Universidad de Valladolid

Escuela de Doctorado Universidad de Valladolid

PROGRAMA DE DOCTORADO EN INGENIERÍA QUÍMICA Y AMBIENTAL

TESIS DOCTORAL:

A techno-economic assessment methodology for the production of polyhydroxyalkanoates and ectoine from biogas in waste treatment plants

Presentada por **Víctor Pérez Martínez** para
optar al grado de
Doctor por la Universidad de Valladolid

Dirigida por:
Raúl Muñoz Torre
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Metodolología de análisis tecno-económico para la producción de polihidroxialcanoatos y ectoína a partir de biogás en plantas de tratamiento de residuos

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A mi familia y amigos

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List of abbreviations and acronyms

BCB	Bubble column bioreactor
BV	Bed volume
CHP	Combined heat and power generation
CH_4	Methane
CH_4-EC	Methane elimination capacity
CH_4-RE	Methane removal efficiency
CO	Carbon monoxide
CO_2	Carbon dioxide
CSTR	Continuous stirred tank reactor
EBRT	Empty bed residence time
EtOH	Ethanol
k_La	Volumetric mass transfer coefficient
FCF	Free cash flow
H/D	Height to diameter ratio
HRT	Hydraulic residence time
HCl	Hydrochloric acid
H_2S	Hydrogen sulfide
H_2S-EC	Hydrogen sulfide elimination capacity
H_2S-RE	Hydrogen sulfide removal efficiency
H_2SO_4	Sulfuric acid
IEX	Ionic exchange chromatography
IRR	Internal rate of return
MSM	Mineral salt medium
MSW	Municipal solid waste
N	Nitrogen
N_2	Molecular nitrogen
NaCl	Sodium chloride
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
Na_2SO_4	Sodium sulfate
NO_3^-	Nitrate
NO_2^-	Nitrite
NO_x	Nitrogen oxide
NPV_{20}	Net present value at 20 years
O_2	Molecular oxygen
P	Pressure
PEC	Purchased equipment cost
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PP	Payback period
r	Interest rate
S	Sulfur
S^0	Elemental sulfur
SO_2	Sulfur dioxide
SO_4^{-2}	Sulfate
T	Temperature
TIC	Total investment cost
V	Working or liquid volume
VFAs	Volatile fatty acids
VOCs	Volatile organic compounds
V_R	Total reactor volume
VSCs	Volatile sulfur compounds
X	Bacterial biomass
λ	Lambda factor
η	Efficiency factor
η_{el}	Electrical efficiency of CHP
η_{th}	Thermal efficiency of CHP

Resumen

La implementación de la tecnología de digestion anaerobia como método para reducir la cantidad de residuos solidos orgánicos enviados a vertedero ha sido un éxito, con más de 25,000 plantas operadas en todo el mundo en la actualidad. Este éxito es especialmente relevante en Europa, donde existen más de 18,000 instalaciones dedicadas al tratamiento de todo tipo de residuos orgánicos como residuos sólidos urbanos, aguas residuales o residuos agroindustriales. La obtención de biogás en el proceso de digestión anaerobia y su valorización como vector energético en motores de co-generación de electricidad y calor (CHP) ha impulsado en gran parte esta implementación a nivel global. Sin embargo, las plantas de digestión anaerobia se enfrentan a día de hoy a un gran dilema, ya que la producción energética a partir de biogás en sistemas CHP presenta unos elevados costes de producción, comparados con los costes de producción decrecientes de otras energías renovables como la solar y la eólica. El alto coste de la producción eléctrica a partir de biogás se ha asociado tradicionalmente a los altos costes de inversión y de mantenimiento de los motores de CHP, debido principalmente a la presencia de múltiples contaminantes en el biogás que pueden dañar los equipos de combustión, principalmente el sulfuro de hidrógeno (H_2S) y los siloxanos. Este problema se ha visto agravado por la falta de incentivos públicos a la producción de energía renovable a partir de biogás, cuyas rebajas fiscales y bonus a la producción se han reducido considerablemente durante la última década. De hecho, la atención de los actores políticos ha virado hacia la producción de productos de mayor valor añadido a partir de los residuos, en el marco de una economía más limpia, verde y circular, alineándose con unas políticas medioambientales cada vez más restrictivas. Por lo tanto, las plantas de producción de biogás de mediano y gran tamaño deben de reconsiderar sus esquemas económicos y encontrar fuentes de ingresos innovadoras que garanticen su viabilidad económica presente y futura. Como consecuencia, hay una creciente motivación para llevar a cabo la transición desde plantas de tratamiento de residuos lineales donde sólo se produce bioenergía hacia biorrefinerías urbanas mucho más circulares donde toda clase de bioproductos puedan ser ofrecidos al mercado.

En esta transición hacia las biorrefinerías urbanas de última generación, una mejor valorización del biogás jugaría un papel principal. De hecho, la utilización de biogás como fuente de materias primas (principalmente metano (CH_4) y dióxido de carbono (CO_2)) frente a su uso como mero vector energético está atrayendo la atención de la

academia y de la industria. En este contexto, la utilización de bacterias metanótrofas, capaces de utilizar el CH_4 contenido en el biogás como única fuente de carbono y energía, ha surgido como una oportunidad para incrementar el valor actual del biogás. Durante la última década, los académicos han demostrado a escala de laboratorio la habilidad de las bacterias metanótrofas para producir bioproductos que se encuentran mucho más arriba en la pirámide de valorización de los residuos, como los polihidroxialcanoatos (PHA) (reactivos químicos y materiales), proteína unicelular (alimentación humana y animal) y ectoína (productos de química fina). Además, en los últimos años, multitud de proyectos han sido financiados para la validación de estas tecnologías a escala demostrativa: INCOVER y URBIOFIN orientados a la producción de biometano, URBIOFIN enfocado en la producción de PHA, CIRCULAR BIOCARBON dirigido a la producción de bioestimulantes, o DEEP PURPLE y CHEERS para la producción de ectoína.

Sin embargo, la futura sostenibilidad técnica, económica y medioambiental de estos procesos a gran escala, así como su robustez en un contexto económico global cambiante, son todavía una incógnita. Es también de una enorme importancia evaluar las limitaciones biotecnológicas actuales para la comercialización de estos bioproductos y la potencial reducción de costes derivada de futuros avances biotecnológicos para definir la hoja de ruta hacia biorrefinerías de biogás competitivas económicamente. Esta tesis doctoral se ha enfocado en el desarrollo e implementación de una metodología para evaluar la viablidad técnica y económica de los procesos de bioconversión de biogás en productos de valor añadido utilizando bacterias metanótrofas como alternativa a la utilización actual del biogás como vector energético en plantas de tratamiento. Esta metodología ha sido diseñada también para ser capaz de identificar y cuantificar los cuellos de botella biotecnológicos de estas tecnologías innovadoras.

En este contexto, el **Capítulo 3** presenta el desarrollo de una metodología robusta para el análisis del potencial impacto económico y de las limitaciones de los procesos de bioconversión de biogás utilizando bacterias metanótrofas. Los límites de batería y la base de cálculo han sido definidas de acuerdo a las particularidades de las plantas de digestión anaerobia de mediano y gran tamaño. Por este motivo, la metodología incluye una recopilación de datos provenientes de: gestores de residuos, operadores de planta, proveedores nacionales e internacionales de equipos, materias primas y servicios auxiliares, entidades gubernamentales y las investigaciones más pioneras en la temática. Esta metodología de análisis económico ha permitido identificar las partidas de costes de operación más críticas, así como los equipos más relevantes dentro de la instalación. Además, la metodología para el análisis de sensibilidad ha permitido identificar las principales barreras biotecnológicas, de manera que es posible definir la hoja de ruta para futuras investigaciones. Esta metodología ha sido mejorada durante los últimos 5 años de investigación y ha servido como marco para el análisis tecno-económico y de sensibilidad de la producción de PHA, un biopolímero de bajo valor añadido con una gran cuota de mercado (**Capítulos 4 y 5**), y de ectoína, un producto de muy alto valor añadido en la industria farmacéutica y cosmética con una demanda reducida en el mercado (**Capítulos 6 y 7**), comparados con la tradicional combustión del biogás en motores CHP para la producción de energía.

En el **Capítulo 4**, se investigó el potencial de utilizar el biogás generado durante la digestión anaerobia para producir PHA como una alternativa atractiva a la co-generación eléctrica en plantas de tratamiento de residuos urbanos. La combustión de biogás para CHP, la bioconversión de biogás en PHA y una combinación de ambos escenarios fueron comparados en términos de impacto ambiental, economía del proceso y responsabilidad social, de acuerdo con las métricas de sostenibilidad establecidas por IChemE. Aunque la producción de PHA presentó mayores costes de inversión y de operación, debido al mayor valor de mercado de los biopolímeros, todos los escenarios de valorización de biogás mostraron un desempeño económico similar en cuanto a valor presente neto evaluado a 20 años (NPV_{20}) (0.77 M€) y en cuanto a tasa interna de retorno (IRR) (6.4 ± 0.2 %). La producción de PHA conllevó una reducción significativa de la acidificación atmosférica y de la emisión de olores comparado con CHP, no obstante, mostró mayores demandas de terreno, agua, reactivos químicos y energía. La creación de puestos de trabajo asociada a la industria de los biopolímeros y la creciente demanda de bioproductos por parte de los consumidores fueron indentificados como aspectos fundamentales para potenciar la aceptación social y local de las plantas de tratamiento de residuos. Estos resultados demostraron que la producción de PHA a partir de biogás constituye a día de hoy una alternativa realista a la cogeneración en plantas de tratamiento de residuos urbanos, y que los PHA pueden ser producidos a un precio de mercado competitivo (4.2 $€\cdot kg^{-1}$ PHA) cuando el excedente de biogás es utilizado para la producción interna de energía eléctrica.

El **Capítulo 5** se enfocó en estudiar el potencial de aplicación a escala global del uso de biogás como materia prima para su bioconversión en PHA, como alternativa a su utilización actual como fuente de energía. La influencia de la localización geográfica de las instalaciones en el desempeño económico de la valorización del biogás mediante CHP y/o PHA fue evaluada en 13 regiones representativas del mundo. Además, se estudió la sensibilidad de los costes de producción de PHA frente a las actuales limitaciones biotecnológicas y el precio de los *commodities*. El análisis geográfico indicó una gran variabilidad en los valores de NPV_{20} alrededor del mundo (variando desde −7.17 hasta +16.27 M€). Los países con los valores de NPV_{20} más bajos también mostraron los menores costes de producción de PHA (4.1 $€\cdot kg^{-1}$ PHA), lo que sugiere que la producción de biopolímeros representa una ruta de valorización alternativa de biogás en regiones donde CHP no es económicamente viable. En países con altos precios energéticos, el uso del excedente del biogás producido durante la digestión anaerobia para la producción de PHA puede contribuir a potenciar el desempeño económico y a mitigar la actual dependencia del mercado eléctrico de las plantas equipadas con CHP. La capacidad de eliminación de CH_4 en biorreactores y el rendimiento de acumulación de PHA en las células bacterianas fueron identificados como los principales parámetros biotecnológicos que deben de ser optimizados para alcanzar una producción de biopolímeros a un precio competitivo (0.2–1.7 $€\cdot kg^{-1}$ PHA) respecto a sus homólogos derivados del petróleo.

En el **Capítulo 6**, la capacidad de las bacterias metanótrofas haloalcalifílicas para sintetizar ectoína a partir del CH_4 contenido en el biogás fue evaluada a gran escala. Esta ruta de valorización de biogás alternativa representa una oportunidad para las plantas de tratamiento de residuos de incrementar sus ingresos económicos y de alinear sus procesos a las cada vez más restrictivas directivas de economía circular. El análisis tecno-económico y de sensibilidad para la bioconversión de biogás en 10 t ectoína $año^{-1}$ se desarrolló en dos etapas: (I) bioconversión del metano en ectoína en reactores tipo columna de burbujeo y (II) purificación de ectoína mediante un proceso de cromatografía de intercambio iónico. El análisis tecno-económico del proceso mostró unos altos costes de inversión (4.2 M€) y de operación (1.4 $M€\cdot año^{-1}$). Sin embargo, el margen bruto entre el precio de mercado de la ectoína (600–1,000 $€\cdot kg^{-1}$) y los costes de producción estimados (214 $€\cdot kg^{-1}$) resultó en una muy alta rentabilidad del proceso, con valores de NPV_{20} de 33.6 M€. El análisis de sensibilidad de los costes reveló una gran influencia del coste de los equipos y de los consumibles en los costes de producción de ectoína. Al contrario que otras rutas de valorización de biogás como la energética o la producción de bioproductos de bajo valor añadido, la bioconversión de biogás en ectoína demostró una gran robustez frente a cambios en los precios de la energía, el agua, el transporte y la mano de obra. Los peores y mejores escenarios evaluados mostraron precios de equilibrio de la ectoína comprendidos entre 158 y 275 €·kg^{-1}, lo que representa una reducción de los precios de venta actuales de entre 3 y 6 veces.

Finalmente, el **Capítulo 7** se enfocó en el estudio de la influencia de aspectos socio-económicos como la localización de la planta, la economía de escala y las fluctuaciones del mercado en la rentabilidad del proceso. Un análisis tecno-económico y de sensibilidad sobre la bioconversión de biogás en ectoína en reactores tipo columna de burbujeo y su posterior extracción y purificación mediante cromatografía de intercambio iónico fue realizado tomando Madrid como escenario base. El análisis geográfico realizado en 13 ciudades representativas del mundo reveló grandes diferencias en los costes de producción de ectoína, variando entre 158 y 231 €·kg^{-1}. El análisis de economía de escala evidenció una gran dependencia de la economía de escala, con precios unitarios variando entre 782 y 164 €·kg^{-1} para producciones anuales entre 0.1 y 89.6 t ectoína, respectivamente. El análisis tecno-económico mostró también una gran robustez de la rentabilidad de bioconversión de biogás a ectoína frente a futuras fluctuaciones en el mercado, obteniéndose en todos los escenarios evaluados una IRR > 15 % y un periodo de recuperación de la inversión < 10 años. Finalmente, el análisis de sensibilidad identificó la capacidad de eliminación de CH_4 en biorreactores, el desarrollo de cultivos bacterianos de alta eficiencia y la selección de las resinas de intercambio iónico de alta calidad como factores clave en la rentabilidad de la producción de ectoína en las futuras biorrefinerias de biogás

Los resultados obtenidos en esta tesis demuestran la robustez y fiabilidad de la metodología desarrollada y el enorme potencial económico y medioambiental de llevar a cabo la transición desde plantas de tratamiento de residuos lineales donde el biogás es considerado únicamente como vector energético a biorrefinerias más circulares donde el biogás puede ser transformado de forma efectiva en bioproductos con valor añadido como pueden ser los PHA o la ectoína. Estos resultados también apuntan a la mejora de la transferencia gas-líquido del metano y el oxígeno en los biorreactores y al uso de cultivos bacterianos altamente eficientes (desde el punto de vista de bioconversión de metano en bioproductos) como factores clave para el futuro éxito de las biorrefinerías de biogás.

Abstract

The implementation of the anaerobic digestion technology as a method for reducing the amount of solid waste sent to landfill has been a success with more than 25,000 plants in operation in the world. This success is especially relevant in Europe with more than 18,000 facilities devoted to the treatment of all sort of organic residues such as municipal solid waste, wastewater or agro-industrial residues. One of the main factors that has driven this worldwide implementation is the associated production of biogas, further valorized as an energy vector in heat and power co-generation engines (CHP). However, anaerobic digestion plants face nowadays a major dilemma, due to the higher cost of producing energy from biogas in CHP systems, compared to the rapidly declining cost of competing renewable energies such as wind or solar power. The higher cost of electricity produced from biogas has been typically associated to the high investment and maintenance costs of CHP engines, due to the presence of multiple pollutants in biogas that can damage the combustion equipment, mainly hydrogen sulphide (H_2S) and siloxanes. These problems have been aggravated by the lack of policy drivers towards the production of renewable energy from biogas and the reduction of feed-in tariffs and fiscal exemptions during the last decade. In fact, the attention of policy makers has shifted to the production of higher added-value products from waste, in the framework of a cleaner, greener and more circular economy and in line with the growingly restrictive environmental policies. Hence, medium and large-scale biogas production plants must reconsider their economic schemes and find innovative sources of revenue for guaranteeing their present and future economic viability. Therefore, there is a growing motivation for transitioning from linear waste treatment plants, where only bioenergy is produced, to more circular urban biorefineries, where all sort of bioproducts can be commercialized.

In this transition to urban biorefineries, a better valorization of biogas plays a major role. Its utilization as a source of raw materials (mainly methane (CH_4) and carbon dioxide (CO_2)) instead of being merely regarded as an energy vector, has gained attention from both the academia and the industry. In this context, the utilization of methanotrophic bacteria, capable of using CH_4-biogas as their only source of carbon and energy, has emerged as an opportunity for increasing the current value of biogas. During the last decade, academics have widely demonstrated at laboratory scale the ability of methanotrophic bacteria for manufacturing bioproducts which are ranked higher up in the

waste valorization pyramid such as polihydroxyalkanoates (PHA) (chemicals and materials), single cell protein (feed and food) and ectoine (fine chemicals). In addition, in recent years, multiple projects have been financed in order to validate these technologies at demo-scale: INCOVER and URBIOFIN devoted to the production of biomethane, URBIOFIN addressing the production of PHA, CIRCULAR BIOCARBON focused on the production of biostimulants or DEEP PURPLE and CHEERs for the production of ectoine.

However, the future technical and economic sustainability of these processes at large scale as well as their robustness in a global economic context in constant change is still unclear. It is also of paramount importance to evaluate the current biotechnological limitations in bioproducts manufacturing and the potential reduction of production costs derived from future biotechnological advances, thus defining the roadmap to develop cost-competitive biogas biorefineries. This PhD thesis focused on the development and implementation of a methodology for evaluating the technical, economical and environmental feasibility of the bioconversion of biogas into added-value products using methanotrophic bacteria as an alternative to the current utilization of biogas as energy vector in waste treatment plants. This methodology was also designed to identify the biotechnological bottlenecks of these innovative technologies.

In this context, **Chapter 3** featured the development of a robust methodology for assessing the economic potential and the limitations of biogas bioconversion processes using methanotrophic bacteria. The battery limits and calculation basis were defined according to the particular characteristics of current medium- and large-scale anaerobic digestion plants. For this purpose, the methodology has included a compilation of data from waste managers and operators, national and international suppliers of equipment, raw materials and utilities, governmental entities and the most state-of-the-art research in the topic. The techno-economic methodology herein developed allowed the identification of the most significant operational cost shares and equipment cost. Additionally, the methodology for sensitivity analysis identified the most relevant biotechnological barriers and defined the roadmap for future investigations. This methodology has been improved throughout the last 5 years of research and has served as a framework for the techno-economic and sensitivity assessment of the production of PHA, a low added-value biopolymer with a large market share (**Chapters 4 and 5**), and ectoine, a high added-value pharmaceutical and cosmetic product with a small market demand (**Chapters 6 and**

7), compared to the traditional combustion of biogas in CHP engines for producing energy.

Chapter 4 investigated the emerging potential of using the biogas generated from organic waste anaerobic treatment to produce PHA as an attractive alternative to heat and power generation in urban waste treatment plants. The sustainability of biogas combustion for CHP, biogas bioconversion into PHA and a combination of both scenarios was compared in terms of environmental impact, process economics and social responsibility according to the IChemE Sustainability Metrics. Although PHA production presented higher investment and operational costs, a comparable economic performance was observed in all biogas valorization scenarios regarding net present value evaluated at 20 years (NPV_{20}) (0.77 M€) and internal rate of return (IRR) (6.4 ± 0.2 %) due to the higher market value of biopolymers. The PHA production entailed a significant reduction of atmospheric acidification and odor emissions compared to CHP despite showing higher land, water, chemicals and energy requirements. Job creation associated to biopolymer industry and the increasing public demand for bioproducts were identified as fundamental aspects for enhancing social and local acceptance of waste processing facilities. These results demonstrated that PHA production from biogas constitutes nowadays a realistic alternative to CHP in waste treatment plants and that PHA can be produced at a competitive market price when biogas is used for internal energy provision (4.2 $€\cdot kg^{-1}$ PHA).

Chapter 5 was focused on studying the worldwide potential applicability of biogas as raw material for its bioconversion into PHA as an alternative to its current use as energy source. The influence of the geographical location on the economic performance of CHP and/or PHA production from biogas generated in urban waste treatment plants was evaluated in 13 representative regions of the world. In addition, the sensitivity of PHA production costs toward current biotechnological limitations and commodity prices was evaluated. The geographical analysis showed a high NPV_{20} variability around the world (ranging from −7.17 to +16.27 M€). Countries with the lowest NPV_{20} also exhibited the lowest PHA production costs (4.1 $€\cdot kg^{-1}$ PHA), which suggested that biopolymer production represents an alternative biogas valorization pathway in regions where CHP is not economically viable. In countries with high energy prices, the use of biogas surplus from anaerobic waste treatment for PHA production enhanced the economic performance and mitigated the electric market dependence of current CHP plants. CH_4 elimination capacity in bioreactors and PHA accumulation yield in bacterial cells were identified as the main biotechnological bottlenecks for the production of biopolymers competitive in price (0.2–1.7 €·kg^{-1} PHA) with their oil-based counterparts.

Chapter 6 evaluated the capacity of haloalkaliphilic methanotrophic bacteria to synthesize ectoine from CH_4-biogas at large scale. This biogas valorization alternative represents an opportunity for waste treatment plants to improve their economic revenues and align their processes to the incoming circular economy directives. A techno-economic and sensitivity analysis for the bioconversion of biogas into 10 t ectoine y^{-1} was conducted in two stages: (I) bioconversion of CH_4 into ectoine in a bubble column bioreactor and (II) ectoine purification via ion exchange chromatography. The techno-economic analysis showed high investment (4.2 M€) and operational costs (1.4 M€·y^{-1}). However, the high brut margin between the current ectoine market value (600–1,000 €·kg^{-1}) and the estimated ectoine production costs (214 €·kg^{-1}) resulted in a high profitability of the process, with a NPV_{20} of 33.6 M€. The cost sensitivity analysis conducted revealed a great influence of equipment and consumable costs on the ectoine production costs. In contrast to alternative biogas valorization into heat and electricity or into low added-value bioproducts, biogas bioconversion into ectoine exhibited high robustness toward fluctuations in energy, water, transportation, and labor costs. The worst- and best-case scenarios evaluated showed ectoine break-even prices ranging from 158 to 275 €·kg^{-1}, ~3–6 times lower than the current industrial ectoine market value.

Finally, **Chapter 7** addressed the influence of socioeconomic aspects such as the location of the plant, the economy of scale and the market fluctuations on the profitability of the biogas-to-ectoine process. A techno-economic and sensitivity analysis of the bioconversion of CH_4 into ectoine in a bubble column bioreactor and the extraction and purification of ectoine via ionic exchange chromatography was herein conducted using Madrid as base-case scenario. The geographical assessment performed in 13 representative cities revealed high differences in the ectoine production costs, ranging from 158 to 231 €·kg^{-1}. The economy of scale analysis evidenced a high dependence of the ectoine production costs on the production scale, amounting to 782 and 164 €·kg^{-1} when manufacturing 0.1 and 89.6 t ectoine·y^{-1}, respectively. The sensitivity study also showed a high robustness of CH_4-ectoine profitability towards future market fluctuations, with all the scenarios analyzed guaranteeing IRR > 15 % and payback periods <10 y. In addition, the sensitivity analysis identified the improvement of CH_4 elimination capacity in bioreactors, the development of highly efficient microbial strains and the selection of the highest quality ionic exchange resins as key factors impacting the profitability of future biogas-to-ectoine biorefineries.

The results herein obtained demonstrated the robustness and reliability of the featured methodology and the huge economic and environmental potential of transitioning from linear waste treatment plants where biogas is merely regarded as an energy vector to more circular urban biorefineries where biogas can be effectively transformed into added-value products such as PHA or ectoine. The results have also pointed at the enhancement of methane and oxygen gas-liquid mass transfer in bioreactors and the use of highly efficient bacterial cultures (in terms of methane bioconversion into biproducts) as the key factors for the future success of biogas-biorefineries.

List of publications

The followings publications are presented within the scope of this thesis.

All of them (Manuscripts I to IV) have been published in international journals indexed in Clarivate Analytics' Web of Science (WoS).

All of them have been published under open access.

Pérez, V., Mota, C.R., Muñoz, R., Lebrero, R., 2020. Polyhydroxyalkanoates (PHA) production from biogas in waste treatment facilities: Assessing the potential impacts on economy, environment and society. Chemosphere 255. https://doi.org/10.1016/j.chemosphere.2020.126929

Pérez, V., Lebrero, R., Muñoz, R., 2020a. Comparative Evaluation of Biogas Valorization into Electricity/Heat and Poly(hydroxyalkanoates) in Waste Treatment Plants: Assessing the Influence of Local Commodity Prices and Current Biotechnological Limitations. ACS Sustain. Chem. Eng. 8, 7701–7709. https://doi.org/10.1021/acssuschemeng.0c01543

Pérez, V., Moltó, J.L., Lebrero, R., Muñoz, R., 2021. Ectoine production from biogas in waste treatment facilities: a techno-economic and sensitivity analysis. ACS Sustain. Chem. Eng. 9, 17371–17380. https://doi.org/https://doi.org/10.1021/acssuschemeng.1c06772

Pérez, V., Moltó, J.L., Lebrero, R., Muñoz, R., 2022. Ectoine production from biogas: a sensitivity analysis. Effect of local commodity prices, economy of scale, market trends and biotechnological limitations. J. Clean. Prod. 369. https://doi.org/https://doi.org/10.1016/j.jclepro.2022.133440

Contribution to the articles included in the thesis

Manuscript I. In this research, I was responsible for the design of the methodology for the sustainability and the techno-economic assessments. I was in charge of compiling all the data necessary for the calculations. I was responsible for the formal analysis of the results and writing of the original manuscript with the collaboration of Dr. César R. Mota in the discussion section. The review and edition of the original manuscript was conducted by Dr. Raquel Lebrero and Dr. Raúl Muñoz.

Manuscript II. In this work, I was in charge of developing the methodology for the techno-economic and sensitivity analysis. I was responsible for the data compilation and for the calculations. I was in charge of the formal analysis of the results and writing the original manuscript. The review and edition of the original manuscript was conducted by Dr. Raúl Muñoz and Dr. Raquel Lebrero.

Manuscript III. In this investigation, I was responsible for the design of the methodology for the techno-economic assessment and the sensitivity analysis. I was assisted by Jose Luis Moltó in the compilation of all the data necessary for the calculations. I was in charge of the formal analysis of the results and writing the original manuscript. The review and edition of the original manuscript was conducted by Dr. Raúl Muñoz and Dr. Raquel Lebrero.

Manuscript IV. During this research, I was in charge of the design of the methodology for the techno-economic assessment and the sensitivity analysis. I collaborated with Jose Luis Moltó in the compilation of all the data necessary for the calculations and I was in charge of the formal analysis of the results and writing the original manuscript. The review and edition of the original manuscript was conducted by Dr. Raúl Muñoz and Dr. Raquel Lebrero.

Chapter 1

Introduction

1.1 Short history of the discovery of anaerobic digestion

The idea of using flammable gases for producing energy has been associated to humankind for at least 3,000 years, since the first dated use for heating thermal baths in Assiria in the year 900 BC (WBA, 2022). However, it was not until the scientific revolution in the 17th century that scientists started to investigate this process carefully. The Belgian alchemist Jan Baptiste van Helmot, the pioneer on using the word *gas* in the scientific community, was also the first to describe the production of a flammable mixture of gases during the degradation of organic matter in the absence of air (Pooya et al., 2022). Interestingly, two of the most recognized fathers of modern electricity, Benjamin Franklin and Alessandro Volta, were tremendously interested in the gases produced in swamps and marshes. In the late 17th century, Franklin reported the flammability of the gas bubbles produced in these water bodies, describing it as *flammable air* (Wyndham, 1956). Shortly after, in 1777, Volta pioneered the isolation of methane (CH_4) and detailed the first quantitative study relating the amount of organic residues treated and the volume of gas produced (Volta, 1777). It was only at the beginning of the 19th century that John Dalton and Humphrey Davy identified CH_4 as the main constituent of these mysterious gases that were concurrently observed in coal mines, farms and humid environments (Thomas, 2015; Thomson, 1810). In 1886, the German scientist August Van Hoffman baptized this gas as methane, as a derivation from the already well-known methanol (Haug, 2019).

In 1861, the father of microbiology, Louis Pasteur, identified for the first time that certain microorganisms only thrive in the absence of air (Haug, 2019). Finally, at the end of that century, Bechamp and Omielanski connected the formation of CH_4 and carbon dioxide (CO_2) to the action of different biological communities, laying the foundation for the current understanding of the anaerobic degradation of organic residues (Abbasi et al., 2012). Nowadays, it is well established that the anaerobic digestion of organic residues comprises a complex matrix of bacterial communities that interact synergistically for the formation of biogas. This process has been typically described as a 4-step sequential process: (i) hydrolysis, (ii) acidogenesis, (iii) acetogenesis and (iv) methanogenesis (Figure 1.1) (Verma, 2002).

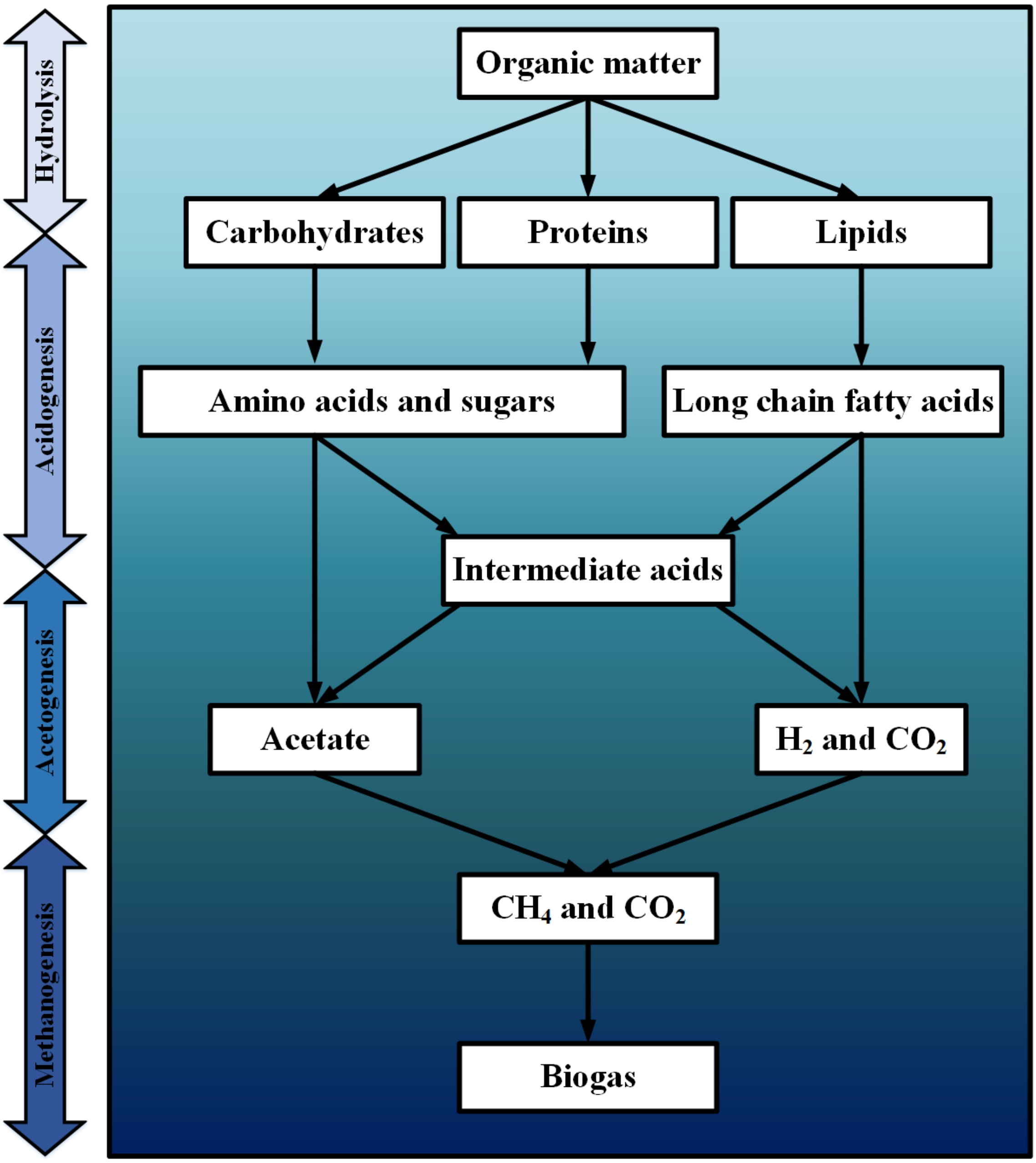

Figure 1.1. 4-step process for the anaerobic degradation of organic waste into biogas.

Motivated by the observations of chemists and biologists, engineers around the world started to conceive multiple concepts of proto-anaerobic digesters: Mouras in France for the treatment of wastewater (1860) (Figure 1.2), Cameron in England with the first septic tank (1895) and James in India for the disposal of leper asylum effluents (1902). In the 1930s, the intensification of the natural gas industry and the identification of bacterial communities responsible for the anaerobic digestion of organic matter triggered the construction of numerous biogas facilities associated to wastewater and sewage sludge treatment (IRENA, 2018). The fuel shortage during World War II boosted the utilization of biogas as energy source, with the construction of more than 1 million biological digesters according to some estimates (Rufai, 2010). But it was not until another energy shortage, during the oil crisis in the 1970s, coupled to a new-born environmental concern regarding water pollution, that the construction of modern anaerobic digestion plants devoted to the production of energy from biogas was consolidated.

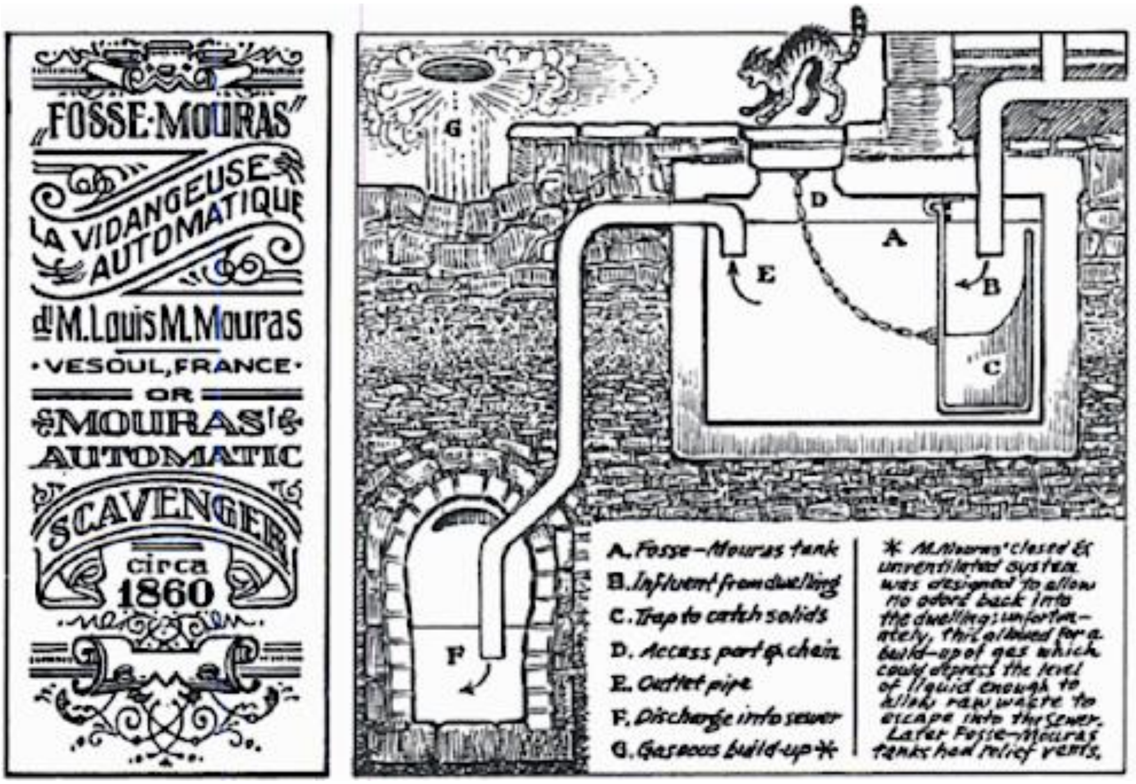

Figure 1.2. Drawing of the first reported anaerobic digester: the automatic scavenger (Mouras, France, 1860).

1.2 Utilization of biogas as an energy vector

Nowadays, the implementation of this platform technology, capable of reducing the amount of organic waste sent to landfills while producing heat and electricity can be considered a story of success with the operation in the world of more than 25,000 medium and large scale anaerobic digestion plants devoted to the treatment of all sort of organic waste: wastewater, agroindustrial residues, municipal solid waste or sewage sludge among others (WBA, 2019). Much of this success is due to the energy valorization of what we know today as biogas, a gas mixture composed of CH_4 (40–75 %), CO_2 (15–60 %) and minor components such as H_2S (0.005–2 %), N_2 (0–2 %), O_2 (0–1 %), NH_3 (< 1 %), CO (< 0.6%), siloxanes (0–0.2%) and halogenated hydrocarbons (VOC < 0.6%) (Ryckebosch et al., 2011). The predominant presence of CH_4, the main constituent of natural gas, provides biogas with a significant calorific value (4.4–6.5 $kWh{\cdot}Nm^{-3}$) that can be industrially exploited via direct combustion in boilers for heat production or in gas engines and turbines for the combined generation of electricity and heat (CHP) (SGC, 2012).

Particularly, gas engines, the most convenient system for the combined production of heat and electricity in medium and large scale biogas plants, have evolved to an overall energy recovery efficiency of 70-80 %, with specific recovery efficiencies of 30-42 % for electricity and 40-50 % for heat, respectively (Wellinger et al., 2013). Despite the maturity of CHP systems after years of development and optimization, these biogas valorization units still suffer from large investment (400-1,000 $€{\cdot}kW_{el}^{-1}$) and operational and maintenance costs (0.01-0.02 $€{\cdot}kWh^{-1}$) (Wellinger et al., 2013). The limited lifespan (10 y) derived from the presence of multiple pollutants in biogas that can damage the combustion equipment, mainly H_2S and siloxanes, reduces significantly the economic viability of biogas-to-energy schemes in waste treatment plants (FNR, 2012). Likewise, the distance to other plants capable of using the high volume of heat generated typically entail that anaerobic digestion plants rarely operate with district heating systems for harnessing the surplus of heat produced and therefore, more than half of the energy potential of biogas is wasted (EPA, 2018).

These technological obstacles have rendered renewable energy production from biogas less competitive against other rapidly developing renewable energies such as solar and wind power, and have limited its worldwide implementation. A recent report indicated that besides the great potential for energy production from waste and the widespread installation of anaerobic digestion plants, this technology has only reached 2 % of the global potential for energy production from waste (WBA, 2019). In the last decade, the cost of renewable energy production from biogas has decreased only by 13.9 % while renewable energy production costs from sun and wind have been reduced by 88.5 % and 62.8 %, respectively (IRENA, 2022a). Thus, the current estimated energy production costs from biogas (0.065 €·kWh^{-1}) are higher compared to the most popular renewable energies such as hydroelectric (0.046 €·kWh^{-1}), wind (0.051 €·kWh^{-1}) and solar (0.046 €·kWh^{-1}) power (Figure 1.3).

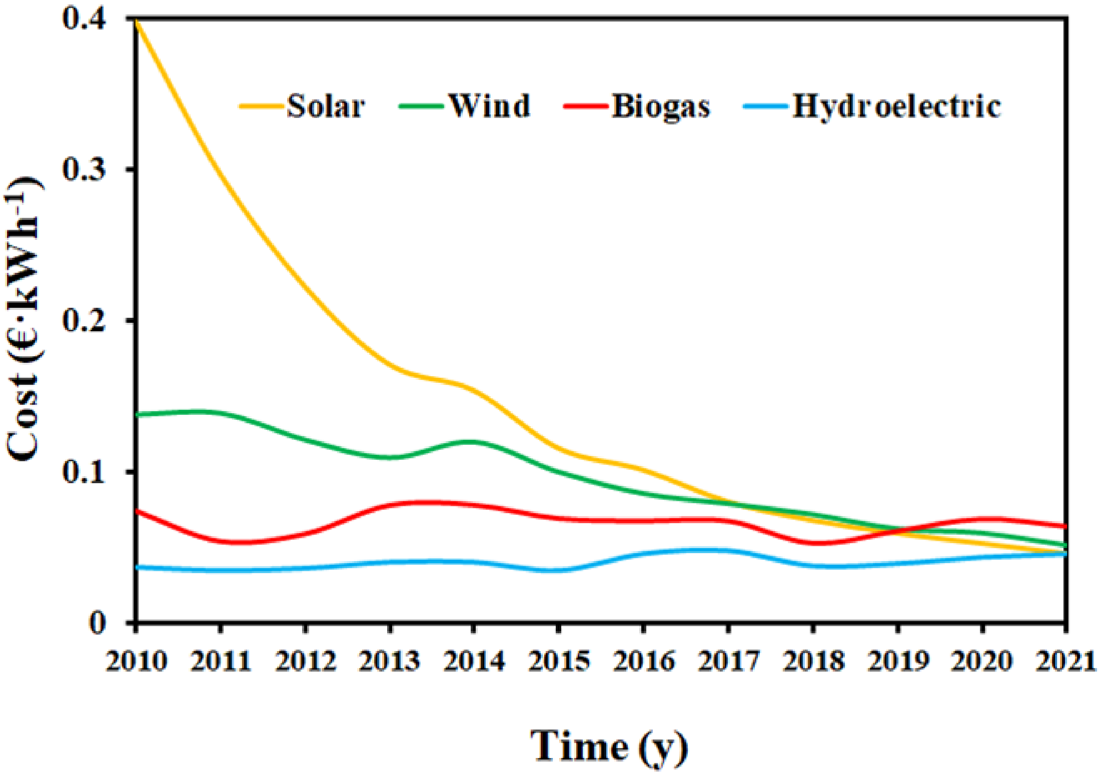

Figure 1.3. Time course of renewable energy production costs. Adapted from (IRENA, 2022a)

Overall, the increase in the installed capacity of renewable energy has doubled over the last decade from 1,444 GW in 2012 to 3,064 GW in 2021 (IRENA, 2022b). This increase in the total energy capacity has been mainly motivated by the increase in solar and wind power from 104 GW and 267 GW in 2012, respectively, to 850 GW and 825 GW in 2021, respectively. In contrast, the installed capacity of biogas has only increased from 13 GW to 22 GW in the same period. Regarding the relative share of each technology in the renewable energy pool, the combined solar and wind power has increased from 25.7 % in 2012 to 54.6 % in 2021, while biogas share has decreased from 0.9 % to 0.7 % in the same period (Figure 1.4) (IRENA, 2022b).

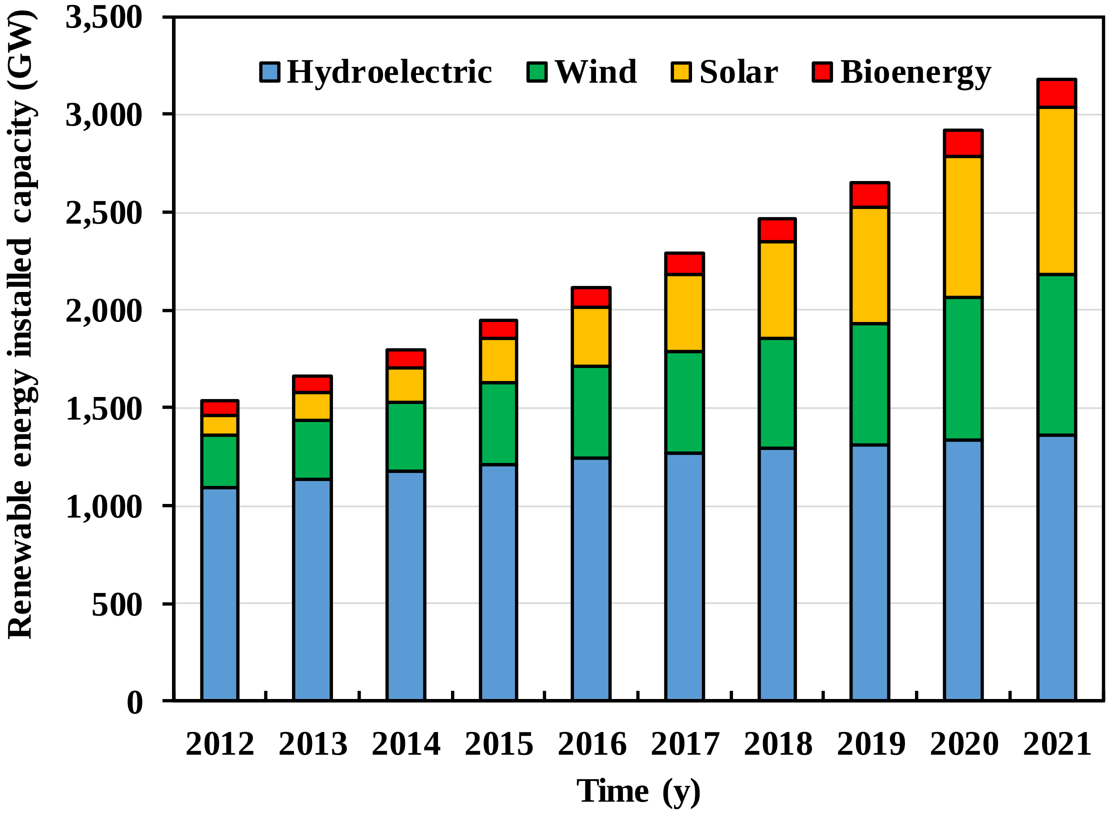

Figure 1.4. Individual installed capacity of renewable energies. Adapted from (IRENA, 2022b)

The implementation of the anaerobic digestion technology has been especially relevant in Europe, where approximately two thirds of the global production of biogas takes place (15 bcm in 2020) (EBA, 2021). This development was particularly vigorous during the first half of the last decade, where the number of biogas plants and the associated energy production from biogas escalated from 12,004 plants to 17,290 plants and from 64 TWh to 149 TWh, respectively, in the period 2011-2015. However, as indicated by the most recent statistical report from the European Biogas Association, this growth has declined severely during the last five years showing a marginal annual biogas market growth of 1.7 % in the number of biogas plants in operation (EBA, 2021) (Figure 1.5). This rapid decline is a consequence of the development of cheaper renewable energies, the limited technological advances in the field of CHP systems, and more importantly to the lack of fiscal incentives towards the production of renewable energy from biogas.

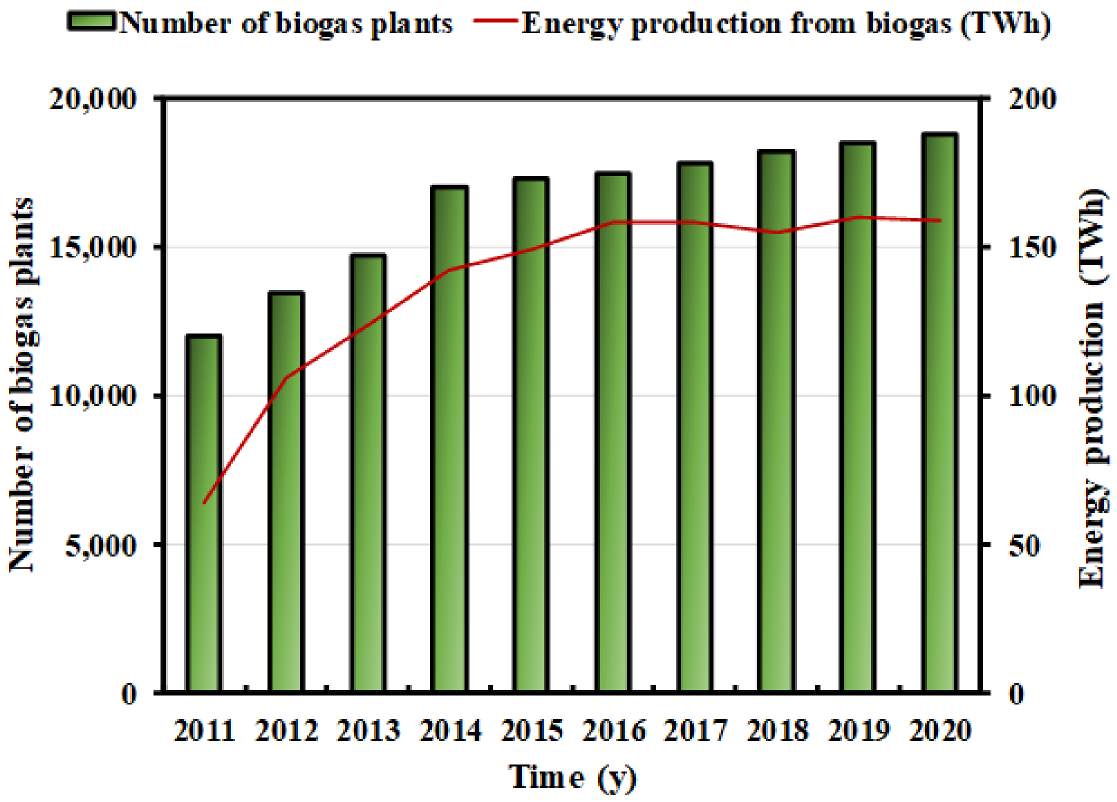

Figure 1.5. Time course of the number of operative biogas plants and of the energy produced from biogas in Europe. Adapted from (EBA, 2021).

1.3 Opportunities for biogas upgrading and reforming into biofuels

In this context, the attention of policy makers has shifted towards the production of higher added-value products from waste in general and from biogas in particular, in the framework of a cleaner, greener and more circular economy and in line with the growingly restrictive environmental policies (European Commission, 2020). Therefore, there is a growing motivation for transitioning from linear waste treatment plants, where only bioenergy is produced, to more circular urban biorefineries, where all sort of biofuels and bioproducts can be commercialized. In this transition to urban biorefineries, a better valorization of biogas plays a major role, and the transformation of its main constituents (CH_4 and CO_2) into high density biofuels instead of being merely regarded as an energy vector for direct combustion, has gained attention from both the academia and the industry. Several alternatives for biogas valorization are currently under investigation such as the purification of biogas constituents via upgrading, the production of syngas via biogas reforming and the transformation of the CH_4 and CO_2 contained in biogas into higher added-value products (Figure 1.6).

The current context of unstability in the energy market constitutes an outstanding opportunity for transitioning from traditional oil-based fuels towards renewable biofuels. Today, more than ever, this transition towards greener fuels represents not only a friendlier alternative from the environmental point of view but also a strategic decision in terms of energy independence. The potential of biogas reforming and upgrading for the production of clean fuels such as H_2 and CH_4 constitute a sustainable valorization alternative to the current utilization of biogas for combustion in CHP. This alternative represents the first approach towards the transformation of current waste treatment plants into sustainable biorefineries, reducing the environmental impact of human activities and also boosting the declining cost-efficiency of these facilities.

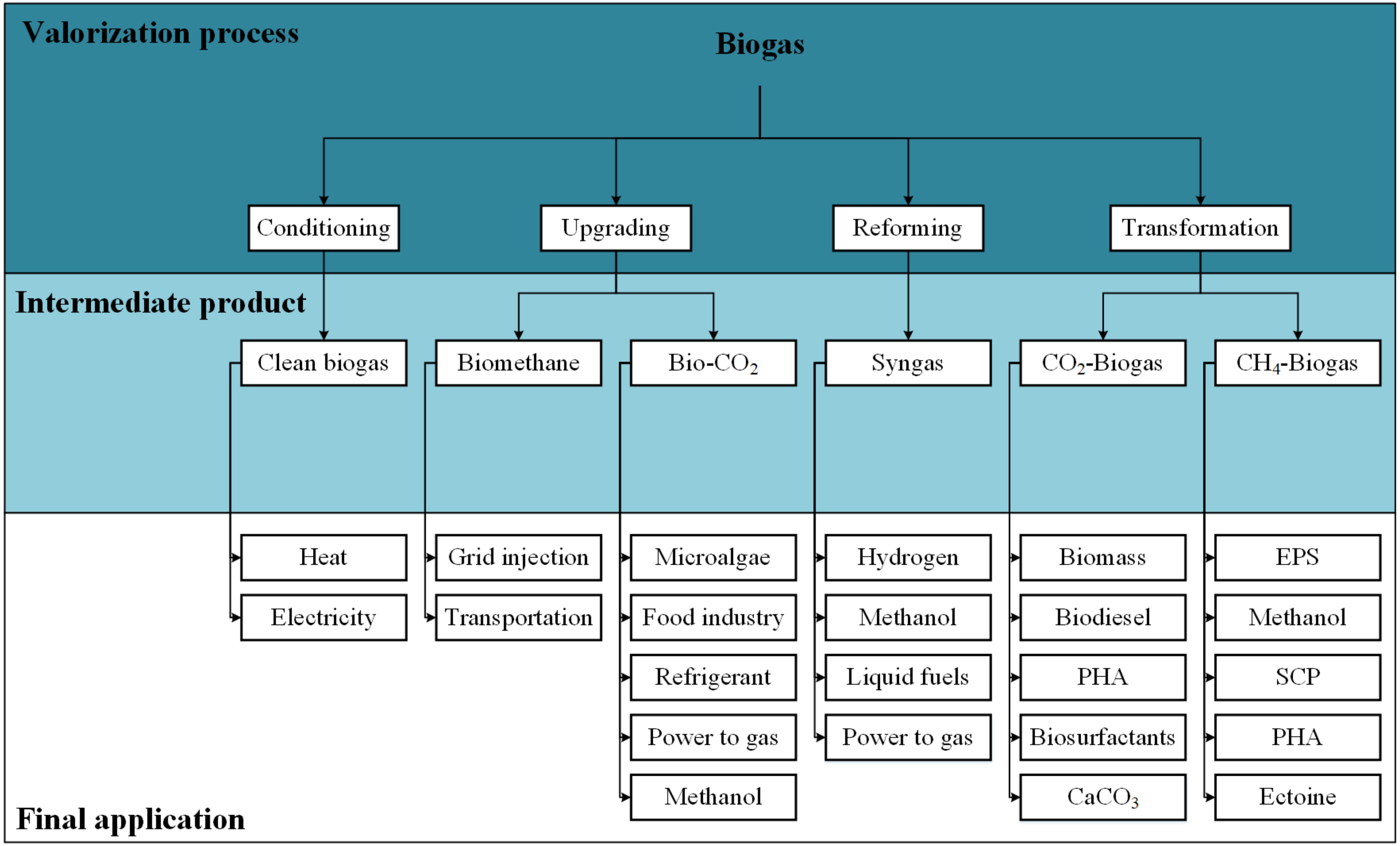

Figure 1.6. Classification of biogas valorization alternatives.

1.3.1 Biogas upgrading into biomethane

Biogas upgrading technologies aim at the purification of biogas for obtaining a high quality biomethane, a green fuel that is comparable to natural gas in terms of properties and functionality. Quality standards on biomethane require a high CH_4 purity in order to be injected into the natural gas grid or to be used as automotive fuel (>80-96 %v·v^{-1}). CO_2 and H_2S are considered the main contaminants in biomethane and their concentration should remain below 2-3 %v·v^{-1} and 5 mg·m^{-3}, respectively, according to different international regulations for these applications (Muñoz et al., 2015). The most ambitious upgrading technologies also aim at the separation and further valorization of the CO_2 contained in biogas, which is often referred in this field as bio-CO_2 (Cordova et al., 2022). Most technologies are only able to remove either CO_2 or H_2S and therefore, a sequential combination of procceses for obtaining a highly pure CH_4 stream is required. A large number of physical-chemical and biological technologies have been developed for the purification and upgrading of biogas (Figure 1.7).

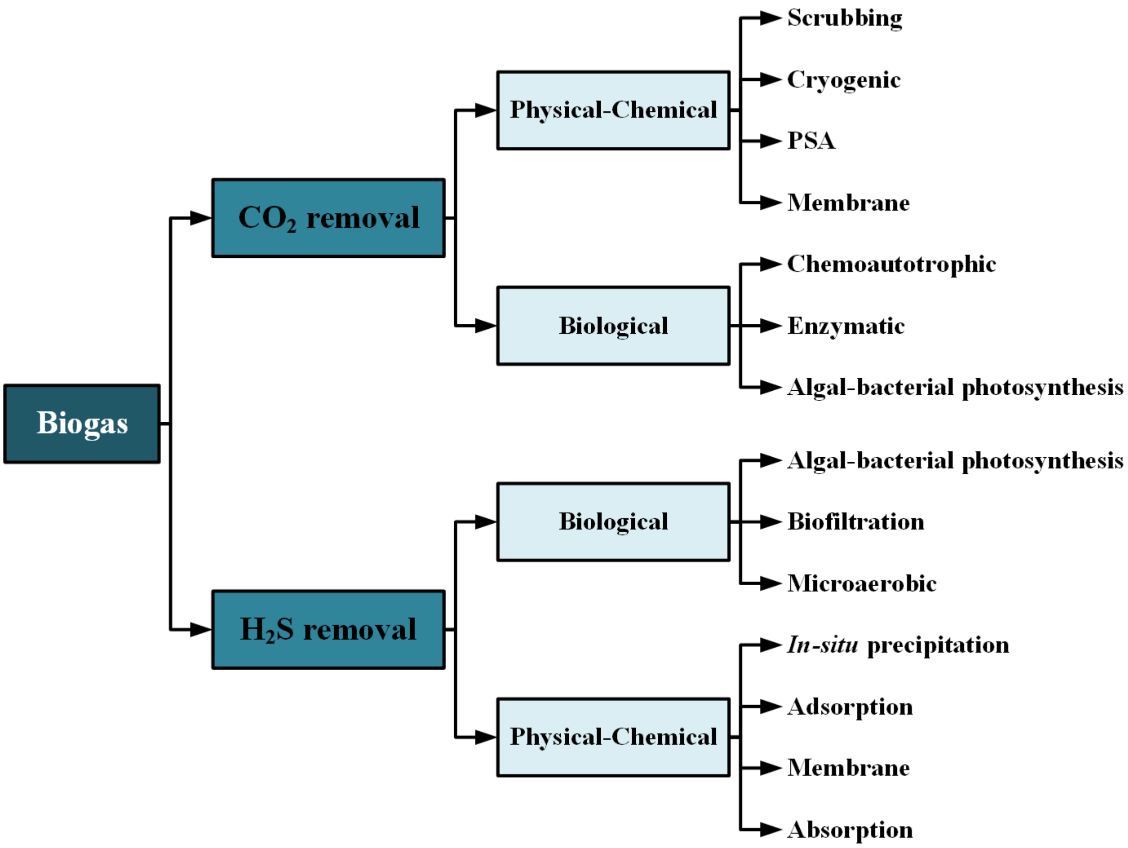

Figure 1.7. Classification of available biogas upgrading technologies for the removal of CO_2 and H_2S from biogas.

Hydrogen sulfide removal from biogas

H_2S presence in biogas is highly dependent on the organic substrate and operating conditions used in the anaerobic digestion process with values ranging from 50 ppm_v to 5,000 ppm_v (Dumont, 2015). Besides its low concentration compared to other contaminants, the presence of H_2S in biomethane entails relevant operational issues, given the formation of sulfuric acid (H_2SO_4) in the presence of water which incurs in severe corrosion and damage in piping and combustion equipment. Physico-chemical technologies dominate the market nowadays, while some biological technologies are currently being tested at semi-industrial scale, showing a better resource utilization and a notable reduction of the operational costs and of the environmental impact (Estrada et al., 2011; Pérez et al., 2020c).

Typically, H_2S removal from biogas has been performed with low complexity technologies that do not require excessive control and automation of the process or a highly specific expertise. The most common method in waste treatment plants is H_2S removal via adsorption and partial oxidation over activated carbon or ferric oxides. This method results in high elimination efficiencies with relatively low investment costs (Ryckebosch et al., 2011). However, the dangerous handling and continuous renewal and regeneration of the adsorbent materials and their treatment as hazardous waste often result in high operational costs (Abatzoglou and Boivin, 2009). Another common alternative in industry is the chemical precipitation of H_2S with ferric salts. This alternative consists of dosing ferric oxides directly to the anaerobic digester (often mixed with the organic waste feed), resulting in the in-situ precipitation of H_2S as FeS and S^0 (Park and Novak, 2013). However, the unhomogeneous mixing in the anaerobic digester and the variability in the chemical composition of the feedstock hamper the optimal dosing of the products, resulting in a high variability of the biogas H_2S concentration and in an increase in the chemical reagents cost (Tomàs et al., 2009).

In this field, multiple biological technologies have been developed at lab-scale and while some of them are being currently optimized at demonstration scale in real environments, only a few have been already implemented at industrial scale. These technologies have shown a lower chemical demand and energy consumption than their physical-chemical counterparts and a high robustness towards changes in the biogas flow and H_2S concentration (Estrada et al., 2012). The main reluctance towards the implementation of biotechnologies is the necessity of a continuous monitoring/maintenance and a deeper understanding of the biological processes by qualified staff.

Aerobic and anaerobic desulfurization in biotrickling filters and photosynthetic biogas upgrading are the most developed biological technologies for the removal of H_2S from biogas (Delhoménie and Heitz, 2005; Lebrero et al., 2016; Toledo-Cervantes et al., 2016). Aerobic and anoxic biofiltration are based on the absorption of H_2S, highly soluble in aqueous media, in a liquid mineral medium which is continuously sprayed over a packed bed in a biotrickling filter. The bacterial communities grown onto the packed bed are able to oxidize the H_2S dissolved in the liquid to sulfate. It has been demonstrated that this biological oxidation can be performed either by aerobic bacteria (aerobic biofiltration) or by anaerobic bacteria (anoxic biofiltration) if oxygen or nitrate are provided as electron acceptors for the oxidation, respectively (Almenglo et al., 2016; Delhoménie and Heitz, 2005). Both technologies have been already implemented in real industrial environments with a high efficiency for the removal of sulfur compounds.

The utilization of microalgae-bacteria consortia has been demonstrated as a low-cost, robust and efficient integral method for biogas upgrading in waste treatment plants at demo-scale (Rodero et al., 2019). In this platform technology, the gas-liquid absorption of H_2S is performed in a bubble column coupled to a photobioreactor where the microalgae-bacteria consortium is grown under optimal conditions. The H_2S transferred to the liquid medium is oxidized to sulfate by the aerobic bacteria present in the cultivation, making use of the oxygen produced by microalgae during photosynthesis. Interestingly, this technology allows an integral biogas upgrading in a single stage, given that CO_2 is also transferred to the liquid media. The solubilized CO_2 is assimilated by microalgae as a source of inorganic carbon for photosynthesis (Bahr et al., 2014). The continuous growth of microalgae in CO_2-biogas allows for a continuous harvesting of microalgal-bacterial biomass, that given its high amino acid and protein content has been demonstrated as a high quality fertilizer and bioestimulant (Kumar and Singh, 2020). This technology has also been optimized at semi-industrial scale and has been tested in real environments producing high quality biomethane. These experiments at industrial scale have confirmed previous techno-economic analysis which indicated a lower energy and resource utilization when compared to their physical-chemical counterparts (Toledo-Cervantes et al., 2017).

Carbon dioxide removal from biogas

CO_2 constitutes the major contaminant in biogas, with a concentration ranging from 35 %v·v^{-1} to 55 %v·v^{-1}, highly dependent on the redox state of the organic matter and type of anaerobic digester (Ghosh et al., 2019). CO_2 in biomethane represents an inert gas during combustion, reducing its calorific value and energy density, and hindering its transportability.

During the last decade, the CO_2 removal market for biogas upgrading was monopolized by physical-chemical scrubbing technologies with an estimated market share over 70 % in 2014 (Thrän et al., 2014). These technologies are characterized by the use of a pressurized liquid (i.e. water, organic solvents or amines) for the selective absorption of CO_2, much more soluble than CH_4 (Bauer et al., 2013; Nock et al., 2014). In a second step, CO_2 is stripped from the solvent with air or heat, recovering a bio-CO_2 rich stream and regenerating the solvent (Kapoor et al., 2020). Scrubbing technologies have demonstrated a high efficiency in the removal of CO_2, consistently achieving a highly concentrated biomethane stream (>98 %v·v^{-1}) that complies with current regulations for biomethane injection and use as automotive fuel. However, this technology presents substantial drawbacks like the recommendation of a preliminary H_2S removal stage and the high operational costs derived from the regeneration of the solvents and the high pressures employed. Despite these negative aspects and the development of competing biogas upgrading technologies, scrubbing platforms still account for 30-40 % of the current market share for biogas upgrading technologies (WBA, 2019).

The second most popular technology for biogas upgrading was Pressure Swing Adsorption (PSA), which represented 21 % of the total market share in 2014 (Thrän et al., 2014). This technology is based on the selective adsorption of CO_2 over activated carbon or zeolite materials. This process is performed in a 4-column system and is based on a sequential adsorption, blow-down, purge and pressurization (Bauer et al., 2013). This process is well-known for its low energy and fixed cost requirements and the high concentration of the biomethane obtained (96-98 %v·v^{-1}), but it presents severe CH_4 losses that might impact negatively the revenue stream of the process (Patterson et al., 2011). The rapid popularization of membrane technologies has decreased the market share of PSA platforms, which are estimated between 5-10 % of the currently installed biomethane production alternatives (WBA, 2019).

In 2014, membrane separation accounted for less than 10 % of the biogas upgrading market (Thrän et al., 2014). This technology is based on the selective permeability of CH_4 and CO_2 accross different membrane materials and entails a very efficient separation of bio-CH_4 and bio-CO_2, encouraging an integral valorization of the biogas constituents (Bauer et al., 2013). Membrane separation has developed rapidly over the last decade and has demonstrated enormous advantages in terms of decreasing energy consumption, compactness and environmental impact reduction, compared to more traditional biogas upgrading techniques (Comesaña-Gándara et al., 2022). The recent advances in material science have triggered the implementation of membrane separation systems which currently dominate the market with >50 % of the total market share (WBA, 2019).

Another interesting technology, although with a residual market share (<1 %), is the cryogenic liquefaction of the CH_4 contained in biogas under extreme conditions of pressure and temperature (80 bar and -110 ºC) (Comesaña-Gándara et al., 2022; Yousef et al., 2017). The intensive energy requirements of this technology incur in prohibitive operational costs for general applications. However, this technology is leader in the niche market of landfill biogas upgrading, given its capacity to produce liquefied natural gas from low-quality biogas with high concentrations of O_2 and N_2.

Evolution of the biomethane market

In the past decades, the level of development of biogas upgrading technologies has not allowed in many cases to obtain a biomethane competitive in price against fossil natural gas. However, the rapid development of these technologies, the decrease in the use of raw biogas for renewable electricity production, the fiscal impulse towards renewable energies and the sudden increase in natural gas prices, have opened a window of opportunity for the implementation of biogas upgrading technologies in waste treatment plants. In fact, the number of anaerobic digestion plants incorporating the production of biomethane has increased exponentially during the last decade in Europe, escalating from 182 in 2011 to 880 in 2020, in contrast to the declining trend in anaerobic digestion plants devoted to the direct combustion of biogas into heat and energy in CHP engines. Concomitantly, the energy production from biomethane has multiplied by 640 % in the last decade, from 5 TWh in 2011 to 32 TWh in 2021 (Figure 1.8) (EBA, 2021).

However, the data retrieved from the latest European Biogas Association report does not depict the current situation derived from the shortage of external natural gas in Europe. Given the patent dependency of foreign gas resources, the European Commission has recently recognized the production of biomethane from organic residues as a strategic objective for achieving energy independence. In this context, this European organism has recently announced an important investment in the biomethane industry with the objective of reaching the production of 35 bcm of biomethane by 2030, which would increase by an order of magnitude the current production of 3 bcm in 2020 (European Comission, 2022).

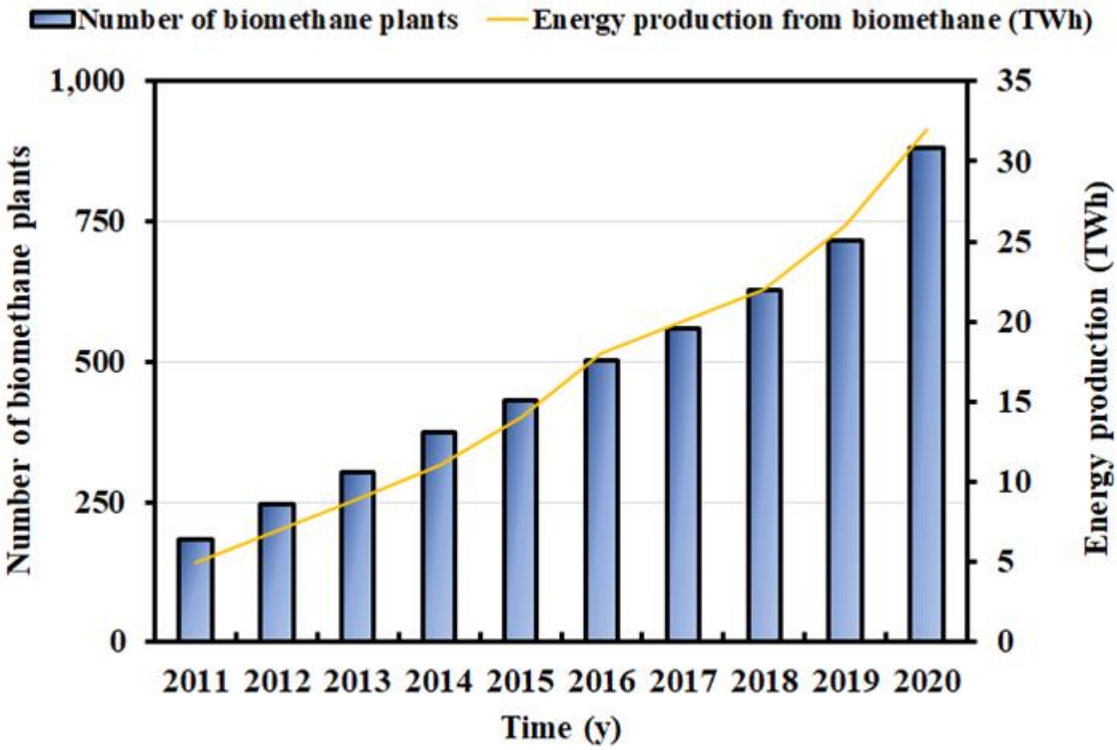

Figure 1.8. Time evolution of the number of operative biomethane plants and of the energy produced from biomethane in Europe. Adapted from (EBA, 2021).

1.3.2. Biogas reforming into syngas

In the last decade, biogas reforming has gained attention as an alternative valorization pathway able to extract the whole biogas energy potential in the form of environmentally-friendly fuels. Biogas reforming aims at the transformation of biogas CH_4/CO_2 mixtures into synthesis gas or syngas, a gas typically formed by 40-70 %v·v^{-1} H_2, 15-25 %v·v$^{-1,}$ CO and 1-2 % v·v^{-1} CO_2 (Schiaroli et al., 2022). The syngas produced can be further use as a H_2-rich stream in fuel cells for the production of electricity, converted into bio-diesel and bio-gasoline through Fischer-Tropsch processes, used as platform chemical in the methanol manufacturing or transformed into higher alcohols via fermentation (Kapoor et al., 2020). Another potential valorization route is the separation of syngas constituents, obtaining high quality H_2 and CO streams. Bio-H_2 is considered the green fuel par excellence given its zero carbon footprint and its high energy density and caloric value. Typically, H_2 produced from natural gas reforming is referred to as blue-H_2, and its competitiveness is higly reliant on the valorization of by-products such as CO and CO_2 (Jens et al., 2021). The recent increase in natural gas prices has rendered blue-H_2 less competitive against other forms of H_2, however, the production of blue-H_2 from biogas has gained attention given its price stability. On the other hand, CO is considered as a relevant intermediate molecule in the production of bulk chemicals with wide applications in the metallurgy, polymer, chemical and biofuel industries (Elschenbroich and Salzer, 2006). Recently, CO has also attracted the attention of bio-technologists as a carbon source for its biological conversion into ethanol, acetic acid or 2,3-butanediol through gas fermentation (Köpke et al., 2011). In this field, the American company LanzaTech Inc. (www.lanzatech.com) pioneered the first industrial commercialization of the biological conversion of syngas compounds into liquid fuels in 2017.

The conversion process of biogas into syngas has been widely studied since the 1920s. This thermo-chemical process is governed by a complex series of sequential and parallel equilibrium reactions that involve CH_4, H_2, CO, CO_2, H_2O and C formation (Equation 1.1-Equation 1.6) (Zhao et al., 2020). Given the high activation energy of the reaction of CH_4 and CO_2 molecules, the use of high temperatures (500-900 ºC) and specific catalysts, being niquel-based ones the most popular, is mandatory. All reaction schemes aim at maximizing the conversion of CH_4 and CO_2, and at enhancing the selectivity towards the conversion into H_2, being CO an interesting by-product.

The first process to be investigated was dry reforming, based on the direct reaction of CH_4 and CO_2 to form CO and H_2 (Equation 1.1). However, the occurrence of simultaneous side reactions and coke deposition over the catalysts limit the conversion and selectivity of the process (Charisiou et al., 2019; Jabbour et al., 2019). A less energy intensive alternative, which is a well-stablished technology at industrial level, is steam reforming based on the reaction of H_2O and CH_4 (Equation 1.2). Interestingly, the combination of these two processes, commonly referred to as bi-reforming, reduces the overall energy requirements and the deactivation of the catalysts by coke formation (Roy et al., 2018). The third known route for syngas production is the partial oxidation reforming, based on the exothermic reaction of CH_4 oxidation with air (Equation 1.3). The combination of the three aforementioned methods, commonly known as tri-reforming, is the best suited for biogas transformation and combines the advantages of low environmental impact, high CO_2 utilization and reduction of catalysts deactivation (Yoo et al., 2015). The comprehensive study of this process in the last decade has helped identifying most of the side reactions (Equation 1.4-Equation 1.6) (Walker et al., 2012). Numerous studies have worked on optimizing this process, maximizing CH_4 (>97 %) and CO_2 (>90 %) conversion values and H_2:CO selectivity (2:1) (Singha et al., 2016; Vita et al., 2014).

$$CH_4 + CO_2 \leftrightarrow 2CO + 2H_2 \qquad \text{Equation 1.1}$$

$$CH_4 + H_2O \leftrightarrow CO + 3H_2 \qquad \text{Equation 1.2}$$

$$CH_4 + \frac{1}{2}O_2 \leftrightarrow CO + 2H_2 \qquad \text{Equation 1.3}$$

$$CO + 2H_2O \leftrightarrow CO_2 + H_2 \qquad \text{Equation 1.4}$$

$$C + H_2O \leftrightarrow CO + H_2 \qquad \text{Equation 1.5}$$

$$C + O_2 \leftrightarrow CO_2 + H_2 \qquad \text{Equation 1.6}$$

1.3.3 Power-to-gas

The current transition towards renewable energies like solar and wind power, that as previously discussed, is displacing the use of biogas as energy vector for electricity production, is not exent of technological obstacles. The most urgent issue of this transition is the decoupling between energy demand and production derived from the exploitation of variable natural phenomena such as sun and wind. This unbalance leads to periods of overproduction of energy that cannot be easily stored for using during underproduction periods. This is a hot topic in the industry and academia today and at the moment, no definitive energy storage technologies are available and feasible at a large scale. Nowadays, four strategies for energy storage are under study: (i) gravitational/mechanical, (ii) electrochemical, (iii) thermal and (iii) chemical (Dodds and Garvey, 2022).

Gravitational/mechanical strategies are based on the transformation of the energy surplus on kinetic, potential or pressure energy transmitted to solid or fluid substances. The most common strategy in this field, and the only strategy currently applied at large scale, is the use of 2-level hydroelectric facilities. These facilities are built as a two-stage water reservoirs at different height levels. This configuration allows using the surplus of energy generated by renewable energies to pump water to the upper level, storing energy in the form of potential energy. This potential energy can be released to the lower level through turbine systems in order to transform the potential energy into electricity when the demand exceeds the renewable energy production (Uria-Martinez et al., 2021). Nevertheless, this system is not applicable worldwide as it is restricted to very specific geographical relief and water availability conditions.

Electrochemical processes are based on the storage of the renewable energy surplus in large-scale lithium-ion batteries. However only a few experiences have been implemented at large scale given its prohibitive investment costs and reduced lifespan (IRENA, 2019a). Thermal strategies aim at the preservation of energy in the form of heat that can be further removed or released to produce energy. The use of molten salts has been applied at large scale given its particular properties regarding boiling point, viscosity and heat capacity (Dodds and Garvey, 2022). However, this strategy suffers from high energy losses given the difficulty of preventing temperature changes in heated/cooled materials.

Chemical strategies involve the storage of surplus renewable energy in the form of chemical energy in high density fuels. When the fuel produced for energy storage is CH_4 or H_2, this technology is referred to as Power-to-Gas. A promising option, which is currently under study and that is gaining great attention is the production of H_2 via water electrolysis. This process, which is highly energy intensive and therefore not cost-efficient by itself, becomes extremely attractive when renewable energy surplus is used. The storage of energy as H_2 is a great alternative from an environmental point of view as only water is required as raw material and oxygen is the only by-product of the process.

Additionally, this technology has substantial potential sinergies with the aforementioned biogas valorization routes such as upgrading and reforming, that could help guaranteeing energy efficiency and energy independence in these facilities. Interestingly, methanation processes that can be performed either thermo-chemically or biologically, consisting of the reaction of CO and CO_2 with H_2 to form CH_4, allow closing the cycle of renewable energy, biogas, biomethane and hydrogen (Paniagua et al., 2022). CO_2 produced during biogas upgrading or direct biogas combustion could be transformed into biomethane by reacting with the bio-H_2 produced during periods of renewable energy overproduction. The H_2 supply during the renewable energy valley production periods could be substituted by hydrogen produced from biogas catalytic reforming. This strategy could help to the transition towards energy-independent waste biorefineries, guaranteeing a versatile portfolio of biofuels (H_2, CH_4), bioproducts (CO, O_2) and bio-energy (direct combustion). This versatility would provide waste treatment plants with improved economic revenues and a paramount independency from energy and natural gas prices. Additionally, the use of biogas as energy vector, instead of competing in production costs with other renewable energies, can play a crucial role for the energy market acting as buffering agent and contributing to the stabilisation of the global energy bulk unbalances between production and demand.

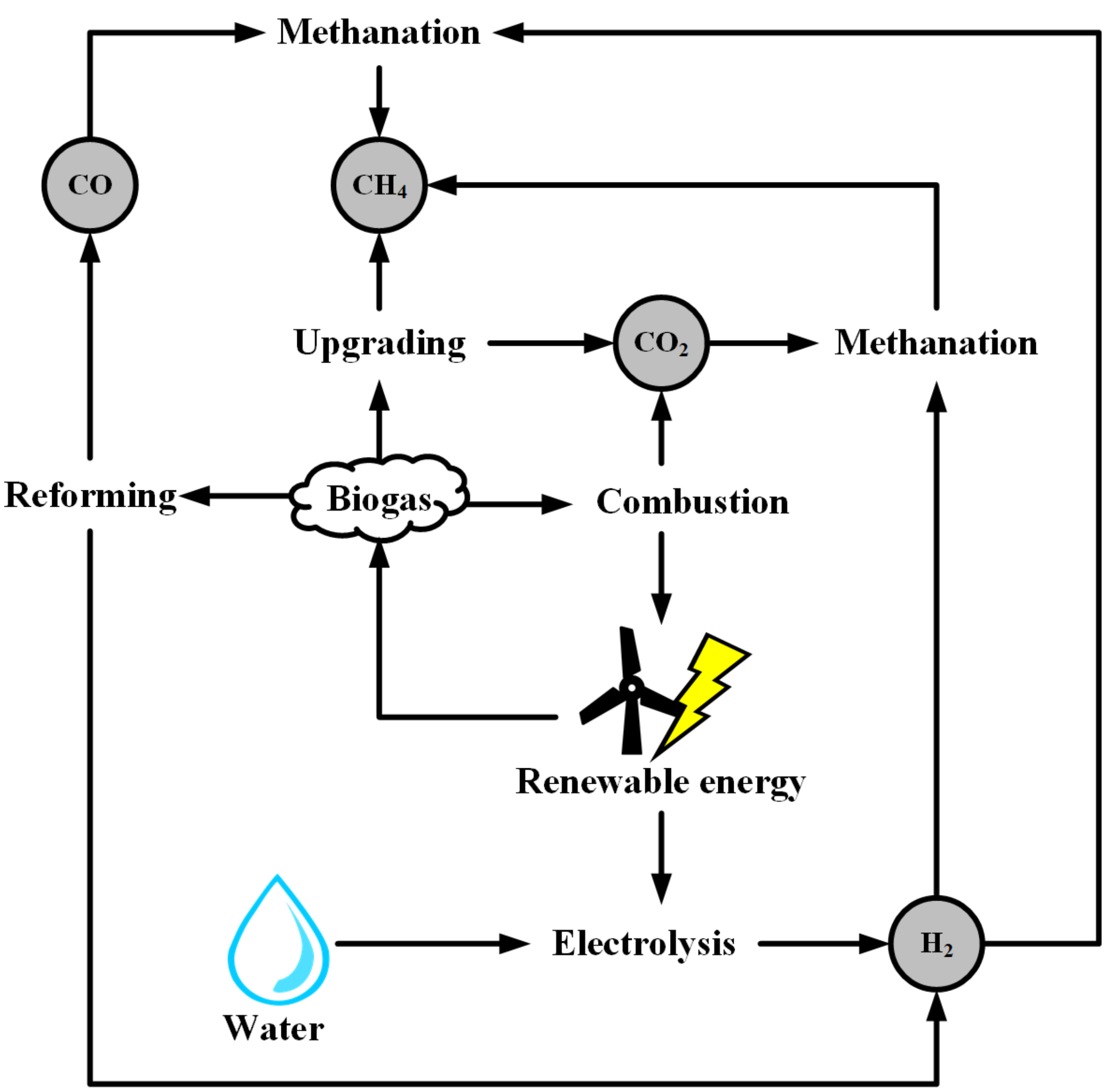

Figure 1.9. Schematic representation of the sinergies between biogas valorization technologies and power-to-gas energy storage.

1.4 Biological transformation of biogas into added-value products

To a smaller extent than the direct combustion of biogas into bioenergy, the cost-efficiency of the production of biofuels from biogas is still dictated by the energy and natural gas prices. The “Innovation for sustainable growth” report indicated clearly the steps towards a smarter use of waste resources: direct energy production and biofuel production are at the bottom of the added-value scheme, while the synthesis of higher-added-value chemicals from biomass are at the top (European Commission, 2012; Ganzevles et al., 2015). In this context, and with the recent adoption of newer directives towards a more circular and sustainable bioeconomy, a great number of biological technologies focused on a better utilization of waste resources have gained attention given its unparalleled transformative potential (European Commission, 2020). Hereby, the implementation of biological technologies within the biorefineries concepts, capable of offering a wide portfolio of higher added-value products than biofuels and bioenergy, is key for the future development of a viable circular bioeconomy.

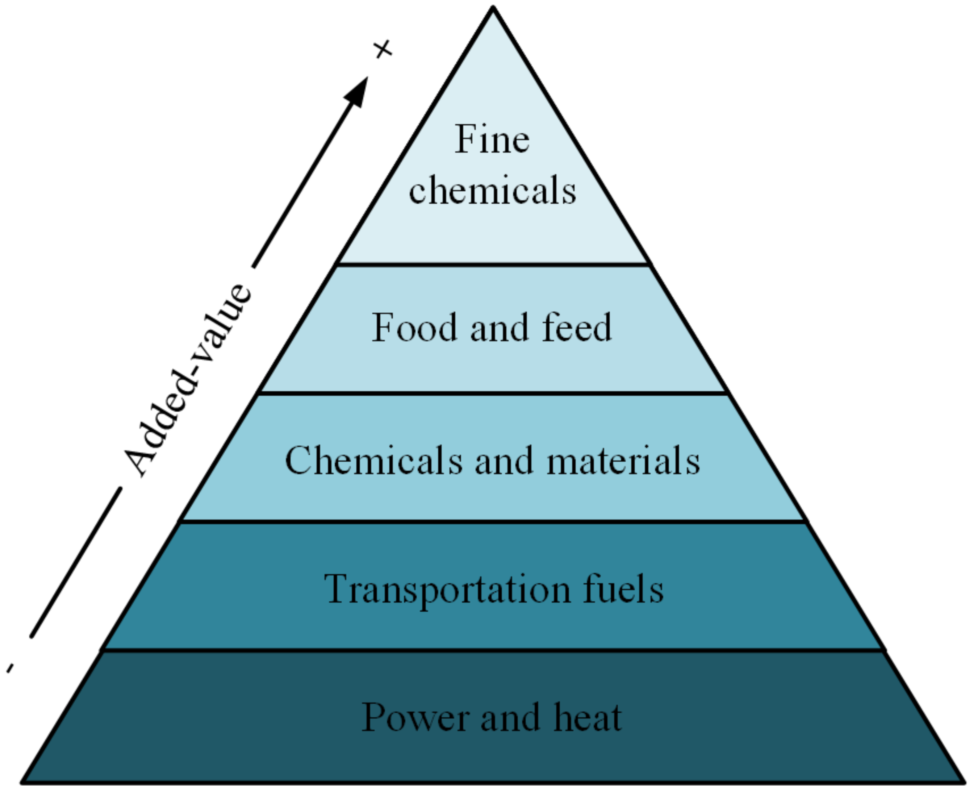

Figure 1.10. Pyramid of added-value for the valorization of waste into bioproducts. Adapted from (Ganzevles et al., 2015).

1.4.1 Biological valorization of CO_2- biogas into added-value products

Rationale and biological aspects for CO_2 biological valorization

According to the World Bank, more than 3,000 Mt of CO_2-equivalents are being yearly emitted by waste treatment plants, as a cause of the underutilization of biogas via flaring or venting to the atmosphere (World Bank, 2019). Avoiding CO_2 emission to the atmosphere, and particularly the CO_2 contained in biogas, is critical from an environmental point of view, given the growing global climatic crisis. As previously mentioned, the use of CO_2 or bio-CO_2 as raw material for the production of biofuel through methanation is undoubtedly one of the technologies that is generating more interest, given its potential to close the carbon cycle regarding biofuels (Paniagua et al., 2022). The upgrading of biogas and the subsequent use of bio-CO_2 has demonstrated its viability in a wide range of applications in the agricultural and food industries. The commercialization of the bio-CO_2 separated from biogas can be a relevant supplement to the revenue stream of biorefineries dedicated to biomethane production. However, the CO_2 market is extremely competitive as concentrated CO_2-streams are generated as byproduct in a great number of industries at much larger quantities, hampering the CO_2-biogas competitiveness in terms of product quality and production costs (Wang et al., 2021). In this sense, the capture and valorization of the CO_2 contained in biogas by means of biological processes has gained attention in the last few years given its capacity to offer a wide alternative portfolio of higher added-value products.

Nonetheless, the use of CO_2 for its biological transformation is less attractive and more inefficient than the valorization of CH_4, given the oxidation state of the CO_2 molecule. This chemical characteristic entails a significant obstacle towards CO_2 bioconversion, given the continuous requirement of an electron input in the form of electricity in fuel cells, photosynthesis or hydrogen. A great number of microorganisms, known as autotrophs, have been recognized as able to metabolize CO_2 into different forms of biomass. The assimilation of CO_2, and its subsequent transformation into bioproducts of high industrial interest, have been widely studied in different families of microalgae, cyanobacteria and bacteria. In this sense, four different CO_2 assimilation pathways have been described in nature: the Calvin cycle, the reductive TCA cycle, the reductive acetyl CoA pathway and the 3-hydroxypropionate cycle (Kumar et al., 2018).

CO_2-biogas biological transformation into added-value products

Traditionally, biological technologies for CO_2-biogas valorization have aimed at carbon sequestration in the form of biomass. The simplest valorization pathway for this biomass is based on its utilization as feedstock for anaerobic digestion processes and the subsequent production of biogas, closing the cycle of CH_4 and CO_2 production (Carrillo-Reyes et al., 2021). However, the anaerobic digestion of bacterial and microalgal cultures has been shown as extremely unefficient given the high water content and the recalcitrant cell wall of microalgae. Some studies have evaluated the capacity of autotrophic bacteria and microalgae to fix CO_2 in the form of intracellular and extracellular lipids, which could be further converted into biodiesel (Bharti et al., 2014a; Mata et al., 2010). However, the economics of the biomass-to-biofuels production is highly constrained by the fossil-fuel market and the economy of scale. In addition, the production of other low added-value products such as biosurfactants or $CaCO_3$ has been reported in literature by CO_2-fixating microorganisms (Bharti et al., 2014b; Maheshwari et al., 2017).

In parallel, the combined utilization of CO_2-biogas and nutrient-rich digestate for microalgal biomass growth has gained great attention given its outstanding properties as high-quality bioestimulant and biofertilizer (Kumar and Singh, 2020; Toledo-Cervantes et al., 2016). As previously mentioned, this fixation strategy would be especially interesting if it is coupled with the production of biomethane via photosynthetic biogas upgrading. This strategy would allow the transformation of two anaerobic digestion byproducts such as digestate and biogas into two added-value products such as fertilizers and biomethane (Toledo-Cervantes et al., 2017). Similarly, a recent work has described the use of the CO_2 fixating bacterium *Actinobacillus succinogenes* for the combined biogas upgrading and succinic acid production (Gunnarsson et al., 2014).

During the last decade, a large number of publications have indicated the great potential for CO_2 assimilating microorganisms to produce higher added-value products. Particularly, Kumar and coworkers have consistently reported the versatility of *Serratia sp.* to produce a wide portfolio of bioproducts of interest such as extracellular polysaccharides (EPS) and polyhydroxyalkanoates (PHA) (Kumar et al., 2016, 2019). However, the potential industrial applications of this microorganism is still unknown. Interestingly, a recent study explored the environmental impact of the utilization of CO_2 as substrate for single cell protein (SCP) production, showing a negative carbon footprint of the process (-1.1 kg $CO_2 \cdot kg^{-1}$ SCP) (Van Peteghem et al., 2022). More recently, Cantera and colleagues have presented the first proof-of-concept for the autotrophic production of ectoines, the biological product with the highest retail market price (600-1000 €·kg^{-1}) (Cantera et al., 2022).

1.4.2 Biological valorization of CH_4-biogas into added-value products

Biological aspects of CH_4-biogas transformation

The taxonomy of methanotrophic bacteria and their potential for CH_4 bioremediation have been known already for over 50 and 40 years, respectively (Semrau et al., 2011; Whittenbury et al., 1970). Despite a great number of microorganisms such as green algae, fungi and yeasts have demonstrated high CH_4 abatement capacities, the utilization of methanotrophic bacteria, and most particularly of aerobic methanotrophic bacteria, has been the most popular among researchers given their capacity to transform CH_4 in a wide range of bioproducts of industrial interest such as methanol, polyhydroxyalkanoates (PHA), single cell protein (SCP) or ectoines (Pieja et al., 2017).

Aerobic methanotrophic bacteria are methylotrophic microorganisms with the ability to perform the oxidation of CH_4 in the presence of O_2 as their sole source of carbon and energy. Traditionally, aerobic methanotrophs have been classified into (i) type I, (ii) type II and (iii) type X methanotrophic bacteria based on their physiological and morphological characteristics. In very general terms, for carbon assimilation, type I are characterized by the use of the ribulose monophosphate pathway, type II by the use of the serine pathway and type X by the combined use of the ribulose monophosphate pathway for formaldehyde assimilation and of the serine pathway for CO_2 assimilation (Karthikeyan et al., 2015).

CH_4 biodegradation is a complex and sequential process that initiates with the oxidation of CH_4 to methanol mediated by the monooxygenase enzyme (which can be either soluble or particulate). The second step consists of the transformation of methanol into formaldehyde, which acts as a metabolic intermediate in the subsequent reactions. Formaldehyde is then converted either to formic acid and through a series of enzyme-catalyzed reactions into CO_2, or either used as building block for the synthesis of new cell materials (López et al., 2013). Thus, a fraction of CH_4 is mineralized to form CO_2 and the remaining fraction is used for the construction of new cells and/or the production of the bioproducts of interest.

On the other hand, O_2 plays two differentiated roles in the methanotrophic CH_4 oxidation (Rostkowski et al., 2013). First, energy is produced by the reduction of O_2 into H_2O using a fraction of the CH_4 electrons (f_e) (Equation 1.7).

$$f_e(\frac{1}{4}O_2 + H^+ + e^- \rightarrow \frac{1}{2}H_2O) \qquad \text{Equation 1.7}$$

And second, O_2 is employed as a reactant in the oxidation reaction of CH_4 (Equation 1.8).

$$\frac{1}{4}CH_4 + \frac{1}{4}O_2 \rightarrow \frac{1}{4}CO_2 + H^+ + e^- \qquad \text{Equation 1.8}$$

Nitrate and ammonium have been demonstrated as the preferred nitrogen sources for aerobic methanotrophs over nitrite (Dunfield and Knowles, 1995). The most recent experiments have demonstrated a superior pH and long-term cultivation stability with the use of nitrate as nitrogen source, and therefore it is preferred for the potential scale-up of the process (Rodríguez et al., 2020a). Selecting nitrate as the nitrogen source, the remaining fraction of CH_4 electrons (f_s) that is employed in the synthesis of new cell is adjusted with Equation 1.9.

$$f_s\left(\frac{1}{28}[5CO_2 + NO_3^- + 29H^+ + 28e^- \rightarrow C_5H_7O_2N + 11H_2O]\right) \qquad \text{Equation 1.9}$$

Therefore, the overall stoichoimetric reaction of CH_4 oxidation via methanotrophic bacteria using nitrate as nitrogen source is obtained as the sum of previous reactions (Equation 1.10).

$$\left(\frac{1}{4}\right)CH_4 + \left(\frac{f_e+1}{4}\right)O_2 + \left(\frac{f_s}{28}\right)NO_3^- + \left(\frac{29}{28}\cdot f_s + f_e - 1\right)H^+ \rightarrow$$

$$\left(\frac{1}{4} - \frac{5f_s}{28}\right)CO_2 + \left(\frac{f_e}{2} + \frac{11f_s}{28}\right)H_2O + \left(\frac{f_s}{28}\right)C_5H_7O_2N \qquad \text{Equation 1.10}$$

Optimal cultivation conditions for methanotrophs have been widely studied in literature and it has been observed that parameters such as the $O_{2:}CH_4$ ratio, temperature, pH and the presence of certain micronutrients are determinant (Rodríguez, 2022). It must be stressed out that optimal conditions for methanotrophic growth are higly strain-dependent and they must be re-considered for each bioconversion technology proposed.

According to the overall stoichiometric reaction presented in Equation 1.9, $O_{2:}CH_4$ ratios ranging from 1:1 to 2:1 are required for an appropriate CH_4 oxidation. In this regard, $O_{2:}CH_4$ ratios of approximately 1.5 have been typically reported in literature as optimal for maximizing CH_4 biodegradation in methanotrophic bioreactors (López et al., 2018a). Methanotrophic growth has been typically assumed to be optimized under mesophilic conditions of temperature (25-30 ºC). However, recent works devoted to the exploration of the bioconversion of CH_4 into PHA and ectoine have demonstrated a high strain-dependency of the temperature. Thus, a mixed methanotrophic culture enriched from activated sludge and *Sphagnum mosses* presented significantly higher PHA accumulation yields at temperatures ranging from 30 ºC to 37 ºC than the cultures enriched at 25 ºC (Pérez et al., 2019). On the contrary, two different mixed haloalkaliphilic methanotrophic cultures obtained from saline natural environments devoted to the production of ectoine showed optimal growth conditions ranging from 15 ºC to 25 ºC (Carmona-Martínez et al., 2021). In addition, mild pH conditions (6.5-7.5) have generally shown to be optimal for growing most methanotrophic cultures. However, some methanotrophic species from *Methylocella* and *Methylocapsa* genera have been isolated from acidic environments (pH 2-2.5) (López et al., 2013). On the contrary, *Methylomicrobium alcaliphilum 20z*, extensively investigated for its capacity to accumulate ectoine, grows optimally at pH values near 9 (Kalyuzhnaya et al., 2013).

The extensive work performed with haloalkaliphilic microorganisms has also showed the paramount importance of micronutrients concentration in the mineral medium and its high strain-dependency. High copper concentrations promoted an undesired excretion of intracellular ectoine during methanotrophic growth (Cantera et al., 2017a). The lack of tungsten in the mineral medium entailed a significant reduction of the CH_4 abatement efficiency, given the accumulation of formic acid (Akberdin et al., 2018). In addition, high magnesium concentrations showed an enhancing effect on the ectoine yields but inhibited PHA accumulation in a mixed methanotrophic culture (Cantera et al., 2018b).

CH_4-biogas biological transformation into bio-based chemicals: Methanol

Methanol, with a global demand of more than 95 million $m^3 \cdot y^{-1}$, is considered one of the most important and versatile platform chemicals in the chemical industry. Methanol is used as an intermediate molecule in the production of gasoline and biodiesel additives, anti-freezing agents and a wide range of solvents (Hobson et al., 2018; IEA-ETSAP and IRENA, 2013). The synthetic route for methanol production is dominant in the market and consists of the catalytic conversion of syngas. Unfortunately, more than 97 % of the global methanol production is based on the previous gasification of fossil fuels for the obtention of the required syngas. Particularly, gasification of natural gas is behind 70 % of the total methanol production, with the subsequent adverse environmental impact (Methanol Institute, 2022). Traditionally, the methanol market has been stable with prices ranging 100-200 $€ \cdot t^{-1}$, against which biotechnological alternatives could not compete. However, the current context of unstability of natural gas prices has boosted methanol prices (500 $€ \cdot t^{-1}$ in Europe in 2022) and as a consequence has revitalized the interest of the industry for bio-methanol alternatives.

The potential of different carbon substrates for its bioconversion into methanol has been described consistently in literature. However, the methanol production from CH_4 with methanotrophic bacteria constitutes one of the most interesting candidates given the high efficiency, simplicity and high selectivity of the process (Bjorck et al., 2018). Some investigations have been performed for biomethanol production with methanotrophs at laboratory scale. Experiments with *Methylosinus tricosporium* has shown promising results with a methanol concentration of 1.1 $g \cdot L^{-1}$ and a conversion efficiency of 64 % (Duan et al., 2011). The main disadvantage encountered of the methanotrophic transformation of CH_4 into methanol is the subsequent oxidation of methanol by the enzyme methanol dehydrogenase which decreases the overall methanol selectivity. Different inhibitors of this enzyme such as chelating agents, sodium chloride or iodoaceate are currently being investigated for boosting the CH_4 conversion efficiency into methanol. In this context, Han and coworkers obtained excellent conversion results (80 %) when using NaCl and NH_4Cl as inhibition agents for a mixed methanotrophic culture (Han et al., 2013).

CH_4-biogas biological transformation into bio-based materials: PHA

Polyhydroxialkanoates (PHA) constitute a family of biopolymers that have gained focus from the academia and the industry given their biodegradability nature and their physical-chemical properties, comparable to those of oil-based plastics (Rodríguez et al., 2020c). A wide range of prokaryotic microorganisms have demonstrated the ability to produce intracellular PHA inclusions under carbon sufficient and nutrient limiting conditions (Castilho et al., 2009). The natural accumulation of PHA serves bacteria as a carbon and energy storage compound (Strong et al., 2016). The first industrial production of PHA dates back to the 1980s and currently over 30 companies are devoted to the industrial manufacturing of PHA. The current global PHA production is estimated in more than 50,000 $t \cdot y^{-1}$ and the market growth trends are ascending in view of a demand that far exceeds the current production (Rodríguez, 2022).

Poly(3-hydroxybutyrate) (PHB) is the most well-established and popular member of the PHA family due to its great potential for substituing propylene in plastic blends (Koller et al., 2010). The most common PHB production processes employ sugar or beet molasses as carbon feedstock for commercial scale production (Bugnicourt et al., 2014). However, the cost of these raw materials accounts for 40-50 % of the total production costs, making PHA compounds less competitive against their oil-based counterparts with general prices ranging 4-20 $€ \cdot kg^{-1}$ (Castilho et al., 2009). The utilization of waste sugar residues for PHA production has been thoroughly investigated with outstanding results for decreasing production costs, however, the unstable composition of waste feedstock hinders the industrial implementation of these biorefinery concepts (Pérez et al., 2020c).

Using the CH_4 produced during anaerobic digestion of organic waste as substrate for the production of PHA would be very advantageous from an economic point of view, given its bulk and ubiquous availability, its constant and uniformous composition and its low cost. Interestingly, a recent life cycle assessment demonstrated the favorable environmental potential for integrating PHB production in waste treatment plants, coupled to the production of energy from biogas (Rostkowski et al., 2012).

In this context, type II methanotrophic bacteria, capable of using CH_4-biogas as their only carbon and energy source, have demonstrated the capacity to accumulate high amounts of PHA under nitrogen feast-famine cycles (López et al., 2018a). Notable efforts have been made in the optimization of these processes at laboratory and semi-industrial scale. In recent years, researchers from the Institute of Sustainable Processes of the University of Valladolid (Spain) have consistently demonstrated the feasibility of the biogas-to-PHA production process in bubble column bioreactors implementing a gas recirculation strategy. These studies have shown high PHB accumulation yields of 0.4 $gPHB \cdot g^{-1}$ biomass and CH_4-EC of 74.4 $g \cdot m^{-3} \cdot h^{-1}$ (García-Pérez et al., 2018; Rodríguez et al., 2020b). Based on these investigations, the same research group has implemented this technology in a 9 m^3 bubble column bioreactor, within the biorefinery project URBIOFIN, aimed at the demonstration at semi-industrial scale of different biological technologies for biogas valorization. Similarly, two companies are currently exploring the industrial production of PHB from biogas, namely Newlight Technologies (www.newlight.com) and Mango Materials (www.mangomaterials.com). Nonetheless, and similarly to other methanotrophic-based products, the scalability and the real reduction costs by the use of biogas are still to be unveiled.

CH_4-biogas biological transformation into feed and food: Single cell protein

The CH_4 bioconversion process for the production of SCP to be used as animal or human feed constitutes the paradigm of the industrial application of methanotrophic bacteria. The first industrial production of SCP for animal feed dates back to the 1960s (Gęsicka et al., 2021). Currently, four companies are leading the market of the industrial production of SCP from the CH_4 contained in natural gas: Calysta Inc. (www.calysta.com), Unibio A/S (www.unibio.dk), Circe Biotechnologie GmbH (www.circe.at) and String Bio Pvt Ltd (www.stringbio.com). The whole bioprocess is relatively simple and is based on the supply of natural gas and pure oxygen streams to a high-mass transfer bubble column, airlift or U-Loop bioreactor for the growth of pure bacterial methanotrophic cultures. The use of mixed methanotrophic cultures is under investigation, given the high protein content of methanotrophs, ranging between 50 % and 80 % in dry weight. Industrially, pure cultures of *Methylococus capsulatus* have been commonly used given their high protein content (70 % in dry weight) and the favorable aminoacid profile to be used as feed for fish, pigs, chickens and domestic animals such as cats and dogs (Gęsicka et al., 2021; Pieja et al., 2017). Besides the high protein content and benign aminoacid profile, methanotrophic SCP has demonstrated benefitial properties for the digestibility and the improvement of animal health (Øverland et al., 2010). Downstream processing consists of a preliminar dewatering step, a thermal pretreatment for reducing the nucleic cid content and a final drying step in order to obtain the solid product. Despite methanotrophic SCP production is currently allocated to fish, rumiants and domestic animal feed, future prospects on a global protein and food shortage make methanotrophic SCP a potential candidate for human food production (Yazdian and Hajizadeh, 2005).

Recently, and in view of the global crisis of biogas, several studies have analysed the possibility of using biogas instead of natural gas for SCP production with methanotrophic bacteria. These studies have demonstrated that a preliminary desulfurization step should be recommended as its presence might impact negatively the protein yields and aminoacid profile of the product (Tsapekos et al., 2020; Xu et al., 2020). In fact, a recent work performed with upgraded biogas has demonstrated comparable protein contents (70 % in dry weight) compared to the natural gas-based process (Acosta et al., 2020).

CH_4-biogas biological transformation to fine chemicals: Ectoine

Ectoine, with a retail market price of 600-1000 €·kg^{-1}, is recognized as the highest added-value product that is produced with bacteria in current times (Strong et al., 2015). Chemically, ectoine is a cyclic imino-acid (2-methyl-1,4,5,6-tetrahydropyrimidine-4-carboxylic acid) that is used for bacteria as osmotic agent, preventing cell lysis under high salinity and pH conditions (Czech et al., 2018). The market of this fine chemical, with an estimated global demand of 20 t·y^{-1}, is growing rapidly given its outstanding properties in pharmaceutical and cosmetic applications (Liu et al., 2019; Strong et al., 2015). The ectoine market is nowadays monopolized by the German company Bitop AG (www.bitop.de), responsible for 90 % of the global ectoine production. However, new actors have announced recently their interest in expanding the ectoine market, such as the Chinese corporation Bloomage Biotechnology (www.bloomagebioactive.com).

The traditional process for ectoine production is based on long fermentations (~120 h) with *Halomonas elongata*, using sugar-based raw materials as the main carbon source. The use of specialized carbon sources and large hydraulic residence times incurs in high capital and operational costs, which impact on the the final cost of the product (Becker and Wittmann, 2020; Strong et al., 2015). The release of the intracellular ectoine to the culture media is mediated by a sudden change in salinity, known as hypo-osmotic shock. This process present a high advantage over similar bioconversion processes, as biomass can be repeteadly recycled to the bioreactor as the so-called “bacterial-biomilking” does not have a negative effect on bacterial cells (Chen et al., 2017; Pastor et al., 2010). However, the high salinity concentration and the low ectoine titer hinders a cost-efficient downstream process (Kunte et al., 2014). A great effort has been made in this field for the use of genetically modified bacteria that naturally excrete ectoine under mild saline conditions, increasing the ectoine-to-salt ratio. However the use of genetically modified microorganisms raises some legal and ethic concerns, particularly for products intended for human consumption (Becker and Wittmann, 2020).

The use of biogas as a widely available and cost-free carbon source for ectoine production has been investigated for a decade. The extensive work performed by Cantera and coworkers demonstrated the feasibility of pure and mixed methanotrophic bacterial cultures for bioconverting CH_4 into ectoines such as ectoine and hydroxyectoine (Cantera et al., 2020). This work was followed by Rodero and coworkers, which demonstrated the robustness of this biogas bioconversion process under long term and continuous operation in bubble column bioreactors equipped with internal gas recirculation (Rodero et al., 2022). In addition, the most recent investigations revealed that the biomilking process in methanotrophic bacteria was extremely effective with > 70 % of the intracellular ectoine released under very low empty bed residence times (EBRT) (<10 min) (Rodero and Muñoz, 2021).

Hence, exploring the scalability of biogas-to-fine chemicals schemes like the one herein presented would definitely impact positively the implementation of biogas bioconversion processes, showing that processes based on methanotrophic bacteria are not only restricted to the production of low added-value products. In particular, the Institute of Sustainable Processes is currently working on the semi-industrial implementation of this technology integrated in a larger waste treatment facility in the framework of two European projects: DEEP PURPLE and CHEERS.

1.5 Challenges in the industrial application of methanotrophs

Notwithstanding the comprehensive research on aerobic methanotrophic processes and its envisaged benefitial applications in the field of biogas valorization, the industrial application of these technologies and the commercialization of methanotrophic bioproducts still faces serious challenges. Most of these questions are common to all the aforementioned products and are similar to those found in other applications in the biological gas fermentation field (Strong et al., 2015). While all the challenges could be presented in isolation, the different issues must often be considered as a whole as they are interconnected. The main biotechnological barriers towards the full scale implementation of methanotrophic bioproducts can be classified into (i) enhancement of CH_4 and O_2 solubilization, (ii) improvement of the bacterial methanotrophic yields and (iii) development of more cost-efficient downstream methodologies.

1.5.1 Enhancement of CH_4 and O_2 solubilization

The major obstacle towards the implementation of CH_4-biogas bioconversion processes based on methanotrophic bacteria is the limited mass transport of CH_4 and O_2 from the gas phase to the cultivation media, where the bioconversion reaction takes place. It is well-established that gas-liquid mass transfer is the key limiting step in the bioconversion kinetics and therefore, limits the CH_4-elimination capacity (CH_4-EC) and subsequently slows down biomass growth and bioproduct productivity. The low solubility of these gaseous compounds is motivated by the low partition coefficients of CH_4 and O_2 in aqueous media. Typically, CH_4-EC is calculated with Equation 1.11, where k_La_{CH4} stands for the volumetric mass transfer coefficient of CH_4, C_{CH4in} represents the inlet concentration of CH_4 in the gas phase, $C_{L,CH4}$ indicates the aqueous concentration of CH_4 in the liquid phase and H_{CH4} is the Henry law dimensionless constant (28.7 at 30 ºC) (Equation 1.11) (Muñoz et al., 2018; Sander, 2015). In biological processes limited by mass transfer such as the ones presented in this thesis, $C_{L,CH4}$ is considered negligible and the calculation can be simplified into Equation 1.12.

$$CH_4 - EC = k_L a_{CH_4} \cdot (C_{\frac{CH_4,in}{H_{CH4}}} - C_{L,CH_4}) \qquad \text{Equation 1.11}$$

$$CH_4 - EC = \frac{k_L a_{CH_4} \cdot C_{CH_4,in}}{H_{CH_4}} \qquad \text{Equation 1.12}$$

Therefore, three variables can be modified in order to increase the CH_4–EC rate: (i) the partition coefficient, (ii) the inlet concentration of CH_4 and (iii) the volumetric mass transfer coefficient of the bioreactor.

Influence of the partition coefficient

The Henry law constant depends on the compound and the temperature and pressure conditions (Sander, 2015). A decrease in the operational temperature would enhance the solubilization of gas compounds in the liquid phase, but it would decrease severely the biomass growth rate and concomitantly the process volumetric productivity, as it has been stated in literature that methanotrophic biomass is tremendously sensitive to temperature (with optimal growth conditions between 15 ºC and 25 ºC) (Pérez et al., 2019). An increase in pressure would significantly decrease the value of the Henry law constant, enhancing the CH_4 mass transfer. However, this would incur a significant increase in the capital and operational costs of the processes. This trade-off between increasing solubility and process performance, and increasing capital and operational expenditures, has not yet been studied carefully in literature. Another strategy that influences the partition coefficient is the use of a secondary non-aqueous phase (NAP) with a higher affinity for CH_4. This strategy would allow a secondary flux of CH_4 from the gas phase to the NAP, and from the NAP to the liquid (Lebrero et al., 2019). This strategy has been thoroughly studied for CH_4 abatement technologies and the use of silicone oil as NAP has shown improvements of 145 % in the CH_4-EC (Rocha-Rios et al., 2010). However, given the viscous nature of silicone oil and its tendency to adhere to the bioreactor walls and to the biomass, its utilization greatly hampers the biomass harvesting and would impact negatively the bioproduct downstream processing.

Influence of the inlet gas concentration

A higher CH_4 gradient between the liquid and gas phases would significantly increase the CH_4-EC. However, this parameter is constrained by biological and safety factors (Pieja et al., 2017). Most studies assessing the bioconversion of CH_4-biogas into bioproducts using methanotrophic bacteria have demonstrated that O_2:CH_4 ratios higher than 1.5:1 are required for an stoichometric degradation of CH_4 and for the long-term operation of these bacterial cultures (López et al., 2018a). When using biogas as CH_4-source, and assuming a 60 $\%v \cdot v^{-1}$ CH_4 concentration, the utilization of a minimum 1.5:1 O_2:CH_4 molar ratio already restricts the CH_4 inlet concentration to 12 $\%v \cdot v^{-1}$ (Figure 1.11.A). The use of enriched air or pure oxygen instead of air for supplying O_2 to the gas mixture could help increasing the CH_4 inlet concentration to over 30 $\%v \cdot v^{-1}$ (Figure 1.11.B). However, the use of pure oxygen or enriched air increases significantly the operational costs and its utilization could be restricted to the production of higher added-value products from CH_4. Notwithstanding, this option has not been studied carefully in literature and should be taken into account as it would significantly reduce the volume of the bioreactors and enhance the productivity of the process. On the other hand, most popular methods for bioconversion of biogas into bioproducts have avoided the explosion range of CH_4, which is often considered to be between 5 and 15 $\%v \cdot v^{-1}$ in the mixture with air (Rodríguez et al., 2020b). Therefore, this constraint limits even more the inlet CH_4 concentration to 4-4.5 $\%v \cdot v^{-1}$ (Figure 1.11). Similar to the use of pure oxygen, this point has not been reviewed carefully in literature and it would be interesting to study in detail the compromise between the increase in capital and operational costs derived from the use of explosive mixtures, in comparison to the potential increases in CH_4-EC and bioproduct production.

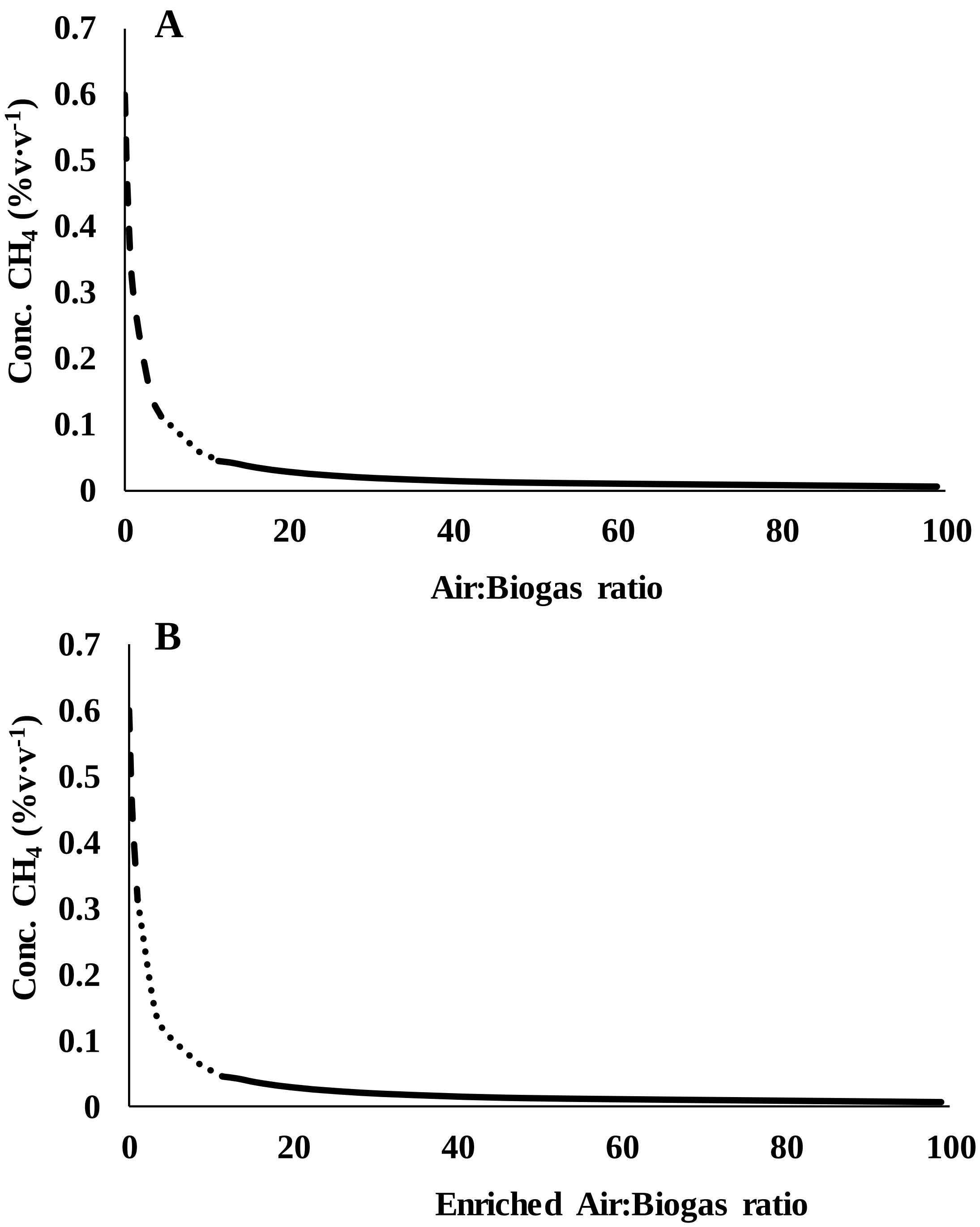

Figure 1.11. Operational ranges for biogas bioconversion into added value products using (A) air and (B) enriched air (98 %v·v^{-1} O_2) for supplying oxygen to the gas mixture. Continuous line represents the operational region below the explosion limit of CH_4. Dotted line represents the operational region within the explosion limits of CH_4. Dashed line represents the non-operational region with O_2:CH_4 ratios below 1.5:1.

Influence of the k_La

The most currently studied and viable way of incrementing the gas-liquid mass transfer efficiency is the enhancement of k_La in bioreactors. k_La is defined as the volumetric mass transfer coefficient and is highly dependent on the turbulence of the system, the configuration of the gas-liquid contact, the bubble size and the fluid dynamics. Therefore, in general, it can be considered as a mechanical factor depending on the configuration of the reactor and the bubble diffusers used for the sparging of the gas mixture.

The use of different bubble diffusers such as fine bubbles, micro bubbles and membrane diffusers is under current investigation for increasing to the maximum the specific volumetric surface of the bubbles (Marín et al., 2020). Reducing the bubble size guarantees a higher k_La, however, there is again a trade-off with the pressure drop in the diffusers, which might have a negative impact on the overall economics of the process. Despite preliminary investigations have been carried out at laboratory scale, additional experiences at pilot and industrial scale are required for fully understanding this compromise between mass transfer efficiency and pressure drop.

A hot topic in the current research is the development of innovative mass transfer reactors. However, the achievement of superior gas-liquid mass transfer rates is not the only requirement for these bioreactors, and since the possibility of an easy harvesting of the biomass is a crucial parameter for the downstream processing and the industrial scale implementation of these processes. Therefore, packed bed bioreactors, membrane biofilm bioreactors or horizontal flow bioreactors, which have been tested with outstanding results for CH_4 abatement, are not considered suitable for bioconversion applications given the difficulties to harvest the biomass (López et al., 2019). Thus, the use of suspended growth bioreactors is the most suitable option for this application (Stone et al., 2017). In this regard, the most widely used reactors in the industry are stirred tank reactors, however, the excessive energy consumption of mechanical stirrers make them unsuitable for the required cost-competitiveness of these biotechnological processes (Kraakman et al., 2011). Bubble column bioreactors are one of the most promising alternatives given their high turbulence and low operating costs. Modifications of the bubble column bioreactors such as airlift bioreactors have also demonstrated an increased CH_4 elimination performance (López et al., 2013).

In recent years, highly specific bioreactor configurations based on the concept of high mass transfer efficiency and effective biomass harvesting such as the U-loop and the Taylor flow bioreactors have been developed and are under current investigation at lab and pilot scale (Cattaneo et al., 2022; Kraakman et al., 2011; Petersen et al., 2017). These reactor configurations include two differentiated sections, one devoted to maximize mass transfer from the gas to the liquid phase and another to separate gas and liquid streams, thus allowing for an easy biomass harvesting. In U-loop bioreactors, the gas-liquid mass transfer section is characterized by its "U" shape and is based on an external recirculation pipe equipped with extra fittings to enhance gas-liquid mass transfer such as static mixers, nozzles for gas side streams or additional liquid pumping systems. U-loop bioreactors were first patented in 2009 by Larsen and are currently under exploitation by Unibio A/S (www.unibio.dk), one of the few companies dedicated to the bioconversion of CH_4 into added value products, for protein production from natural gas (Larsen, 2011). This operation has already been implemented at large scale with outstanding outcomes of 4 kg biomass$\cdot$m^{-3} h^{-1}. However, the footprint of these bioreactors compared to the operating reactor volume could be limiting its implementation. Another example are Taylor flow bioreactors, based on the maximized gas-liquid mass transfer obtained by a bubble train of alternating liquid and small gas bubbles flowing upwards or downwards in the same direction in thin cappilaries. These systems are under study at laboratory scale for the production of different bioproducts from CH_4-biogas, however their performance at large-scale is still to be unveiled (Cattaneo et al., 2022).

Besides the development of new bioreactor configurations, another strategy that has attracted the attention of researchers is the use of an internal gas recirculation for enhancing CH_4 gas-liquid mass transfer and the overall gas residence time. The recirculation of the outlet gas stream allows decoupling the EBRT from the turbulence in the cultivation broth, thus achieving enhanced CH_4-removal efficiencies (CH_4-RE) in reduced reactor volumes (García-Pérez et al., 2018). This technology has showed a substantial enhancement of CH_4-RE and CH_4-EC in biofilters and bubble column bioreactors at laboratory scale (Estrada et al., 2014; García-Pérez et al., 2018). However, high gas recirculation rates might incur a compromise between the enhancement in mass transfer and the increase in the operational costs due to the high energy consumption of the biogas/air blower.

It should be highlighted, that a deeper understanding of the potential impact of these biotechnologies would require of more ambitious tests at pilot and industrial scale given the influence on the mass transfer of parameters such as the reactors height or height-to-diameter ratio. In contrast to the scale-up of other relevant operation parameters, the CH_4 mass transfer efficiency could be positively influenced when increasing the scale of the biogas conversion process. Higher reactors would present a combined effect of a longer contact time between gas bubbles and the liquid bulk but also a higher pressure at the bottom of the reactor set by the liquid height. The improved performance of industrial scale bubble column bioreactors has not been openly disclosed by biotechnological companies devoted to gas transformation but CH_4 elimination capacities of at least 148 $g \cdot m^{-3} \cdot h^{-1}$ can be extrapolated from the available information, which triples the commonly reported values in literature for experiments at laboratory scale (54.4-74 $g \cdot m^{-3} \cdot h^{-1}$) (García-Pérez et al., 2018; Rodríguez et al., 2020b).

1.5.2 Improvement of the bacterial methanotrophic yields

Culture selection and its influence on bioproduct titer have been identified in literature as key limiting factors towards the development of cost-competitive biogas bioconversion processes (Choi and Lee, 1999). Bioproduct productivity (P) can be defined as the amount of bioproduct produced per reactor volume unit and per time unit, and can be expressed as the product of bioproduct yield (Y), specific growth rate (μ) and the biomass concentration (X), according to Equation 1.13.

$$P\left(\frac{kg_P}{m^3 \cdot h}\right) = Y\left(\frac{kg_p}{kg_X}\right) \cdot \mu\left(\frac{1}{h}\right) \cdot X\left(\frac{kg_x}{m^3}\right) \qquad \text{Equation 1.13}$$

In contrast to the above mentioned technological barriers, biological obstacles are far more difficult to generalize as they are highly dependent on the microorganisms employed and the bioproduct addressed. However, for example in the field of PHA production from biogas, methanotrophic growth rate (0.3-0.4 h^{-1}), PHB productivity (0.4 g $PHB \cdot gX^{-1}$) and cell density (<5 $g \cdot L^{-1}$) have shown to be significantly lower than that of other bacteria such as genetically modified *Escherichia coli* (0.4-0.7 h^{-1}; 0.9 $gPHB \cdot gX^{-1}$; 100 $gX \cdot L^{-1}$) (Aljuraifani et al., 2019; Pieja et al., 2017; Van Wegen et al., 1998). In this context, the field is open to the cultivation of genetically modified microorganisms, capable of certainly increasing the bioproduct productivity.

Under non mass transfer limiting conditions, the use of high density cultures would significantly increase the overall bioproduct productivity, which would have a major impact on the reduction of the bioreactor volume and therefore on the capital and operational expenditures of the process. More indirectly, the use of high density cultures has shown benefitial effects on the gas-liquid mass transfer efficiency of the process (Choi and Lee, 1999). However, this effect should be studied for every particular case. Recently, Rodero and colleagues showed a negligible effect of the biomass concentration for the bioconversion of biogas into ectoine, given the limited amount of CH_4 transferred for biomass growth and for ectoine accumulation (Rodero and Muñoz, 2021). Notwithstanding, the use of high density cultures has a direct impact on the downstream processing costs. The reduction of the water content in the culture facilitates the extraction of the bioproducts and reduces significantly the size of the downstream equipment.

On the other hand, literature has consistently pointed at the use of pure cultures of specialized bacteria as a crucial strategy for maximizing the overall bioproduct productivity. However, the necessity of thorough sterilization processes for the equipment and the mineral media incurs in prohibitive operational costs for certain bioproducts. This is especially true for moderate added-value products such as SCP, PHA or EPS. The production of these bulk chemicals from biogas is characterized by the low margin between production costs and selling price, making sterilization processes unfeasible at large scale (Pérez et al., 2020a). Therefore, the use of sterilization procedures should be restricted to the production of fine bio-chemicals such as ectoine. The most recent studies based on the production of high added-value chemicals from biogas have shown wide margins between bioproduct selling prices and production costs. Therefore, there is room for using pure cultures and even genetically modified bacteria (Pérez et al., 2022). Overall, the use of mixed methanotrophic cultures is highly recommended for the production of moderate and low added-value products such as PHA, EPS and SCP. In addition to the reduced operational costs while avoiding sterilization, these processes have shown a great robustness under long-term operation given their inherent prevention of culture contamination (Pérez et al., 2019).

In this context, tailoring mineral medium composition and the use of nutrient stress techniques that inhibit the growth of undesired microorganisms have become popular. Indeed, the accumulation of certain bioproducts act as an internal natural selector for the growth of the specific desired groups of bacteria. For example, in the case of PHB accumulation in methanotrophic bacteria, which has been described in literature as a nutrient storage advantage under nutrient deprivation conditions, the use of nitrogen feast-famine cycles has been demonstrated as an effective technique for promoting the growth of type II methanotrophic bacteria, responsible for PHB accumulation against competitive type I methanotrophs, not able to accumulate PHB (López et al., 2018b). Similarly, intracellular ectoine is accumulated by certain groups of haloalkaliphilic bacteria in order to protect cells against extreme salinity conditions. Thus, the optimization of salinity conditions in the culture for promoting high yields of ectoine production also prevents the proliferation of competing bacteria and guarantees the presence of ectoine-acumulating microorganisms such as *Methylomicrobium japanense* and *Methylomicrobium butyarense* (Carmona-Martínez et al., 2021).

1.5.3 Development of more cost-efficient downstream methodologies

The development of more cost-efficient downstream processes is of paramount importance for the economic viability of biogas bioconversion. In fact, it has been estimated that downstream processing can represent up to 50 % of the total bioproduct production costs (Castilho et al., 2009). In this context, downstream processes for methanotrophic bioproducts should be carefully evaluated for each process. However, the enhancement of the bioproduct titer and cell density in the methanotrophic cultures typically significantly facilitates downstream processes (Choi and Lee, 1999). A higher concentration of biomass and intra- or extra-cellular bioproduct yields entails a significant reduction of the chemical reagents and energy demand, given the inherent simplification of product dewatering and concentration (Strong et al., 2015). Similarly, the reduction of the water content leads to a size reduction of the downstream equipment, and therefore, a significant reduction on the associated capital expenditures.

In addition, downstream processes for low and moderate added-value products such as SCP, EPS and PHA require simpler and more cost-efficient purification methodologies, as the margin between the bioconversion process and the selling price of the product is limited (Pérez et al., 2020a). In this regard, it can be highlighted the work performed by Lopez-Abelairas and coworkers in 2015, screening different methods for the extraction and purification of PHB. In this study, several downstream processes are compared in terms of product recovery, product purity, environmental impact and cost-effectiveness (López-Abelairas et al., 2015). As a result, a NaOH digestion method was proposed as the most cost-efficient and environmentally friendly method, showing product recovery and purity of 80 % and 92 %, respectively.

On the other hand, the extraction and purification of high added-value products, such as ectoine, can be focused on the maximization of the purity and recovery of the bioproduct, given the wider margin between biogas bioconversion and market selling price (Pérez et al., 2022). Particularly for ectoine, it is well-established that the two-step purification process consisting of the utilization of high performance ionic resins and subsequent methanol crystallization constitutes a significant share of the total price of the product (Chen et al., 2017; Fülberth et al., 2002). In this sense, this process makes use of expensive pieces of equipment and consumables, given the high quality of the product required, typically used as a fine chemical in the pharmaceutical and cosmetic industry.

1.6 Techno-economic assessment of methanotrophic processes

A crucial milestone in the study of all innovative and emerging technologies is the execution of feasibility and techno-economic analyses. These methodologies allow narrowing down the distance between the current development of the technology and a future potential industrial implementation. They also provide reliable information on the biotechnological bottlenecks that must be overtaken in order to obtain cost-competitive bioproducts and thus, help defining the roadmap for future investigations. Overall, in the academic field, techno-economic analyses present low levels of accuracy given the novelty of the processes involved and the limitations of the data used as input for the methodology. Typically, lab-scale results regarding bacterial yields and kinetics and preliminary mass and energy balances are extrapolated to real-scale scenarios. However, the lack of industrial expertise for these bioprocesses and the low applicability of general techno-economic methodologies to these biological technologies limit the potential impact and interest of these studies for newborn technologies. At this point, it should be stressed that a closer collaboration between industry and academia is essential for boosting the development of these biotechnologies.

In the field of project assessment, these techno-economic evaluations are classified in 5 categories depending on the level of detail of the inputs and the range of accuracy of the estimations. An additional zero class can be defined also for proof-of-concept estimations that do not aim at obtaining an estimation of the whole industrial process (Table 1.1). Class 0 and 1 estimates are mainly focused on the comparison of general capital and operational costs between currently established technologies and innovative technologies. Some other analyses implement more sophisticated methodologies for the calculation of the capital costs and include sensitivity analysis in order to provide a higher reliability of the results obtained, attending at the market circumstances and the potential variability of the inputs (Class 2).

Table 1.1 Classification of project investment estimations. Adapted from (Dysert, 2003). The methodology for techno-economic assessment presented in this thesis can be categorized as a class 2 estimate.

Estimate class	**Project definition (%)**	**Purpose of estimate**	**Estimating method**	**Accuracy range (%)**	**Preparation effort relative to project cost**
Class 0	0	Proof-of-concept	Semi-detailed calculation of operational costs for a single scenario	-	-
Class 1	0-2	Screening	Capacity-factored, parametric models	Low = -20 to -50 % High = 30 to 100 %	1
Class 2	**1-15**	**Feasibility**	**Equipment-factored, parametric models**	**Low = -15 to -30 %** **High = 20 to 50 %**	**2-4**
Class 3	10-40	Budget authorization or cost control	Semi-detailed unit cost estimations with assembly-level items	Low = -10 to -20 % High = 10 to 30%	3-10
Class 4	30-70	Control of bid or tender	Detailed unit-cost estimation with forced, detailed takeoff	Low = -5 to -15 % High = 5 to 20 %	4-20
Class 5	50-100	Check estimate, bid or tender	Semi-detailed unit cost estimation with detailed takeoff	Low = -3 to -10 % High = 3 to 15 %	5-100

Notwithstanding the growing interest in the use of aerobic methanotrophy for the bioconversion of biogas into added-value products and the great number of studies aimed at the optimitization of different bioproducts, the scientific literature focused on the study of the economics of these bioprocesses is very scarce. Table 1.2 summarizes the literature review of techno-economic analyses of the use of stranded CH_4 emissions, biogas, landfill gas or natural gas as substrate for the biological production of added-value bioproducts.

In 2007, Listewnik and coworkers performed the first comprehensive techno-economic analysis on the emerging production of PHA biopolymers with methanotrophic bacteria. The calculation of the investment and operational costs allowed the quantification of the PHB production costs when natural gas was used as carbon source and energy source (Listewnik et al., 2007). Likewise, Levett and colleagues performed a real-scale scenario analysis devoted to the production of 100,000 t PHB$\cdot$y^{-1}. This study also integrated a sensitivity analysis that allowed the identification of the main equipment and operational costs, as well as their influence on the final production costs of the biodegradable polymer (Levett et al., 2016).

The production of SCP for feed and food applications has gained attention during the last decade. For instance, Abbadi and coworkers, in 2022, studied the potential application from stranded CH_4 emissions to produce SCP to be used as animal feed. The outcomes of the analysis evidenced that SCP could already be produced at competitive prices against current protein sources. In addition, this study evidenced that the reduction of labor and cooling costs was of paramount importance for the implementation of this technology at large scale (El Abbadi et al., 2022). Likewise, García-Martínez and coworkers performed a comprehensive analysis assessing the potential use of SCP from methanotrophic bacteria for human consumption under catastrophic food shocks employing natural gas and biogas as CH_4 sources. Interestingly, this study also implemented a methodology for the evaluation of the limiting steps towards the rapid construction of these facilities (García Martínez et al., 2022).

In 2020, Fei and coworkers conducted an ambitious analysis studying the influence of certain biotechnological factors on the production costs of lactic acid from natural gas, using methanotrophic bacteria. This analysis concluded that the CH_4 conversion efficiency, the lactic acid product titer and productivity and the gas flow rate were the key factors limiting the cost-efectiveness of the process (Fei et al., 2020). Similarly, Liang and coworkers, in 2022, compared different production scenarios for biogas valorization into iso-butanol. The scenarios evaluated the sequential use of cyanobacteria and methanotrophic bacteria for the combined transformation of CO_2 and CH_4 into iso-butanol, respectively (Liang et al., 2022).

Cantera and coworkers were the first to quantify the operational costs and the potential revenues of integrated CH_4-biorefineries. This research proposed a multi-product biorefinery aiming at the simultaneous production of various methanotrophic products such as PHB, SCP, EPS and ectoine. This investigation represented the first proof-of-concept of the economic and environmental potential of these future biorefineries (Cantera et al., 2018a) .

In summary, the rapid development of innovative biogas valorization alternatives that respond to the declining use of biogas as energy vector and the recently discovered capacity of methanotrophic bacteria to add value to biogas while reducing the environmental impact of waste treatment schemes, have motivated a great effort in the study of these systems at laboratory and semi-industrial scale. However, the economic potential and the process bottlenecks of these bioconversion strategies have yet to be systematically explored.

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Table 1.2 Compilation of articles describing the techno-economic aspects of CH_4 bioconversion into added-value products with methanotrophic bacteria.

CH_4 source	**Product**	**Costs evaluated**				**Estimate class**	**Reference**
		Capital	**Operational**	**Product**	**Sensitivity**		
Landfill gas	SCP EPS PHB Ectoine	-	×	-	-	0	(Cantera et al., 2018a)
Natural gas Biogas	SCP	×	×	×	-	1	(García Martínez et al., 2022)
Natural gas Biogas	Isobutanol	×	×	×	-	1	(Liang et al., 2022)
Natural gas	PHB	×	×	×	-	1	(Listewnik et al., 2007)
Natural gas	Lactic acid	×	×	×	×	2	(Fei et al., 2020)
Natural gas	PHB	×	×	×	×	2	(Levett et al., 2016)
Landfill gas Wastewater	SCP	×	×	×	×	2	(El Abbadi et al., 2022)

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Chapter 2

Aim and scope of the thesis

2.1 Justification of the thesis

The successful implementation of anaerobic digestion as a method for reducing the amount of organic solid waste sent to landfill has resulted in more than 25,000 plants in operation in the world. In Europe, more than 18,000 facilities devoted to the treatment of all sort of organic residues such as municipal solid waste, wastewater or agro-industrial residues, are currently in operation. A large part of this success has been due to the associated production of biogas and digestate-based fertilizer. However, these plants face nowadays a major dilemma due to the higher cost of producing energy from biogas in co-generation engines, compared to the rapid decrease in the cost of competing renewable energies such as wind or solar power. This higher cost of electricity produced from biogas has been typically associated to the high investment and maintenance costs of combined heat and power generation engines, mainly due to the presence of multiple pollutants in biogas that can damage the combustion equipment such as hydrogen sulphide (H_2S) and siloxanes. These problems have been worsened by the lack of policy drivers towards the production of renewable energy from biogas and the gradual reduction of feed-in tariffs and fiscal exemptions during the last decade. In fact, the attention of policy makers has been focused on the production of higher added-value products from waste, in the framework of a cleaner, greener and more circular economy, and in line with the increasing restrictive environmental policies. Hence, medium and large-scale biogas production plants must reconsider their economic schemes and find innovative sources of revenue for guaranteeing their present and future economic viability. Therefore, there is a growing motivation for transitioning from linear waste treatment plants where only bioenergy is produced to more circular urban biorefineries, where all sort of bioproducts can be commercialized.

In this transition to urban biorefineries, a better valorization of biogas plays a major role, and its utilization as source of raw materials (mainly methane (CH_4) and carbon dioxide (CO_2)) instead of being merely regarded as energy vector, has gained attention from both academia and industry. In this context, the utilization of methanotrophic bacteria, capable of using CH_4 as their only source of carbon and energy, has emerged as an opportunity for increasing the current value of biogas. During the last decade, academics have widely demonstrated at laboratory scale the ability of methanotrophic bacteria for manufacturing bioproducts that are ranked higher up in the waste valorization pyramid such as polihydroxyalkanoates (chemicals and materials), single cell protein (feed and food) and ectoine (fine chemicals). In addition, in recent years, multitude of projects have been financed in order to validate these technologies at demo-scale: INCOVER and URBIOFIN devoted to the production of biomethane, URBIOFIN addressing the production of PHA, CIRCULAR BIOCARBON focused on the production of biostimulants or DEEP PURPLE and CHEERs for the production of ectoine.

However, the future technical, economic and environmental sustainability of these processes at large scale, as well as their robustness in a global economic context in constant change, is still unclear. It is also of paramount importance to evaluate the current biotechnological limitations in bioproducts manufacturing and the potential reduction of production costs derived from future biotechnological advances, thus defining the roadmap to develop cost-competitive biogas biorefineries.

2.2 Main objectives

The overall objective of this research was the development and implementation of a methodology for evaluating the technical and economic feasibility of the bioconversion of biogas into added-value products using methanotrophic bacteria as an alternative to the current utilization of biogas as an energy vector in urban waste treatment plants. This methodology was used for assessing the economic potential of producing polyhydroxyalkanoates, a low added-value product with a large market share, and ectoine, a high added-value product with a small market demand, compared to the traditional combustion of biogas in co-generation engines for producing energy. More particularly, the specific objectives accomplished in this thesis are:

I. Development of a robust methodology for the techno-economic and sensitivity analysis of biogas bioconversion into bioproducts using methanotrophic bacteria in waste treatment plants.

II. Evaluation of the economic, environmental and social sustainability of polyhydroxyalkanoates production from biogas using methanotrophic bacteria.

III. Assessment of the economic feasibility of biogas bioconversion into low added-value polyhydroxyalkanoates compared to the current utilization of biogas as energy vector.

IV. Identification of the current biotechnological limitations of biogas-based polyhydroxyalkanoates and analysis of the influence of the geographical location and the commodity prices on economic feasibility of the process.

V. Study of the techno-economic feasibility of producing ectoine, a high added-value product, from biogas using methanotrophic bacteria.

VI. Analysis of the influence of the economy of scale, market and biotechnological limitations on the bioconversion of biogas into ectoine.

2.3 Thesis outline

In the present thesis work, the techno-economic feasibility of biogas bioconversion into low and high added-value products was compared to its current utilization as energy vector in CHP generation. More precisely:

Chapter 1 presents an exhaustive literature review of the technologies available for biogas valorization in urban waste treatment plants, emphasizing the potential advantages and bottlenecks of the biogas bioconversion into added-value bioproducts using methanotrophic bacteria. The current chapter (**Chapter 2**) describes the motivation of the work and its main objectives. **Chapter 3** features the development of a robust methodology for assessing the economic impact and the biotechnological limitations of the biogas bioconversion processes using methanotrophic bacteria in order to fulfil objective I. This methodology has been improved throughout the last 5 years of research and serves as the basis for the techno-economic and sensitivity studies featured in **Chapters 4 to 7**. **Chapters 4 and 5** address the production of polyhydroxyalkanoates, a low added-value biopolymer, from biogas in urban waste treatment plants, according to objectives II, III and IV. In **Chapter 4**, the three pillars of sustainability (economic viability, environmental concern and social responsibility) of the biogas bioconversion into polyhydroxyalkanoates are compared to the current combustion of biogas in co-generation engines. In **Chapter 5**, the techno-economic analysis is extended by evaluating the influence of the geographical location, the cost of electricity, water and raw materials and the biotechnological limitations on the polyhydroxyalkanoates production costs. Objectives V and VI are addressed in **Chapters 6 and 7** with a techno-economic and sensitivity analysis of the production of ectoine via biogas bioconversion in urban waste treatment plants. **Chapter 6** is devoted to the techno-economic analysis of ectoine production, as an example of a high-added value bioproduct that can be produced from biogas with methanotrophic bacteria. **Chapter 7** assesses the potential impacts of the economy of scale and the ectoine market price, and identifies the most critical biotechnological barriers of the technology. **Chapter 8** summarizes the fundamental results and the concluding remarks of this investigation and provides a list of topics of interest for future research. Finally, **Chapter 9** is devoted to the motivations and academic background of the author, listing his contributions and collaborations during his work as a pre-doctoral researcher.

Chapter 3

Methodology for the techno-economic assessment of biogas bioconversion processes

3. Methodology

Different methodologies for the development of techno-economic analyses have been described in literature. However, each of them present specific characteristics for the particular processes studied and are not completely applicable to the biogas bioconversion processes targeted in this PhD thesis. Techno-economic assessments can be performed for a single process proof-of-concept study, but the results are optimal when several processes are compared using the same methodology. In contrast, the comparison between different techno-economic assessments can often lead to incorrect evaluations and misleading results as the objectives, the items considered or the level of approximation might differ from one to another. Techno-economic assessments cannot be considered as a stand-alone procedure and their validity and robustness rely on associated sensitivity analyses that evaluate the influence of certain critical inputs on the results obtained. It is also relevant to mention that techno-economic assessment methodologies like the one presented in this chapter often entail a 20-30 % level of uncertainty (Table 1.1). This chapter carefully describes the methodology developed for the realization of this investigation in order to guarantee a good replicability of the results obtained and also as a tool for future investigations focused on the production of bioproducts in waste treatment plants. This methodology aims at addressing the challenges described in the last section of the introduction: evaluating the economic performance of the production of low added-value and high added-value bioproducts with methanotrophic bacteria compared to the current utilization of biogas as energy vector, identifying the current biotechnological bottlenecks of biogas bioconversion processes and defining the roadmap of future investigations towards cost-competitive biogas-biorefineries. This methodology has been structured as a 6-step sequential process: (I) definition of the battery limits, (II) selection of a calculation basis, (III) definition of the process under analysis including mass balance and equipment design, (IV) calculation of the energy balance, (V) economic evaluation and (VI) confirmation of the results with a sensitivity analysis (Figure 3.1). This methodology has been continuously improved in order to guarantee the highest standards of reliability in terms of data compilation, process design and results obtained. Note that the current chapter describes the most up-to-date version of the methodology and that the content of some of the chapters, especially 4 and 5 might differ from the methodology presented here. Notwithstanding, each chapter summarizes the particularities of the associated methodology and calculations performed.

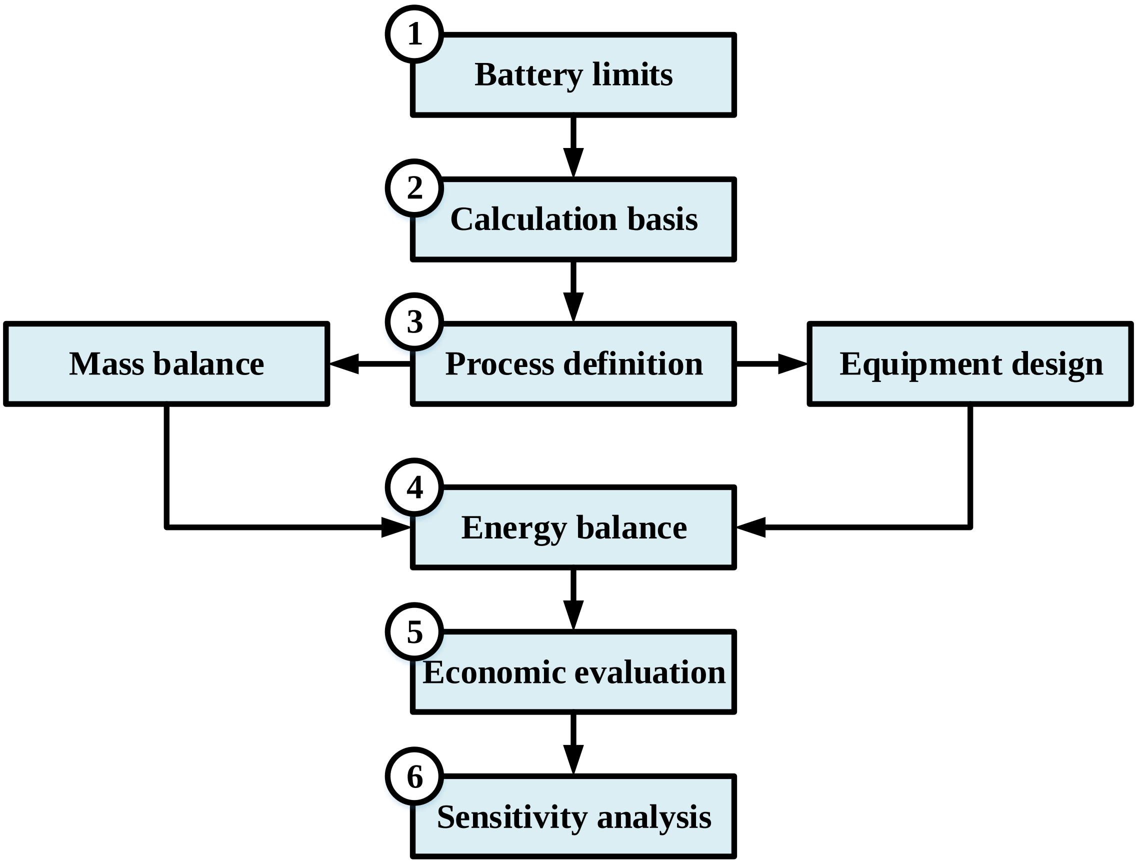

Figure 3.1. 6-steps methodology for the techno-economic and sensitivity assessment.

3.1 Definition of battery limits

The first step in the methodology is the definition of the scope of the techno-economic analysis. This scope is intimately related to the socioeconomic context of the study and to the potential implementation of the technology in a real industrial environment. More specifically, this section aims at defining which steps of the bioproduct production process, from the production of raw materials to the distribution and commercialization of a marketable bioproduct, are to be considered inside the battery limits of the study.

In this research, we have focused on the production and purification of the bioproducts, considering the plant as an annex to a previously built medium- or large-scale anaerobic digestion plant, and therefore the anaerobic digestion process and the post-processing of the bioproduct remained out of the battery limits (Figure 3.2). Since the motivation of this study was the evaluation of the potential positive economic impacts of transitioning from the valorization of biogas as an energy vector to the production of bioproducts, biogas from the anaerobic digestion plant was considered completely available at zero cost. This point should be re-considered when the studied biogas valorization pathway modifies the design or the operation of a currently operating anaerobic digestion process. The production of raw materials, utilities and services were considered out of the scope of the analysis and they were accounted as operational costs for the annex biogas bioconversion plant. This point should be re-evaluated depending on the process investigated as some raw materials and utilities, just like biogas, could be *in-situ* produced by the anaerobic digestion plant. It is worth mentioning that transportation cost of raw materials are often included in the raw materials purchase price. Given the low volume and complexity of the solid waste and wastewater produced in the biogas bioconversion process, their treatment was considered as a subcontracted service to the anaerobic digestion plant at a reduced cost. This point should also be evaluated for each biogas valorization process considered, as the volumetric flow and the organic loads of the waste streams could be significant in some scenarios. At the other end, the post-processing of the bioproduct into a marketable product in a secondary industry and its distribution to the consumers were considered out of the battery limits in this methodology. The transportation costs from the biogas bioconversion plant to the secondary industry were considered, since some techno-economic assessments often include these transportation costs in the selling price of bioproducts.

The current methodology differentiates between the production of the bioproduct by biological conversion of biogas and the extraction and purification of the bioproduct in order to better identify the individual cost shares and the biotechnological bottlenecks of each stage of the process. In addition, it should be pointed out that the logistic structure of future biorefineries is still to be defined and it could be possible that biogas bioconversion into bacterial biomass and product purification might be carried out in different facilities.

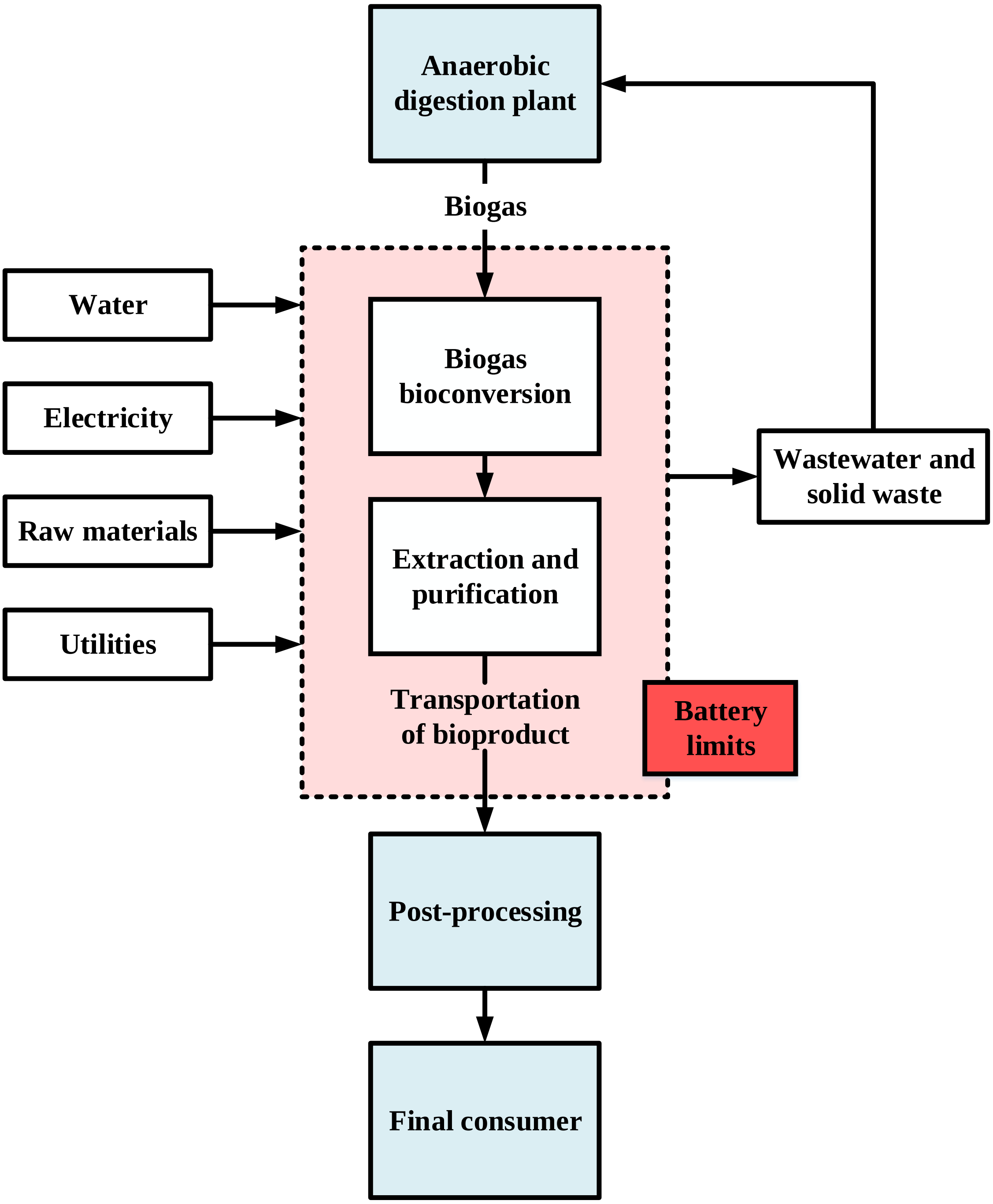

Figure 3.2. Definition of battery limits for the techno-economic assessment.

3.2 Calculation basis

The second step in the methodology is to define the outcome of the calculation. The calculation basis is the pivotal parameter from which the process and the calculations are defined and it strongly depends on the objectives and nature of the techno-economic assessment. For biogas bioconversion into bioproducts, two different calculation basis approaches were considered: the biogas treatment capacity and the bioproduct production capacity. Therefore, the calculation basis must be selected taking into account the biogas availability from the associated anaerobic digestion plant and also the bioproduct market demand. Despite a wide range of process simulation tools are currently available for the design and optimization of biotechnological process, all calculations were performed in Excel Sheets in this thesis. The characteristics of this software provide the user an absolute control of the definition of input and output variables, crucial for the design and modelling of benchmark technologies like the ones described in this thesis. All calculations have been confirmed with global and elemental mass balances. The simulations were in all cases performed assuming an ideal gas behavior given the low pressure and temperature of the streams. The text includes all the relevant parameters for a reliable reproduction of the process design, economic calculations, and sensitivity analysis herein performed.

In Chapters 4 and 5, devoted to the comparative evaluation of the utilization of biogas for bioconversion into PHA and for energy production in CHP engines, a flow rate of 1,000 Nm^3 biogas$\cdot h^{-1}$ was considered as calculation basis. This value was defined considering the typical production of biogas in medium and large scale anaerobic digestion plants devoted to the treatment of municipal solid waste in medium and large municipalities in Europe. The calculation basis was based on the objective of evaluating the economic performance of substituting bioenergy production in urban waste treatment plants with PHA production. The total biogas production was selected as calculation basis given the wide portfolio of marketable biopolymers and more specifically the high demand of PHA at a global scale. Low added-value products like PHA can be considered as bulk products and the global market demand (1.5 million tonnes in 2021, according to the European Bioplastics) exceeds by far the production capacity of a single biogas bioconversion plant (681 t PHA$\cdot y^{-1}$) (European Bioplastics, 2021; Pérez et al., 2020a).

In Chapters 6 and 7, where the potential ectoine productivity from biogas at large scale was unknown and taking into account that the demand for fine chemicals is significantly less than that for bulk products, the calculation basis was based on a market analysis. It is true, that the global demand of ectoine is highly unknown with some reports varying between 20 and 200 $t \cdot y^{-1}$. In addition, given the fact that only a few companies are responsible for the whole global ectoine production, a high market share could be considered for a single biogas bioconversion plant. In these chapters, an ectoine production capacity of 10 $t \cdot y^{-1}$ was set as calculation basis, which translated into a market share ranging from 50 % to 5 %, corresponding to an estimated global demand for ectoine of 20 $t \cdot y^{-1}$ and 200 $t \cdot y^{-1}$, respectively. The implementation of this biogas valorization pathway in a previously built medium-to-large scale municipal waste treatment plant was considered similar to PHA, with the particularity that in this scenario, only a fraction of the biogas produced should be devoted to the production of bioproducts and the biogas surplus can be still dedicated to the production of energy in CHP.

3.3 Process definition

3.3.1 Mass balance

All processes presented in Chapters 4-7 were simulated considering continuous operation under steady-state. Batch processes were defined neglecting storage and filling times. Maintenance and shutdowns were considered negligible and a total operation time of 8,760 $h\cdot y^{-1}$ was established for the analysis (24 $h\cdot d^{-1}$ × 365 $d\cdot y^{-1}$). Mass and energy balances were performed assuming an ideal gas behavior given the low pressure and temperature of the streams. Biogas thermodynamic properties such as density, calorific power and Wobbe index were obtained from the Swedish Gas Technology Center report (SGC, 2012). Relevant design parameters for desulfurization (e.g. H_2S removal efficiency (H_2S-RE), nitrate requirements), CHP (e.g. air excess, power efficiency), PHA extraction (e.g. pH, product recovery and purity) and ectoine purification (e.g. ionic exchange resin characteristics, methanol extraction) were obtained from the literature (Almenglo et al., 2016; Brito et al., 2018; Chen et al., 2017; Fülberth et al., 2002; López-Abelairas et al., 2015; López et al., 2017; Wellinger et al., 2013). CH_4-RE, CH_4 elimination capacity (CH_4-EC), PHA and ectoine productivity and other operational parameters for the production of bioproducts from biogas were obtained from previous experimental studies conducted in our laboratory (Cantera et al., 2017b, 2017a; Carmona-Martínez et al., 2021; García-Pérez et al., 2018; López et al., 2018a). The reaction kinetics for CH_4 bioconversion into biomass, bioproducts and mineralization to CO_2 were simulated as parallel equations considering a steady-state scenario. All calculations were performed in Excel Sheets and have been double-checked with global and elemental mass balances. The text includes all the relevant parameters for a reliable reproduction of the process design, economic calculations, and sensitivity analysis herein performed.

3.3.2 Equipment design

Each equipment was designed individually based on well-stablished design parameters. This section summarizes the equations and parameters required for the sizing of the equipment presented in this thesis (Table 3.1).

The working volume V (m^3) of continuous stirred tank reactors (CSTR) and crystallizers was calculated with the inlet volumetric flow Q (m^3·h^{-1}) and the hydraulic retention time HRT (h) according to Equation 3.1. The dimensioning of the vessel was performed considering a cylindrical configuration, a height-to-diameter ratio H/D of 1 when not indicated otherwise, and an overhead H_0 of 20 %. The outcomes of the calculation were the liquid volume (V), the total reactor volume (V_R), the liquid height (H), the reactor height (H_T) and the reactor diameter (D).

$$HRT = \frac{V}{Q} \qquad \text{Equation 3.1}$$

The liquid volume V (m^3) in bubble column bioreactors (BCB) for biogas bioconversion into bioproducts was calculated based on the inlet volumetric gas flow Q_{in} (Nm^3·h^{-1}), the outlet volumetric gas flow Q_{out} (Nm^3·h^{-1}), the inlet concentration of CH_4 $C_{CH4,in}$ (g·Nm^{-3}), the outlet concentration of CH_4 $C_{CH4,out}$ (g·Nm^{-3}) and the CH_4 elimination capacity $CH_4\text{-}EC$ (g·m^{-3}·h^{-1}) according to Equation 3.2. $C_{CH4,out}$ was calculated using the CH_4 removal efficiency $CH_4\text{-}RE$ (%) (Equation 3.3). The reactor was dimensioned considering a cylindrical configuration, with the height-to-diameter ratio H/D and an overhead H_0 of 20 %. The outcomes of the calculation were the liquid volume (V), the total reactor volume (V_R), the liquid height (H), the reactor height (H_T) and the reactor diameter (D).

$$CH_4 - EC = \frac{Q_{in} \cdot C_{CH_4,in} - Q_{out} \cdot C_{CH_4,out}}{V} \qquad \text{Equation 3.2}$$

$$CH_4 - RE = \frac{Q_{in} \cdot C_{CH_4,in} - Q_{out} \cdot C_{CH_4,out}}{Q_{in} \cdot C_{CH_4,in}} \qquad \text{Equation 3.3}$$

The bed volume BV (m^3) in packed columns was calculated with the inlet volumetric flow Q ($m^3 \cdot h^{-1}$ for liquids and $Nm^3 \cdot h^{-1}$ for gases) and with the empty bed residence time *EBRT* (h) or with the *HRT* (h), for gas and liquid streams, respectively (Equation 3.4). The packed column was dimensioned considering a cylindrical configuration, a height-to-diameter ratio *H/D* of 2 for biotrickling filters and 10 for ionic exchange chromatography when not otherwise indicated, and with an overhead H_0 of 20 %. The outcomes of the calculation were the bed volume (*BV*), the total column volume (V_R), the height of the packed bed (*H*), the reactor height (H_T) and the reactor or bed diameter (*D*).

$$HRT\ or\ EBRT = \frac{BV}{Q} \qquad \text{Equation 3.4}$$

The area of ultrafiltration membranes S (m^2) was calculated considering a permeate flux Fp of 15 $L \cdot m^{-2} \cdot h^{-1}$, a water recovery efficiency W_R of 90 % and a solid recovery efficiency S_R of 99 %. Permeate volumetric flow Q_P ($m^3 \cdot h^{-1}$), permeate solid concentration C_P ($g \cdot L^{-1}$), retentate volumetric flow Q_R ($m^3 \cdot h^{-1}$), retentate solid concentration C_R ($g \cdot L^{-1}$) and membrane area S were calculated from the inlet volumetric flow Q ($m^3 \cdot h^{-1}$) and the inlet solid concentration C_{in} ($g \cdot L^{-1}$) according to Equation 3.5-Equation 3.9.

$$S_R = \frac{Q_R \cdot C_R}{Q \cdot C_{in}} \qquad \text{Equation 3.5}$$

$$W_R = \frac{Q_P}{Q} \qquad \text{Equation 3.6}$$

$$Q \cdot C_{in} = Q_R \cdot C_R + Q_P \cdot C_P \qquad \text{Equation 3.7}$$

$$Q = Q_R + Q_P \qquad \text{Equation 3.8}$$

$$S = \frac{Q_P}{F_p} \qquad \text{Equation 3.9}$$

The area of electrodialysis membranes S (m^2) was calculated considering a permeate flux F_P of 45 $L\cdot m^{-2}\cdot h^{-1}$, a water recovery efficiency W_R of 90 % and a salt concentration factor F_S of 25. The permeate volumetric flow Q_P ($m^3\cdot h^{-1}$), permeate solid concentration C_P ($g\cdot L^{-1}$), retentate volumetric flow Q_R ($m^3\cdot h^{-1}$), retentate solid concentration C_R ($g\cdot L^{-1}$) and membrane area S were calculated from the inlet volumetric flow Q ($m^3\cdot h^{-1}$) and the inlet solid concentration C_{in} ($g\cdot L^{-1}$) according to Equation 3.10-Equation 3.14.

$$S_R = \frac{C_R}{C_{in}} \qquad \text{Equation 3.10}$$

$$W_R = \frac{Q_P}{Q} \qquad \text{Equation 3.11}$$

$$Q \cdot C_{in} = Q_R \cdot C_R + Q_P \cdot C_P \qquad \text{Equation 3.12}$$

$$Q = Q_R + Q_P \qquad \text{Equation 3.13}$$

$$S = \frac{Q_P}{F_p} \qquad \text{Equation 3.14}$$

Centrifuges were designed considering a solid retention efficiency S_R of 95 %, and a solid concentration in the thickened stream C_T of 200 $g\cdot L^{-1}$ unless otherwise stated. The thickened volumetric flow Q_T ($m^3\cdot h^{-1}$), the supernatant volumetric flow Q_S ($m^3\cdot h^{-1}$) and the supernatant concentration C_S ($g\cdot L^{-1}$) were calculated from the inlet volumetric flow Q ($m^3\cdot h^{-1}$) and the inlet solid concentration C_{in} ($g\cdot L^{-1}$) using Equation 3.15-Equation 3.17:

$$S_R = \frac{Q_T \cdot C_T}{Q \cdot C_{in}} \qquad \text{Equation 3.15}$$

$$Q \cdot C_{in} = Q_T \cdot C_T + Q_S \cdot C_S \qquad \text{Equation 3.16}$$

$$Q = Q_T + Q_S \qquad \text{Equation 3.17}$$

The tray dryer surface S (m^2) was calculated from the inlet mass flow $\dot{m}$ (kg·h^{-1}) and the bioproduct density ρ (kg·m^{-3}) assuming a cake layer height H_C of 2 cm and a HRT of 1 h (Equation 3.18). The bioproduct was dried from the initial moisture W_I (%w·w^{-1}) to a final moisture W_F of 5 %w·w^{-1} with warm air at 20ºC, considering a water content in air $S_{water\text{-}air}$ of 0.015 kg water·kg air^{-1}. Air requirements Q_{Air} (kg·h^{-1}) were calculated using Equation 3.19.

$$S = \frac{\dot{m} \cdot HRT}{\rho \cdot H_C} \qquad \text{Equation 3.18}$$

$$Q_{Air} = \dot{m} \cdot \frac{W_I - W_F}{S_{water-air}} \qquad \text{Equation 3.19}$$

The spray dryer volume V (m^3) was calculated from the inlet mass flow $\dot{m}$ (kg·h^{-1}) and a specific evaporation rate E_R of 100 kg·h^{-1}·m^{-3} according to Equation 3.20. The bioproduct was dried from the initial moisture W_I (%w·w^{-1}) to a final moisture W_F of 5 %w·w^{-1} with warm air at 20ºC, considering a water content in air $S_{water\text{-}air}$ of 0.015 kg water·kg air^{-1}. Air requirements Q_{Air} (kg·h^{-1}) were calculated with Equation 3.21.

$$V = \frac{\dot{m}}{E_R} \qquad \text{Equation 3.20}$$

$$Q_{Air} = \dot{m} \cdot \frac{W_I - W_F}{S_{water-air}} \qquad \text{Equation 3.21}$$

Pumps and compressors were dimensioned with the inlet volumetric flow Q (m^3·h^{-1} or Nm^3·h^{-1}) and the pressure difference between the inlet and the outlet ΔP (bar) obtained from the mass and energy balances.

Table 3.1 Summary of process design parameters and outcomes for the equipment dimensioned in this thesis.

Equipment	**Design parameters**	**Outcome**
Reactor (CSTR)	HRT, H/D, H_0	V, V_R, H, D, H_T
Reactor (BCB)	H/D, H_T, CH_4-EC, CH_4-RE, Q_{in}, Q_{out}, $C_{CH4,in}$, $C_{CH4,out}$	V, V_R, H, D, H_T
Packed Column	EBRT or HRT, H/D, H_T	BV, V_R, H, D, Ht
Centrifuge	Q, C_{in}, C_T, S_R	Q_T, Q_S, C_S
Membrane	F_P, W_R, S_R, Q, C_S	S (m^2), Q_P, Q_R, C_P, C_R
Electrodialysis	F_P, W_R, F_S, Q, C_S	S (m^2), Q_P, Q_R, C_P, C_R
Tray dryer	$\dot{m}$, ρ, HRT, H_C, W_F, W_I, $S_{water\text{-}air}$	S, Q_{air}
Spray dryer	$\dot{m}$, E_R, W_F, W_I, $S_{water\text{-}air}$	V, Q_{air}
Pump	Q, ΔP	
Blower/compressor	Q, ΔP	

3.4. Energy balance

The process was considered to operate at ambient conditions of temperature (15 ºC) and absolute pressure (1 atm) unless otherwise stated in the description of the equipment. Constant atmospheric conditions of temperature and pressure were assumed throughout the year for calculation purposes. Heat losses in equipment and piping and pressure drop in the piping were considered negligible. Operating pressure and pressure drop in the different equipment were specified in the equipment description. The energy (electricity and heat) balance was based on the estimation of individual equipment energy consumption rates.

Pumps, blowers and compressors were the main energy demanding equipment in the biogas bioconversion processes herein reported. Energy requirements for pumps, blowers and compressors were calculated from power consumption, assuming a continuous operation. For pumps, the power consumption was estimated according to Equation 3.22, where P_{Pump} stands for the power consumption (kW), Q represents the inlet volumetric flow ($m^3 \cdot s^{-1}$), ΔP is the pressure drop (kPa) and η is the electrical efficiency of pumps (70 %). Power requirements for blowers and compressors were estimated according to Equation 3.23 and Equation 3.24, where P_{Blower} stands for the power requirements (kW), P_{is} is the isentropic power (kW), η represents the electrical blower efficiency (70 %), γ refers to the adiabatic coefficient (dim.), T_{out} is the gas isentropic outlet temperature (K), T_{in} stands for the gas inlet temperature (K), Pm represents the gas molecular weight ($g \cdot mol^{-1}$) and Q stands for the inlet volumetric flow ($Nm^3 \cdot h^{-1}$). T_{out} in blowers and compressors was estimated from T_{in}, the suction pressure P_{in} (atm) and the discharge pressure P_{out} (atm) with Equation 3.25.

$$P_{Pump} = \frac{Q \cdot \Delta P}{\eta} \qquad \text{Equation 3.22}$$

$$P_{Blower} = \frac{P_{is}}{\eta} \qquad \text{Equation 3.23}$$

$$P_{is} = 2.31 \cdot \frac{\gamma}{\gamma - 1} \cdot \frac{T_{out} - T_{in}}{Pm} \cdot Q \qquad \text{Equation 3.24}$$

$$\frac{P_{in}}{P_{out}} = \left(\frac{T_{in}}{T_{out}}\right)^{\frac{\gamma}{\gamma-1}} \qquad \text{Equation 3.25}$$

Energy requirements for less common equipment such as centrifuges (1 $kWh \cdot m^{-3}$), mixers (0.2 $kWh \cdot m^{-3}$) and electrodialysis membranes (7 $kWh \cdot m^{-3}$) were calculated as suggested in specific literature (Acién et al., 2012; Szepessy and Thorwid, 2018; Turek, 2003).

Given that the bioconversion processes herein presented typically operate at ambient conditions of temperature, the heating (low pressure steam) and cooling (cooling water) requirements in the processes described were marginal compared to the electricity requirements. Heating/cooling demand calculation was based on an enthalpy analysis considering an optimal heat transfer efficiency. Enthalpy values for low pressure steam ($P = 2$ bar, $T_{in}= 120.6$ ºC, $T_{out} = 80.0$ ºC) and cooling water ($T_{in} = 15$ ºC, $T_{out} = 50$ ºC) were obtained from the NIST-FLUIDS database (NIST, 2022). This method should be comprehensively improved for processes that are more heat demanding as it could lead to non-negligible deviations from the results obtained.

3.5. Economic evaluation

3.5.1. Calculation of total capital investment

The cost of the individual equipment was calculated based on the sizing of the equipment designed. The cost of most equipment was calculated with Matches online estimation tool (Matches, 2022). This cost estimation tool is based on a contributive database which includes prices for more than 275 types of equipment. The calculation is based on an extrapolation that depends on fundamental design parameters. In Chapter 7, the equations from the online estimation tool were obtained in order to provide an automatic calculation of the economy of scale. These equations followed a 2-factor exponential trend (Equation 3.26).

$$Equipment\ cost\ (€\,) = a \cdot x^{b} \qquad \text{Equation 3.26}$$

Table 3.2 summarizes the relevant variables (x) for the costing of the equipment represented in this methodology and the associated factors (a, b) of the equation. The values obtained from Matches were compared with a similar estimation tool provided by Peters, Timmerhaus and West, and with specific quotations from national and international suppliers (Peters et al., 2003). Prices from equipment not included in Matches' database were obtained from quotations of national and international companies and literature review. The use of concrete as construction material for the BCBs was considered in Chapters 4-5 due to its low cost (190 $€\cdot m^{-3}$ including civil work and construction) compared to traditional materials such as stainless steel that would result in economically unsustainable prices (3,000 $€\cdot m^{-3}$) for PHA production (Humbird et al., 2017). In Chapters 6-7, given the future use of ectoine in human cosmetic and pharmaceutical products, the use of stainless steel BCBs was recommended. Costing for CHP was calculated according to Wellinger and colleagues, assuming an investment cost of 1,000 $€\cdot kW_{el}^{-1}$ and operation and maintenance costs of 0.015 $€\cdot kWh^{-1}$ (Wellinger et al., 2013).

Table 3.2 Summary of the design variables and the factors for the estimation of equipment cost.

Type of equipment	Relevant variable (x)	Factors
Blower	Flow ($m^3 \cdot d^{-1}$)	a = 6 b = 0.79
Reactor CSTR	Volume (m^3)	a = 18,756 b = 0.53
Centrifuge	Flow ($m^3 \cdot d^{-1}$)	a = 1,117 b = 0.55
Pump	Flow ($m^3 \cdot d^{-1}$)	a = 339,20 b = 0.51
Column	Vessel weight (kg)	a = 354 b = 0.62
Tray dryer	Surface (ft^2)	a = 7,893 b = 0.38
Crystallizer	Volume (m^3)	a = 48,070 b = 0.33
Condenser	Surface (m^2)	a = 9,694 b = 0.24
Spray dryer	Water removal ($kg \cdot s^{-1}$)	a = 481,922 b = 0.21

The total investment cost (TIC) was calculated using the Lang's method. The Lang's method is a well-stablished factorial methodology for the calculation of the TIC in industrial plants by extrapolation from the individual value of purchased equipment cost (PEC) (Equation 3.27). The Lang's method allows the estimation of indirect costs that are not typically included in the PEC such as the installation and construction expenses, the potential civil works and the engineering and supervision. The method provides a set of ranges for the indicators that are summed to obtain the total Lang's Factor (Lf), 4.09 in this methodology. In this methodology, the values of the Lang's multipliers were selected according to the recommendations for solid-liquid processes in similar chemical industries (Table 3.3) (Levett et al., 2016; Ulrich and Vasudevan, 2006a). Note that in Chapter 7, several Lang's factors were identified as wage dependent parameters and were modified accordingly in order to study the effect of the economy of scale on the indirect labor costs.

$$TIC = Lf \cdot PEC \qquad \text{Equation 3.27}$$

Table 3.3 Summary of Lang’s factor selected for this methodology.

	Selected values	**Range**[2]
Equipment	1.00	1.00
+ Equipment installation labor[1]	0.38	0.39-0.47
+ Instrumentation and controls	0.12	0.09-0.18
+ Piping	0.31	0.16-0.66
+ Electrical installations	0.10	0.10-0.11
+ Buildings	0.29	0.18-0.29
+ Yard improvements[1]	0.10	0.10-0.13
+ Service facilities	0.54	0.40-0.70
+ Land	0.06	0.06
Direct plant cost	2.90	2.48-3.58
+ Engineering and supervision[1]	0.32	0.32-0.33
+ Construction expenses[1]	0.34	0.34-0.41
Direct and indirect costs	3.56	3.14-4.32
+Contractor's fee	0.18	0.17-0.2
+Contingency	0.36	0.34-0.42
Total depreciable costs (Lang’s Factor)	4.09	3.65-4.94

[1] Wage dependent parameters

[2] Individual factor ranges extracted from (Peters et al., 2003)

3.5.2 Calculation of operational costs

Operational costs were calculated as the sum of consumable and commodities costs (raw materials, water, electricity and utilities), maintenance costs, labor costs, transportation costs of raw materials and products, and waste treatment costs. Given the high geographical variability in consumable and commodity prices, and unless otherwise stated, Madrid (Spain) has been selected as the model scenario for the operational costs estimation. Throughout the development of this methodology, Madrid has demonstrated to exhibit a reliable worldwide average purchase price of consumables and commodities.

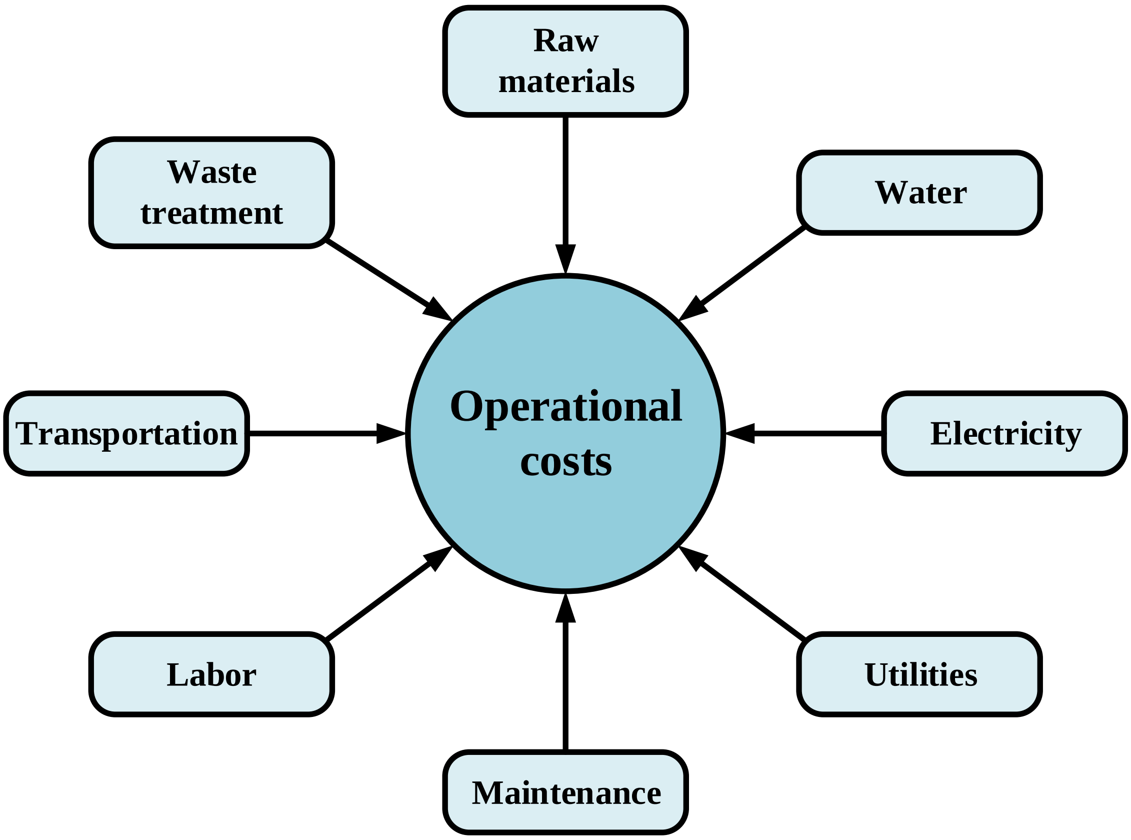

Figure 3.3. Graphical representation of the cost items included in the calculation of the operational costs.

Raw materials and chemical reagents

Raw materials requirements were calculated according to mass balances. The cost of raw materials, mainly chemical reagents, was gathered from quotations with national and international suppliers. Biogas was considered as a waste stream of the anaerobic digestion process and therefore, its acquisition cost was set equal to zero. If international companies are addressed, it is relevant to identify if transportation costs are included or not in the selling price as they cannot be considered negligible in certain situations. If national companies are addressed, and given the difficulty of finding reliable sources for local prices of chemical reagents in different regions of the world, the purchase price of chemicals can be extrapolated from region to region with a price level factor. Different parameters can be used for estimating the price level factor, however this methodology suggests using widely and publicly available indexes for the calculation of the price level factor. In this research, the Cost of Living Index (CLI) and the Annual Average Wage (Aw) expressed in €·y^{-1} in each region have been used for calculation, using Madrid (Spain) as the reference city (Numbeo.es Free Database, 2020; Worlddata.info, 2021). Subindex *i* stands for the value of the parameter evaluated in each region while subindex *R* srepresents the value of the parameter evaluated in the reference city. The selection of the parameter relies on the consideration of chemical reagents price as a wage-dependent or price-dependent parameter. The price level factor can be calculated according to Equation 3.28 and Equation 3.29:

$$Price\ level = \frac{CL_i}{CL_R} \qquad \text{Equation 3.28}$$

$$Price\ level = \frac{Aw_i}{Aw_R} \qquad \text{Equation 3.29}$$

Water, electricity and utilities

Water, electricity and utilities demand were calculated according to the mass and energy balances. Water and electricity costs were obtained from national and international industrial suppliers. The cost of utilities, mainly low pressure steam and cooling water, were estimated with the coefficient method provided by Ulrich and Vasudevan for process modules (Ulrich and Vasudevan, 2006b). Steam cost was calculated with Equation 3.30, where *Csteam* is the steam cost calculated in $\$\cdot kg^{-1}$, $\dot{m}_s$ represents the steam requirements in $kg\cdot s^{-1}$ and p indicates the steam pressure in barg. Cooling water cost was calculated with Equation 3.31, where C_{cw} stands for the cooling water cost calculated in $\$\cdot m^{-3}$ and q represents the cooling water requirements in $m^3\cdot s^{-1}$. *CEPCI* stands for the inflation parameters in US countries (596.2 in 2020) and $C_{S,f}$ is the price of fuel in $\$\cdot GJ^{-1}$.

$$C_{steam} = (2.7 \cdot 10^{-5} \cdot \dot{m}_s^{-0.9}) \cdot CEPCI + 0.0034 \cdot p^{0.05} \cdot C_{S,f} \qquad \text{Equation 3.30}$$

$$C_{cw} = (0.0001 + 3 \cdot 10^{-5} \cdot q^{-1}) \cdot CEPCI + 0.003 \cdot C_{S,f} \qquad \text{Equation 3.31}$$

Wastewater and solid waste treatment

Wastewater and solid waste treatment were considered as potentially relevant cost items in this analysis. Given the low organic load and volumetric flow of the wastewater produced in the biogas bioconversion processes herein described, compared to the typical digestate concentration and flowrate of liquid waste streams in urban waste treatment plants, the wastewater treatment costs were considered comparable to those of domestic wastewater (0.2 $€\cdot m^{-3}$). On the other hand, based on the organic nature and the low amounts of biomass purged in the processes, no additional charge was considered for this waste, as it could be easily recirculated to the first stages of the anaerobic digestion process (thus constributing to generate more biogas). At this point it should be stressed that small amounts of potentially hazardous waste are produced in these bioconversion and purification processes such as the ionic exchange resins for ectoine adsorption and the activated carbon used as packing material in the desulfurization biotrickling filter. A typical incineration cost for this waste of 500 $€\cdot t^{-1}$ was considered. Often, the management cost of this type of products is included in the product selling price.

Transportation

Transportation costs for raw materials and bioproducts were considered comparable to other petrochemical products (60 $€\cdot t^{-1}$) according to personal communication with industrial managers. It should be highlighted that transportation costs for raw materials are often included in the selling price and it should be therefore not accounted in the calculation of the transportation costs. In addition, it is worth mentioning that most techno-economic analysis do not include transportation costs into the bioproduct production cost share. Therefore, each scenario should be carefully and individually evaluated in order to prevent comparative advantages in the discussion of the results. In this research, transportation costs were not included in the analysis of PHA production, given that most authors did not include this item in the production costs of similar biopolymers. In the case of ectoine, on the contrary, since no similar techno-economic analysis had been reported and only selling market prices were available for the comparative evaluation, it was decided to include the transportation cost analysis in the techno-economic study.

Labor

To the best of the authors' knowledge, while there exist in literature multitude of methods for the evaluation of labor costs in well-stablished industries, there is not a standard method for evaluating the direct labor cost of biogas upgrading processes integrated in larger facilities. Therefore, direct labor costs were evaluated in each scenario considering the amount of full-time and part-time operators and shifts necessary for the operation, as recommended by industrial waste managers for each scenario evaluated. The average salary in Madrid (14.5 $€\cdot \text{person-h}^{-1}$) was used for the estimation of direct labor costs in the base-case scenario and adjusted to the different locations evaluated with the local value of average wage (Worlddata.info, 2021). It is relevant to note that the variability in the average wage induces a significant change not only in the direct labor costs but also in the calculation of the Lang's Factor, and therefore in the maintenance costs, given the wage-dependent nature of some of the factors considered (Table 3.3). The wage-dependent Lang's multipliers were adjusted to each scenario by multiplying the factor for the price level calculated according to Equation 3.29.

Maintenance

Maintenance costs are highly dependent on the industry and typically range from 1 to 10 % of the total investment costs (Eti and Ogaji, 2006). For this methodology, yearly maintenance costs of 3.5 % over the TIC were selected as recommended by industrial waste operators and in agreement with the relative simplicity of the equipment and control systems required for the biogas bioconversion into bioproducts. In order to confirm this recommendation, a comparative evaluation of the maintenance percentage was performed considering an interest rate of 5 % and an equipment lifespan of 20 y (Figure 3.4). The study, evaluating maintenance cost over the TIC in the range of 1-10 %, highlighted that a maintenance cost rate of 3.5 % over the TIC was sufficient to cover 1.2 times the TIC after the project time period of 20 years, which could be understood as a total renewal of the plant equipment after the equipment lifespan has expired. Note that the maintenance rate should be re-considered if more complex equipment and control or a different time horizon for the project is selected in further techno-economic analyses.

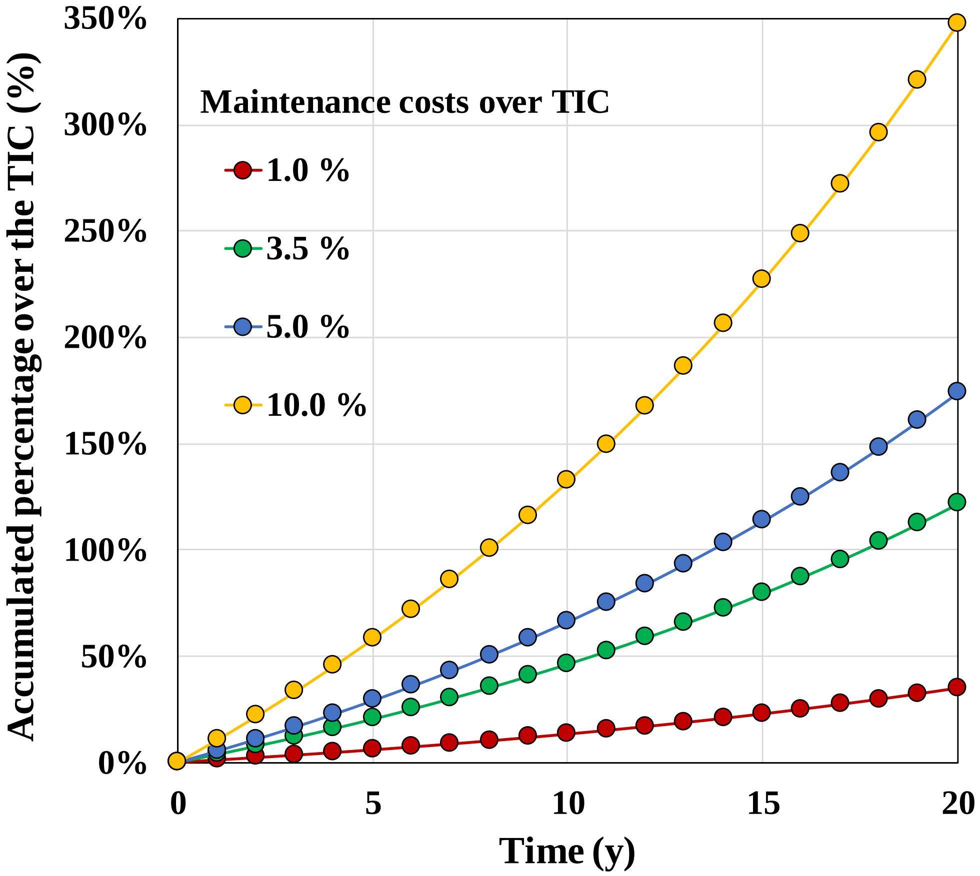

Figure 3.4. Influence of the maintenance cost rate on the time evolution of the accumulated maintenance cost over the TIC.

3.5.3. Determination of the economic performance

The net present value evaluated at 20 years (NPV_{20}), the internal rate of return (IRR), the payback period (PP) and the break-even price were selected for determining the economic performance of the processes evaluated.

The net present value (NPV) is defined as the difference between the present value of cash inflows and the present value of cash outflows over a period of time. NPV is typically used in investment planning in order to analyze the profitability of a projected investment and to find the current value of a future stream of payments. For investment projects in chemical plants, NPV is typically evaluated over a period of 10 or 20 years. For this methodology, 20 years was selected as the optimal period of time for studying the investment, in agreement with the typical lifespan of industrial equipment and the duration of the operation contracts of anaerobic digestion plants. The NPV_{20} was calculated according to Equation 3.32, where FCF_t stands for the free cash flow at time t, r represents the interest rate and TIC accounts for the total investment cost.

$$NPV_{20} = \sum_{t=1}^{t=20} \frac{FCF_t}{(1+r)^t} - TIC \qquad \text{Equation 3.32}$$

The free cash flow (FCF) can be defined as the amount of cash a business has remaining after paying capital expenditures. For the calculation of FCF_t, TIC was assigned to year 0. The interest rate (r) and the tax rate are highly dependent on the economic context, the geographical location of the plant and the risk of the investment. For this methodology, a 5 % interest rate and a 30 % tax rate were selected as standard for techno-economic analysis of biotechnological processes like the ones studied in this research. A linear depreciation of the equipment over the first 10 and 20 years was used for calculating the amortization costs in Chapters 4-5 and Chapters 6-7, respectively. A circulating capital, which is the initial amount of money required for the day-to-day operation of a business, of 2 % and 5 % over the TIC in year 1 was considered for Chapters 4-5 and Chapters 6-7, respectively. Table 3.4 summarizes the cost items and the calculation method of FCF_t followed in this methodology.

Table 3.4 Cost items and calculation method for the free cash flow.

Sign	Item	Description
(-)	A1. Total capital investment	Computed at year 0
(-)	A2. Circulating capital	2-5 % over the TIC in year 1
	A. Funds invested	$A1 + A2$
(+)	B1. Income from sales	Yearly production × Selling price
(-)	B2. Operational costs	Sum of all the operational costs
	B. Brut margin	$B1 + B2$
(-)	C1. Amortization	Linear during the first 10-20 years
	C. Benefit before taxes	$B + C1$
(-)	D1. Taxes	30 % over C (0 if C is negative)
	D. Benefit after taxes	$C + D1$
	E. Free cash flow	$A + D - C1$
	E2. Updated free cash flow	$\frac{E}{(1+r)^t}$
	F. Free cash flow accumulated	$\sum_{t=0}^{t=t} \frac{E}{(1+r)^t} = NPV_t$

The internal rate of return (IRR) is defined as the actual interest yield of an investment. IRR is by definition, the value of the interest rate (r) that would render NPV_{20} equal to zero (Equation 3.33). It is generally used for determining the risk of a projected investment. Risky investments would present an internal rate of return similar to the current interest rate and safe investments would present an internal rate of return notably higher than the interest rate. The payback period (PP) states the first period in which the initial investment is recovered and the NPV_t becomes positive (Equation 3.34). PP must be lower than the time horizon of the project for the investment to be feasible. Typically, PP of 10 years over a project of 20 years are considered acceptable.

$$NPV_{20} = \sum_{t=0}^{t=20} \frac{FCF_t}{(1+IRR)^t} = 0 \qquad \text{Equation 3.33}$$

$$NPV_t = \sum_{t=0}^{t=PP} \frac{FCF_t}{(1+r)^t} \geq 0 \qquad \text{Equation 3.34}$$

Figure 3.5 serves as graphical representation of the economic parameters recently mentioned. Green bars represent the yearly updated free cash flow. Yellow bars indicate the accumulated free cash flow. TIC is represented at t=0 (orange square) and the NPV_{20} at t=20 (purple square). The payback period is highlighted with a blue square and indicates the first time period in which the accumulated free cash flow or NPV becomes positive.

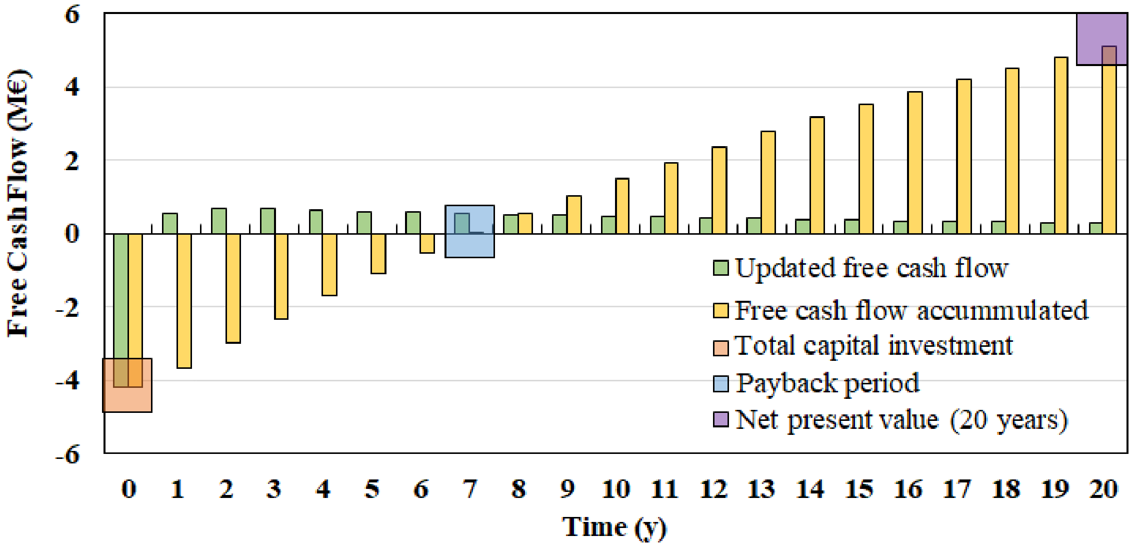

Figure 3.5. Graphical example of the economic parameters considered in this methodology for the economic evaluation of biogas valorization processes.

The income from sales was calculated as the product of the bioproduct selling price and the yearly total production. The bioproduct demand was considered constant throughout the 20-year period of the project and equal to the yearly production of bioproducts for the calculation of NPV_{20}. The break-even price was used for calculating the production costs of the bioproducts considered. The break-even price is considered a standard in techno-economic processes evaluation for the estimation of production costs and it is also typically referred in literature as "minimum selling price". The break-even price can be defined as the value of production costs that renders NPV_{20} equal to zero, IRR equal to the interest rate (5 %) and the PP equal to the time horizon (20 y). A minimum increase or decrease in the bioproduct selling price over the break-even price would render a project feasible or unfeasible. The bioproduct break-even price can be expressed explicitly by re-arranging Equation 3.32 with NPV_{20} equal to zero and was used for estimating the influence of several parameters in the sensitivity analysis. As an example, Figure 3.6 represents the influence of a ±10 % variation in the selling price on the NPV_t.

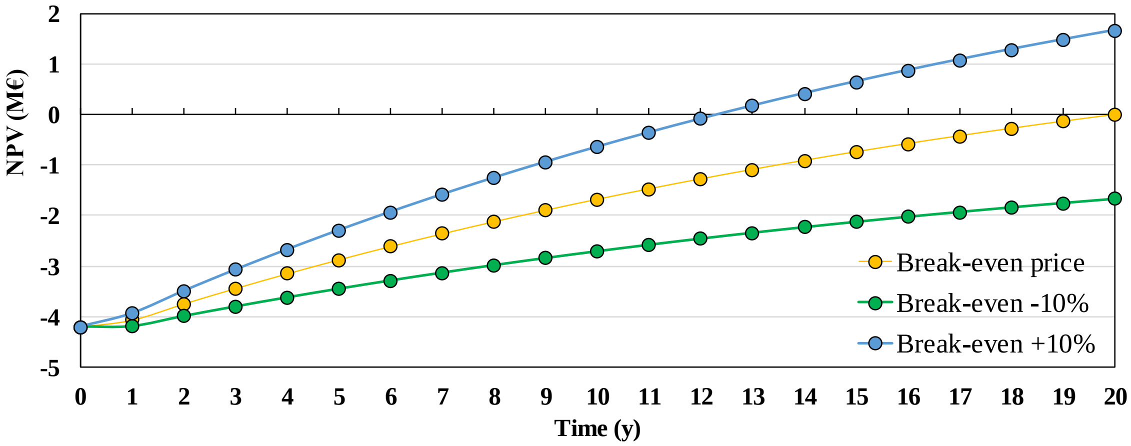

Figure 3.6. Graphical representation of the influence of a ±10 % change in the selling price on the NPV_t. Blue line represents a feasible project, green line represents an unfeasible project and the yellow line represents a project in the equilibrium point.

3.6 Sensitivity analysis

A sensitivity analysis studies the influence of certain input parameters on the target variables of a techno-economic analysis. Sensitivity assessments are accessory documents to techno-economic analysis that have two main goals: first, the determination of the uncertainty of the choices made during the design of the process and, therefore, give an insight of the robustness of the process and the results obtained; and second, and most importantly, indicate which variables and to what extent these variables are relevant for process optimization in order to identify the process bottlenecks. In our case, this will also help define the roadmap for future investigations.

In this methodology, the target variables were mainly the economic parameters considered for the evaluation of the techno-economic analysis, namely the NPV_{20}, the IRR, the PP and the bioproducts break-even price. Given the enormous number of inputs of the models herein presented, the input variables selected for the sensitivity analysis were grouped into two main groups: the cost of utilities and commodities, and the biotechnological parameters.

As previously mentioned, the cost of utilities and commodities is highly variable depending on the socioeconomic context, i.e. the location of the plant and the global economic context. The main variables included in this group are the operational costs such as electricity, water, price level and transportation costs. It is also of paramount importance to perform a sensitivity analysis on the capital costs, in order to estimate the error margin made during costing of the individual equipment. One way of performing the sensitivity analysis for these operational costs is the individual variation of each of the parameters from the values selected for the base-case scenario. This approach is useful for identifying the reliability of the set of costs chosen in the first place and for assessing the level of robustness of the results obtained. However, this approach ignores the potential synergetic variation of combined parameters under real world situations. For this purpose, the authors have proposed a geographical analysis as a more realistic approach for studying the combination of certain variables on the model output. Based on the work of Estrada and co-workers in the field of odor emissions abatement, we selected 13 representative cities to assess the high variability of commodity prices (water, electricity and chemical reagents) and of levels of adoption of anaerobic digestion technologies in different regions of the world (Estrada et al., 2012). Based on a compilation of data from national and international suppliers, the geographical analysis provides a set of inputs for realistic experiences and allows to minimize the local bias when performing a techno-economic analysis.

Regarding the sensitivity analysis of the biotechnological parameters, many authors have agreed that the design of enhanced gas-liquid mass transfer bioreactors, the increase of bacterial product yields and the development of cost-effective and highly efficient extraction and purification processes are nowadays the limiting barriers towards a competitive bioconversion of biogas in bioproducts (Choi and Lee, 1999; López et al., 2019; Pieja et al., 2017). In this context, the sensitivity analysis methodology herein presented addressed the assessment of the influence of the potential improvements in the CH_4-EC, the CH_4-RE, the biomass concentration in bioreactors, the enhancement of bacterial bioproduct yields and the decrease of extraction and purification costs on the final bioproduct production costs. Similar to the sensitivity analysis on commodities and consumable prices, the sensitivity analysis on biotechnological parameters can be performed by individually increasing / decreasing the value of each of the identified variables by a nominal percentage. This approach allows the identification of the most influential parameters on the final production costs. However, this strategy neglects the biological or technical maximum limits that some of these factors might have in reality and as a consequence lead to unrealistic or unfeasible results. In order to overcome this limitation, the authors of this methodology have proposed the use of currently available experimental data obtained at laboratory or demonstration scale for the calculation of the base-case scenario. Then, a study of the potential deviation of each of the variables based on the maximum and minimum values reported in literature and theoretical calculations should be performed. This holistic approach allows to present a reliable base-case scenario based on the current state-of-the-art of the technology but also to assess a realistic scenario for future bioconversion processes, based on the maximum theoretical improvement rates of the technologies.

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Chapter 4

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Polyhydroxyalkanoates (PHA) production from biogas in waste treatment facilities: Assessing the potential impacts on economy, environment and society

https://doi.org/10.1016/j.chemosphere.2020.126929

Chapter 5

Polyhydroxyalkanoates (PHA) production from biogas in waste treatment facilities: Assessing the influence of local commodity prices and current biotechnological limitations

https://doi.org/10.1021/acssuschemeng.0c01543

Chapter 6

Ectoine production from biogas in waste treatment facilities: A techno-economic and sensitivity analysis

https://doi.org/https://doi.org/10.1021/acssuschemeng.1c06772

Chapter 7

Ectoine production from biogas in waste treatment facilities: Assessing the effect of local commodity prices, economy of scale, market trends and biotechnological limitations

https://doi.org/https://doi.org/10.1016/j.jclepro.2022.133440

Chapter 8

Conclusions and future work

Conclusions and future work

A methodology was designed and implemented with the overall aim of evaluating the technical and economic feasibility of the bioconversion of biogas into added-value products using methanotrophic bacteria as an alternative to the current utilization of biogas as energy vector in organic waste treatment plants. The results herein obtained demonstrated the robustness and reliability of the featured methodology and the huge economic and environmental potential of transitioning from linear waste treatment plants, where biogas is merely regarded as an energy vector, to more circular urban biorefineries where biogas can be effectively transformed into added-value products such as PHA or ectoine.

In this context, **Chapter 3** featured the development of a robust methodology for assessing the economic potential and the technical limitations of biogas bioconversion processes using methanotrophic bacteria. The battery limits and calculation basis were defined according to the particular characteristics of current medium- and large-scale anaerobic digestion plants. For this purpose, the methodology included a compilation of data from waste managers and operators, national and international suppliers of equipment, raw materials and utilities, governmental entities and the most state-of-the-art research. This methodology has been improved throughout the last 5 years of research and has served as framework for the techno-economic and sensitivity assessment of the production of PHA, a low added-value biopolymer with a large market share, and ectoine, a high added-value pharmaceutical and cosmetic product with a small market demand, compared to the traditional combustion of biogas in CHP engines for producing energy. The techno-economic methodology herein developed allowed the identification of the most substantial operational cost shares as well as the most significant equipment cost. Additionally, the methodology for sensitivity analysis pointed out the most critical biotechnological barriers and helped defining the roadmap for future investigations.

The methodology was first implemented for the evaluation of the three pillars of sustainability (economic feasibility, environmental concern and social responsibility) of the biogas bioconversion into polyhydroxyalkanoates (PHA) in **Chapter 4**. This study demonstrated that biogas valorization into PHA in urban waste treatment plants constitutes already a competitive alternative to its current utilization for heat and power production. Although PHA production showed higher investment and operational costs, the higher market value of PHA overcame this limitation and both processes supported similar net present values evaluated at 20 years (NPV_{20}) and internal rates of return (IRR). This investigation indicated that PHA can be already produced from biogas at a competitive market price (8.6-8.8 €·kg^{-1} PHA) in medium- and large-size waste treatment plants, regardless of the economy of scale and the level of technology readiness. However, this research revealed that the optimal scenario for biogas valorization within a urban waste treatment plant involves the utilization of biogas-fuelled CHP units for providing the power and heat necessary for PHA production, extraction and purification, which would render PHA selling prices of 4.2-4.6 €·kg^{-1} PHA. In parallel, the environmental analysis showed that PHA production entails a significant reduction of atmospheric acidification and odor emissions compared to traditional CHP, while both processes showed similar impacts on global warming and water eutrophication and acidification. In contrast, biopolymer production exhibited higher ecotoxicity to aquatic life and ozone depletion potentials, and demanded more land, water, energy and chemical reagents than CHP. Finally, the increasing public demand for bioproducts and the job creation associated to this new biopolymer industry could potentially enhance social and local acceptance of urban waste treatment facilities, traditionally facing *Nimbyism* issues.

The robustness and sensitivity of the results obtained during this investigation were evaluated in **Chapter 5.** This research showed that biogas constitutes already a worldwide available and suitable feedstock for PHA production in waste treatment plants. The geographical analysis indicated that the economic performance of anaerobic digestion plants devoted to CHP exhibited a strong dependence on local commodity prices. Particularly, the variability in electricity acquisition costs determined the economic feasibility of biogas transformation into heat and electricity with NPV_{20} values ranging from −7.17 to +16.27 M€. The geographical analysis also demonstrated that the production costs of biogas-based PHA were comparable to the production costs of PHA from other waste carbon substrates. The regions with the lowest electricity prices exhibited the lowest PHA production costs (4.1 €·kg^{-1}), providing an opportunity for the widespread implementation of anaerobic digestion in these regions. In contrast, in countries with high energy prices the combination of CHP generation and PHA production from biogas is recommended for producing cost-competitive PHA (1.5 €·kg^{-1}). The sensitivity analysis showed a significant impact of methane elimination capacity and PHA accumulation yield on the total investment cost (TIC) and the PHA annual production. Overcoming these biotechnological limitations will allow reducing PHA production costs down to 0.2−1.9 €·kg^{-1}, which would render PHA competitive in price with their oil-based counterparts.

Chapter 6 constituted the first techno-economic study of the large-scale production of ectoine from biogas in waste treatment plants. The results indicated a high profitability of the process with payback times below 3 years in all the scenarios evaluated. Ectoine break-even prices in the best- and worst case scenarios considered entailed a 3- to 6-fold decrease in the ectoine production costs when compared to the current production via long-time fermentation with *Halomonas elongate*, mainly due to the use of CH_4-biogas as a low-cost carbon substrate for the growth of haloalkaliphilic bacteria. The process showed a high sensitivity toward the purchase cost of equipment and consumables (chemical reagents, raw materials, and utilities). On the contrary, the sensitivity analysis revealed a high robustness toward fluctuations on water and energy prices, labor and transportation costs. In summary, this study demonstrated that large-scale production of high added-value products from biogas represents a highly profitable alternative to the current utilization of biogas as energy source, but also a much more feasible valorization pathway than the production of low added-value bioproducts.

Chapter 7 showed that the utilization of methane-biogas as a feedstock for the production of high added-value products such as ectoine represents a highly profitable alternative to energy production in waste treatment facilities, in terms of economic sustainability. The results of this techno-economic assessment predicted the substitution of the current industrial ectoine production processes by the production of ectoine with methanotrophic haloalkaliphilic bacteria, given the 3-6-fold lower production costs herein reported. The sensitivity analysis showed a high profitability regardless of the commodity prices, the economy of scale and the fluctuations in the ectoine retail market. The analysis of the current biotechnological limitations showed that the improvement in methane elimination capacity in high-mass transfer bioreactors, the research on highly efficient microorganisms and the selection of the highest quality ionic exchange resins are critical parameters for the future development of biogas-based biorefineries.

Despite the substantial advances and promising results obtained in this thesis on the techno-economic analysis of the biogas bioconversion into added-value bioproducts in urban waste treatment plants, there is still much room for the improvement of the methodology and the results. In brief, further research on the techno-economic analysis on this topic should focus on:

- Comparative evaluation of the environmental impact of CHP, PHA and ectoine production from biogas with a comprehensive life cycle assessment.
- Enhancement of the methodologies for the assessment of the social impact of transitioning from energy-focused waste treatment plants to bioproduct-focused biorefineries.
- Improvement of the data collection by creating a database with updated purchase prices for raw materials, services and utilities.
- Development of a specific methodology for the estimation of labor costs of alternative biogas valorization schemes integrated in waste treatment plants.
- Broadening the understanding of the bioproducts market dynamics: total global demand, market shares, production trends, operational margins and potential stakeholders.
- Validation of the results obtained with experimental data at demo and industrial scale.

Chapter 9

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About the author

Víctor Pérez Martínez (Valladolid, 1992) studied a bachelor degree in Chemical Engineering at the University of Valladolid, Valladolid (Spain). He was awarded with an Erasmus+ mobility grant to conduct his final project at the Åbo Akademi University, Turku (Finland) under the supervision of Professor Henrik Grénman. This research investigated the hot water liquid extraction of hemicelluloses from lignocellulosic biomass and their further hydrolysis over heterogeneous catalysts as a profitable source of rare sugars.

Then, he studied a master in Chemical Engineering at the University of Valladolid, Valladolid (Spain) and was awarded with a second Erasmus+ mobility grant for developing his master thesis at the Technical University of Hamburg-Harburg, Hamburg (Germany) under the supervision of Dr. Wienke Reynolds. His research focused on the modeling and simulation of a pilot plant for the pretreatment of lignocellulosic biomass with the final purpose of producing chemical products and bio-fuels.

In 2016 he was selected by OAN International, a student-based non profit organization, as a volunteer for participating in its cooperation to development programme in Nikki (Benin). After that, he was elected for a two-year period as President of the Committee of Energy, Water and Infraestructures of the organization. During this time, he was in charge of several projects related to the improvement of water supply and sanitation in rural areas in Benin.

In 2017, he joined the VOC and Microalgae Research group in the Environmental Technology Research Group (Institute of Sustainable Processes – University of Valladolid). During this time, he has partitipated in two biorefinery demonstration projects (URBIOFIN and DEEP PURPLE) where he has been in charge of the preliminary design, detailed engineering, start-up and operation of three pilot plants devoted to the bioconversion of biogas into: biomethane, polyhydroxyakcanoates (PHA) and ectoine. In parallel, he has developed his PhD research under the supervision of Professor Raul Muñoz and Associate Professor Raquel Lebrero. This research has been focused on the techno-economic and sensitivity assessment of biogas valorization routes for the production of added-value products, focusing specially on the bioconversion of CH_4-biogas into PHA and ectoine using methanotrophic bacteria. During his period as pre-doctoral researcher, he has co-authored 10 publications in ISI-indexed journals and has contributed to the publication of 5 book chapters.

Publications in ISI-indexed journals

Within the scope of this PhD thesis

Pérez, V., Mota, C.R., Muñoz, R., Lebrero, R., 2020. Polyhydroxyalkanoates (PHA) production from biogas in waste treatment facilities: Assessing the potential impacts on economy, environment and society. Chemosphere 255. https://doi.org/10.1016/j.chemosphere.2020.126929

Pérez, V., Lebrero, R., Muñoz, R., 2020. Comparative Evaluation of Biogas Valorization into Electricity/Heat and Poly(hydroxyalkanoates) in Waste Treatment Plants: Assessing the Influence of Local Commodity Prices and Current Biotechnological Limitations. ACS Sustain. Chem. Eng. 8, 7701–7709. https://doi.org/10.1021/acssuschemeng.0c01543

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Out of the scope of this PhD thesis

Schmidt, L.M., Pérez Martínez, V., Kaltschmitt, M., 2018. Solvent-free lignin recovered by thermal-enzymatic treatment using fixed-bed reactor technology – Economic assessment. Bioresour. Technol. 268. https://doi.org/10.1016/j.biortech.2018.07.107

Marín, D., Posadas, E., Cano, P., Pérez, V., Blanco, S., Lebrero, R., Muñoz, R., 2018. Seasonal variation of biogas upgrading coupled with digestate treatment in an outdoors pilot scale algal-bacterial photobioreactor. Bioresour. Technol. 263. https://doi.org/10.1016/j.biortech.2018.04.117

Marín, D., Posadas, E., Cano, P., Pérez, V., Lebrero, R., Muñoz, R., 2018. Influence of the seasonal variation of environmental conditions on biogas upgrading in an outdoors pilot scale high rate algal pond. Bioresour. Technol. 255, 354–358. https://doi.org/10.1016/j.biortech.2018.01.136

Rodríguez, Y., Firmino, P.I.M., Pérez, V., Lebrero, R., Muñoz, R., 2020. Biogas valorization via continuous polyhydroxybutyrate production by *Methylocystis hirsuta* in a bubble column bioreactor. Waste Manag. 113, 395–403. https://doi.org/10.1016/j.wasman.2020.06.009

Rodero, M.R., Herrero-Lobo, R., Pérez, V., Muñoz, R., 2022. Influence of operational conditions on the performance of biogas bioconversion into ectoines in pilot bubble column bioreactors. Bioresour. Technol. 358. https://doi.org/10.1016/j.biortech.2022.127398

Severi, C.A., Pérez, V., Pascual, C., Muñoz, R., Lebrero, R., 2022. Identification of critical operational hazards in a biogas upgrading pilot plant through a multi-criteria decision-making and FTOPSIS-HAZOP approach. Chemosphere 135845. https://doi.org/https://doi.org/10.1016/j.chemosphere.2022.135845

Book chapters

López, J.C., Rodríguez, Y., Pérez, V., Lebrero, R., Muñoz, R., 2019. CH4-based polyhydroxyalkanoate production: A step further towards a sustainable bioeconomy, in: Kalia, V.C. (Ed.), Biotechnological Applications of Polyhydroxyalkanoates. https://doi.org/10.1007/978-981-13-3759-8

Lebrero, R., Osvaldo, D.F., Pérez, V., Cantera, S., Estrada, J.M., Muñoz, R., 2019. Biological treatment of gas pollutants in partitioning bioreactors, in: Advances in Chemical Engineering. https://doi.org/10.1016/bs.ache.2018.12.003

Pérez, V., Pascual, A., Rodrigo, A., García Torreiro, M., Latorre-Sánchez, M., Coll Lozano, C., David-Moreno, A., Oliva-Dominguez, J.M., Serna-Maza, A., Herrero García, N., González Granados, I., Roldan-Aguayo, R., Ovejero-Roncero, D., Molto Marin, J.L., Smith, M., Musinovic, H., Raingué, A., Belard, L., Pascual, C., Lebrero, R., Muñoz, R., 2020c. Integrated innovative biorefinery for the transformation of municipal solid waste into biobased products, in: Waste Biorefinery. pp. 41–80. https://doi.org/10.1016/b978-0-12-818228-4.00002-2

Rodríguez, Y., Pérez, V., López, J.C., Bordel, S., Igor Firmino, P., Lebrero, R., Muñoz, R., 2020. Coupling biogas with PHA biosynthesis, in: Koller, M. (Ed.), The Handbook of Polyhydroxyalkanoates. CRC Press. https://doi.org/10.1201/9780429296611

Rodero, M.R., Ángeles, R., Pérez, V., Gancedo, J., Bolado, S., Lebrero, R., Muñoz, R., 2020. Microalgae-based processes as an energy efficient platform for water reclamation and resource recovery, in: Advances in Science, Technology and Innovation. https://doi.org/10.1007/978-3-030-13068-8_23

Conference proceedings

Oral communications

Víctor Pérez, Raquel Lebrero, Raul Muñoz. “Scale-up of biogas upgrading and valorisation technologies”. 5th Jornada de Doctorandos del Programa de Doctorado en Ingeniería Química y Ambiental. 29 December 2017. Valladolid (Spain). Awarded with best 1st-year student conference prize.

Víctor Pérez, Raquel Lebrero, Raúl Muñoz. “Recent advances in URBIOFIN project: design, scale-up (and bureaucracy…)”. 6th Jornada de Doctorandos del Programa de Doctorado en Ingeniería Química y Ambiental. 3 December 2018. Valladolid (Spain). Awarded with best 2nd-year student conference prize.

Víctor Pérez, Raquel Lebrero, Raúl Muñoz, Rebeca Pérez. “Optimisation of CH_4 bioconversion into high added-value biopolymers: Polyhydroxyalkanoates (PHA)”. Biotechniques for Air Pollution Control & Bioenergy 2019. 30 August 2019. Galway (Ireland).

Víctor Pérez, Celia Pascual, Raquel Lebrero, Raúl Muñoz. “Transformación de biogás en biopolímeros y biometano a escala semi-industrial”. XXXVI Jornadas Nacionales de la Ingeniería Química. 5 September 2019. Zaragoza (Spain).

Víctor Pérez, Celia Pascual, Cristian Alfredo Severi, Raquel Lebrero, Raúl Muñoz. “The role of biogas/anaerobic digestion in the future of Urban Biorefineries: Production of higher-value biostimulats and biopolymers with algae and bacteria”. " 1st URBIOFIN workshop: boosting circular economy in cities through new models of municipal solid waste management. the urbiofin biorefinery concept. 16 June 2020. On-line.

Víctor Pérez. “Tecnologías de tratamiento de aguas residuales en España”. Tecnologias de Tratamento de Efluentes: Uma Visão Brasil – Espanha. 26 August 2020. On-line.

Víctor Pérez, Cristian Alfredo Severi, Celia Pascual, Raquel Lebrero, Raúl Muñoz. “Valorización de biogás en biopolímeros y biometano a escala semi-industrial- Valorization of biogas into biopolymers and biomethane at semi-industrial scale”. III Jornada Internacionales de investigación ciencia y universidad - Universidad Maza (Argentina). 19 October 2020. On-line.

Víctor Pérez, Cristian Alfredo Severi, Celia Pascual, Raquel Lebrero, Raúl Muñoz. “Valorización de biogás en biopolímeros y biometano a escala semi-industrial”. 15º Encuentro Internacional de Ciencias de la Tierra (E-ICES-15). 23-25 November 2020. On-line.

Víctor Pérez, Cristian Alfredo Severi, Celia Pascual, Raquel Lebrero, Raúl Muñoz. “Proyecto URBIOFIN: Transformación de biogás en biometano a escala piloto”. I Curso de verano de biogás: Life Smart Agromobility. 15 July 2021. Soria (Spain).

Víctor Pérez, Raul Muñoz. “Proyecto Deep Purple - Bioconversión de biogás en ectoína a escala de laboratorio, escala demostrativa y escala real”. III Jornada Técnica sobre Biorrefinerías: Soluciones para la transformación integral de residuos orgánicos y emisiones gaseosas en bioproductos: tecnologías y casos de éxito. 15 November 2022. Valencia (Spain).

Poster communications

Víctor Pérez, Raul Muñoz, Raquel Lebrero. “Towards a circular bioeconomy: Urban biorefineries will transform municipal solid waste into bioproducts. How can microalgae be integrated into waste biorefineries?”. 3rd EUAlgae training school. 24-25 November 2018. Florence (Italy).

Víctor Pérez, Raquel Lebrero, Raúl Muñoz. “Is PHA production from biogas a realistic alternative in waste treatment facilities?”. 7th Jornada de Doctorandos del Programa de Doctorado en Ingeniería Química y Ambiental. 2 December 2019. Valladolid (Spain). Awarded with best poster prize.

Víctor Pérez, Cristian Severi, Celia Pascual, Raquel Lebrero, Raúl Muñoz. “One small step for the environment one giant leap for the ISP: Challenges of scaling-up biological biogas valorisation processes”. 8th Jornada de Doctorandos del Programa de Doctorado en Ingeniería Química y Ambiental. 2 December 2020. Valladolid. (Spain).

Víctor Pérez, Raquel Lebrero, Raúl Muñoz. “Can methanotrophs produce high-added value products?”. 9th Jornada de Doctorandos del Programa de Doctorado en Ingeniería Química y Ambiental. 2 December 2021. Valladolid. (Spain).

Víctor Pérez, Maria del Rosario Rodero, Raúl Muñoz. “Proyecto Deep Purple: Bioconversión de biogás en ectoína a escala de laboratorio, escala demostrativa y escala real”. XV Congreso Internacional de Bioenergía. 5-6 October 2022. Valladolid (Spain).

Víctor Pérez, Maria del Rosario Rodero, Raquel Lebrero, Raúl Muñoz. “Deep Purple Project: Biogas bioconversion into ectoine at lab-, demo- and industrial-scale”. 10th Jornada de Doctorandos del Programa de Doctorado en Ingeniería Química y Ambiental 2 December 2022. Valladolid (Spain).

Participation in research projects

INCOVER: Innovative Eco-Technologies for Resource Recovery from Wastewater. Funder: European Union Horizon2020 program. Water 1b-2015. Principal investigators: Raquel Lebrero and Raúl Muñoz. Total budget: 7,209,031 €. University of Valladolid budget: 336,687 €.

URBIOFIN: Demonstration of an integrated innovative biorefinery for the transformation of Municipal Solid Waste (MSW) into new BioBased products. Funder: Programa: H2020-BBI-JTI-2016. Principal investigators: Raquel Lebrero and Raul Muñoz. Total budget: 10,946,366 €. University of Valladolid budget: 1,132,250 €.

DEEP PURPLE: Conversion of diluted mixed urban bio-wastes into sustainable materials and products in flexible purple photobiorefineries. Funder: H2020-BII-JTI-2018. Principal investigator: Raul Muñoz. Total budget: 7,549,300 €. University of Valladolid budget: 590,425 €.

PROCODES: Experimental study of commercial desulphurization products. Funder: Contract signed with the waste management company URBASER. Principal investigators: Raquel Lebrero and Raul Muñoz. Total budget: 3,600 €.

Biological valorization of diluted emissions of CH_4 for the production of bioplastics. Funder: MINECO CTM2015-73228-JIN. Principal investigators: Rebeca Pérez and Raúl Muñoz. Total budget: 205,700 €.

Reviewer experience

Reviewer in Journal of Environmental Technology & Innovation since July 2021 (IF: 7.758).

Teaching and co-supervision

Lecturer at the Master of Environmental Engineering from the University of Valladolid. Course “Environmental engineering laboratory practical courses”. 8 h.

Lecturer at the Master of Environmental Engineering from the University of Valladolid. Course “Prevención y tratamiento de la contaminación atmosférica”. 2 h.

Co-supervisor of Cristina Villamediana Lozano, student of BSc in Chemical Engineering at the University of Valladolid. Project title: “Estudio de la eficacia de remoción de materia orgánica, nutrientes y otros compuestos en un filtro de bioarena para su implantación en países en vías en desarrollo”. Date: 22 July 2019.

Co-supervisor of Christophe Moma, exchange student of MSc in Chemical Engineering at the University of Valladolid. Project title: "Biogas valorization via combined heat and power generation”. Date: 8 July 2020.

Co-supervisor of Gorka Hontiyuelo, student of BSc in Chemical Engineering at the University of Valladolid. Project title: “Techno-economic study on the biogas bioconversion into ectoine”. Date: Planned June 2023.

Committee membership

Member of the Organizing Committee of the “IV Conferencia Internacional Sobre Gestión de Olores y COVs en el medio ambiente”. 20-21 September 2017. Valladolid (Spain).

Member of the Institute of Sustainable Processes (ISP) of the University of Valladolid since 2018.

Member of the Organizing Committee of the “IWA Conference on Algal Technologies and Stabilization Ponds for Wastewater Treatment and Resource Recovery. Iwalgae 2019”. 1-2 July 2019. Valladolid (Spain).

Proposer and member of the committee for the standardization of the “Extraction, production and purification of added value products from urban wastes — Part 1: Production and purification of ectoine obtained from biogas”. CWA Deep Purple prCWA 17897-1:2022 within the framework of the CEN-CENELEC system.

Specialized courses & Workshops

“Odours, science and engineering” (8 h). 20-23 November 2017. School of Industrial Engineering, University of Valladolid, Valladolid (Spain). Lecturer: Selena Sironi (Politecnico di Milano, Italy).

“Escritura de artículos científicos en ingeniería y arquitectura” (4 h). 31 May 2018. School of Industrial Engineering, University of Valladolid. Lecturer: Prof. Raúl Muñoz Torre (University of Valladolid, Spain).

“Taller práctico sobre Técnicas analíticas físico-químicas e instrumentales” (8.5 h). 6-16 November 2018. School of Industrial Engineering, University of Valladolid, Valladolid (Spain). Lecturers: Araceli Crespo, Beatriz Muñoz, Mónica Gay, Isabel Rodríguez and Enrique Marcos (University of Valladolid, Spain).

“Evaluación tecno-económica en biorefinerías de biomasa lignocelulósica” (8 h). 10-11 December 2018. School of Industrial Engineering, University of Valladolid, Valladolid (Spain). Lecturer: Ana Isabel Susmozas (Ciemat, Spain).

“Aprende a trabajar en equipo: metodología Belbin en acción” (4 h). 8 November 2018. School of Industrial Engineering, University of Valladolid, Valladolid (Spain). Lecturer: Leire Juez Prieto (Belbin, Spain).

“3rd EUAlgae training school: Microalgal bioeconomy in modern society”. (17 h). 24-25 November 2018. CNR-Area di Ricerca di Firenze, Istituto per lo Studio degli Ecosistemi, Florence (Italy).

“Life Cycle Assessment. Calc Software” (8 h). 17-19 December 2018. School of Industrial Engineering, University of Valladolid, Valladolid (Spain). Lecturer: Dr. Alejandro Gallego (University of Manchester, UK).

“Biotecnología de microalgas” (35 h). 13 January-11 March 2019. Online, University of Almería, Almería (Spain). Lecturers: Francisco Gabriel Acien, Emilio Molina Grima and colleagues (University of Almería, Spain).

“Comunicación eficaz para el mercado laboral” (8 h). 12-14 February 2019. UVa orienta, Fundacion General de la Universidad de Valladolid. Valladolid. Spain. Lecturer: Inés Moya de la Calle (Fundación General de la Universidad de Valladolid, Spain).

“Gas Chrompatography basic course” (6 h). 14-21 February 2019. School of Industrial Engineering, University of Valladolid, Valladolid (Spain). Lecturer: Dr. Jon Sanz Landaluze (Complutense University of Madrid, Spain)

“Fundamentals of Bioengineering” (8 h). 25-28 March 2019. School of Industrial Engineering, University of Valladolid, Valladolid (Spain). Lecturer: Dr. José Martínez Ruiz (DTU, Denmark).

“How to write great papers: from title to references, from submission to publication”. 23 October 2019. Library of the University of Valladolid, University of Valladolid, Valladolid (Spain). Lecturer: Massimiliano Bearzot (Consumer Consultant, Elsevier Researcher Academy).

“Formación de primeros auxilios en los laboratorios”. (2 h). 12 November 2019. School of Industrial Engineering, University of Valladolid, Valladolid (Spain). Lecturers: Beatriz Bombín Granado and Maria Dolores Villalonga Martín de Aguilera (University of Valladolid, Spain).

“Comités éticos en la investigación en la UVa”. (3 h). 10 December 2019. Escuela de doctorado ESDUVA, University of Valladolid, Valladolid (Spain).

“Protección del conocimiento: patentes, protección intelectual y derechos de autor”. (8 h). 11-12 December 2019. Escuela de doctorado ESDUVA, University of Valladolid, Valladolid (Spain). Lecturer: José Luis Velasco Pérez (Fundación General de la Universidad de Valladolid, Spain).

“Iniciación al análisis de secuencias 16 S Illumina® MiSeq para el estudo de comunidades bacterianas” (14 h). 16-19 December 2019. School of Industrial Engineering, University of Valladolid, Valladolid (Spain). Lecturer: Dr. Sara Cantera (Wageningen University and Research Center, The Netherlands).

“Microalgae biorefineries as multi-product integrated biorefineries: A course on combined modelling and experimental approaches”. (8 h). 21-22 January 2020. School of Industrial Engineering, University of Valladolid, Valladolid (Spain). Lecturer: Constantinos Theodoropoulos (Department of Chemical Engineering and analytical Science, University of Manchester, United Kingdom).

“Eficiencia y viabilidad de nuevas tecnologías en procesos de tratamiento de aguas residuales” (10 h). 9-13 March 2020. School of Industrial Engineering, University of

Valladolid, Valladolod (Spain). Lecturer: Dr. Francesc Hernández (University of Valencia, Spain).

“Funding your research”. 16-17 April 2020. Online, Elsevier Researchers Academy.

“Curso en prevención de riesgos laborales” (50 h). 23 June-8 July 2020. Online, Fundación General de la Universidad de Valladolid, Valladolid (Spain). Lecturer: FREMAP- Quirón Prevención (Spain).

“Valorización de resultados de investigación y creación de EBTS”. (6 h). 25-26 November 2020. Escuela de doctorado ESDUVA, University of Valladolid, Valladolid (Spain). Lecturer: Pedro Ignacio Ruiz de la Loma (Parque científico de la Universidad de Valladolid, Spain).

“Financiación de la investigación”. (8 h). 2-16 December 2020. Escuela de doctorado ESDUVA, University of Valladolid, Valladolid (Spain). Lecturer: Yolanda Calvo Conde (Fundación General de la Universidad de Valladolid, Spain).

“Taller de divulgación científica para personal investigador”. (15 h). 10-16 December 2020. Escuela de doctorado ESDUVA, University of Valladolid, Valladolid (Spain). Lecturer: Antonio Martín (Universidad de Valladolid, Spain).

“Introduction to Next-Generation Sequencing Technologies” (5 h). 10-11 December 2020. School of Industrial Engineering, University of Valladolid, Valladolid (Spain). Lecturer: Dr. Sara Cantera (Wageningen University and Research Center, The Netherlands).

“Enzimas fúngicas”. (8 h). 18-21 October 2021. Online, University of Valladolid, Valladolid (Spain). Lecturer: María Daniela Rodríguez Mendoza (Universidad Nacional de Misiones, Argentina).

“Anaerobic digestion, Quo Vadis?” (4.5 h) 21 October 2021. Institute of Sustainable Processes. University of Valladolid, Valladolid. (Spain).

“Cómo orientar tu carrera académica”. (8 h). 26-27 May 2021. Escuela de doctorado ESDUVA, University of Valladolid, Valladolid (Spain). Lecturer: Javier Blasco (Universidad de Valladolid, Spain).

“¿Cómo realizar una evaluación económica en proyectos de ingeniería?”. (4 h). 24 June 2022. School of Industrial Engineering, University of Valladolid. Lecturer: Segismundo Izquierdo (University of Valladolid, Spain).

“Taller de excel para investigadores”. (2 h) 24 November 2022. School of Industrial Engineering, University of Valladolid. Lecturer: Rafael Mato (University of Valladolid, Spain).

Dissemination activities

Participation in the science dissemination activity “Espacios de Ingenio: creatividad, tecnología y sostenibilidad” held in Valladolid (Spain) on the 15th March 2018.

Participation in the science dissemination activity “La noche europea de los investigadores” held in Valladolid (Spain) on the 29th September 2017.

Participation in the semi-final of the science dissemination contest “Three Minute Thesis” organized by the Escuela de doctorado ESDUVA, University of Valladolid, Valladolid (Spain) in 2021 with a presentation entitled “A grandes males, pequeños remedios: utilización de bacterias para combatir el cambio climático”.

Participation in the final of the science dissemination contest “Three Minute Thesis” organized by the Escuela de doctorado ESDUVA, University of Valladolid, Segovia (Spain) in 2022 with a presentation entitled “Profesor Bacterio, mision ¡Salvar el mundo!”.

Grants and prizes

Awarded with a EU-COST Action mobility grant for participating in the "3rd EUAlgae training school: Microalgal bioeconomy in modern society" held in the CNR-Area di Ricerca di Firenze, Istituto per lo Studio degli Ecosistemi, Florence (Italy) on the 24th and 25th November 2018 with a poster communication entitled "Towards a circular bioeconomy: Urban biorefineries will transform municipal solid waste into bioproducts. How can microalgae be integrated into waste biorefineries?" co-authored by Víctor Pérez, Raul Muñoz and Raquel Lebrero.

Awarded with best 1st-year student conference prize in the 5th Jornada de Doctorandos del Programa de Doctorado en Ingeniería Química y Ambiental organized by the Programa de doctorado en Ingeniería Química y Ambiental at the School of Doctoral Studies from the University of Valladolid, Valladolid (Spain) on the 29th December 2017. The presentation was entitled "Scale-up of biogas upgrading and valorisation technologies" and was co-authored by Víctor_Pérez, Raquel Lebrero and Raul Muñoz.

Awarded with best 2st-year student conference prize in the 6th Jornada de Doctorandos del Programa de Doctorado en Ingeniería Química y Ambiental organized by the Programa de doctorado en Ingeniería Química y ambiental at the School Doctoral Studies from the University of Valladolid, Valladolid (Spain) on the 3rd December 2018. The presentation was entitled "Recent advances in URBIOFIN project: design, scale-up (and bureaucracy…)" and was co-authored by Víctor_Pérez, Raquel Lebrero and Raul Muñoz.

Awarded with best poster prize in the 7th Jornada de Doctorandos del Programa de Doctorado en Ingeniería Química y Ambiental organized by the Programa de doctorado en Ingeniería Química y Ambiental at the School of Industrial Engineering from the University of Valladolid, Valladolid (Spain) on the 2nd December 2019. The poster was entitled "Is PHA production from biogas a realistic alternative in waste treatment facilities?" and was co-authored by Víctor_Pérez, Raquel Lebrero and Raul Muñoz.

Finalist in the science dissemination contest "Three Minute Thesis" organized by the Escuela de doctorado ESDUVA, University of Valladolid, Valladolid (Spain) in 2021 with a presentation entitled "A grandes males, pequeños remedios: utilización de bacterias para combatir el cambio climático".

Granted with a 4-year pre-doctoral research contract by the Regional Government of Castile and León on the 2019 call for pre-doctoral research contracts with a budget of 80,500 € for developing the research entitled “Diseño y escalado de tecnologías para el upgrading y valorización de biogás”.

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A Raquel, por haberme guiado no solo por los caminos de la ciencia, si no también en el respeto, la empatía y la colaboración con los demás. Nunca has dejado de alimentar mi curiosidad ni has eludido un debate conmigo, aunque sea un cabezota y mis preguntas hayan aparecido cuando tú preferías dormir en aviones o autobuses. Has sido un gran ejemplo de cómo se puede ser mi jefa en el despacho y mi amiga en la cafetería. Muchas gracias.

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