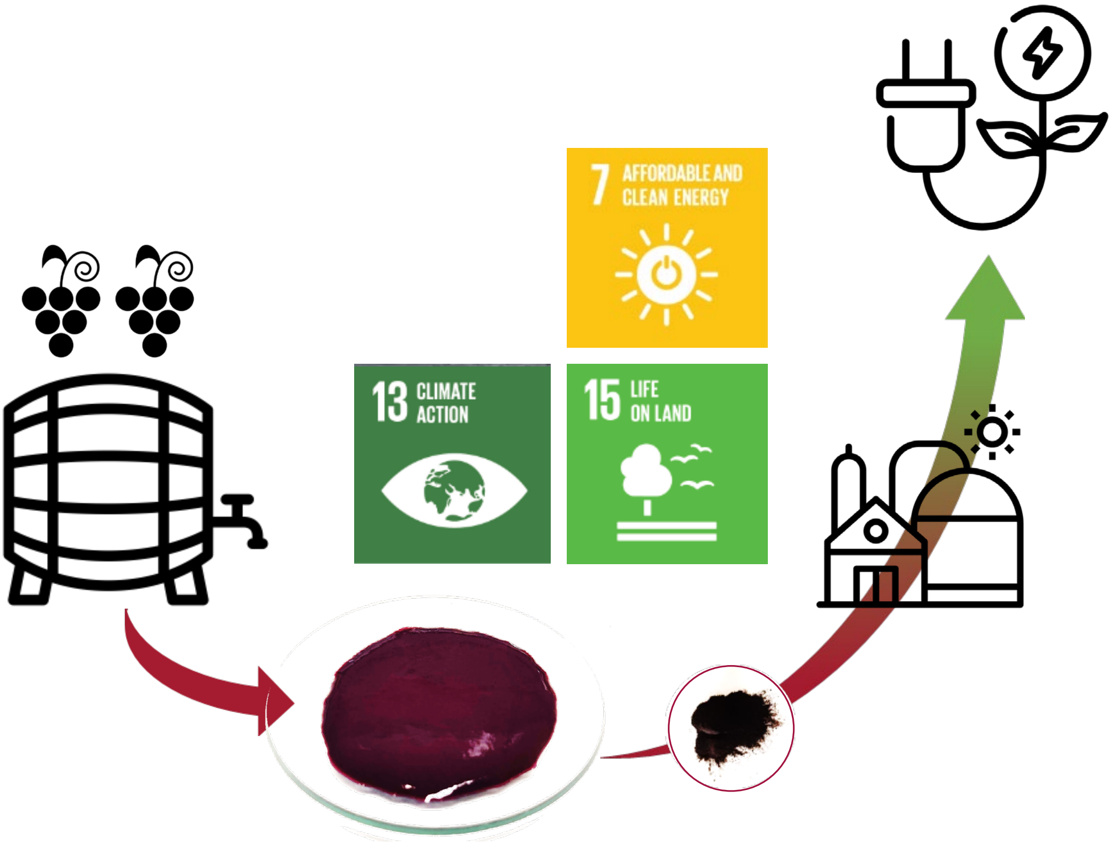
Abstract

Cereal straw is one of the most abundant wastes worldwide, with 30.000 million tons produced per year. Bioconversion of this residual material into carboxylates by anaerobic digestion could potentially replace conventional production based on fossil feedstocks (oil). In this work, fundamental issues of this bioconversion have been explored, including: different kinds of straw (wheat, barley and rye), biomass size reduction, mass balances and modelling of the different steps of the digestion. Under optimum conditions, 44% of the raw material was effectively converted into VFAs (mainly acetate) when barley was used as substrate. Wheat and rye straw presented lower conversion rates due to the higher lignin content compared to barley straw. According to the modelling proposed, methanogenesis and hydrolysis presented very similar reaction rates, which resulted in a simultaneous production of VFAs and biogas. In view of these results, a process integration is proposed where biogas covers the thermal needs of the biotransformation of barley biomass into VFAs.

Chapter 5

Improving the anaerobic digestion process of wine lees by the addition of microparticles



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Improving the anaerobic digestion process of wine lees by the addition of microparticles

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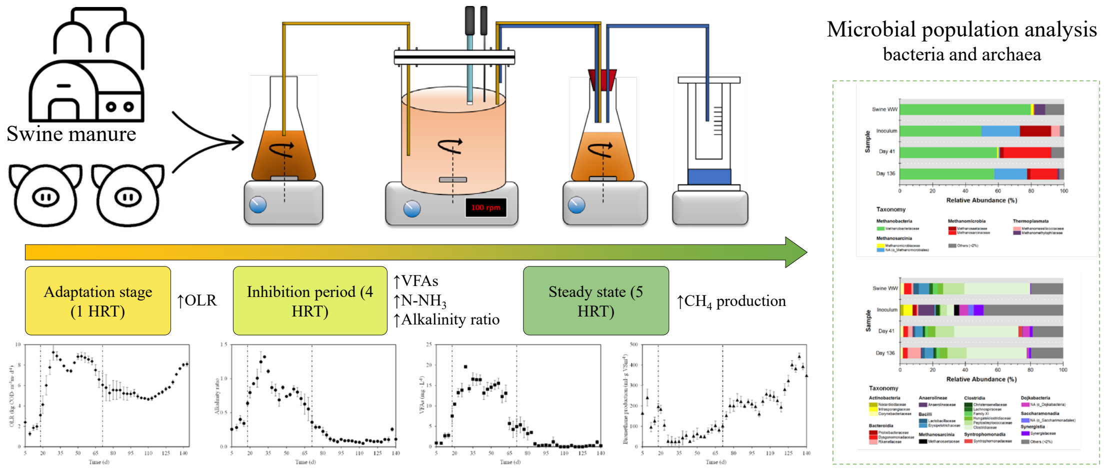
Abstract

Wine lees generation, a by-product of the wine industry, imply economic challenges for producers in terms of management due to its high organic load and low pH value. Biological treatment based on controlled anaerobic digestion may emerge as viable management alternative given their promising potential of biogas production thanks to the organic content of the substrate. However, the complex properties of wine lees may lead to microbial activity inhibition and process kinetics failure. Various solutions have already been explored, including co-digestion with other substrates, or the application of different pre-treatments, to mitigate the effects of the accumulation of phenolic compounds, volatile fatty acids, and antioxidants, or the acidic pH value of the medium. In this study, laboratory-scale batch reactors were established, adding iron (magnetite) or carbon (graphite) based microparticles to assess their impact on the kinetics of the process. The results demonstrate a significant improvement of the 35% in the potential production of biomethane after four days of operation with graphite particles and 40% after six days using magnetite particles. Evidences of acceleration of the methanogenesis phase were detected along the essays. However, the strong inhibition mediated by the carboxylate accumulation was not avoided in any of the conditions tested.

Chapter 6

Microbial analysis of anaerobic digester reveals prevalence of manure microbiota



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Microbial analysis of anaerobic digester reveals prevalence of manure microbiota

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Abstract

Anaerobic digestion of swine manure offers a solution to the environmental impacts of farm greenhouse gases emissions and provides a renewable gas in the agricultural sector. The particular composition of manure, with high concentrations of ammoniacal nitrogen and volatile fatty acids, often threatens the stability of the process through inhibition of methanogens. In this work, continuous production of biogas was tested under relative short hydraulic retention time (15 days) using swine manure as substrate. Although a strong inhibition period was detected, adaptation of the microbiota and displacement of bacteria and archaea present in the inoculum by microorganisms present in the animal manure resulted in biogas production close to the values found in standardized batch tests in absence of inhibitions (410 ml CH_4 per g organic matter). The findings suggest that inoculation of digesters in farms is unnecessary and it may even outcome in long inhibition periods characterized by low biogas productivities.

Keywords: Anaerobic digestion, inhibition, microbial community, swine manure, waste to energy.

1. Introduction

The production of gases of renewable origin plays a crucial role in the transition to a sustainable, carbon-free energy future, becoming instrumental in shaping the energy mix [1]. These gases, such as biogas and biomethane, are generated through the anaerobic digestion of organic matter and waste, providing a clean energy source. Agricultural residues have significant potential to lead this transformation due to their abundance and constant availability [2]. The agricultural industry produces a large amount of organic waste, such as crop residues, animal manure and spoiled products. These substrates are susceptible to be converted into a valuable source of energy by processing them in anaerobic digestion plants [3]. In case of swine production, there is an increasing trend in the global production which is expected to increase by more than 10% between 2020 and 2030 [4]. The high environmental impact associated with this activity and the legal requirements force the sector to implement sustainable practices in the proper management of the by-products (mainly manure), efficiency in the use of resources and the adoption of cleaner technologies [5,6]. The biological process of anaerobic digestion offers an efficient and sustainable technical solution for livestock manure management, generating renewable energy in the form of biogas and a liquid organic fertilizer. In addition, greenhouse gas (GHG) diffuse emissions in the form of CH_4 and NO_2 are reduced when agricultural wastes are digested and methane is recovered. According to some estimations, GHG savings in case of wet animal manure can reach 240 % when applying anaerobic digesters and using the biogas generated for replacement of fossil-based fuels [7]. Therefore, an increasing interest in the implementation of agricultural digesters has been experimented in the recent years and foster the fossil natural gas replacement. While some European countries have achieved biogas/biomethane production that represents more than 15 % of the gas consumption, the percentage of replacement in this continent is expected to reach between 35 and 62 % by the year 2050, depending on the different projections based on the organic substrate availability [8]. At this point, it must be stressed that all the projections highlight the significance of animal manure in this transformation [9]. The production of biogas directly depends on the optimal operation of digesters in order to guarantee the stability and maximizes bioconversion.

The detection of operational problems due to different inhibitions (ammonia, sulfide and heavy metals ion among others) has been studied through the different anaerobic digestion stages [10–12]. However, there is a lack of research that specifically investigates these effects associated with a simultaneous study of the microbiological populations together with a physical-chemical monitoring [13,14]. The stability and efficiency of anaerobic digestion of complex substrates such as swine manure, relies on the tight equilibrium between the microbial populations responsible of the hydrolytic, acidogenic, acetogenic, and methanogenic stages [15]. This equilibrium is based on the microbial symbiotic relationships that may be importantly affected by changes in the environmental conditions (temperature, pH, nutrient content), intermediate compounds and toxic substances. In this sense, the very slow growth rates of methanogenic archaea and the lower tolerance towards some chemicals has been identified as the critical step [16,17]. The selection and maintenance of adequate anaerobic inoculum for starting the digestion processes of complex substrates such as swine manure is normally viewed as a decisive strategy for a successful process [18]. Little importance has been addressed to the manure microbiota which is already adapted to chemical conditions and can play decisive role in the bioprocess.

In the present work, two parallel reactors fed with swine manure working under semi-continuous conditions were operated with the objective of monitoring the relevant physicochemical parameters for a successful anaerobic digestion process working under a short hydraulic retention time (HRT) in order to identify possible inhibitions. Changes in the microbial populations based on 16s rRNA-based relative abundances were studied and correlated with the performance in terms of biometanization. The yields of bioconversion and rates were compared with normalized batch anaerobic test with and without anaerobic inoculum.

2. Material and methods

2.1 Substrate and inoculum

Pig manure from a feeder farm placed in Sauquillo de Boñices (Soria, Spain) was used as substrate. Average content of volatile solids (VS) was between 13.41 ± 0.35 g· kg^{-1} and 62.43 ± 0.12 g· kg^{-1}, respectively. This large difference in the organic content is due to the fact that the manure is stored in an open lagoon and exposed to ambient conditions. The manure was sieved to prevent clogging and provide efficient mixing and pumping and stored at 4 °C before usage. Two anaerobic inoculums were used in the batch experiments: one sampled from a local urban wastewater treatment plant treating mixed sludge (namely WWT) in Soria (Spain) and other withdrawn from the anaerobic digester placed in the pig farm (namely farm digester, FD). The WWT inoculum presented a content of volatile solids (VS) value of 12.8 ± 0.1 g· kg^{-1} and the FD of 20.7 ± 0.1 g· kg^{-1}.

2.2 Biomethane potential test

To determine the potential biogas production of the manure, standardized BMP (biomethane potential tests) were performed in triplicate for 60 days. In order to evaluate the effect of the microbiota present in the waste, three different essays were performed: with WWT inoculum, with FD inoculum and uninoculated. Glass serum bottles with a total working volume of 120 mL were used as batch reactors for BMP experiments with an inoculum/substrate ratio of 1:1 [19]. Temperature conditions were maintained at 35 ± 0.5 °C in an incubator (Selecta, Hotcold-GL) provided with an orbital stirring plate (Selecta, Rotabit). Control tests with the inoculum were included to measure the endogenous production of biogas in inoculated essays. 0.5 g· L^{-1} of $CaCO_3$ were introduced as a buffer to prevent alterations in pH and to ensure anaerobic conditions. Bottles were flushed with N_2 gas (99.9% purity) and immediately sealed with butyl rubber stoppers and aluminium crimps [20,21]. The rates of biogas production and its composition were measured by water displacement and biogas composition was periodically determined.

2.3 Continuously stirred tank reactors set up

The experimental set up consisted of two continuously stirred tank reactors (CSTR) with a total volume of 1.5 L and 0.75 L of liquid. Mesophilic conditions were used for the digestion (35 ± 1 °C) using a thermal bath (Selecta, Termotronic-100). The reactor was operated for a period of 5 months with an HRT of 15 days. A 1-L gas trap containing water was used to measure the biogas produced. The swine manure feeding was done manually every 24 hours. The reactor was mixed continuously with a magnetic stirrer at 100 rpm (Barnstead Thermolyne, SP131320-33). Final effluent from the digesters were collected to measure total volatile fatty acids (VFAs) concentration, chemistry oxygen demand (COD), total and partial alkalinity, pH and ammoniacal nitrogen. Biogas composition was also measured with a gas analyser once per week. **Fig. 1** shows a scheme of the experimental setup.

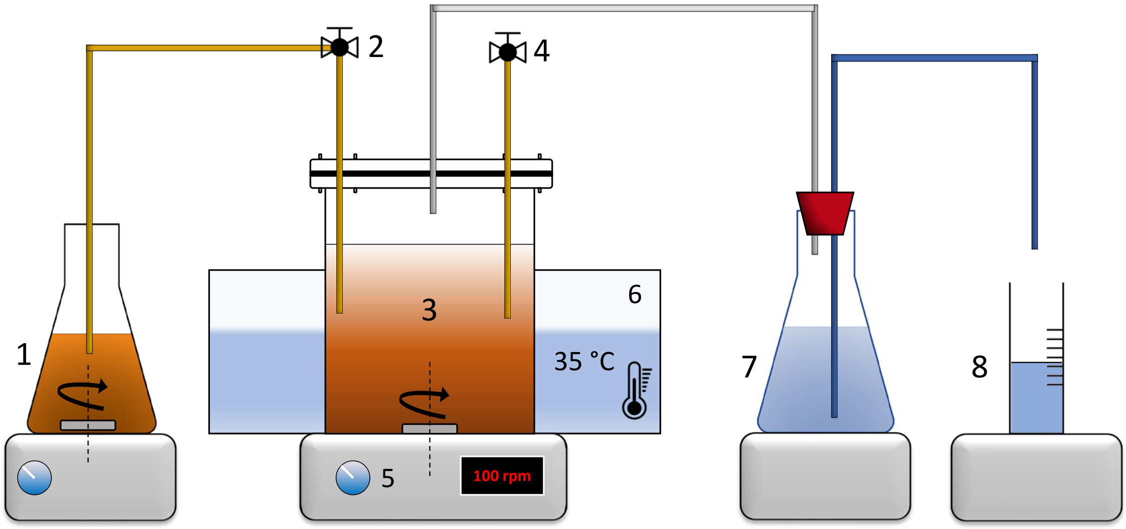


Fig. 1. Schematic representation of the experimental set-up and components: (1) Swine manure (substrate); (2) Feeding inlet valve; (3) Anaerobic reactor; (4) Digestate outlet valve; (5) Stirring plate; (6) Thermal bath; (7) Water trap; (8) Measuring cylinder

2.4 Analytical methods

The characterization of the substrate and inoculum was carried out following the standardized methodology by The American Public Health Association (APHA) in order to measure total solids (TS), volatile solids (VS), pH, total and partial alkalinity, total nitrogen (TN) and ammoniacal nitrogen and PO_4^{3-} [22]. Samples for volatile fatty acids (VFA) determination were prepared following the procedure described in [23] and determined through a gas chromatograph (Agilent 7820). Biogas composition was analysed with a (GeoTech, Biogas 5000) gas analyser. The characteristics of inoculum and diluted substrates used in the experiments are presented in **Table 1**.

Table 1. Characteristics of substrates, inoculum and digestate

Analitic parameter	**Pig manure**	**Inoculum**	**Digestate**
Total solids (g· kg^{-1})	45.3±28.9	17.9±0.1	35.8± 12.2
Volatile solids (g· kg^{-1})	33.3±23.1	12.8±0.1	25.01± 8.6
Total Nitrogen (g· L^{-1})	3.3±1.3	4941.1±854.8	3.1± 0.8
Mineral Nitrogen (%)	63.5±4.6		71.7± 2.7
Total Phosphorus $PO4^{3-}$ (g· L^{-1})	1024.5±819.7		751.25± 317.2
Electrical conductivity (mS)	13.9±2.8		16.3± 2.7
Chemistry oxygen demand (g· L^{-1})	95.3±37.6		72.1± 31.0
Total Alkalinity (g $CaCO_3$· L^{-1})	13.8±6.8		13.5± 2.8
Alkalinity ratio	1.10±0.20		0.41± 0.35
pH	7.5±0.4		7.9± 1.4

2.5 Microbial communities

Microbial characterization was performed in samples taken from the bioreactors at the different stages identified during the operation: after 41 days of operation, corresponding to a period of low biogas productivity and after 136 days of operation, when biogas production reached maximum levels. Additionally, the microbial composition was also analysed in samples of the swine manure and the WWT inoculum. Samples were preserved at -20 °C until DNA extraction procedures. After DNA extraction, a total of 50 ng of high-quality DNA was amplified following the 16S metagenomic sequencing library Illumina 15044223 B protocol (ILLUMINA). Two sets of primers were used to amplify 16S regions. The V3-V4 region of the 16S rRNA gene was amplified using the universal 341F-805R set of primers [24], additionally, the V4 region of 16S was amplified to study archaeal communities, using the primer pair combination 344F-1041R/519F-806R [25].

The retrieved 16s rRNA sequences were analysed using the software package DADA2 v1.6 in the R environment [26]. Forward and reverse reads were filtered and truncated to 290 and 220 nucleotides, respectively, and then paired reads were assembled.

Subsequently, paired-end reads underwent denoising, and singleton and chimera sequences were removed. Taxonomy was assigned to representative sequences taken from an amplicon sequence variant (ASV) table using the Naïve Bayesian classifier trained against the SILVA database release v132. Data analysis was performed using R through RStudio software (R Core Team, 2021). The sequences obtained in this work were deposited in the Genbank Sequence Read Archive under BioProject number PRJNA1025111.

3. Results and discussion

3.1 BMP tests

The biomethane production data was monitored over a time span extending for 60 days shown in **Fig. 2**. Significant discrepancies emerged between the trials concerning the rate of production and the total biogas output. In the case of tests inoculated with WWT sludge, biogas production showed a rapid increase without lag phase increasing until it peaked at 435 ml CH_4· g VS^{-1} after 18 days of incubation. The trial with only pig sludge (uninoculated) showed a slower production during the first 10 days of the essay with a notable lag phase. However, a higher accumulated biomethane production was reached with 517.5 ml CH_4· g VS^{-1} on day 55. The flasks inoculated with FD (taken from the farm digester) showed an intermediate rate of biogas production but the higher bioconversion of organic matter to methane with a value of 534 ml· g VS^{-1} by day 57. Taking in consideration that the same substrate was employed in the three essays, these variations between the trials should be attributed to the inoculum use. Although the WWT anaerobic inoculum presented a high rate and lack of latency, the yield of biomethanization was considerable higher (up to 18 %) in essays without inoculum or with a manure adapted inocula (FD test). Even though higher rates were achieved in WWT inoculated tests during the first 10 days, this fact could be negligible since anaerobic processes are designed to operate for years.

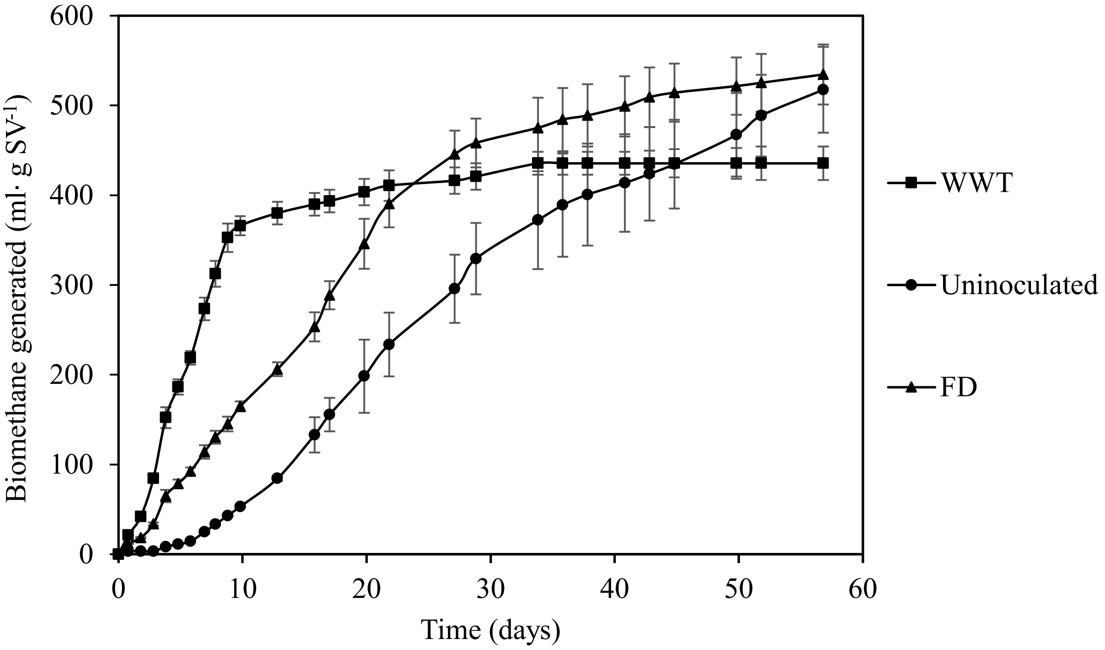


Fig. 2. Biogas production in the BMP tests: ■ (WWT- Swine manure with WWT sludge); ● (Uninoculated- Swine manure without inoculum); ▲ FD- (Swine manure with farm digester digestate)

3.2. Biogas production in CSTR

Three different phases were observed through the experiment: an initial short-duration adaptation stage (stage 1), followed by a stage 2 marked by a minimal biogas production, and finally, a third stage when biogas production gradually increased reaching the yields achieved in the BMP tests (stage 3) (**Fig. 3b**). Stage 1 was characterized by large fluctuation in methane production from 164.47 mL· g VS^{-1} on day 5 to a peak of 240.64 mL· g VS^{-1} on day 9. These unstable conditions could be attributed to microbial adaptation and substrate availability still present in the WWT inoculum. A significant decline in biogas production commenced after day 15 (stage 2), when a period corresponding to one HRT was completed. This inhibition period was marked by a very low biogas production, decreasing from initial values of about 196.57 mL· g VS^{-1} to only 25.20 mL· g VS^{-1} and corresponded to 55 days of continuous operation (until day 70) (**Fig. 3b**). This decline in organic matter conversion into biogas was associated with the accumulation of inhibitory factors and attributed to the elevated organic load rate (OLR) entering the reactor and the inhibition of methanogens due to the relative reduced HRT applied of 15 days.

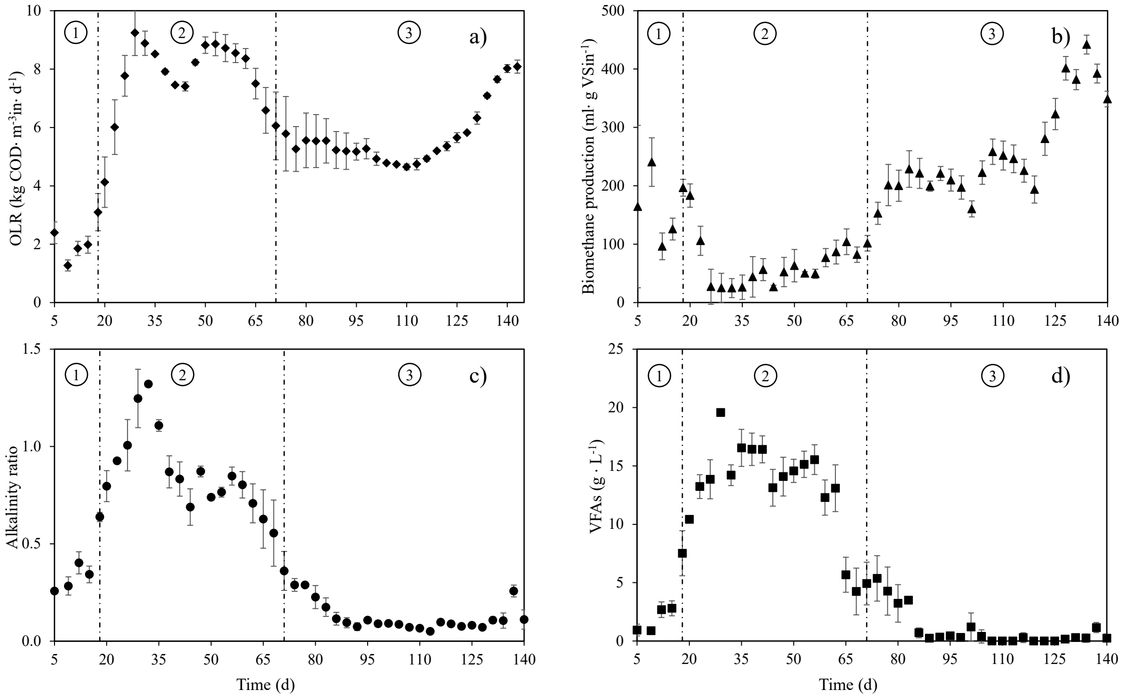


Fig. 3. Time course of the operational parameters during the experiment inside the anaerobic reactors: a) Organic loading rate (OLR); b) Biomethane production; c) Alkalinity ratio; d) Volatile fatty acids (VFAs)

From day 70 onwards (stage 3), biogas production experienced a significant increase which lasted until the end of the experiment with an average value of biogas production of 375 mL· g VS^{-1} during the second half of the stage (**Fig. 3b**). This increase in biogas production was concomitantly detected with a decrease in VFA concentration in the reactor and notably decrease of the alkaline ratio, suggesting that the systems have reached a steady state period after 5 HRTs. The biomethanization levels at the end of stage 3 were close to the values achieved in the BMP tests (410, *vs.* 435 mL· g VS^{-1}). At this point it must be highlighted that under the conditions of BMP essays no inhibitions are likely to occur due the optimum dosages of inoculum and substrate, suggesting that inhibition phenomena detected in CSTRs during stage 2 was overcome.

The inhibition of the methanogenic process was evidenced by the VFA accumulation and the considerable high values of the alkalinity ratio. In case of VFA, average concentration of the stage 2 was above the inhibition thresholds with average values of 14.6 ± 2.2 g L^{-1} of total VFA per litre. In this sense, concentration above 5 g· L^{-1} can potentially inhibit microbial activity in the anaerobic digestion process, and subsequently reducing the capacity to produce biogas [27]. VFAs, such as acetic acid, propionic acid, and butyric acid, are intermediate products of anaerobic fermentation of organic matter. At low concentrations, these VFAs are utilized by methanogenic bacteria to produce methane, the primary component of biogas. However, at elevated concentrations, VFAs can have a toxic effect on methanogenic bacteria, reducing their activity and resulting in decreased biogas production [28]. Yeole T. (1996) and Yuan H. (1999) stablished the VFA concentrations of 5-6 g· L^{-1} as inhibitory level using cattle dung and sewage sludge as substrate [29,30]. Alkalinity ratio is often used to monitor the digester stability [31]. Stage 2 was characterised by values above 0.4, and reaching values beyond 1, which are normally associated with inhibition processes and the insufficient capacity to neutralize or remove organic acids and maintain an appropriate pH for microbial activity. A high alkalinity ratio, above 0.3, may indicate an excess of alkalinity, signifying an imbalance of the anaerobic digestion. In the same manner that VFA concentration, the alkalinity ratio showed a remarkable decrease along stage 3, evidencing the increase of the methanogenic activity.

Animal waste products, such as swine manure, frequently contain exceptionally high concentrations of total ammoniacal nitrogen due to the presence of ammonia, as well as proteins and urea that readily release ammonia after anaerobic treatment [32,33]. While sudden increases in ammonia concentration in the feedstock are uncommon [34], stored feed slurries, like the substrate under study, often contain elevated levels of ammonia released during the organic nitrogen decomposition process. Ammoniacal nitrogen together with the VFA are main inhibitors found during swine manure treatment in digesters.

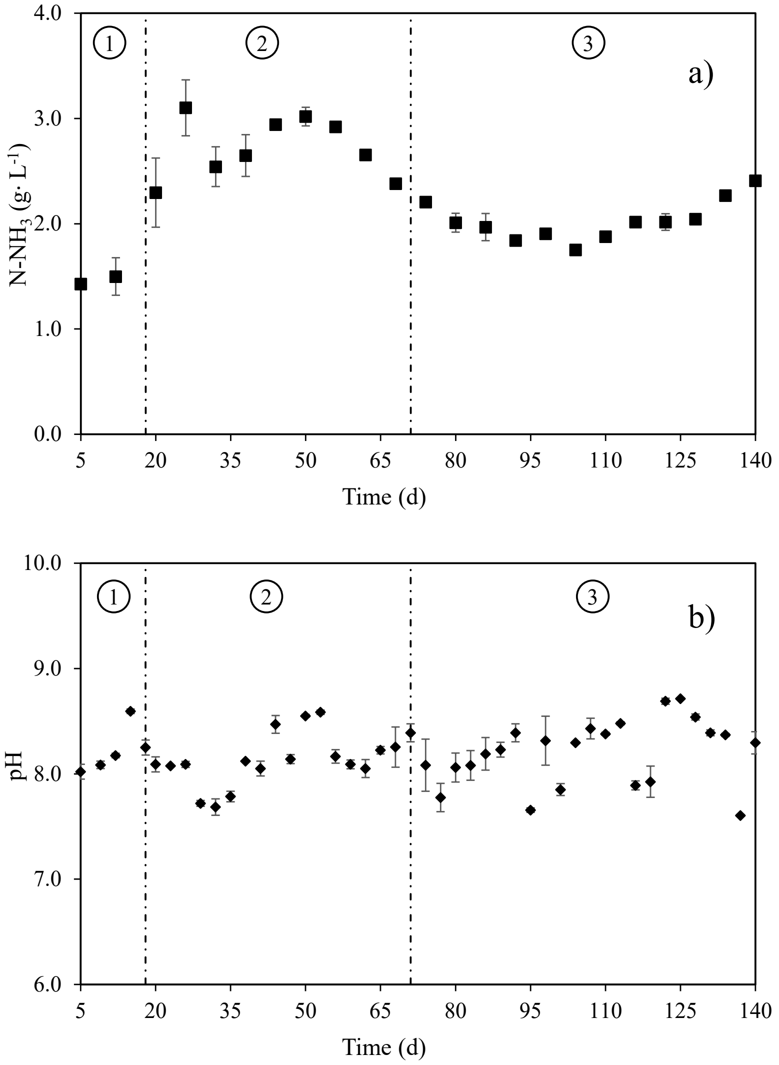


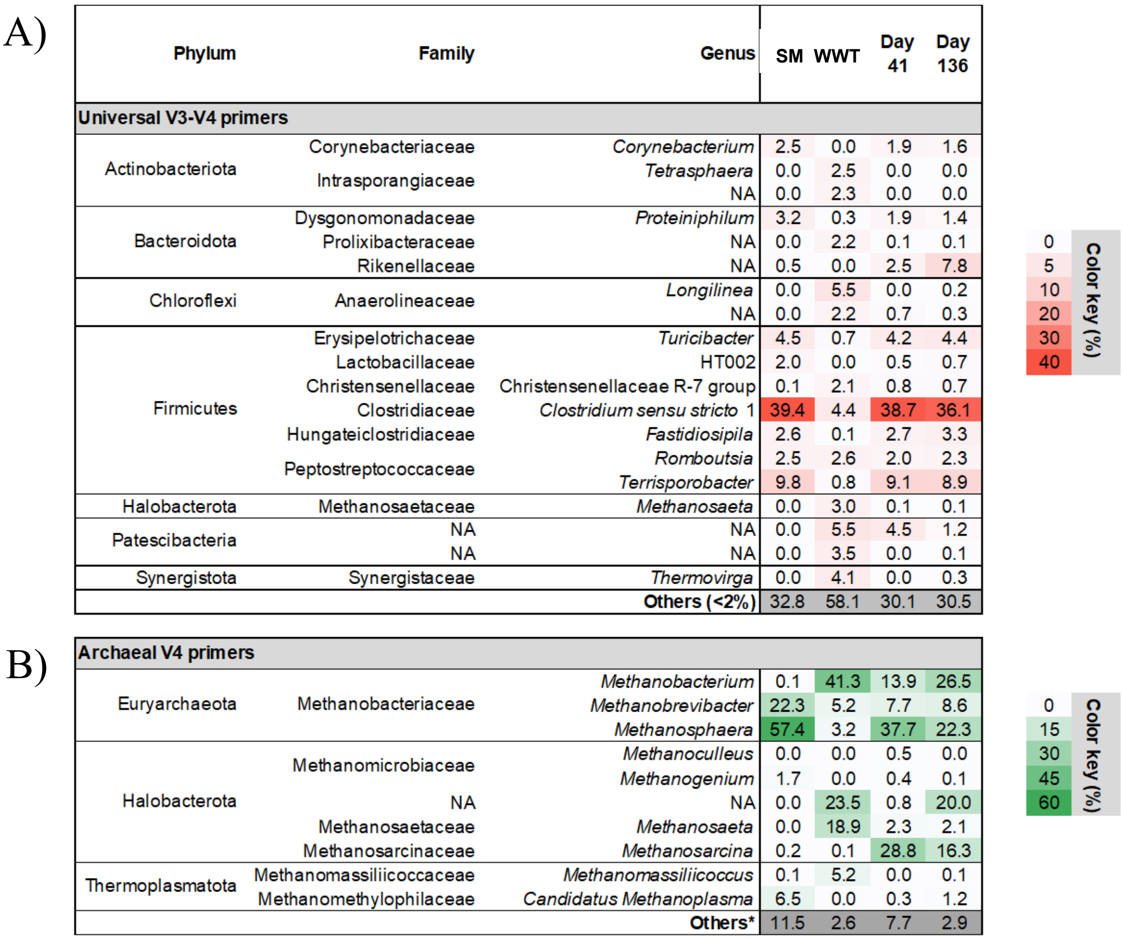
Fig. 4. Time course of the operational parameters during the experiment inside the anaerobic reactors: a) N-NH_3; b) pH;

Fig. 4 illustrates the evolution in total ammonia nitrogen (TAN) and pH in the CSTRs over the course of the experiment. In stage 2, the TAN concentration averaged in 2.72 ± 0.29 g· L^{-1}, while slightly lower concentrations were detected in stage 3, 2.03 ± 0.19 g· L^{-1}. These conditions were detected together with mild basic conditions, which increases the free ammonia form. Stable pH values of 8.21 ± 0.3 and 8.14 ± 0.25 were measured in stages 2 and 3, respectively. Ammonia accumulation often triggers process instability through inhibition of the methanogenesis, leading to the accumulation of VFAs, which subsequently causes a decrease in pH and reduces the free ammonia concentration. The interplay between free ammonia, VFAs, and pH can result in what is termed an "inhibited steady state" - a situation where the process operates steadily but with a reduced methane yield [35,36] and corresponding to the conditions described in stage 2. Nevertheless, microbial acclimatization to these conditions can lead to a resume of the biomethane production. Such adaptation may arise from internal changes within the dominant fermentative or methanogenic species or shifts in the population [37]. In this experiment, the adaptation period seemed to occur within a duration spanning between 4 to 5 HRT. Among the four types of anaerobic microorganisms involved in the anaerobic digestion process, methanogens are the least resilient and are prone to halting their growth in response to ammonia inhibition [38]. Numerous studies have examined this issue, suggesting that a concentration of 4 g NH_3-N· L^{-1} is already sufficient to affect certain methanogenic microorganisms. Once acclimated, microorganisms can maintain their viability even at concentrations well above the initially inhibitory levels. Koster & Koomen, (1988) reported that non-acclimated methanogens failed to produce methane at ammonia concentrations of 1.9-2 g NH_3-N· L^{-1}, but they did resume methane production at a concentration of 11 g NH_3-N· L^{-1} after the adaptation process [39]. Experiments have unequivocally demonstrated the feasibility of achieving stable manure digestion at ammonia concentrations exceeding 5 g NH_3-N· L^{-1} following an initial adaptation period.

3.3 Microbial communities in CSTR

Microbial community analysis based on universal primers (V3-V4) revealed a significant difference in the composition of microbial lineages between swine manure and the WWT used as inoculum (**Supplementary Information**, **Fig. S1A** and **Fig. 5)**. While the swine manure was mainly composed of three phyla: Firmicutes, Bacteroidota, and Actinobacteriota, accounting for approximately 82, 8 and 6 % of the microbial community, respectively, the WWT inoculum displayed a broader microbial diversity. Firmicutes and Actinobacteriota were the dominant groups each comprising approximately 17 % of the microbial community, followed by Bacteroidota, Patescibacteria and Chloroflexi, each representing 12 to 13 % of the community found in the WWT sample. Regarding archaeal members of the communities, only members of the Haloarchaeota phylum were detected by the V3-V4 primers in the inoculum, making up 3.7% of the microbial community. However, no archaea were detected in the swine manure by these primers (**Fig. S1A**). Conversely, when archaea-targeted V4 primers were employed, Euryarchaeota were predominant in the swine manure (80 %), while the WWT sample was also dominated by Euryarchaeota (50 %) followed by Halobacterota, comprising 42 % of the archaeal community (**Fig. S1B**).

Interestingly, bacterial groups originally found in the swine manure showed stability and resilience throughout the anaerobic digestion process, whereas those originally found in the anaerobic sludge tended to diminish over time (**Fig. 5A**).



Phylum	Family	Genus	SM	WWT	Day 41	Day 136
Universal V3-V4 primers						
Actinobacteriota	Corynebacteriaceae	*Corynebacterium*	2.5	0.0	1.9	1.6
	Intrasporangiaceae	*Tetrasphaera*	0.0	2.5	0.0	0.0
		NA	0.0	2.3	0.0	0.0
Bacteroidota	Dysgonomonadaceae	*Proteiniphilum*	3.2	0.3	1.9	1.4
	Prolixibacteraceae	NA	0.0	2.2	0.1	0.1
	Rikenellaceae	NA	0.5	0.0	2.5	7.8
Chloroflexi	Anaerolineaceae	*Longilinea*	0.0	5.5	0.0	0.2
		NA	0.0	2.2	0.7	0.3
Firmicutes	Erysipelotrichaceae	*Turicibacter*	4.5	0.7	4.2	4.4
	Lactobacillaceae	HT002	2.0	0.0	0.5	0.7
	Christensenellaceae	Christensenellaceae R-7 group	0.1	2.1	0.8	0.7
	Clostridiaceae	*Clostridium sensu stricto* 1	39.4	4.4	38.7	36.1
	Hungateiclostridiaceae	*Fastidiosipila*	2.6	0.1	2.7	3.3
	Peptostreptococcaceae	*Romboutsia*	2.5	2.6	2.0	2.3
		Terrisporobacter	9.8	0.8	9.1	8.9
Halobacterota	Methanosaetaceae	*Methanosaeta*	0.0	3.0	0.1	0.1
Patescibacteria	NA	NA	0.0	5.5	4.5	1.2
	NA	NA	0.0	3.5	0.0	0.1
Synergistota	Synergistaceae	*Thermovirga*	0.0	4.1	0.0	0.3
		Others (<2%)	32.8	58.1	30.1	30.5

Archaeal V4 primers						
Euryarchaeota	Methanobacteriaceae	*Methanobacterium*	0.1	41.3	13.9	26.5
		Methanobrevibacter	22.3	5.2	7.7	8.6
		Methanosphaera	57.4	3.2	37.7	22.3
Halobacterota	Methanomicrobiaceae	*Methanoculleus*	0.0	0.0	0.5	0.0
		Methanogenium	1.7	0.0	0.4	0.1
	NA	NA	0.0	23.5	0.8	20.0
	Methanosaetaceae	*Methanosaeta*	0.0	18.9	2.3	2.1
	Methanosarcinaceae	*Methanosarcina*	0.2	0.1	28.8	16.3
Thermoplasmatota	Methanomassiliicoccaceae	*Methanomassiliicoccus*	0.1	5.2	0.0	0.1
	Methanomethylophilaceae	*Candidatus Methanoplasma*	6.5	0.0	0.3	1.2
		Others*	11.5	2.6	7.7	2.9

Fig. 5. Heatmap displaying the microbial community analysis employing 16s-based universal (Panel A) and archaeal (Panel B) primers. For the V3-V4 universal primers, all taxa with relative abundances of less than 2% were summarized in the "others" group. *For the V4 archaeal primers, all taxa not belonging to the archaea kingdom were summarized in the "others" group. SM stands for swine manure and WWT stands for wastewater treatment plant inoculum. Day 41 and 136 columns correspond to the samples taken from CSTRs.

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For instance, members of the Peptostreptococcaceae and Clostridiaceae families (specifically *Terrisporobacter* and *Clostridium sensu stricto* 1 genera) from the Firmicutes demonstrated minimal fluctuations in their relative abundance maintaining levels of approximately 10 % and 40 %, respectively throughout the entirety of the anaerobic digestion process, that means in stages 2, 3 and swine manure (**Fig. 5A**).

Furthermore, an unidentified member of the Rikenellaceae family (Bacteroidota), increased its relative abundance from 0.5 in the swine manure, to 2.5, and 8 % in days 41 and 136 of the anaerobic digestion operation. These facts prove that the microorganisms originally found in the swine manure possess metabolic capabilities contributing to anaerobic digestion. Supporting this fact, several recent studies have detected *Terrisporobacter* and *Clostridium sensu stricto* 1 as key bacteria responsible of fermentation reactions during the digestion of swine manure [40–42]. Historically, members of the Firmicutes phyla have been recognized due to their fermentative metabolism which allows them to provide acetoclastic and methanogenic microbes with substrates for biogas production [43,44]. Furthermore, members of the Rikeneallaceae family have been reported as H_2 producing microorganisms and thus they could have contributed to hydrogenotrophic methanogenesis in the studied system [45,46].

In contrast, members of *Longilinea*, *Tetrasphaera*, unclassified *Intrasporangiaceae*, and *Thermovirga* genera, that were present in the WWT inoculum, significantly diminished their relative abundance by 9, 6, and 5 % respectively by the end of the anaerobic digestion process (**Fig. 6**). This suggests a less prominent role in the anaerobic digestion of the swine manure, even though members of these genera have been previously reported to play significant roles in digesters [47–49].

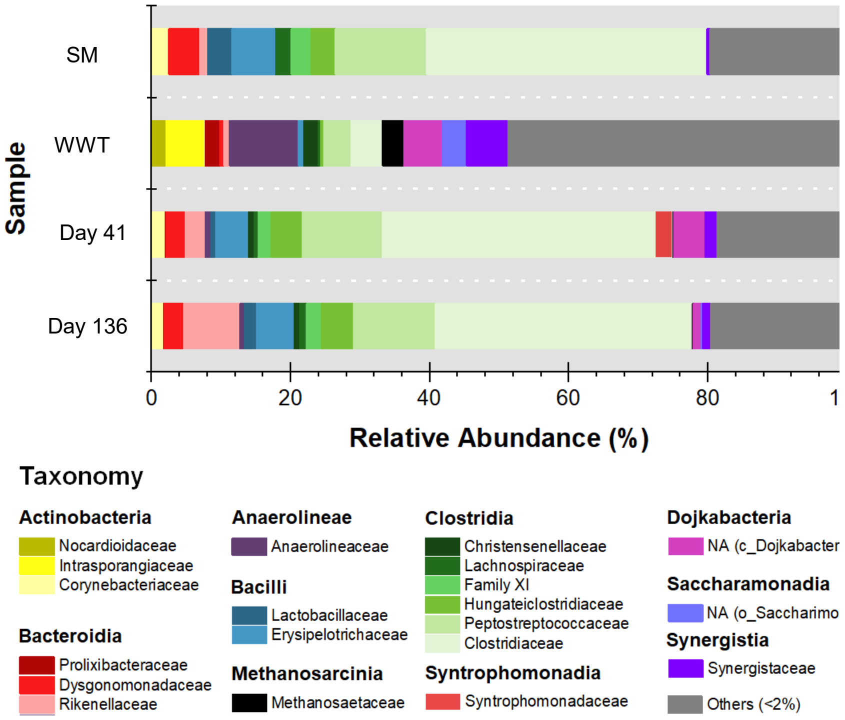

Fig. 6. 16s-based rRNA analysis showing the relative abundance of bacteria retrieved by V3-V4 universal primers from biomass and swine wastewater samples. Taxa not identified at the family level include the next identified taxonomic level in parenthesis. All taxa with relative abundances of less than 2% were summarized in the “others” group. *Abbreviations: o_: order, c_:class.

From the archaeal counterpart of the microbial communities, taxa originally identified in both, the swine manure and WWT sludge, showed prevalence during anaerobic digestion (**Fig. 7**). From the swine manure, members from the *Methanobrevibacter* and *Methanosphaera* genera reached relative abundances of 9 and 22 %, respectively, at stage 3 when biogas production reached its maximum value, albeit their dominance was considerable higher in the manure, 22 and 57 %, respectively. From the WWT sludge, members of *Methanobacterium*, and unclassified Halobacterota, comprised 27 and 20 % of the archaeal community in stage 3, respectively. However, the practical absence of the unclassified member of the Halobacterota phylum in the sample of the stage 2 (inhibition period) suggest that the inoculated organisms belonging to this group were probably inhibited by high concentrations of ammonia and/or VFA. The subsequent adaptation of these organisms probably conducted to the most favourable conditions detected during stage 3. The genus *Methanosaeta*, strongly present in the WWT inoculum (up to 19 %) were practically absent in the steady state conditions of stage 3 (2 %). Interestingly, the most drastic increase in relative abundance was observed for *Methanosarcina* (final abundance of 16 %) which was present at very low levels (<0.2 %) in both the swine manure and in the inoculum.

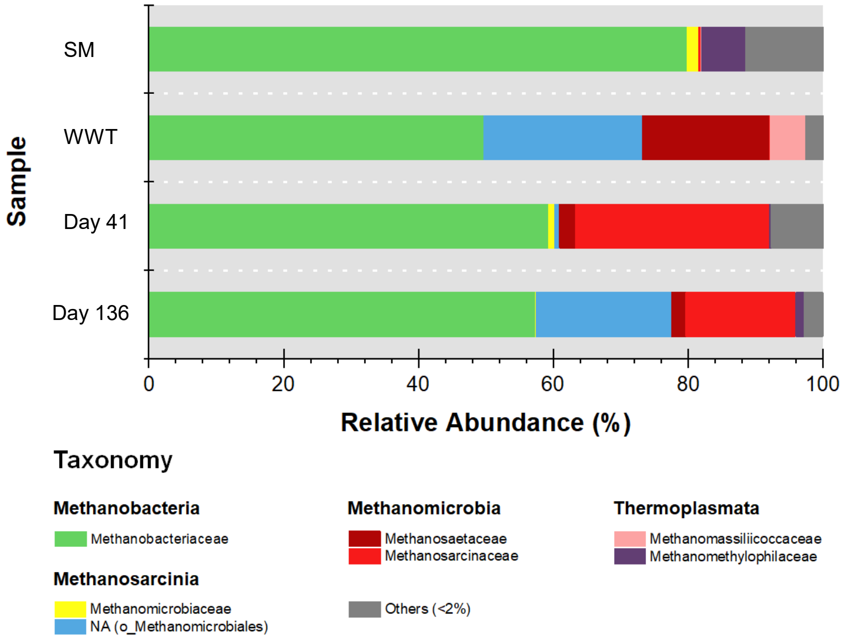

Fig. 7. 16s-based rRNA analysis showing the relative abundance of archaea retrieved by V4-V5 archaeal primers from biomass and swine wastewater samples. Taxa not identified at the family level include the next identified taxonomic level in parenthesis. All taxa with relative abundances of less than 2% were summarized in the "others" group. *Abbreviations: o_: order.

Overall, the microbial community analysis suggests that bacterial taxa with fermentative metabolism originally present in the swine manure do prevail during anaerobic digestion and provide to the hydrogenotrophic and acetoclastic methanogens from both the swine manure and the inoculum with substrates for biogas production. In case of archaeal, responsible of the methane production, the intermediate microbial population found in the steady state of the reactors, comprising organisms present in both manure and the WWT inoculum, suggested that inoculation could played a relative minor role. This observation is in agreement with the results found in the BMP tests, where uninoculated tests showed similar levels or even higher levels of biomethane production.

4. Conclusions

In summary, biomethane production in continuous mode showed a strong inhibition due to high ammonia and VFA concentrations that was overcome after more than 70 days of operation. These trends indicate changing reactor conditions over time and underscore the importance of careful monitoring and control of system parameters (chemical and biological) to optimize biogas production in anaerobic digestion applications. Furthermore, the 16s analyses performed revealed that clades of bacteria and archaea intrinsic to the swine manure display resilience and might play key roles during the anaerobic digestion process presumably due to acclimation to the substrates found in the swine manure. This suggests that microbial population find in this substrate might be sufficient for the quick start-up of anaerobic digestion without the need of the addition of external sources of microorganisms (i.e., anaerobic sludge).

Acknowledgements

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5. References

[1] A. Nemmour, A. Inayat, I. Janajreh, C. Ghenai, Green hydrogen-based E-fuels (E-methane, E-methanol, E-ammonia) to support clean energy transition: A literature review, Int J

Hydrogen Energy. 48 (2023) 29011–29033. https://doi.org/https://doi.org/10.1016/j.ijhydene.2023.03.240.

[2] L. Deng, H.H. Ngo, W. Guo, S.W. Chang, D.D. Nguyen, A. Pandey, S. Varjani, N.B. Hoang, Recent advances in circular bioeconomy based clean technologies for sustainable environment, Journal of Water Process Engineering. 46 (2022) 102534. https://doi.org/https://doi.org/10.1016/j.jwpe.2021.102534.

[3] P.G. Kougias, I. Angelidaki, Biogas and its opportunities—A review, Front Environ Sci Eng. 12 (2018) 14. https://doi.org/10.1007/s11783-018-1037-8.

[4] Food and Agriculture Organization of the United Nations, OECD Agriculture Statistics, 2022. https://doi.org/https://doi.org/10.1787/13d66b76-en (accessed June 1, 2023).

[5] C.R. Palomar, A. García-Alvaro, V. de Almeida Guimarães, E.B. Hedo, R. Muñoz, I. de Godos Crespo, Biofuels in Low Carbon Economies and Societies, in: S.A. Bandh, F.A. Malla (Eds.), Biofuels in Circular Economy, Springer Nature Singapore, Singapore, 2022: pp. 31–58. https://doi.org/10.1007/978-981-19-5837-3_3.

[6] A.G. Álvaro, C.R. Palomar, R.M. Torre, D.H. Redondo, I. de Godos Crespo, Hybridization of anaerobic digestion with solar energy: A solution for isolated livestock farms, Energy Conversion and Management: X. (2023) 100488. https://doi.org/https://doi.org/10.1016/j.ecmx.2023.100488.

[7] X. Flotats, Gestión de purines y emisión de gases contaminantes, 193 (2022) 8–14.

[8] European Biogas Association, EBA Statistical report 2022, 2022.

[9] F. Haque, C. Fan, Y.Y. Lee, From waste to value: Addressing the relevance of waste recovery to agricultural sector in line with circular economy, Journal of Cleaner Production. 415 (2023) 137873. https://doi.org/10.1016/j.jclepro.2023.137873.

[10] X. Meng, D. Yu, Y. Wei, Y. Zhang, Q. Zhang, Z. Wang, J. Liu, Y. Wang, Endogenous ternary pH buffer system with ammonia-carbonates-VFAs in high solid anaerobic digestion of swine manure: An alternative for alleviating ammonia inhibition?, Process Biochemistry. 69 (2018) 144–152. https://doi.org/10.1016/J.PROCBIO.2018.03.015.

[11] Y. Xiao, H. Yang, D. Zheng, Y. Liu, L. Deng, Alleviation of ammonia inhibition in dry anaerobic digestion of swine manure, Energy. 253 (2022) 124149. https://doi.org/10.1016/J.ENERGY.2022.124149.

[12] Y. Jiang, E. McAdam, Y. Zhang, S. Heaven, C. Banks, P. Longhurst, Ammonia inhibition and toxicity in anaerobic digestion: A critical review, Journal of Water Process Engineering. 32 (2019) 100899. https://doi.org/https://doi.org/10.1016/j.jwpe.2019.100899.

[13] X. Li, J. Tong, M. Yuan, M. Song, J. Gao, J. Zhu, Y. Liu, Demonstrating the application of batch anaerobic digestion recirculating slurry inoculation of food waste engineering from a microbiological perspective, Renew Energy. (2023) 119216. https://doi.org/https://doi.org/10.1016/j.renene.2023.119216.

[14] R. Azarmanesh, M. Zarghami Qaretapeh, M. Hasani Zonoozi, H. Ghiasinejad, Y. Zhang, Anaerobic co-digestion of sewage sludge with other organic wastes: A comprehensive review focusing on selection criteria, operational conditions, and microbiology, Chemical Engineering Journal Advances. 14 (2023) 100453. https://doi.org/https://doi.org/10.1016/j.ceja.2023.100453.

[15] M.C. Nelson, M. Morrison, Z. Yu, A meta-analysis of the microbial diversity observed in anaerobic digesters, Bioresour Technol. 102 (2011) 3730–3739. https://doi.org/https://doi.org/10.1016/j.biortech.2010.11.119.

[16] Y. Chen, J.J. Cheng, K.S. Creamer, Inhibition of anaerobic digestion process: A review, Bioresour Technol. 99 (2008) 4044–4064. https://doi.org/https://doi.org/10.1016/j.biortech.2007.01.057.

[17] J. Palatsi, M. Viñas, M. Guivernau, B. Fernandez, X. Flotats, Anaerobic digestion of slaughterhouse waste: Main process limitations and microbial community interactions, Bioresour Technol. 102 (2011) 2219–2227. https://doi.org/https://doi.org/10.1016/j.biortech.2010.09.121.

[18] H.B. Nielsen, I. Angelidaki, Strategies for optimizing recovery of the biogas process following ammonia inhibition, Bioresour Technol. 99 (2008) 7995–8001. https://doi.org/https://doi.org/10.1016/j.biortech.2008.03.049.

[19] V. Kavan Kumar, R. Mahendiran, P. Subramanian, S. Karthikeyan, A. Surendrakumar, Optimization of inoculum to substrate ratio for enhanced methane yield from leather fleshings in a batch study, Journal of the Indian Chemical Society. 99 (2022) 100384. https://doi.org/https://doi.org/10.1016/j.jics.2022.100384.

[20] P. Ghofrani-Isfahani, H. Baniamerian, P. Tsapekos, M. Alvarado-Morales, T. Kasama, M. Shahrokhi, M. Vossoughi, I. Angelidaki, Effect of metal oxide based TiO2 nanoparticles on anaerobic digestion process of lignocellulosic substrate, Energy. 191 (2020) 116580. https://doi.org/10.1016/J.ENERGY.2019.116580.

[21] J.A. Magdalena, C. González Fernández, Anaerobic digestion of microalgae biomass for volatile fatty acids production, 2019.

[22] A.W. APHA, Standard Methods for the Examination of Water & Wastewater, 22nd Edition. (2012).

[23] A.G. Alvaro, C.R. Palomar, D.H. Redondo, R.M. Torre, I. de Godos Crespo, Simultaneous production of biogas and volatile fatty acids through anaerobic digestion using cereal straw as substrate, Environ Technol Innov. (2023) 103215. https://doi.org/10.1016/J.ETI.2023.103215.

[24] A. Klindworth, E. Pruesse, T. Schweer, J. Peplies, C. Quast, M. Horn, F.O. Glöckner, Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies, Nucleic Acids Res. 41 (2013) e1–e1. https://doi.org/10.1093/nar/gks808.

[25] M.R. Pausan, C. Csorba, G. Singer, H. Till, V. Schöpf, E. Santigli, B. Klug, C. Högenauer, M. Blohs, C. Moissl-Eichinger, Exploring the Archaeome: Detection of Archaeal Signatures in the Human Body, Front Microbiol. 10 (2019). https://doi.org/10.3389/fmicb.2019.02796.

[26] B.J. Callahan, P.J. McMurdie, M.J. Rosen, A.W. Han, A.J.A. Johnson, S.P. Holmes, DADA2: High-resolution sample inference from Illumina amplicon data, Nat Methods. 13 (2016) 581–583. https://doi.org/10.1038/nmeth.3869.

[27] F. Hu, S. Zhang, S. Liu, L. Wan, G. Gong, T. Hu, X. Wang, L. Xu, G. Xu, Y. Hu, Alleviating acid inhibition via bentonite supplementation during acidulated swine manure anaerobic digestion: Performance enhancement and microbial mechanism analysis, Chemosphere. 313 (2023) 137577. https://doi.org/https://doi.org/10.1016/j.chemosphere.2022.137577.

[28] Y. Wang, Y. Zhang, J. Wang, L. Meng, Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria, Biomass Bioenergy. 33 (2009) 848–853. https://doi.org/https://doi.org/10.1016/j.biombioe.2009.01.007.

[29] T.Y. Yeole, S. Gokhale, S.R. Hajarnis, D.R. Ranade, Effect of brackish water on biogas production from cattle dung and methanogens, Bioresour Technol. 58 (1996) 323–325. https://doi.org/https://doi.org/10.1016/S0960-8524(96)00119-8.

[30] H. Yuan, N. Zhu, Progress in inhibition mechanisms and process control of intermediates and by-products in sewage sludge anaerobic digestion, Renewable and Sustainable Energy Reviews. 58 (2016) 429–438. https://doi.org/https://doi.org/10.1016/j.rser.2015.12.261.

[31] L. Martín-González, X. Font, T. Vicent, Alkalinity ratios to identify process imbalances in anaerobic digesters treating source-sorted organic fraction of municipal wastes, Biochem Eng J. 76 (2013) 1–5. https://doi.org/https://doi.org/10.1016/j.bej.2013.03.016.

[32] J. Zhang, C. Buhe, D. Yu, H. Zhong, Y. Wei, Ammonia stress reduces antibiotic efflux but enriches horizontal gene transfer of antibiotic resistance genes in anaerobic digestion, Bioresour Technol. 295 (2020) 122191. https://doi.org/https://doi.org/10.1016/j.biortech.2019.122191.

[33] T. Müller, B. Walter, A. Wirtz, A. Burkovski, Ammonium Toxicity in Bacteria, Curr Microbiol. 52 (2006) 400–406. https://doi.org/10.1007/s00284-005-0370-x.

[34] H. V Hendriksen, B.K. Ahring, Effects of ammonia on growth and morphology of thermophilic hydrogen-oxidizing methanogenic bacteria, FEMS Microbiol Ecol. 8 (1991) 241–245. https://doi.org/10.1111/j.1574-6941.1991.tb01729.x.

[35] C. Liu, Y. Chen, H. Huang, X. Duan, L. Dong, Improved anaerobic digestion under ammonia stress by regulating microbiome and enzyme to enhance VFAs bioconversion: The new role of glutathione, Chemical Engineering Journal. 433 (2022) 134562. https://doi.org/https://doi.org/10.1016/j.cej.2022.134562.

[36] I. Angelidaki, B.K. Ahring, Thermophilic anaerobic digestion of livestock waste: the effect of ammonia, Appl Microbiol Biotechnol. 38 (1993) 560–564. https://doi.org/10.1007/BF00242955.

[37] G. Zeeman, W.M. Wiegant, M.E. Koster-Treffers, G. Lettinga, The influence of the total-ammonia concentration on the thermophilic digestion of cow manure, Agricultural Wastes. 14 (1985) 19–35. https://doi.org/https://doi.org/10.1016/S0141-4607(85)80014-7.

[38] M. Kayhanian, Performance of a high-solids anaerobic digestion process under various ammonia concentrations, Journal of Chemical Technology & Biotechnology. 59 (1994) 349–352. https://doi.org/https://doi.org/10.1002/jctb.280590406.

[39] I.W. Koster, E. Koomen, Ammonia inhibition of the maximum growth rate (µm) of hydrogenotrophic methanogens at various pH-levels and temperatures, Appl Microbiol Biotechnol. 28 (1988) 500–505. https://doi.org/10.1007/BF00268222.

[40] Y. Xiao, H. Yang, D. Zheng, Y. Liu, C. Zhao, L. Deng, Granular activated carbon alleviates the combined stress of ammonia and adverse temperature conditions during dry anaerobic digestion of swine manure, Renew Energy. 169 (2021) 451–460. https://doi.org/https://doi.org/10.1016/j.renene.2021.01.021.

[41] H. Chen, J. Yuan, Q. Xu, E. Yang, T. Yang, L. Shi, Z. Liu, H. Yu, J. Cao, Q. Zhou, J. Chen, Swine wastewater treatment using combined up-flow anaerobic sludge blanket and anaerobic membrane bioreactor: Performance and microbial community diversity, Bioresour Technol. 373 (2023) 128606. https://doi.org/https://doi.org/10.1016/j.biortech.2023.128606.

[42] B. Yang, C. Wang, X. Zhao, J. Liu, F. Yin, C. Liang, K. Wu, J. Liu, H. Yang, W. Zhang, Effects of environmental factors on low temperature anaerobic digestion of pig manure, Environ Res Commun. 4 (2022) 125006. https://doi.org/10.1088/2515-7620/aca647.

[43] H.D. Ariesyady, T. Ito, S. Okabe, Functional bacterial and archaeal community structures of major trophic groups in a full-scale anaerobic sludge digester, Water Res. 41 (2007) 1554–1568. https://doi.org/https://doi.org/10.1016/j.watres.2006.12.036.

[44] C. Sundberg, W.A. Al-Soud, M. Larsson, E. Alm, S.S. Yekta, B.H. Svensson, S.J. Sørensen, A. Karlsson, 454 pyrosequencing analyses of bacterial and archaeal richness in 21 full-scale biogas digesters, FEMS Microbiol Ecol. 85 (2013) 612–626. https://doi.org/10.1111/1574-6941.12148.

[45] X.-L. Su, Q. Tian, J. Zhang, X.-Z. Yuan, X.-S. Shi, R.-B. Guo, Y.-L. Qiu, Acetobacteroides hydrogenigenes gen. nov., sp. nov., an anaerobic hydrogen-producing bacterium in the family Rikenellaceae isolated from a reed swamp, Int J Syst Evol Microbiol. 64 (2014) 2986–2991. https://doi.org/https://doi.org/10.1099/ijs.0.063917-0.

[46] M.-T. Li, L. Rao, L. Wang, M. Gou, Z.-Y. Sun, Z.-Y. Xia, W.-F. Song, Y.-Q. Tang, Bioaugmentation with syntrophic volatile fatty acids-oxidizing consortia to alleviate the ammonia inhibition in continuously anaerobic digestion of municipal sludge, Chemosphere. 288 (2022) 132389. https://doi.org/https://doi.org/10.1016/j.chemosphere.2021.132389.

[47] S. Wang, X. Hou, H. Su, Exploration of the relationship between biogas production and microbial community under high salinity conditions, Sci Rep. 7 (2017) 1149. https://doi.org/10.1038/s41598-017-01298-y.

[48] L. Zhang, Q. Ban, J. Li, Microbial community dynamics at high organic loading rates revealed by pyrosequencing during sugar refinery wastewater treatment in a UASB reactor, Front Environ Sci Eng. 12 (2018) 4. https://doi.org/10.1007/s11783-018-1045-8.

[49] J. Wang, C. Zhang, P. Li, H. Xu, W. Wang, W. Yin, J. Wu, Z. Hu, Bioaugmentation with Tetrasphaera to improve biological phosphorus removal from anaerobic digestate of swine wastewater, Bioresour Technol. 373 (2023) 128744. https://doi.org/https://doi.org/10.1016/j.biortech.2023.128744.

Supplementary material

Microbial analysis of anaerobic digester reveals prevalence of manure microbiota

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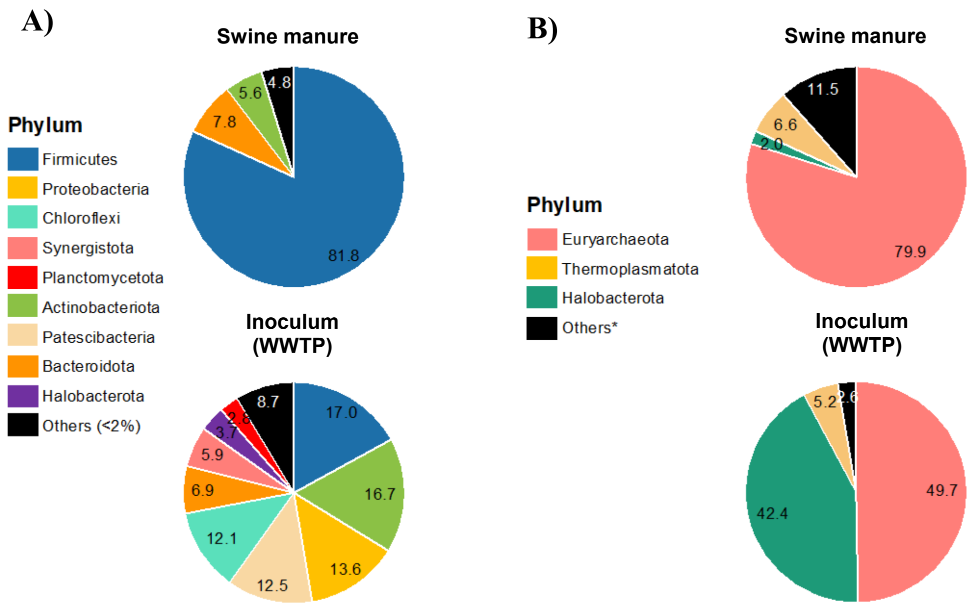
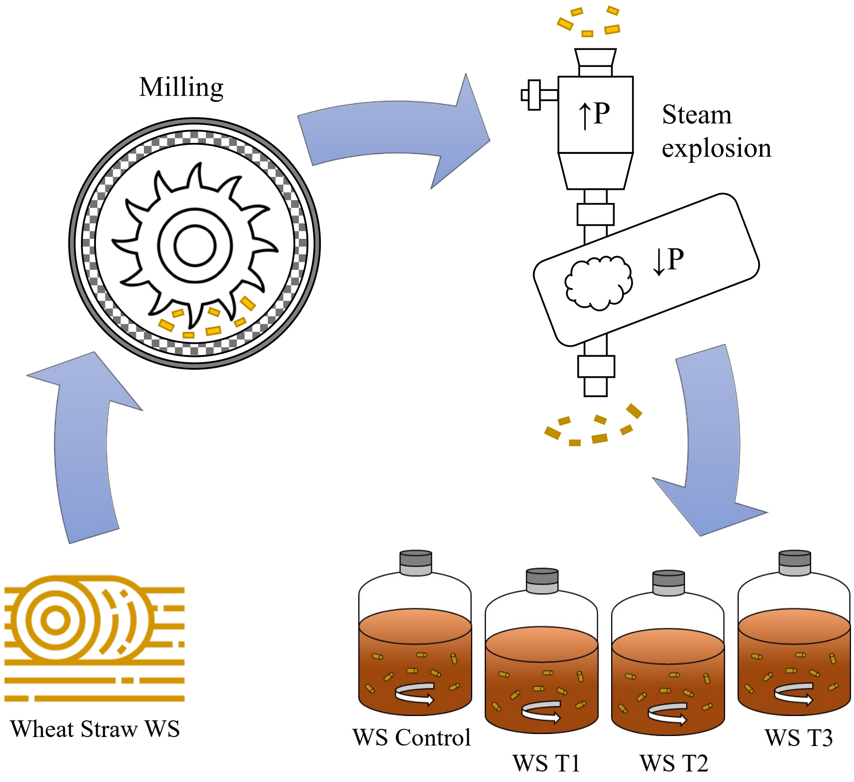


Fig. S1. Pie plot displaying the microbial community analysis based on 16s-based universal (Panel A) and archaeal (Panel B) primers at the phylum level. For the V3-V4 universal primers, all taxa with relative abundances of less than 2% were summarized in the “others” group. *For the V4 archaeal primers, all taxa not belonging to the archaea kingdom were summarized in the “others” group.

Chapter 7

Improving biogas yield from wheat straw with thermal pretreatment. Energy integration study



This chapter is based on the article:

Alfonso García Álvaro, César Ruiz Palomar, Israel Díaz Villalobos, Daphne Hermosilla Redondo, Raúl Muñoz Torre, Ignacio de Godos Crespo (2023). Improving biogas yield from wheat straw with thermal pretreatment. Energy integration study. *Energies.* (In review)

Improving biogas yield from wheat straw with thermal pretreatment. Energy integration study

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Abstract

Cereal straw stands out as one of the most abundant and globally distributed agricultural residues. Traditional applications cope with a limited amount of the production, leaving the remainder in the field for its natural decomposition. Managing cereal straw through controlled biological transformation under anaerobic conditions holds the potential to generate added value in the form of bioenergy. However, the lignocellulosic composition of these substrates poses challenges for organic degradation, often requiring energy-intensive pretreatments. Detailed research with a comprehensive calculation of the overall energy balance of the integrated process, aiming to provide real added value and replicability to such studies. Three scenarios for wheat straw transformation were investigated, incorporating two preliminary pre-treatment stages—mechanical milling and physicochemical steam explosion—under varying conditions based on experimental data. The subsequent energy integration analysis reveals that the process can be optimized by up to 15% in the final energy balance.

Keywords: Anaerobic digestion, Biogas, Cereal straw, Energy integration, Thermal pretreatment, Waste to energy

1. Introduction

There is a growing interest in the use of agricultural residues as an alternative to fossil fuels in energy production. There are several reasons for this trend. First, agricultural residues, such as crop residues, straw and manure, are abundant and renewable, making them a sustainable source of energy [1]. In addition, their use reduces dependence on fossil fuels and helps mitigate greenhouse gas emissions [2]. Likewise, the use of agricultural residues for energy generation can provide economic benefits to agricultural communities by generating additional income and promoting the diversification of their activities [3]. Anaerobic digestion (AD) is introduced as a biological process that yields high-calorific-value biogas, primarily composed of biomethane [4]. This biogas serves as a substitute for traditional natural gas, thereby promoting a more environmentally friendly balance in greenhouse gas emissions during its use [5]. Along with the aspects mentioned above, the growing interest in the use of agricultural residues to replace fossil fuels in energy production has also been backed by legal and political support.

Many countries and regions have implemented policies and regulations that encourage the use of renewable energy sources, including agricultural residues [6]. This is reflected in financial incentives, subsidies and feed-in tariff schemes that promote power generation from agricultural biomass. Legal and political support must play a crucial role in promoting the use of agricultural residues as a viable and sustainable alternative to fossil fuels in energy production [7,8]. Several countries around the world have implemented regulations and policies to encourage the use of agricultural residues in energy production e.g. Germany, United States, Brazil or China where there are regulations and incentive programs to encourage power generation from agricultural biomass in order to reduce its dependence on fossil fuels and mitigate air pollution [9]. Spain has regulations and provides support for the advancement of technologies that transform agricultural waste into energy [10]. The Electricity Sector Law 24/2013 establishes the legal foundation for promoting renewable sources, including agricultural biomass. Economic incentives like feed-in tariffs and premiums are available, as well as financing programs and subsidies for renewable energy initiatives [11]. Furthermore, both national and European-funded research and development programs are in place to bolster research and the adoption of innovative technologies in agricultural waste management for energy production [12].

Within the agricultural residues produced, there exists a category characterized by its substantial lignocellulosic content, which, owing to its limited degradability, presents a significant hurdle in the biogas production process [13]. These wastes primarily consist of three distinct polymers: cellulose, hemicellulose, and lignin. Cellulose, which is characterized by its rigid crystalline structure, makes up a significant percentage. Hemicellulose, on the other hand, has a lower molecular weight and short side chains, making it a polymer that can be easily hydrolyzed. Lignin, a complex and amorphous heteropolymer, comprises three different phenylpropane units and is insoluble in water [14]. Wheat straw is principally composed of cellulose (30-45 %), hemicellulose (20-35 %), and lignin (7-22 %) [15–17]. Both the cellulose and hemicellulose fractions can be broken down into monomeric sugars through hydrolysis. Anaerobic digestion is presented as an alternative to convert these wastes into biogas, but their low biodegradability blocks this process [18]. Composting, enzymatic hydrolysis and pretreatments are key techniques to improve biodegradability and increase biogas production [19]. Overcoming microbial inhibition and optimizing mass balances are crucial challenges to obtain optimal performance in biogas production from lignocellulosic wastes.

Thermal pretreatment is an effective strategy to improve the degradability of lignocellulosic wastes [20]. It consists of subjecting the waste to high temperatures, typically above 150 ˚C, for a given time [21]. This process breaks down the lignocellulose structure, increasing the accessibility of enzymes and facilitating the release of the components of interest. Thermal pretreatment can be performed by different methods, such as steam explosion, pyrolysis and or in combination with chemical processes such as a thermal-acid treatment [22], being the most widely employed pretreatment method for lignocellulosic biomass due to its cost-effectiveness and efficiency [23]. These methods modify the chemical composition and physical properties of the residues, making them more susceptible to enzyme action during the fermentation stage [24]. As a result, a higher conversion of waste to biogas is achieved, thus improving the efficiency and profitability of the process of producing renewable energy from lignocellulosic waste [25]. **Table 1** shows the main results found in works where steam explosion pretreatment of lignocellulosic materials has been studied to improve biogas production. This process involves exposing the material to high temperatures by directly injecting saturated steam and subsequently, the material undergoes rapid depressurization, which generates shear forces capable of separating the fibres. However, it is important to note that while steam explosion consumes 70 % less energy compared to mechanical treatments, it has some drawbacks. [26] These include the partial destruction of xylans from hemicelluloses, incomplete breakdown of the lignin-carbohydrate matrix, and the production of fermentation inhibitors [27].

Table 1. Summary of the anaerobic digestion effect of steam explosion pretreatment on AD of lignocellulosic biomass

Substrate	Pretreatment conditions	Anaerobic Digestion effect	Reference
Wheat Straw (WS)	175, 5 min	26.6 % increase methane production	
WS	175, 30 min	62.9 % increase methane production	[28]
WS	178, 30min	85.7 % increase methane production (day 10)	[29]
WS	170, 5 min	40.5 % increase methane yield	
WS	170, 15 min	59.4 % increase methane yield	[30]
WS	160, 10 min	13.9 % increase methane production	
WS	180, 10 min	12.8 % increase methane production	
WS	180, 15 min	20.0 % increase methane production	[31]
WS	160, 60 min	42.1 % increase methane production	
WS	180, 60 min	57.3 % increase methane production	[32]
Spent grain	170, 30 min	40.2 % increase methane production	[33]
Corn stover	180, 15 min	12.7 % increase methane production	[34]
Rice straw	174, 30 min	6.7 % increase methane yield	[35]

On that path, the economic viability of applying thermal pretreatment depends on several factors. The cost of pretreatment, which includes energy consumption and necessary equipment, must be weighed against the benefits obtained. These benefits may include a significant increase in biogas production and an improvement in the efficiency of the anaerobic fermentation process. In addition, the economic value of the substrate used without pretreatment, such as its potential use as fodder or direct sale, must be considered. Other factors to consider are the availability of local resources, government incentives or subsidies, and energy market conditions [36]. In general, an economic analysis, based on detailed mass and energy balances, is required to assess the specific feasibility of applying thermal pretreatment to wheat straw in a particular context. The current study explores the utilization of wheat straw, as a widely available lignocellulosic by-product [37], as a substrate for energy generation via anaerobic digestion, which includes a steam water explosion pretreatment conducted under varying conditions. Batch experiments were established to evaluate the potential benefits following the wheat straw pretreatment. The production rate of biogas was assessed and modelled, followed by an energy assessment of the entire process to determine its feasibility on a full-scale basis.

2. Materials and Methods

2.1 Feedstocks and anaerobic inoculum

Agricultural residues, specifically wheat straw, were collected in the Soria province of Spain following the harvest season and employed as a substrate. To prepare the substrate, a physical pretreatment process was performed using a Moulinex grinder, resulting in particle sizes of 1 mm [38]. Inoculum for the anaerobic reactor, operated under mesophilic conditions, was sourced from the anaerobic digester of sewage sludge of the wastewater treatment (WWT) plant in Soria, Spain. The inoculum used required an adaptation period of four weeks under continuous conditions feeding with wheat straw in a 1.2-liter reactor at 35 ± 0.5 ˚C degrees and magnetic stirring. **Table 2** offers detailed characteristics of both the substrates and the inoculum.

Table 2. Substrate and inoculum characterization.

Parameter	Units	WWT inoculum	Wheat straw
Total Solids	$g \cdot kg^{-1}$	14.9 ± 0.1	928.2 ± 7.3
Volatile Solids	$g \cdot kg^{-1}$	10.2 ± 0.2	851.6 ± 15.9
COD	$g \cdot L^{-1}$	19.2 ± 0.4	1422.4 ± 15.5
Cellulose	%	-	33.9 ± 0.4
Hemicellulose	%	-	23.8 ± 0.2
Lignin	%	-	22.3 ± 0.1

2.2 Thermal hydrolysis pretreatment

The laboratory-scale hydrolysis system consists of a 2 L reactor that receives the wheat straw (WS) substrate and is heated using steam until the desired temperature is attained. Additionally, there is a 5 L flash tank where the steam explosion takes place once the hydrolysis reaction time has passed (**Fig. 1**). A detailed description of the thermal hydrolysis TH equipment can be found elsewhere [39]. The operating conditions remained constant, maintaining a temperature of 170 ˚C and a pressure of 7 ± 0.2 bar until the depressurization, following the approach by [40], and varying the hydrolysis times to 5, 10, and 15 minutes. These specific operating conditions were chosen with the aim of optimizing methane production and enhancing the maximum kinetics, as indicated by BMP tests.

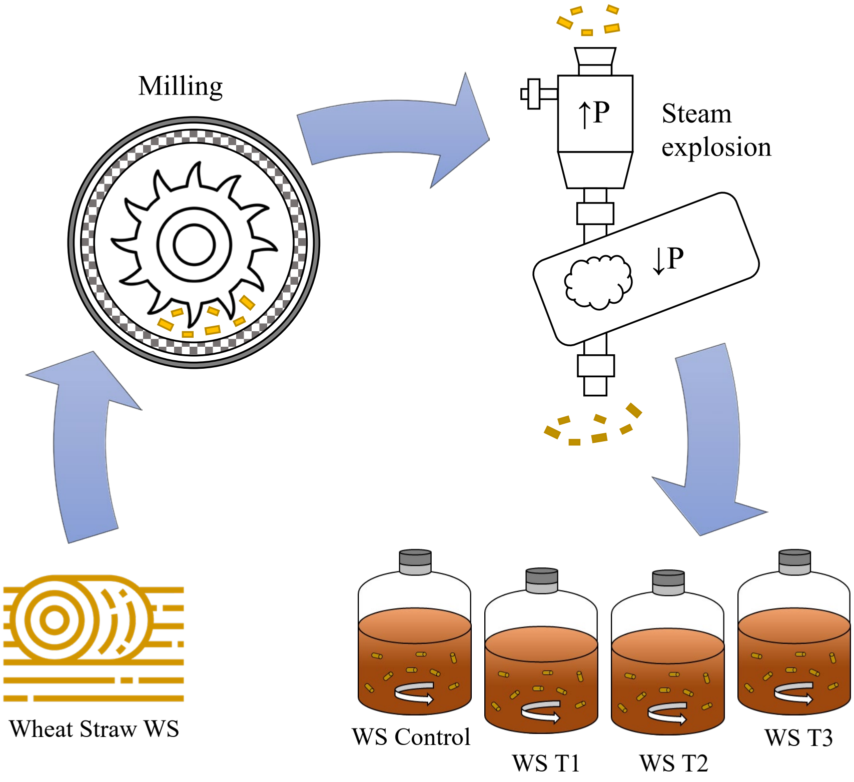

Fig. 1. Schematic diagram of the wheat straw transformation from the field until the AD processing trough mechanical and thermal pretreatment.

2.3 BMP tests

2.3.1 Anaerobic digestion tests

The anaerobic biodegradation of the pretreated substrates was assessed in batch mode over a 40-day period. The tests were initiated following the BPM (Biochemical Methanogenic Potential) methodology, in accordance with the standard protocol outlined by [41].

For these experiments, 120 mL serological glass bottles with a working volume of 70 ml and a headspace of 50 mL were prepared. The temperature was maintained at 35 ± 0.5 ˚C in an incubator (Hotcold-GL, Selecta), and mixing was achieved using an orbital stirring plate (Rotabit, Selecta). The inoculum-to-substrate ratio was set at 1.5:1 g SV, and 1.5 g of $CaCO_3$ L^{-1} was added as a buffering agent to prevent pH fluctuations [42,43]. To establish anaerobic conditions, the bottles were purged with nitrogen to remove any residual air at the start of the experiment.

Three parallel tests were conducted for each pretreatment conditions, namely: Wheat straw standalone (WS Control), wheat straw with a 5-minutes period of steam explosion pretreatment as explained in section 2.2 (WS T1), with a 10-minutes pretreatment period (WS T2) and 15 minutes (WS T3); as well as a blank test with only the inoculum were included to measure the endogenous production of biogas originating from the inoculum. The measurements were adjusted by subtracting the endogenous production. Daily biogas production was quantified using a water displacement method, and the biogas volume was corrected for standard conditions, accounting for ambient pressure and operating temperature. Biogas composition was also measured by a gas analysis equipment (Biogas 5000, GeoTech).

2.3.2 Analytical procedure

American Public Health Association's standard methods were employed for the analysis of Total Solids (TS), Volatile Solids (VS), and Chemical Oxygen Demand (COD) [44]. The determination of carbon (C) and nitrogen (N) content was carried out using a Leco CNS-928 elemental analyser. This analyser employs a process that involves the total combustion of the sample, followed by the determination of the percentage of total weight for each element.

The lignocellulosic fractions (comprising hemicellulose, lignin, and cellulose) in both the pre- and post-pretreatment samples were analysed using the lignocellulosic biomass analysis methods established by the National Renewable Energy Laboratory [45].

2.4 Modelling

2.4.1 Biogas production

The experimental data acquired from the tests were applied to the modified Gompertz mathematical model to adjust the biomethane production for each test and applied for evaluating the anaerobic digestion (AD) performance following the Eq. 1 [46–48]. This model segments bacterial growth curves into three phases: the lag and stationary phases, where the specific growth rate remains at zero, and the exponential phase, where the natural logarithm of the bacterial population increases linearly with time.

$$B\,(t) = B \cdot e^{\left\{-e^{\left[\frac{R\cdot e}{P}\cdot(\lambda-\mathrm{t})+1\right]}\right\}} \qquad (1)$$

In this equation, *B(t)* represents the cumulative methane production at standard temperature and pressure mL CH_4· g VS^{-1}. *B* stands for the potential methane production in mL CH_4· g VS^{-1}, *R* represents the maximum methane production rate mL CH_4· g VS^{-1}

d^{-1}, λ signifies the lag phase duration in days, and t denotes the elapsed time in days. The model was fitted to the experimental data using the least squares methodology.

2.5 Energy Integration study

A scenario has been created based on the configuration shown in **Fig. 2**, which integrates two pretreatment methods: mechanical and thermal. The energy requirements for both pretreatment processes have been determined using the electrical consumption for substrate grinding and the steam requirements based on Cano et al. (2014) [31] prior research [33]. To achieve this, the scenario has been applied in a full-scale plant, with a thorough analysis of energy inputs and outputs.

The potential biomethane generated from the BMP tests was assessed using an energy content of 10 $kWh\cdot Nm^{-3}$ [4,49]. Initially, all raw substrates and cold water were assumed to be at 20 ˚C, with a consistent heat capacity equivalent to that of water (4.18 $kJ\cdot kg^{-1}\cdot {}^{\circ}C^{-1}$) assigned to them to achieve hydrolysis conditions at 170 ˚C and anaerobic digestion at 35 ˚C. Furthermore, the process considered the grinding of straw in a 30-kW knife mill. This grinding operation had a specific flow rate per dry matter (DM) of 2.6 $kg\ DM\cdot h^{-1}\cdot kW^{-1}$ and consumed 140 $kWh\cdot t\ DM^{-1}$[38,50,51]. The net energy balance of the process was determined as the difference between the energy produced from renewable sources and the cost associated with natural gas as can be observed in Eq. 2-5.

$$E_{Balance}\left(\frac{kW\cdot h}{t}\right) = E_{Generation\ (CH_4)}\left(\frac{kW\cdot h}{t}\right) - E_{Consumption\ (PreT)}\left(\frac{kW\cdot h}{t}\right) + E_{Saving}\left(\frac{kW\cdot h}{t}\right) \qquad (2)$$

Where $E_{(generation)}$ is the energy produced in the anaerobic digestion process and $E_{(consumption)}$ is the energy consumed including the pretreatments.

$$E_{Generation\,(CH_4)}\left(\frac{kW\cdot h}{t}\right) = MP\ \left(\frac{m^3}{t\,VS}\right) * VS\ \left(\frac{ton\ Vs}{t}\right) * CV\left(\frac{kW\cdot h}{m^3}\right) \qquad (3)$$

Where MP is the methane production per ton of substrate, VS is the volatile solids of the substrate and *CV* the calorific value of the methane.

$$E_{Consumption\,(Pretreatments)}\left(\frac{kW\cdot h}{t}\right) = E_{(Miller)}\left(\frac{kW\cdot h}{t}\right) + E_{(Steam)}\left(\frac{kW\cdot h}{t}\right) \qquad (4)$$

Where $E_{(miller)}$ is the energy consumed in the mechanical pretreatment of cutting the substrate to 1 mm size with a value of 140 kWh· t^{-1} and $E_{(steam)}$ is the energy consumed in the thermal pretreatment.

$$E_{Steam}\left(\frac{kW\cdot h}{t}\right) = \ Steam\ \left(\frac{m^3}{t}\right) * \eta bo\ (\%) * CV\left(\frac{kW\cdot h}{m^3}\right) \qquad (5)$$

Where *Steam* is the steam needed in the pretreatment per ton, η_{bo} is the boiler efficiency (90 %) and *CV* the calorific value of the natural gas used.

In addition to improve this balance, there are energy savings in heating consumption achieved by increasing the temperature of the substrate as it enters in the anaerobic digester. This avoids losses within the digester since, following pretreatment, the substrate is already at a high temperature (≈100 ˚C), eliminating the need for additional heat during AD. Energy savings are defined in Eq. 6:

$$E_{Savings}\left(\frac{kW\cdot h}{t}\right) = \ WS\ substrate\ (tn) * Sh\ (\frac{kW\cdot h}{°C\cdot t}) * \Delta T(°C) \qquad (6)$$

Where *WS substrate* is the biomass entering in the anaerobic digester, *Sh* is the specific heat of the substrate and ΔT is the temperature difference between the outdoor conditions and the inside reactor conditions (15 ˚C)

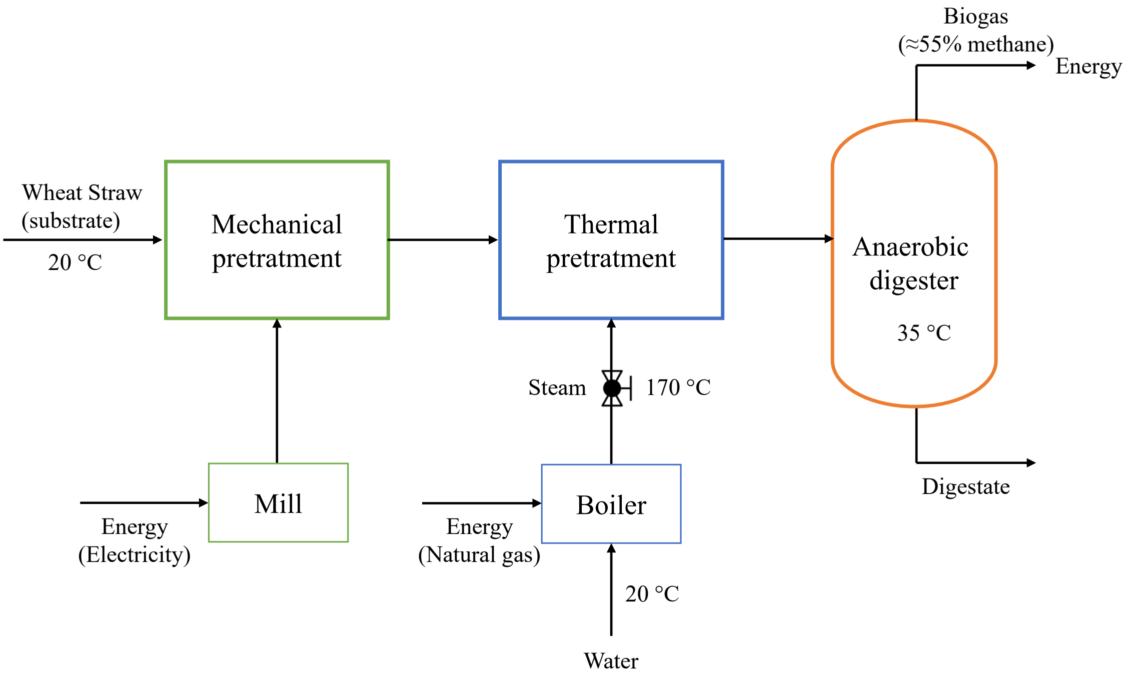

Fig. 2. Energy flow diagram configuration. Mechanical and thermal hydrolysis pretreatments.

3. Results and discussion

3.1 Effect of substrate particle size on biogas production.

Fig. 3 shows the daily and cumulative biomethane generation outcomes from a 25-day experimental regimen. Over this period, notable fluctuations in biomethane production were observed, corresponding with distinct growth phases.

It can be observed that biogas production trends exhibit similarity in the three tests where prior thermal pretreatment was applied. The most prolific production levels occurred during the initial five days, where the experiments surpassed the threshold of 50 mL biomethane per gram of volatile solids per day (g $VS^{-1} \cdot d^{-1}$). Subsequently, from the fifth day onwards, daily production displayed fluctuations, alternating between decline and ascent, within the range of 10 to 25 mL biomethane g $VS^{-1} \cdot d^{-1}$. However, an average biogas production level was sustained between days 10 and 25, marking the stationary phase of the process. Beyond the 13th day, data gradually diminished due to reduced availability of convertible organic substrates. Ultimately, the cumulative production reached 277, 316, and 340 ml biomethane g VS^{-1} for pretreatment trials WS T1, WS T2, and WS T3, respectively. In contrast, the control trial, WS Control, exhibited a more gradual biogas production pattern, maintaining values within the range of 10-15 ml biomethane g $VS^{-1}\cdot d^{-1}$ for the first 10 days. Subsequently, it entered a stationary production phase, yielding between 5 and 10 mL biomethane mL· g $VS^{-1}\cdot d^{-1}$ until the conclusion of the trial with a cumulative production of 212 mL biomethane g VS^{-1}. Different authors have reported values between 204 and 285 mL· g VS^{-1} using raw wheat straw as substrate in the AD process [52–54].

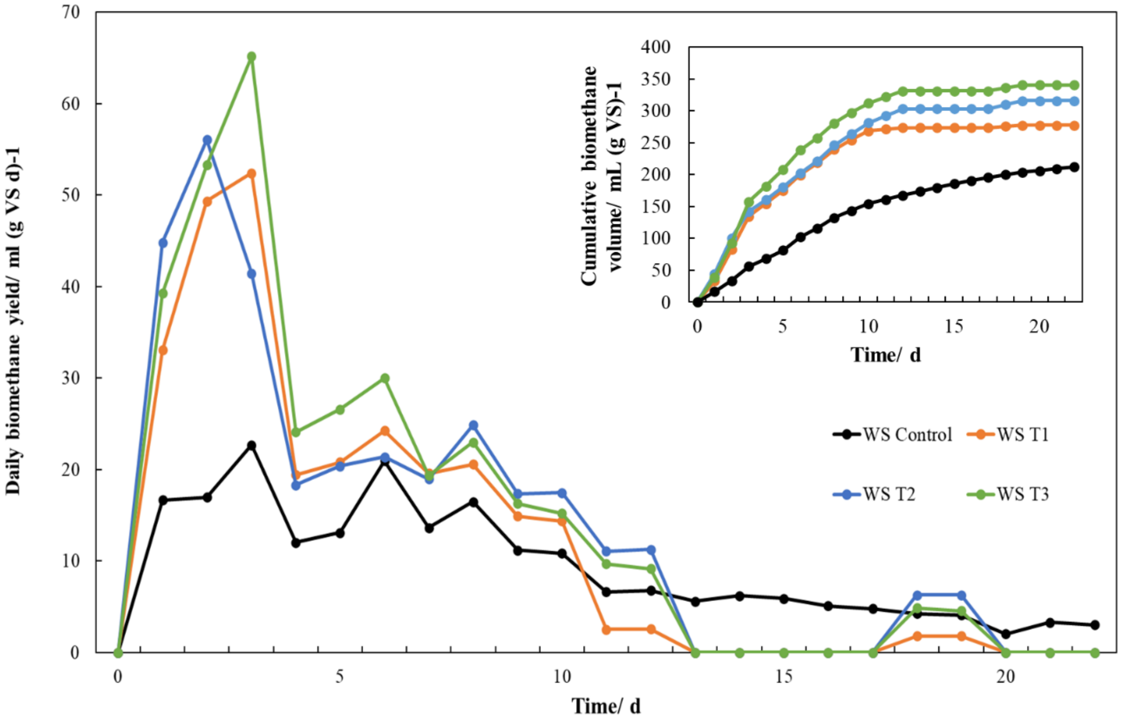

Fig. 3. Daily and cumulative biomethane production from the anaerobic digestion of wheat straw with steam explosion pretreatment at different conditions along the experiment. WS Control stands for Wheat straw standalone, WS T1, WS T2 and WS T3 for wheat straw with a 5-minutes, 10-minutes and 15-minutes period of steam explosion pretreatment respectively.

Table 3 provides an overview of the increment in biomethane production observed throughout the trials involving the three pretreatments when compared to the control. Remarkably, the most substantial disparity was discerned after the initial five days, owing to the effect of thermal pretreatment, which expedited the hydrolysis phase of the anaerobic digestion process. Over an extended period, the pretreatment effect remained prominent, ultimately finishing in a 35 %, 53 %, and 65 % augmentation in biomethane production in comparison to the control test for pretreatments WS T1, WS T2, and WS T3. This rise also described by other authors as described in **Table 1**, using wheat straw as substrate under various steam water pretreatment conditions in AD process [23,29–31,34,35].

Table 3. Increase in cumulative methane production in comparison with control experiment (%) from the anaerobic digestion of wheat straw with steam explosion pretreatment at different conditions.

Essay	Increase in cumulative methane production in comparison with control experiment (%)			
	Day 5	Day 10	Day 15	Day 20
WS T1	114.9 %	73.9 %	47.5 %	34.7 %
WS T2	122.2 %	81.9 %	63.5 %	53.5 %
WS T3	155.8 %	102.1 %	78.3 %	65.4 %

Regarding the biogas composition in each trial, it can be observed that it is quite similar across all four trials, with slightly over 50 % methane content in **Fig. 4**. This methane content is within the typical range for such substrates [31,55]. There is a slight trend of decreasing methane concentration, observed from the control trial with a recorded 52 % to the trial with more pretreatment time, which shows a biogas content of 50 %. This trend aligns with findings reported by [29].

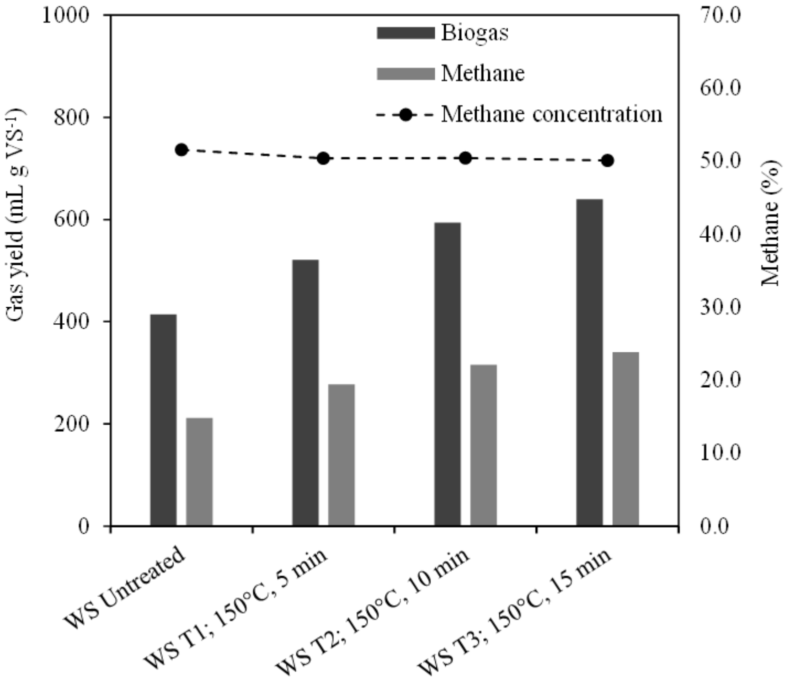

Fig. 4. Biogas composition and biogas total production from the anaerobic digestion of wheat straw with steam explosion pretreatment at different conditions.

3.2 Modelling

The thermal pretreatment positive impacted biomethane yields and anaerobic digestion rates, a pattern evidenced across all three experimental conditions. Employing the Gompertz model to analyse biomethane production across the four trials yielded the fitting results illustrated in **Fig. 5** and the corresponding parameter values detailed in **Table 4**. Higher rates of biogas production, denoted as *R* in the model, signify a mitigation of the rate-limiting step within the hydrolysis phase. Specifically, WS T1 achieved a value of 38.89 ml biomethane g $VS^{-1}\cdot d^{-1}$, while WS T2 achieved 40.19, and WS T3 reached 43.68.

Table 4. Kinetic parameters of methane production for the Gompertz modelling from the AD of wheat straw with steam explosion pretreatment at different conditions.

	Gompertz			
	P∞ (mL CH_4· g VS^{-1})	Rm (mL CH_4· g VS^{-1}· d^{-1})	λ (d)	R^2
Control	212	16.73	0.09	0.996
WS T1	277	38.89	0.06	0.992
WS T2	316	40.19	0.00	0.985
WS T3	340	43.68	0.00	0.993

Likewise, the reduction in the lag phase was evident, as indicated by values approaching zero in all trials. This outcome is attributed to the adaptation of anaerobic sludge to the WS substrate, as expounded in section 2.1.

This substantial variation found in the biomethane production rates, ranging from 130 % to 160 % according to the Gompertz modelling for the three distinct pretreatment conditions respect the control experiment, suggests the potential operational benefits of integrating it into the AD process of wheat straw in an anaerobic reactor. This integration could optimize, on the one hand, the biomethane production and on the other hand, the potential reduction of the hydraulic residence time (HRT) by accelerating the organic decomposition of the substrate. Consequently, this acceleration could lead to a smaller digester size, lower HRT, decreased investment costs for the operator, and ultimately, a more sustainable process.

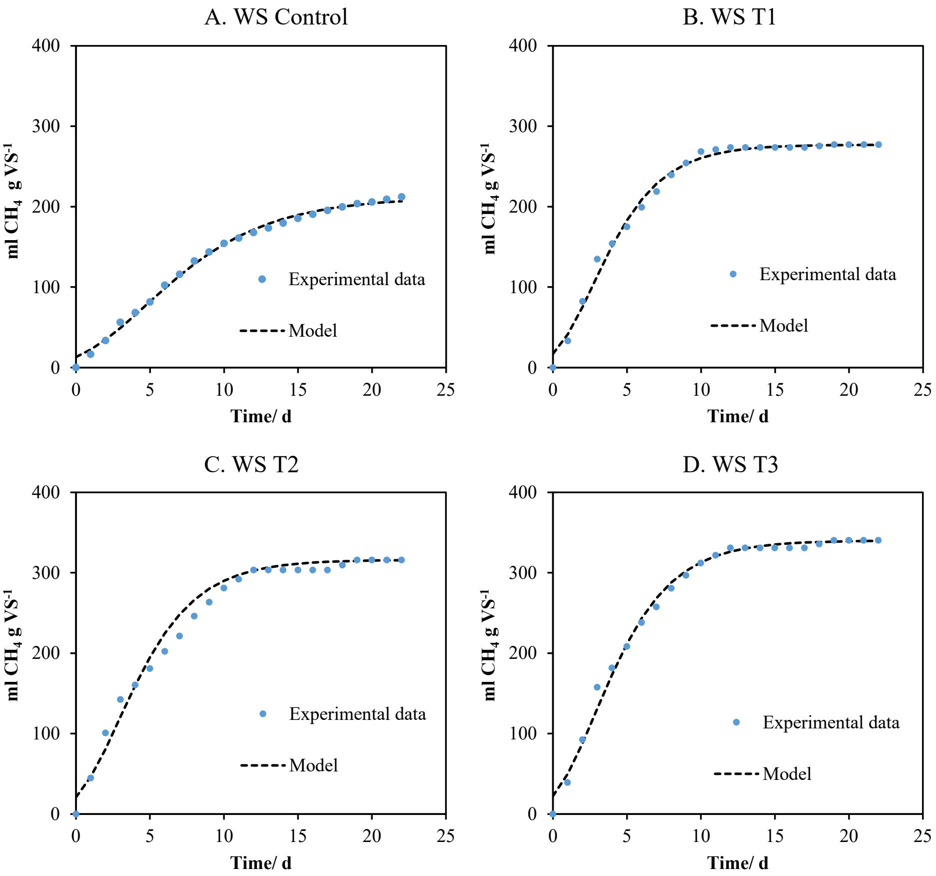

Fig. 5. Biomethane production per amount of volatile solid for wheat straw under three different pretreatment conditions. Gompertz modelling.

3.4 Lignocellulose components decomposition

Table 5 shows the alterations in the chemical composition of the samples, comparing those with and without thermal pretreatment. In the control test, the dry matter content of the ground sample, without thermal pretreatment, stands at 92.3 %. However, this percentage decreases to 58.5 % as the intensity of pretreatment increases due to the use of steam to maintain the pretreatment temperature, which partly condenses in the flash tank. The proportion of volatile solids within the dry matter remains consistent, ranging from 91.7 % to 95.5 %. To ensure comparability between the biomass before anaerobic digestion, it was necessary to calculate the relative proportion of the analysed components, excluding the water-soluble fractions. It can be observed that as pretreatment severity increases, there is a reduction in hemicellulose content. This outcome is anticipated since hemicellulose becomes soluble at 150 ˚C, and, therefore, its decrease can be attributed to both temperature and pretreatment duration [56].

The degradation of hemicellulose in pretreated wheat straw showed an increase of 4.5 % to 9.2 % as the retention time increased, aligning with findings from previous studies [23,29]. Prolonged exposure time accelerated the rate of hemicellulose hydrolysis, which ultimately had a positive impact on AD. Conversely, the degradation rate of lignin and cellulose exhibited the opposite effect. In the case of lignin, as previously reported by Brownell and Saddler in 1987, this may be attributed to the formation of pseudolignin under severe conditions or the creation of cross-linked compounds resulting from reactions of sugars released during the hydrolysis of the hemicellulose fraction [57,58]. More severe treatment, involving higher temperatures or longer exposure times, of lignocellulosic biomass can lead to lower methane yield due to the breakdown of lignin can release phenolic and heterocyclic compounds from the degradation of hemicellulose and cellulose, such as furfural and hydroxymethylfurfural [59,60]. These compounds can inhibit the activity of fermenting microorganisms in the process, as noted by Hendriks and Zeeman in 2009 [61].

Table 5. Chemical composition of untreated and steam-exploded wheat straw

Sample			Components mass fraction		
	Dry matter (%)	Volatile solids (% DM)	Cellulose (% VS)	Hemicellulose (% VS)	Lignin (%VS)
WS Untreated	92.8 ± 0.1	91.7 ± 1.8	31.2 ± 0.4	21.8 ± 0.2	20.5 ± 0.1
WS T1; 170 ˚C, 5 min	61.78 ± 0.2	94.5 ± 0.1	36.0 ± 1.1	17.3 ± 0.4	23.3 ± 1.0
WS T2; 170 ˚C, 10 min	60.12 ± 0.4	95.8 ± 0.1	36.6 ± 0.5	14.0 ± 0.2	24.3 ± 0.2
WS T3; 170 ˚C, 15 min	58.46 ± 0.3	95.5 ± 0.1	38.2 ± 0.4	12.6 ± 0.	23.5 ± 1.4

3.4 Energy integration

The viability of employing mechanical-thermal hydrolysis pretreatment in a continuous processing facility has been assessed using the configuration outlined in Section 2.5. To extend the findings from BMP tests to continuous operations, the biogas yields have been maintained as constants. Meanwhile, the energy consumption associated with both mechanical and thermal pretreatment has been quantified using empirical data resulting from actual operational processes. These calculations are based on the processing of one metric ton of raw wheat straw within the facility.

Table 6 represents the primary outcomes of the study. The predominant energy consumption arises from the grinding process in the knife mill to achieve the desired 1-4 mm-size for wheat straw digestion [62,63], and this remains reliable across all four trials at 140 kWh· t^{-1}, while the natural gas requirements for steam generation increase with prolonged thermal pretreatment times. Energy production is related to biomethane generation under each operational condition. It is important to note that all trials involving thermal pretreatment exhibit a positive energy balance, resulting in a net benefit. However, after a 10-minutes period of thermal pretreatment, the enhancement of the energy balance starts to decline because of the increased consumption associated with extended exposure times and the heightened biomethane production. The highest percentage improvement (15.1 %) in energy balance was achieved for the 10-minute pretreatment. Furthermore, it is known that prolonged exposure times can lead to process inhibition, potentially resulting in a detrimental impact on biomethane production.

Table 6. Energy integration results expressed per ton of wheat straw fed.

Hydrolysys conditions		Energy generation		Energy consumption				Energy savings	Energy balance	
Temperature (°C)	Time (min)	Methane ($m^3 \cdot t\ VS^{-1}$)	Energy (kWh)	Miller ($kWh \cdot t^{-1}$)	Steam ($kg \cdot t^{-1}$)	Energy (kWh)	Energy (kWh)	Energy (kWh)	Energy ($kWh \cdot t^{-1}$)	%
Raw	-	212	1876.4	140.0	0	0	140	0	1736.4	-
170	5	277	2361.2	140.0	31.5	283.8	423.8	16.9	1954.4	12.5
170	10	316	2689.5	140.0	63.1	567.6	707.6	16.9	1998.8	15.1
170	15	340	2897.9	140.0	94.6	851.4	991.4	16.9	1923.4	10.8

Substrates with high VS content like wheat straw, ideally above 110 $g \cdot kg^{-1}$, have demonstrated positive outcomes applying a steam water pretreatment [33]. Conversely, it is important to highlight that other waste materials, such as fatty wastes, may require no pretreatment and can yield exceptionally high energy consumption levels exceeding 3000 $kWh \cdot t^{-1}$. This underscores the substantial potential for anaerobic digestion in this substrate category [33]. On the other hand, waste materials with greater availability but lower volatile solids content, such as livestock waste or sewage sludge, do necessitate pretreatment to achieve positive net energy balances in the process [64,65].

4. Conclusions

The high availability of lignocellulosic organic residues from the agricultural sector presents a novel opportunity in its management for energy valorization through anaerobic digestion. This potential that can be maximized by applying pretreatments such as the combination of mechanical grinding and physic-chemical steam explosion. Under controlled conditions, this approach has the capability to double the biomethane production rate and optimize the energy balance by over 15% in a real-scale process integration. The preliminary steam explosion stage has demonstrated significant efficacy in the hydrolytic stage of the process primarily breaking down hemicellulose in wheat straw and increasing the substrate's biodegradability.

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5. References

[1] S. Singh, K.K. Pant, M. Krishania, Current perspective for bio-oil production from agricultural residues in commercialization aspect: A review, J Anal Appl Pyrolysis. 175 (2023) 106160. https://doi.org/https://doi.org/10.1016/j.jaap.2023.106160.

[2] P. Gołasa, M. Wysokiński, W. Bieńkowska-Gołasa, P. Gradziuk, M. Golonko, B. Gradziuk, A. Siedlecka, A. Gromada, Sources of Greenhouse Gas Emissions in Agriculture, with Particular Emphasis on Emissions from Energy Used, Energies (Basel). 14 (2021). https://doi.org/10.3390/en14133784.

[3] S. Babu, S. Singh Rathore, R. Singh, S. Kumar, V.K. Singh, S.K. Yadav, V. Yadav, R. Raj, D. Yadav, K. Shekhawat, O. Ali Wani, Exploring agricultural waste biomass for energy, food and feed production and pollution mitigation: A review, Bioresour Technol. 360 (2022) 127566. https://doi.org/https://doi.org/10.1016/j.biortech.2022.127566.

[4] Dieter Deublein & Angelika Steinhauser, Biogas from Waste and Renewable Resources, Focus on Catalysts. 2011 (2011) 8. https://doi.org/10.1016/S1351-4180(11)70392-0.

[5] K. Stamatelatou, G. Antonopoulou, G. lyberatos, 12 - Production of biogas via anaerobic digestion, in: R. Luque, J. Campelo, J. Clark (Eds.), Handbook of Biofuels Production, Woodhead Publishing, 2011: pp. 266–304. https://doi.org/https://doi.org/10.1533/9780857090492.2.266.

[6] A. Khatri-Chhetri, C. Costa Junior, E. Wollenberg, Greenhouse gas mitigation co-benefits across the global agricultural development programs, Global Environmental Change. 76 (2022) 102586. https://doi.org/https://doi.org/10.1016/j.gloenvcha.2022.102586.

[7] P. Morone, L. Cottoni, F. Giudice, Chapter 3 - Biofuels: Technology, economics, and policy issues, in: R. Luque, C.S.K. Lin, K. Wilson, C. Du (Eds.), Handbook of Biofuels Production (Third Edition), Woodhead Publishing, 2023: pp. 55–92. https://doi.org/https://doi.org/10.1016/B978-0-323-91193-1.00012-3.

[8] A.G. Álvaro, C.R. Palomar, V. de Almeida Guimarães, E.B. Hedo, R.M. Torre, I. de Godos Crespo, Developmental Perspectives of the Biofuel-Based Economy, in: S.A. Bandh, F.A. Malla (Eds.), Biofuels in Circular Economy, Springer Nature Singapore, Singapore, 2022: pp. 133–156. https://doi.org/10.1007/978-981-19-5837-3_9.

[9] N.S. Bentsen, D. Nilsson, S. Larsen, Agricultural residues for energy - A case study on the influence of resource availability, economy and policy on the use of straw for energy in Denmark and Sweden, Biomass Bioenergy. 108 (2018) 278–288. https://doi.org/https://doi.org/10.1016/j.biombioe.2017.11.015.

[10] MITECO. Ministerio para la Transición Ecológica y el Reto Demográfico de España, Ley 7/2022, de 8 de abril, de residuos y suelos contaminados para una economía circular, 2022.

[11] MITECO. Ministerio para la Transición Ecológica y el Reto Demográfico de España, Hoja de ruta del biogas, 2022.

[12] European Parliament, Directive (EU) 2023/2413 of the European Parliament and of the Council of 18 October 2023 amending Directive (EU) 2018/2001, Regulation (EU) 2018/1999 and Directive 98/70/EC as regards the promotion of energy from renewable sources, and repealing Council Directive (EU) 2015/652, 2023.

[13] R.A. Dar, M. Parmar, E.A. Dar, R.K. Sani, U.G. Phutela, Biomethanation of agricultural residues: Potential, limitations and possible solutions, Renewable and Sustainable Energy Reviews. 135 (2021) 110217. https://doi.org/https://doi.org/10.1016/j.rser.2020.110217.

[14] X. Jin, W. Ai, W. Dong, Lignocellulose degradation, biogas production and characteristics of the microbial community in solid-state anaerobic digestion of wheat straw waste, Life Sci Space Res (Amst). 32 (2022) 1–7. https://doi.org/https://doi.org/10.1016/j.lssr.2021.09.004.

[15] V. Passoth, M. Sandgren, Biofuel production from straw hydrolysates: current achievements and perspectives, Appl Microbiol Biotechnol. 103 (2019) 5105–5116. https://doi.org/10.1007/s00253-019-09863-3.

[16] C. Xu, J. Li, X. Zhang, P. Wang, B. Deng, N. Liu, Q. Yuan, Effects of segmented aerobic and anaerobic fermentation assisted with chemical treatment on comprehensive properties and composition of wheat straw, Bioresour Technol. 362 (2022) 127772. https://doi.org/https://doi.org/10.1016/j.biortech.2022.127772.

[17] S. Kim, B.E. Dale, Life cycle assessment of various cropping systems utilized for producing biofuels: Bioethanol and biodiesel, Biomass Bioenergy. 29 (2005). https://doi.org/10.1016/j.biombioe.2005.06.004.

[18] C. Sawatdeenarunat, K.C. Surendra, D. Takara, H. Oechsner, S.K. Khanal, Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities, Bioresour Technol. 178 (2015) 178–186. https://doi.org/10.1016/J.BIORTECH.2014.09.103.

[19] B. Hashemi, S. Sarker, J.J. Lamb, K.M. Lien, Yield improvements in anaerobic digestion of lignocellulosic feedstocks, J Clean Prod. 288 (2021). https://doi.org/10.1016/j.jclepro.2020.125447.

[20] P. Tsapekos, P.G. Kougias, A. Frison, R. Raga, I. Angelidaki, Improving methane production from digested manure biofibers by mechanical and thermal alkaline pretreatment, Bioresour Technol. 216 (2016) 545–552. https://doi.org/https://doi.org/10.1016/j.biortech.2016.05.117.

[21] A.A. Rajput, Zeshan, C. Visvanathan, Effect of thermal pretreatment on chemical composition, physical structure and biogas production kinetics of wheat straw, J Environ Manage. 221 (2018) 45–52. https://doi.org/https://doi.org/10.1016/j.jenvman.2018.05.011.

[22] F.P. Camargo, C.A.B.S. Rabelo, I.C.S. Duarte, E.L. Silva, M.B.A. Varesche, Biogas from lignocellulosic feedstock: A review on the main pretreatments, inocula and operational variables involved in anaerobic reactor efficiency, Int J Hydrogen Energy. 48 (2023) 20613–20632. https://doi.org/https://doi.org/10.1016/j.ijhydene.2023.02.073.

[23] Y. Yu, J. Wu, X. Ren, A. Lau, H. Rezaei, M. Takada, X. Bi, S. Sokhansanj, Steam explosion of lignocellulosic biomass for multiple advanced bioenergy processes: A review, Renewable and Sustainable Energy Reviews. 154 (2022) 111871. https://doi.org/https://doi.org/10.1016/j.rser.2021.111871.

[24] Z. Song, G. Yang, X. Liu, Z. Yan, Y. Yuan, Y. Liao, Comparison of seven chemical pretreatments of corn straw for improving methane yield by anaerobic digestion, PLoS One. 9 (2014). https://doi.org/10.1371/journal.pone.0093801.

[25] G.P. Naik, A.K. Poonia, P.K. Chaudhari, Pretreatment of lignocellulosic agricultural waste for delignification, rapid hydrolysis, and enhanced biogas production: A review, Journal of the Indian Chemical Society. 98 (2021) 100147. https://doi.org/10.1016/J.JICS.2021.100147.

[26] S. Periyasamy, V. Karthik, P. Senthil Kumar, J.B. Isabel, T. Temesgen, B.M. Hunegnaw, B.B. Melese, B.A. Mohamed, D.-V.N. Vo, Chemical, physical and biological methods to convert lignocellulosic waste into value-added products. A review, Environ Chem Lett. 20 (2022) 1129–1152. https://doi.org/10.1007/s10311-021-01374-w.

[27] I. Ballesteros, M.J. Negro, J.M. Oliva, A. Cabañas, P. Manzanares, M. Ballesteros, Ethanol Production From Steam-Explosion Pretreated Wheat Straw, in: J.D. McMillan, W.S. Adney, J.R. Mielenz, K.T. Klasson (Eds.), Twenty-Seventh Symposium on Biotechnology for Fuels and Chemicals, Humana Press, Totowa, NJ, 2006: pp. 496–508. https://doi.org/10.1007/978-1-59745-268-7_41.

[28] G. Shang, C. Zhang, F. Wang, L. Qiu, X. Guo, F. Xu, Liquid hot water pretreatment to enhance the anaerobic digestion of wheat straw—effects of temperature and retention time, Environmental Science and Pollution Research. 26 (2019) 29424–29434. https://doi.org/10.1007/s11356-019-06111-z.

[29] F. Theuretzbacher, J. Lizasoain, C. Lefever, M.K. Saylor, R. Enguidanos, N. Weran, A. Gronauer, A. Bauer, Steam explosion pretreatment of wheat straw to improve methane yields: Investigation of the degradation kinetics of structural compounds during anaerobic digestion, Bioresour Technol. 179 (2015) 299–305. https://doi.org/10.1016/J.BIORTECH.2014.12.008.

[30] L.C. Ferreira, A. Donoso-Bravo, P.J. Nilsen, F. Fdz-Polanco, S.I. Pérez-Elvira, Influence of thermal pretreatment on the biochemical methane potential of wheat straw, Bioresour Technol. 143 (2013) 251–257. https://doi.org/https://doi.org/10.1016/j.biortech.2013.05.065.

[31] A. Bauer, P. Bösch, A. Friedl, T. Amon, Analysis of methane potentials of steam-exploded wheat straw and estimation of energy yields of combined ethanol and methane production, J Biotechnol. 142 (2009) 50–55. https://doi.org/https://doi.org/10.1016/j.jbiotec.2009.01.017.

[32] A.A. Rajput, Zeshan, C. Visvanathan, Effect of thermal pretreatment on chemical composition, physical structure and biogas production kinetics of wheat straw, J Environ Manage. 221 (2018) 45–52. https://doi.org/10.1016/J.JENVMAN.2018.05.011.

[33] R. Cano, A. Nielfa, M. Fdz-Polanco, Thermal hydrolysis integration in the anaerobic digestion process of different solid wastes: Energy and economic feasibility study, Bioresour Technol. 168 (2014) 14–22. https://doi.org/10.1016/j.biortech.2014.02.007.

[34] J. Lizasoain, A. Trulea, J. Gittinger, I. Kral, G. Piringer, A. Schedl, P.J. Nilsen, A. Potthast, A. Gronauer, A. Bauer, Corn stover for biogas production: Effect of steam explosion pretreatment on the gas yields and on the biodegradation kinetics of the primary structural compounds, Bioresour Technol. 244 (2017) 949–956. https://doi.org/https://doi.org/10.1016/j.biortech.2017.08.042.

[35] D. Steinbach, D. Wüst, S. Zielonka, J. Krümpel, S. Munder, M. Pagel, A. Kruse, Steam Explosion Conditions Highly Influence the Biogas Yield of Rice Straw, Molecules. 24 (2019). https://doi.org/10.3390/molecules24193492.

[36] I. Renewable Energy Agency, Renewable capacity statistics 2020 statistiques de capacité renouvelable 2020 estadísticas de capacidad renovable 2020, 2020. www.irena.org.

[37] V. Wyman, J. Henríquez, C. Palma, A. Carvajal, Lignocellulosic waste valorisation strategy through enzyme and biogas production, Bioresour Technol. 247 (2018) 402–411. https://doi.org/10.1016/j.biortech.2017.09.055.

[38] P.P. Dell'Omo, V.A. Spena, Mechanical pretreatment of lignocellulosic biomass to improve biogas production: Comparison of results for giant reed and wheat straw, Energy. 203 (2020) 117798. https://doi.org/https://doi.org/10.1016/j.energy.2020.117798.

[39] I. Díaz, A. Díaz-Curbelo, N. Pérez-Lemus, F. Fdz-Polanco, S.I. Pérez-Elvira, Traceability of organic contaminants in the sludge line of wastewater treatment plants: A comparison study among schemes incorporating thermal hydrolysis treatment and the conventional anaerobic digestion, Bioresour Technol. 305 (2020) 123028. https://doi.org/https://doi.org/10.1016/j.biortech.2020.123028.

[40] F. Fdz-Polanco, R. Velazquez, S.I. Perez-Elvira, C. Casas, D. del Barrio, F.J. Cantero, M. Fdz-Polanco, P. Rodriguez, L. Panizo, J. Serrat, P. Rouge, Continuous thermal hydrolysis and energy integration in sludge anaerobic digestion plants, Water Science and Technology. 57 (2008) 1221–1226. https://doi.org/10.2166/wst.2008.072.

[41] C. Holliger, M. Alves, D. Andrade, I. Angelidaki, S. Astals, U. Baier, C. Bougrier, P. Buffière, M. Carballa, V. de Wilde, F. Ebertseder, B. Fernández, E. Ficara, I. Fotidis, J.-C. Frigon, H.F. de Laclos, D.S.M. Ghasimi, G. Hack, M. Hartel, J. Heerenklage, I.S. Horvath, P. Jenicek, K. Koch, J. Krautwald, J. Lizasoain, J. Liu, L. Mosberger, M. Nistor, H. Oechsner, J.V. Oliveira, M. Paterson, A. Pauss, S. Pommier, I. Porqueddu, F. Raposo, T. Ribeiro, F. Rüsch Pfund, S. Strömberg, M. Torrijos, M. van Eekert, J. van Lier, H. Wedwitschka, I. Wierinck, Towards a standardization of biomethane potential tests, Water Science and Technology. 74 (2016) 2515–2522. https://doi.org/10.2166/wst.2016.336.

[42] F. Romagnoli, L. Pastare, A. Sabūnas, K. Bāliņa, D. Blumberga, Effects of pre-treatment on Biochemical Methane Potential (BMP) testing using Baltic Sea Fucus vesiculosus feedstock, Biomass Bioenergy. 105 (2017) 23–31. https://doi.org/https://doi.org/10.1016/j.biombioe.2017.06.013.

[43] E. Rouches, R. Escudié, E. Latrille, H. Carrère, Solid-state anaerobic digestion of wheat straw: Impact of S/I ratio and pilot-scale fungal pretreatment, Waste Management. 85 (2019) 464–476. https://doi.org/https://doi.org/10.1016/j.wasman.2019.01.006.

[44] A.W. APHA, Standard Methods for the Examination of Water & Wastewater, 22nd Edition. (2012).

[45] National Renewable Energy Laboratory, Biomass Compositional Analysis Laboratory Procedures, 2022.

[46] Y. Zhao, F. Sun, J. Yu, Y. Cai, X. Luo, Z. Cui, Y. Hu, X. Wang, Co-digestion of oat straw and cow manure during anaerobic digestion: Stimulative and inhibitory effects on fermentation, Bioresour Technol. 269 (2018) 143–152. https://doi.org/https://doi.org/10.1016/j.biortech.2018.08.040.

[47] W. Li, H. Khalid, Z. Zhu, R. Zhang, G. Liu, C. Chen, E. Thorin, Methane production through anaerobic digestion: Participation and digestion characteristics of cellulose, hemicellulose and lignin, Appl Energy. 226 (2018) 1219–1228. https://doi.org/https://doi.org/10.1016/j.apenergy.2018.05.055.

[48] O. Meneses Quelal, D. Pilamunga Hurtado, Anaerobic Fermentation of Slaughterhouse Waste—Codigestion with Wheat Straw to Determine Methane Biochemical Potential and Kinetic Analysis, Fermentation. 9 (2023). https://doi.org/10.3390/fermentation9080726.

[49] P. Schley, M. Beck, M. Uhrig, S.M. Sarge, J. Rauch, F. Haloua, J.-R. Filtz, B. Hay, M. Yakoubi, J. Escande, A. Benito, P.L. Cremonesi, Measurements of the Calorific Value of Methane with the New GERG Reference Calorimeter, Int J Thermophys. 31 (2010) 665–679. https://doi.org/10.1007/s10765-010-0714-z.

[50] T. Jiradechakorn, S. Chuetor, S. Kirdponpattara, M. Narasingha, M. Sriariyanun, Performance of combined hydrochemo-mechanical pretreatment of rice straw for bioethanol production, Energy Reports. 9 (2023) 180–185. https://doi.org/https://doi.org/10.1016/j.egyr.2023.08.081.

[51] G. Ji, W. Xiao, C. Gao, Y. Cao, Y. Zhang, L. Han, Mechanical fragmentation of wheat and rice straw at different scales: Energy requirement in relation to microstructure properties and enzymatic hydrolysis, Energy Convers Manag. 171 (2018) 38–47. https://doi.org/https://doi.org/10.1016/j.enconman.2018.05.087.

[52] S. Menardo, P. Balsari, An Analysis of the Energy Potential of Anaerobic Digestion of Agricultural By-Products and Organic Waste, Bioenergy Res. 5 (2012) 759–767. https://doi.org/10.1007/s12155-012-9188-0.

[53] C. Sambusiti, F. Monlau, E. Ficara, H. Carrère, F. Malpei, A comparison of different pre-treatments to increase methane production from two agricultural substrates, Appl Energy. 104 (2013) 62–70. https://doi.org/https://doi.org/10.1016/j.apenergy.2012.10.060.

[54] C. Eskicioglu, F. Monlau, A. Barakat, I. Ferrer, P. Kaparaju, E. Trably, H. Carrère, Assessment of hydrothermal pretreatment of various lignocellulosic biomass with CO2 catalyst for enhanced methane and hydrogen production, Water Res. 120 (2017) 32–42. https://doi.org/https://doi.org/10.1016/j.watres.2017.04.068.

[55] Y.-R. Kang, Y. Su, J. Wang, Y.-X. Chu, G. Tian, R. He, Effects of different pretreatment methods on biogas production and microbial community in anaerobic digestion of wheat straw, Environmental Science and Pollution Research. 28 (2021) 51772–51785. https://doi.org/10.1007/s11356-021-14296-5.

[56] J. Wang, D. Ma, Y. Lou, J. Ma, D. Xing, Optimization of biogas production from straw wastes by different pretreatments: Progress, challenges, and prospects, Science of The Total Environment. 905 (2023) 166992. https://doi.org/https://doi.org/10.1016/j.scitotenv.2023.166992.

[57] M.C. Nelson, M. Morrison, Z. Yu, A meta-analysis of the microbial diversity observed in anaerobic digesters, Bioresour Technol. 102 (2011) 3730–3739. https://doi.org/https://doi.org/10.1016/j.biortech.2010.11.119.

[58] M. Yadav, V. Vivekanand, Combined fungal and bacterial pretreatment of wheat and pearl millet straw for biogas production – A study from batch to continuous stirred tank reactors, Bioresour Technol. 321 (2021) 124523. https://doi.org/https://doi.org/10.1016/j.biortech.2020.124523.

[59] S. Akizuki, H. Suzuki, M. Fujiwara, T. Toda, Impacts of steam explosion pretreatment on semi-continuous anaerobic digestion of lignin-rich submerged macrophyte, J Clean Prod. 385 (2023) 135377. https://doi.org/https://doi.org/10.1016/j.jclepro.2022.135377.

[60] P. Li, X. Wei, M. Wang, D. Liu, J. Liu, Z. Pei, F. Shi, S. Wang, X. Zuo, D. Li, H. Yu, N. Zhang, Q. Yu, Y. Luo, Simulation of anaerobic co-digestion of steam explosion pulping wastewater with cattle manure: Focusing on degradation and inhibition of furfural, Bioresour Technol. 380 (2023) 129086. https://doi.org/https://doi.org/10.1016/j.biortech.2023.129086.

[61] A.T.W.M. Hendriks, G. Zeeman, Pretreatments to enhance the digestibility of lignocellulosic biomass, Bioresour Technol. 100 (2009) 10–18. https://doi.org/https://doi.org/10.1016/j.biortech.2008.05.027.

[62] A.G. Alvaro, C.R. Palomar, D.H. Redondo, R.M. Torre, I. de Godos Crespo, Simultaneous production of biogas and volatile fatty acids through anaerobic digestion using cereal straw as substrate, Environ Technol Innov. (2023) 103215. https://doi.org/10.1016/J.ETI.2023.103215.

[63] M. Garuti, E. Sinisgalli, M. Soldano, F.G. Fermoso, A.J. Rodriguez, M. Carnevale, F. Gallucci, Mechanical pretreatments of different agri-based feedstock in full-scale biogas plants under real operational conditions, Biomass Bioenergy. 158 (2022) 106352. https://doi.org/10.1016/J.BIOMBIOE.2022.106352.

[64] M. Vanegas, F. Romani, M. Jiménez, Pilot-Scale Anaerobic Digestion of Pig Manure with Thermal Pretreatment: Stability Monitoring to Improve the Potential for Obtaining Methane, Processes. 10 (2022). https://doi.org/10.3390/pr10081602.

[65] F.L. Kakar, H. Aqeel, S.N. Liss, E. Elbeshbishy, Impact of Hydrothermal Pretreatment Parameters on Mesophilic and Thermophilic Fermentation and Anaerobic Digestion of Municipal Sludge, Fermentation. 9 (2023). https://doi.org/10.3390/fermentation9060508.

Chapter 8

Conclusions and future work

Conclusions and future work

The optimisation of the anaerobic digestion process was successfully carried out based on independent works looking for new technology development insights to improve the yield of biogas production or other by-products of AD using agricultural waste as substrate.

The hybrid solution of integrating anaerobic digesters with hybrid solar panels to enhance biogas and biomethane production could generate savings of up to 65% as studied in **Chapter 3**. This configuration will ensure a higher energy yield and access to bioenergy in isolated communities. The innovative system proposed in a pig farm was evaluated at different climate conditions. The high temperatures and particularly, the availability of sunlight, will favour this hybrid system in terms of energy efficiency and conversion to biomethane being particularly favourable in tropical climates, achieving up to 83.3% of its potential and minimum in cold temperate climates, less than 40 % in annual average. In temperate regions, biomethane production is limited to warm periods. The economic study revealed a Levelized Cost of Electricity (LCOE) for the AD/S system between 0.042- 0.055 USD/kWh, making it economically competitive compared to other energy technologies.

In **Chapter 4**, the AD process was evaluated using crop straw from cereal cultivation for the simultaneous production of biogas and volatile fatty acids (VFAs) through the modelling of the process stage kinetics. The organic conversion rates for all substrates were highly significant, ranging from 45% to 70%, with the highest value corresponding to barley straw due to its lower lignocellulosic content which facilitates its biodegradability. The obtained VFAs concentration varied between 3.0 and 4.6 $g \cdot L^{-1}$, resulting in biomethane production ranging from 288 to 317 ml $CH_4 \cdot g\ VS^{-1}$. Finally, the process integration study in a 3000 m^3 anaerobic digester demonstrated that the proposed system's energy demand is covered with a daily potential production over 3 tons of VFAs.

Chapter 5 confirmed that the use of microparticles in the anaerobic digestion process can have a positive impact on biomethane production rates. Specifically, the addition of Fe_3O_4 and graphite particles resulted in an improvement in biomethane quality and production by up to 40% compared to control experiments using wine lees from the wine industry as a biodegradable substrate. The particularity of this high-organic-load and phenolic-content residue also represented an operational challenge. As a result, after 10 days of the experiment, the process was inhibited, showing a significant accumulation of volatile fatty acids (VFAs) and biogas with a high H_2S content.

The microbiological study carried out in **Chapter 6,** focusing on semi-continuous anaerobic reactors fed with swine wastewater, contributed to understand their connection and dependence on the abiotic parameters of the AD process. After a strong inhibition period in the biogas production, the system was recovered, demonstrating the high adaptability of microorganisms. This inhibition was attributed to high concentrations of VFAs and ammonia when operating with an Organic Loading Rate (OLR) above 5 Kg COD· m^{-3} d^{-1}. Microorganism analysis using 16S rRNA analysis at different stages of the process revealed the prevalence of bacterial and archaeal species from swine wastewater, suggesting that external inoculation, such as anaerobic sludge, may not be necessary. This was confirmed through BMP-type experiments. After 140 days of reactor operation and under seasonal conditions, biomethane production reached approximately 400 ml CH_4· g VS^{-1}, aligning with the studied potential production.

In **Chapter 7**, wheat straw again used as substrate, aiming to enhance substrate biodegradability through a combination of mechanical and thermal pretreatment to break down the structure of lignocellulosic compounds in the substrate and improve biogas production. Milling the sample to a size of 1 mm, coupled with steam explosion at 170 ˚C for 5-15 minutes, resulted in biogas production improvements ranging from 35% to 65%, with higher efficiency observed at longer exposure times. A second phase of process integration at an industrial scale demonstrated that these pretreatments could enhance the process's energy balance by up to 15%. The optimal steam exposure time was found to be 10 minutes, as beyond this point, the energy consumption was higher than the improvement in biomethane production efficiency.

The representative results of these studies demonstrate that technological advancements in the process can enhance biogas production performance, particularly when using agricultural residues as a feedstock, which, as shown, is a very abundant and widespread resource throughout the world. This technology holds a triple advantage over others: (i) it addresses the global issue of waste management, (ii) generates renewable energy by capturing potentially harmful CH_4 and CO_2 as greenhouse gases, and (iii) exhibits competitive implementation and operational costs per kWh. With these considerations, future work should be directed toward defining strategic priorities, focusing on:

1. Explore novel strategies to accelerate and enhance the performance of the anaerobic digestion process, making its implementation more cost-effective.
2. Conduct in-depth studies on bacterial and archaeal species with optimal productivity for each substrate independently, analyzing their interactions with abiotic factors (primarily temperature, Hydraulic Retention Time, and pH).
3. Develop innovative approaches to increase the degradability of more complex substrates, such as combining pretreatments or codigestion strategies.
4. Create simple models for each stage of operational strategies to each substrate or substrate mixture. Make use of neural networks or data analysis for improved interpretation.
5. Investigate new substrates of diverse origins and composition that could add value to various processes or activities (e.g., lavender distillation residues, effluents from palm oil industry activities).
6. Full-scale implementation of the models and experimental laboratory studies.

About the author

Biography

Alfonso García Álvaro (Soria, 1987) grew up in Soria where he completed his basic studies and the bachelor. He studied a degree in Environmental Sciences in the University of Alcalá -UAH- in Alcalá de Henares (Spain) between 2005 and 2011 including an academic year at the University of Life Sciences in Tartu (Estonia). In 2012 he spent 6 months at the University of Natural Resources and Life Sciences in Vienna (Austria) and returned to Soria to work in two private engineering companies until 2018. During these years he also completed the Forestry Engineering Degree and a Master's degree in Bioenergy and Energy Sustainability at the University of Valladolid -UVa-. In November 2018 he joined the Department of Chemical Engineering and Environmental Technology at the University of Valladolid in the Soria campus thanks to a UVa pre-doctoral contract. During these more than three years, he has developed his line of research in the area of anaerobic digestion led by Dr. Ignacio de Godos Crespo and Dra. Daphne Hermosilla Redondo, period in which he made three research stays in (i) Querétaro (Mexico) with the research group of Dr Guillermo Quijano Govantes of the National Autonomous University of Mexico -UNAM-, in (ii) Suchitoto (El Salvador) in the implementation of the project Innovation 2020/ACDE/000266 of the AECID for the supply of drinking water through photovoltaic technologies and in (iii) Copenhagen (Denmark) in the Technical University of Denmark -DTU- in the research group of Dr. Irini Angelidaki. He has also been an active participant in the implementation and operation of the LIFE Smart Agromobility project of the LIFE CCM/ES/001206 programme in the province of Soria where a biomethane plant is working thanks to the anaerobic digestion of the pig slurry generated in a farm with a photosynthetic upgrading system.

Publications in International Indexed Journals

1. **Alfonso García Álvaro**, César Ruiz Palomar, Daphne Hermosilla Redondo, Raúl Muñoz Torre, Ignacio de Godos Crespo (2023). *Hybridization of anaerobic digestion with solar energy: A solution for isolated livestock farms*. Energy Conversion and Management: X, 100488. https://doi.org/https://doi.org/10.1016/j.ecmx.2023.100488
2. **Alfonso García Álvaro**, César Ruiz Palomar, Daphne Hermosilla Redondo, Raúl Muñoz Torre, Ignacio de Godos Crespo (2023). *Simultaneous production of biogas and volatile fatty acids through anaerobic digestion using cereal straw as substrate*. Environmental Technology & Innovation, 103215. https://doi.org/10.1016/J.ETI.2023.103215
3. **Alfonso García Álvaro,** César Ruiz Palomar, Daphne Hermosilla Redondo, Raúl Muñoz Torre, Ignacio de Godos Crespo (2023). *Improving the anaerobic digestion process of wine lees by the addition of microparticles*. *Water*, *16*(1). https://doi.org/10.3390/w16010101
4. **Alfonso García Álvaro**, Edgardo Iván Valenzuela, Alejandro Vargas, Ignacio de Godos, Raúl Muñoz, Miguel Vital-Jácome, Guillermo Quijano (2022). *Wastewater treatment potential, light penetration profile and biomass settling performance of a photo-sequencing batch reactor*. Journal of Chemical Technology & Biotechnology, 98(2), 346–356. https://doi.org/https://doi.org/10.1002/jctb.7249
5. Armando Aguilar-Jiménez, Luis Hernández-Callejo, Macías Suástegui, Víctor Alonso Gómez, **Alfonso García Álvaro**, Raúl Maján Navalón, Lilian Johana Obregón (2023). *Energy and Economic Analysis of Renewable Energy-Based Isolated Microgrids with AGM and Lithium Battery Energy Storage: Case Study Bigene, Guinea-Bissau*. Urban Science. https://doi.org/10.3390/urbansci7020066
6. Alfonso García Álvaro, César Ruiz Palomar, Edgardo I. Valenzuela, Daphne Hermosilla Redondo, Raúl Muñoz Torre, Ignacio de Godos Crespo (2023). *Simultaneous production of biogas and volatile fatty acids through anaerobic digestion using cereal straw as substrate*. Journal of Water Process Engineering. (In review)
7. **Alfonso García Álvaro,** César Ruiz Palomar, Israel Díaz Villalobos, Daphne Hermosilla Redondo, Raúl Muñoz Torre, Ignacio de Godos Crespo (2023). *Improving biogas yield from wheat straw with thermal pretreatment. Energy integration study.* Energies. (In review)

8. Blanca Castell, **Alfonso García Álvaro**, Isabel Amez, David León, Antonio Gascó, Marcelo Ortega, Daphne Hermosilla Redondo (2023). *Comparison of pyrolysis and anaerobic digestion processes for wheat straw biomass.* Energy Reports. (In review)

Book chapters

1. **Alfonso García Álvaro**, César Ruiz Palomar, Vanessa de Almeida Guimarães, Eva Blasco Hedo, Raúl Muñoz Torre, Ignacio de Godos Crespo (2022). Developmental Perspectives of the Biofuel-Based Economy. In S. A. Bandh & F. A. Malla (Eds.), *Biofuels in Circular Economy (pp. 133–156). Springer Nature Singapore.* https://doi.org/10.1007/978-981-19-5837-3_9
2. Ignacio de Godos Crespo, **Alfonso García Álvaro**, César Ruiz Palomar, Felix Gaspar Ibrahim, Raúl Muñoz Torre (2023). Algal-Bacterial Consortiums, from Fundamental Interactions to Environmental Applications. In T. Encarnação & A. Canelas Pais (Eds.), Marine Organisms: A Solution to Environmental Pollution? *Uses in Bioremediation and in Biorefinery (pp. 65–77). Springer International Publishing.* https://doi.org/10.1007/978-3-031-17226-7_4
3. César Ruiz Palomar, **Alfonso García Álvaro**, Vanessa de Almeida Guimarães, Eva Blasco Hedo, Raúl Muñoz Torre, Ignacio de Godos Crespo (2022). Biofuels in Low Carbon Economies and Societies. In S. A. Bandh & F. A. Malla (Eds.), *Biofuels in Circular Economy* (pp. 31–58). *Springer Nature* Singapore. https://doi.org/10.1007/978-981-19-5837-3_3
4. **Alfonso García Álvaro**, César Ruiz Palomar, Laura Sánchez, Marcelo Ortega, Ignacio de Godos Crespo (2023). From farm to fuel: microalgae technology to produce novel and sustainable fuel. *Circular Economy on Energy and Natural Resources Industries. Springer International Publishing*. (In press)

Conference participation

1. **Alfonso García Álvaro**, César Ruiz Palomar, Raúl Muñoz Torre, Bernardo Llamas Moya, Ignacio de Godos Crespo. *Transformación de subproductos de cereal en ácidos grasos volátiles mediante digestión anaerobia. Balances de materia y modelización*. XI Congreso Ibérico de Agroingeniería. 11-12 November 2021. Online. - Oral presentation.
2. **Alfonso García Álvaro**, César Ruiz Palomar, Octavio Depraect, Raúl Muñoz Torre, Bernardo Llamas Moya, Ignacio de Godos Crespo. *Low cost biomethane production in swine farms: A solution for agriculture greenhouse gases emissions.* 17th World Congress on Anaerobic Digestion. June 2022. Michigan (United States of America) - Oral presentation.

3. **Alfonso García Álvaro**, Guillermo Quijano, Miguel Ángel Vital, Daphne Hermosilla, Ignacio de Godos Crespo. *Sistema biológico avanzados de depuración de aguas residuales con microalgas*. XIV Congreso Español de Tratamiento de Aguas. 1-3 June 2022. Sevilla (Spain) - Poster
4. **Alfonso García Álvaro**, César Ruiz Palomar, Luis Hernández Callejo, Faphene Hermosilla Redondo, Ignacio de Godos Crespo. *Anaerobic digester for biogas production in isolated environments with solar energy support.* ASABE Sustainable Energy of a Sustainable Future. 24-26 October 2022. San José (Costa Rica) – Oral presentation.
5. **Alfonso García Álvaro**, María Nafría Martín, Víctor Alonso Gómez, Raúl Muñoz Torre, Daphne Hermosilla Redondo, Ignacio de Godos Crespo. *Solar-driven drinking water supply in rural areas under different climate scenarios*. V Iberoamerican Conference of Smart Cities. 28-30 November 2022. Cuenca (Ecuador) - Oral presentation.
6. **Alfonso García Álvaro**, Henrry Pizarro Guazha, César Ruiz Palomar, Antonio Gascó Guerrero, Raúl Muñoz Torre, Daphne Hermosilla Redondo, Ignacio de Godos Crespo. *Efecto de la adicción de partículas en el proceso de digestión anaerobia de paja de trigo para la mejora de la producción de metano*. XII Congreso Ibérico de Agroingeniería. 4-6 September 2023. Universidad de Sevilla (España) - Oral presentation.
7. César Ruiz Palomar, **Alfonso García Álvaro**, Ignacio de Godos Crespo, Raúl Muñoz Torre. *Comparativa de biofertilizantes para el cultivo de Helianthus annuus*. XII Congreso Ibérico de Agroingeniería. 4-6 September 2023. Universidad de Sevilla (España) - Oral presentation.
8. **Alfonso García Álvaro**, Henrry Pizarro Guazha, Isrrael Díaz Villalobos, Antonio Gascó Guerrero, Daphne Hermosilla Redondo, Raúl Muñoz Torre, Ignacio de Godos Crespo. *Effect of the particle's addition in the anaerobic digestion process in wheat straw for the improvement of methane production.* XIV Latin American workshop and symposium on Anaerobic Digestion. 24-27 October 2023. Juriquilla (Mexico) - Oral presentation.

Research stays

- Instituto de Ingeniería de la UNAM, National Autonomous University of Mexico. August 2021 – October 202. Supervisor: Professor Guillermo Quijano Govantes.
- DTU Chemical Engineering, Technical University of Denmark. February 2022. Supervisor: Professor Irini Angelidaki

Participation in research projects

- Processing of livestock waste, for the production of biomethane for use in agricultural vehicles and biofertilizers (LIFE Smart AgroMobility). Life Climate change mitigation CCM/ES/001206. P.I. Ignacio de Godos Crespo.
- Garantizando el acceso al agua potable por medio de tecnológicas fotovoltaicas con enfoque de cuenca en comunidades de Jutiapa, El Salvador. Convocatoria de subvenciones a acciones de cooperación para el desarrollo para la realización de acciones de innovación para el desarrollo 2020, AECID. P.I. Luis Hernández Callejo.
- Integración de paneles solares híbridos y producción de biogás en el edificio de I+D+i del Campus de Soria. University of Valladolid. P.I. Ignacio de Godos Crespo, Luis Hernández Callejo.
- Programa Prometeo: Digestor anaerobio para producción de biogás en entornos aislados con apoyo de energía solar. University of Valladolid. P.I. Alfonso García Álvaro.
- Catedra Caja Rural: Generación de biometano por digestión anaerobia a partir de residuos ganaderos en entornos aislados con apoyo de energía solar. Aplicación en la provincia de Soria. University of Valladolid. P.I. Alfonso García Álvaro.
- Catedra Caja Rural: Producción energética sostenible a partir de residuos generados en la industria vitivinícola y su potencial en la provincia de Soria. University of Valladolid. P.I. Alfonso García Álvaro.

Teaching and student mentoring

- Biogas. Assistant Professor, 3.5 ECTS. Degree in Agricultural and Energy Engineering. 1st course, academic year 2020/2021 and 2023/2024, University of Valladolid (Spain).
- Chemical analysis of forestry products. Assistant Professor, 2.5 ECTS. Degree in Forest Engineering: Forest Industries. 1st course, academic year 2020/2021 and 2021/2022, University of Valladolid (Spain).
- Chemistry. Assistant Professor, 1.4 ECTS. Degree in Forest Engineering: Forest Industries / Degree in Agricultural and Energy Engineering. 1st course, academic year 2023/2024, University of Valladolid (Spain).

- Co-supervisor of students: *End of Degree Project* in Agricultural and Energy Engineering at Valladolid University.
    - Student: Adrián del Castillo Llorente. Title: Proyecto de explotación porcina de cebo con autosuficiencia eléctrica y térmica en Fuentetoba (Soria). Course: 2020/2021
    - Student: Patricio Huerta Michilot. Title: Proyecto de construcción y gestión de una planta de producción de biogás en granja de porcino, ubicada en el término municipal de tejado (Soria). Course: 2020/2021
- Co-supervisor of students: *End of Degree Project* in Environmental Technologies Engineering at Polytechnical University of Madrid.
    - Student: Henrry Marcelo Pizarro Guazha. Title: Evaluación de la mejora de la producción de biogás mediante la adición de distintas partículas de hierro y carbono. Course: 2022/2023
- Co-supervisor of students: *End of Master Project* in Master in Bioenergy Engineering and Energy Sustainability at Valladolid University (2018).
    - Student: Miguel Martínez Veraméndiz. Title: Digestor anaerobio sujeto a cambios drásticos de sustratos. Adaptación y rendimiento. Course 2022/2023
    - Student: Aitor Ridruejo Romera. Title: Estudio del upgrading fotosintético de biogás a biometano mediante el uso de columna de absorción y microalgas en una planta de digestión anaerobia en Sauquillo de Boñices (Soria). Course 2022/2023

Peer-review in ISI Web of Knowledge journals

- Chemical Engineering Journal

Attended short-courses and seminars

1. Formación en comunicación y Soft Skills. *University of Valladolid*. 12/11/2020 – 20/11/2020. 8 hours.
2. Valorización de resultados de investigación y creación de EBTs (Ciencias, Ciencias de la Salud, Ingenierías y Arquitectura). *University of Valladolid*. 25/11/2020 – 26/11/2020. 6 hours.
3. Iniciación a la escritura y publicación de artículos científicos (Ingenierías y Arquitectura). *University of Valladolid*. 11/12/2020 – 11/12/2020. 4 hours.

4. Modelado, Control y Optimización de la Producción de Microalgas en Fotobioreactores. *University of Valladolid*. 11/01/2021 – 12/01/2021. 8 hours.
5. Introducción a las tecnologías de secuenciación y bases de datos genómicas. *University of Valladolid*. 02/03/2021 – 13/03/2021. 6 hours.
6. Cooperación universitaria para el desarrollo. *University of Valladolid*. 02/03/2021 – 13/03/2021. 10 hours.
7. Curso Speaking C1 Inglés. *University of Valladolid*. 04/03/2021 – 07/06/2021. 20 hours.
8. English writing and oral skills for academia. *University of Valladolid*. 02/06/2021 – 07/06/2021. 15 hours.
9. Enzimas fúngicas: producción y aplicaciones. *University of Valladolid*. 18/10/2021 – 21/10/2021. 8 hours.
10. Análisis de Ciclo de vida: Fundamentos y casos prácticos. *University of Valladolid*. 21/10/2021 – 21/10/2021. 5 hours.
11. A happy PHD. Productividad, bienestar y progreso del doctorando. *University of Valladolid*. 10/03/2022 – 31/03/2022. 10 hours.
12. Estadística con R. *University of Valladolid*. 14/03/2022 – 17/03/2022. 20 hours
13. Realización de figuras de calidad para artículos científicos. *University of Valladolid*. 02/06/2022 – 23/06/2022. 8 hours.
14. Seminario "Excel para investigadores"'. *University of Valladolid*. 25/11/2022 – 25/11/2022. 4 hours.
15. Curso "Contar la ciencia". *University of Valladolid*. 15/12/2022 – 19/12/2022. 6 hours.
16. Escritura de artículos científicos. *Asociación Española de Ecología Terrestre*. 23/10/2023 – 27/10/2023. 20 hours.

Agradecimientos/Acknowlegements

Ya que la sección de agradecimientos suele ser la que más lee gente, no voy a perder la oportunidad de hacerla algo diferente y amena,

5:30, suena el despertador, Izana me da patadas, su mamá María duerme. ¡qué sueño, la leche! Enciendo el ordenador, mejor dicho, lo activo porque creo que llevo sin apagarlo semanas. Una taza calentita de té de menta de Marruecos (Gracias Cris) con miel. Es diciembre en Soria y hace frío y tengo mocos y sobre todo sueño. Sueño por un sueño.

Empiezo a escribir donde lo dejé ayer, partículas de magnetita para producir más biometano (qué máquinas son Daphne y Antonio), *"el uso de estas partículas…"*, -Parece que estoy espabilado a estas horas escribiendo, ¡vamos a darle caña!

8:00, toca despertar a las chicas que hay que prepararse para ir cada uno a sus obligaciones, desayuno con roscos de la abuela y al lío. 9:15 y ya en la uni, - *¡Buenos días, chicos!*, saludo a César y Nuski, compañeros y amigos de laboratorio. Hoy toca hacer unos Sólidos y unas DQOs de unas "cacas" que nos han enviado. Paso a ver a Nacho para ver si ha revisado el paper de "metanito" y sigo avanzando con la tesis.

11:30 y marcho con César a Sauquillo a hacer unas pruebas a la planta de biogás del LIFE. – *César, ¿has visto eso?, ¿es un aguilucho cenizo o uno pálido?* Llegamos a la granja, hoy toca cambiar los filtros del gel de sílice y ver la calidad del biometano con el frío. Hemos llegado a un 95 % de metano y en psicrófilo..., llamada a Nacho - *¡Somos la leche!*

14:20, llego tarde a recoger a Izana que me recibe cansada pero alegre, así que, volando a casa a preparar la comida, - *¡pero qué suerte Izana, si tenemos tupper del Ventorro!* Llega María del trabajo y comemos, pero toca volver a la uni que hay que avanzar.

16:05, 55 céntimos, café cappuccino de máquina, cuatro risas con Olga y Elena y a funcionar. Hay que avanzar con una tabla de los trabajos previos que hayan usado pretratamiento térmico en la paja de trigo…

19:30, 46 pestañas abiertas en Google Chrome, unos 80 trabajos revisados después y ya con el cerebro frito parece que ha salido adelante la tabla. Eso sí, el programa *Word* no quiere colaborar con el formato. Voy a ver si mando un par de mails que tengo pendientes.

19:37, e-mail a Raúl con una duda de algún problema o de lo que sea.

19:40, Raúl contesta que ya lo ha solucionado.

20:05, llamo a María, - *¡Holaaa! ¿Qué tal, qué hacéis, cómo está Izana?, ¡voy a casa ya!*

20:30, Bañito a Izana con el cocodrilo, la cebra, el pato y el resto de amigos. *¡Cómo crece nuestra bichilla! … - María, me apetece cena tipo pizza, - ¡A mí también!, - Pero hay unas judías verdes que se van a poner malas, - ¿y qué hacemos? ...* Al final judías verdes, *- así no voy a acabar la tesis nunca ¿eh?*, nos reímos…

22:30, ya toca irse a dormir o a intentarlo, pero voy a ver si termino una figura que tengo a medias que así me la quito de encima ya.

23:32, ahora sí que sí, un día más y un día menos. ¡qué afortunado soy de hacer lo que más me gusta rodeado de los que más me gustan!

Aunque de manera informal haya relatado un día de un doctorando en su última etapa y estoy seguro de que muchos se sentirán identificados, no quiero dejar pasar la oportunidad de agradecer, de manera formal, esta maravillosa etapa de aprendizaje y crecimiento en mi vida que se plasma en este documento, a todos y todas los que de alguna forma han sido parte del camino.

Quiero y debo empezar por dar las gracias a mi mentor el Dr. de Godos o Nacho a secas que ha sido el faro de esta travesía con su gran conocimiento y capacidad de compartirlo. Además de director, amigo. También a Daphne como codirectora que, a pesar de estar en Madrid, estamos trabajando muy bien juntos. Y creo que lo haremos más. A Raúl, que, en calidad de tutor de la tesis, me ha ayudado siempre, sumado a su buena mano en la escritura científica desatascando trabajos.

Y cómo no, a todos los compañeros de departamento, César, Nuski, Ana y Félix, al grupo de Cambium y el de Luis y Víctor. A los compañeros de Valladolid, en especial a Isra y Enrique y las mil y una muestras de AGVs. Al fantástico grupo de la UPM con Antonio y Daphne al frente. A mi gente de Lipata, Guillermo, Lalo, Antonio, Edgardo, Marcos, Agus, ¡Viva México! A Irini de la DTU. A los socios del LIFE y a la memoria de Bernardo

A todos, ¡muchas gracias!

No me quiero olvidar de mi familia que poco a poco han ido entendiendo de que va esto de la digestión anaerobia, aunque (however) sin duda a quién más le agradezco y le debo haber llegado aquí es a María por ser y por estar y nuestra hija Izana que es el mejor regalo de la vida.