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ESTRATEGIAS PARA LA MEJORA DE LA DIGESTIÓN ANAEROBIA: INTEGRACIÓN DE PROCESOS ELECTROQUÍMICOS Y BIOELECTROQUÍMICOS

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STRATEGIES FOR IMPROVING ANAEROBIC DIGESTION: INTEGRATION OF ELECTROCHEMICAL AND BIOELECTROCHEMICAL PROCESSES

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LISTA DE ABREVIATURAS/ LIST OF ABBREVIATIONS

En este listado se recogen las siglas pertenecientes a los capítulos experimentales en orden alfabético

AD	Anaerobic Digestion
AOP	Advanced Oxidation Process
BDD	Boron Doped-Diamond
BES	Bioelectrochemical Systems
CF	Carbon Felt
CHP	Combined Heat and Power
COD	Chemical Oxygen Demand
CR	Control Reactor
CST	Capillary Suction Time
EO	Electrooxidation
EPS	Extracellular Polymeric Substance
FID	Flame Ionization Detector
FTIR	Fourier Transform Infrared
HR	Hydrogen reactor
HRT	Hydraulic Retention Time
MEC	Microbial Electrolysis Cell
MFC	Microbial Fuel Cell
MT	Million Tons
OLR	Organic Loading Rate
OTU	Operational Taxonomic Unit

PAS	Pyrolised Almond Shell
PBS	Phosphate Buffer Solution
PS	Primary Sludge
SBP	Standard Biogas Production
SMP	Standard Methane Production
SRF	Specific Resistance Filtration
SS	Sewage Sludge
SSA	Specific Surface Area
STP	Standard Temperature and Pressure
TCD	Thermal Conductivity Detector
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Polyphenols
TS	Total Solids
TSS	Total Suspended Solids
VFA	Volatile Fatty Solid
VS	Volatile Solids
WAS	Waste Activated Sludge
WL	Wine Lees
WWTP	Wastewater Treatment Plant

RESUMEN

El crecimiento de la producción de residuos orgánicos y su gestión, unida a la demanda energética de la población a nivel global, son aspectos importantes a los que tiene que hacer frente la sociedad del s. XXI en las futuras décadas. Poner en práctica políticas encaminadas a la protección del medio ambiente, aprovechar al máximo los recursos naturales, valorizar los residuos generados y apostar por tecnologías renovables será esencial para frenar el cambio climático

La digestión anaerobia es una tecnología madura, robusta y segura. Se trata de una tecnología escalable y puesta en práctica a nivel global. Su practicidad reside en la capacidad de transformar la materia orgánica contenida en los residuos y generar productos de alto interés energético y agrícola. No obstante, la digestión anaerobia encuentra dificultades para degradar componentes complejos y, es por ello, que necesita de estrategias complementarias para mejorar su funcionamiento.

Estrategias como la utilización de pretratamientos permiten reducir la cantidad de compuestos de difícil degradación y tóxicos. No obstante, aquellos que son más eficientes suelen presentar una demanda energética elevada y un coste excesivo. Es por ello, que se necesita buscar nuevas vías que sean igualmente eficaces pero que permitan obtener beneficios económicos.

Este trabajo está enfocado en el empleo de diversas estrategias como son: la inyección de hidrógeno, la mejora de los tratamientos bioelectroquímicos y la aplicación de pretratamientos oxidativos avanzados para aumentar el rendimiento de la digestión anaerobia, lo cual se verá traducido en el aumento de la producción de biogás. Estas estrategias son complementarias al trabajo realizado por el digestor y han sido puestas en práctica para degradar residuos complejos, como son los lodos de depuradora, y con presencia de material recalcitrante como las vinazas y lías de fermentación.

En el capítulo 4 de este trabajo se ha estudiado el impacto que tiene la inyección de hidrógeno en reactores que operan en semicontinuo y el cambio producido sobre las comunidades microbianas. También se realizó una evaluación energética sobre la integración teórica de un sistema bioelectroquímico (BES) en una estación depuradora

de aguas residuales (EDAR). La inyección de hidrógeno supuso un incremento del 12% de la producción de biogás comparado con el control (1353 mL CH_4 d^{-1} a un flujo de inyección de 1938 mL H_2 d^{-1}). La evaluación microbiológica mostró ligeros cambios en la abundancia relativa de las arqueas y notables en la comunidad de eubacterias. La evaluación de una hipotética incorporación de una BES podría servir para la mejora de la producción de biogás del digestor anaerobio, pero está lejos de cubrir todas las necesidades energéticas de una EDAR.

En el capítulo 5 se puso a prueba la capacidad que tiene un material pirolizado de origen vegetal para ser incorporado como electrodo en una BES frente a la utilización de fieltro de carbono. También se hizo una caracterización de las poblaciones residentes sobre ambos tipos de electrodos. Los electrodos se dispusieron en celdas de electrolisis microbiana (MEC) bicamerales —separadas por una membrana— que operaron en discontinuo. La cáscara de almendra pirolizada exhibió un funcionamiento muy similar al mostrado por el electrodo de fieltro de carbono durante los 90 días de la experimentación. Los resultados mostrados abren la posibilidad de ir avanzando en la valorización de subproductos de diversa procedencia para ir sustituyendo parcialmente a los costosos electrodos tradicionales.

En el capítulo 6 se empleó una celda de diamante dopado con boro (BDD) para tratar, antes y después de la digestión anaerobia, los lodos activados de depuradora (WAS) evaluando diversos parámetros —pH, densidades de corriente, tiempo de experimentación, etc. —. El objetivo fue determinar su influencia en la solubilización de la materia orgánica y en la deshidratación. Los resultados obtenidos mostraron una mejora en el rendimiento de producción de metano y una mejora en las propiedades reológicas de los WAS.

En el capítulo 7 también se utilizó una celda BDD como estrategia para el pretratamiento de residuos líquidos de la industria del vino. Se comparó su eficiencia frente la adición de biochar. Después de 47 días, la digestión anaerobia de lías de fermentación presentó una mejora de entre 28.8-50% en la producción de biogás y una reducción de la DQO de un 20%. Por el contrario, la adición de biochar no mostró en este caso efectos sobre la producción de biogás.

ABSTRACT

The growth of the amount of organic waste and its management, along with the increase in energy demand of the global population, are relevant aspects that society of the 21st century needs to confront in future decades. Policies aimed at protecting the environment, making the most of natural resources, recovering waste material and investing in renewable technologies will be essential to curb climate change

Anaerobic digestion is a mature, robust and safe technology. It is an easy scalable technology widely implemented. Its technical advantages lie in the ability to greatly reduce the volume of wastes to transform them into products of high energy content and agricultural interest. However, anaerobic digestion finds it difficult to degrade complex organic materials and therefore requires complementary strategies to improve its performance.

Strategies such as the use of pre-treatments allows for a reduction in the amount of difficult-to-degrade and toxic compounds. However, those that are more efficient tend to be energy-demanding and costly. Therefore, it is necessary to look for new ways of pre-treatments that may be as effective but providing economic benefits to traditional waste treatment processes.

In this work, the focus is on the use of different strategies such as: hydrogen injection, improvement by the use of bioelectrochemical treatments and advanced oxidative pre-treatments to increase the efficiency of anaerobic digestion which will result in increased biogas production. These strategies are complementary to the work carried out by the digester and have been implemented to degrade recalcitrant wastes such as sewage sludge and stillage.

Chapter 4 of this work has studied the impact of hydrogen injection in semi-continuously operating reactors and the change produced on microbial communities. An energy assessment was also carried out on the theoretical integration of a bioelectrochemical system (BES) in a wastewater treatment plant (WWTP). Hydrogen injection resulted in a 12% increase in biogas production compared to the control system (1353 mL CH_4 d^{-1} at an H_2 injection flow rate of 1938 mL H_2 d^{-1}). Microbial analysis showed slight changes in

the relative abundance of archaea and notable changes in the Eubacteria community. The evaluation of a hypothetical incorporation of a BES could serve to improve the biogas production of the anaerobic digester, but it is far from covering all the energy needs of a WWTP.

In Chapter 5, the ability of pyrolysed material from plant origin to be incorporated as an electrode in a BES was tested against the use of carbon felt. A characterisation of microbial populations on both types of electrodes was carried out. Electrodes were arranged in bicameral microbial electrolysis cells (MEC) separated by a membrane operating in batch conditions. The pyrolysed almond shell exhibited a similar performance to that shown by the carbon felt electrode during the 90-day experimental period. The results open up the possibility for advancing in the valorisation of by-products of diverse origin to partially replace the expensive traditional electrodes.

In chapter 6, a boron-doped diamond (BDD) cell was used to treat, before and after anaerobic digestion, waste activated sludge (WAS) evaluating different parameters — pH, current densities, experimental time, etc.—. The aim was to establish their influence on organic matter solubilisation and dewatering. The results obtained showed an improvement in methane yield as well as in rheological properties of WAS.

In chapter 7 a BDD cell was used as a strategy for the pre-treatment of wine lees (WL). Its efficiency was compared to the addition of biochar. After 47 days, the anaerobic digestion of WL achieved an improvement of 28.8 — 50% in biogas production and a reduction of COD by 20% —. In contrast, digesters supplemented which biochar showed no positive effect.

Capítulo/Chapter 1

Introducción General

1.1 INTRODUCCIÓN GENERAL

1.1.1 Situación actual

En 1997, la UE estableció en el Libro Blanco los principios que rigen la seguridad medioambiental y la promoción de las energías renovables (E. Commission 1997). Esta iniciativa fue seguida por la Directiva de Energías Renovables (Directive 2009/28/EC) y la Directiva de Biocombustibles (Directive 2003/30/EC). Así como el Plan Estratégico en Tecnologías Energéticas y el Plan de Acción de Energías Renovables que instaron a cada uno de los Estados Miembros a cumplir con lo legislado, adaptándose a la realidad de cada país (National renewable energy action plans 2020; Strategic Energy Technology Plan 2007). Las políticas medioambientales adoptadas cristalizaron en una cumbre a nivel global conocida como el Acuerdo de Paris de 2015. En dicha cumbre 195 países acordaron reducir las emisiones de gases de efecto invernadero en al menos 40% para el año 2030 y en un 85-90% para el año 2050 con respecto los niveles de emisión de 1990 (E. U. Commission and others 2007; United Nations 2015). El objetivo final fue mantener la temperatura global 2 ˚C por debajo de los niveles pre-industriales. Por lo que, reemplazar los combustibles fósiles por fuentes renovables facilitará de forma importante alcanzar dicho objetivo (Pacesila et al. 2016).

Por otro lado, la proliferación de residuos orgánicos y una insuficiente gestión de los mismos plantea una problemática acentuada en los países desarrollados. En la UE se generan entre 118-138 millones de toneladas de residuos cada año depositándose el 53% en vertederos (Cabbai et al. 2016; Municipal waste statistics- Statistics Explained 2018). Esto plantea una situación de emergencia medioambiental ya que se estima que por cada tonelada de residuos producida se emite el equivalente a 1,9 toneladas de CO_2 (Priefer et al. 2016). Para mejorar la sostenibilidad en la gestión de los desechos sólidos urbanos, la UE ha promovido directivas tales como la Directiva 1999/31/EC (Directiva 1999/31/EC) para reducir la generación, fomentar el reciclado, promover la selección en la fuente y reducir los desechos biodegradables que se envían a los vertederos. Además, en 2015 la Comisión Europea adoptó un plan de acción para contribuir a acelerar la transición de Europa hacia una economía circular, impulsar la competitividad mundial y promover el crecimiento económico sostenible. La economía circular ha sido definida

como aquellas acciones que reemplazan el concepto de residuo al reducir, reciclar y recuperar materiales en los procesos de producción, distribución y consumo. En una economía circular, los residuos orgánicos no se vierten en vertederos. En cambio, forman un recurso para la mejora orgánica de suelos, fertilizantes, componentes de medios de cultivo como los productos de base biológica o se utilizan para la generación de energía. Los residuos generados durante el tratamiento de aguas residuales, así como algunos residuos orgánicos de industrias alimentarias o provenientes del sector agrario, presentan grandes oportunidades para su aprovechamiento en el marco de la economía circular.

La digestión anaerobia es una tecnología que aporta soluciones al reto que supone la reducción de emisión de gases efecto invernadero y la reducción de la cantidad de residuos orgánicos que encuentran el vertedero como su método de disposición final. De este modo, el proceso de digestión se convierte en un pilar importante en el desarrollo de la economía circular. Su mayor ventaja es que es capaz de producir energía en forma de biogás a la vez que favorece la estabilización de los residuos orgánicos, aprovechando las corrientes residuales de diferentes tipos de industrias, mejorando por lo tanto la eficiencia de los recursos (Appels et al. 2011; Pan et al. 2015). En dicho proceso, la materia orgánica es degradada por la acción de un conjunto de microorganismos en ausencia de oxígeno, obteniéndose dos productos: digestato y biogás. El primero se trata de una biomasa rica en nutrientes como potasio, fósforo y nitrógeno que puede ser usado como enmienda orgánica. El segundo es una mezcla de gases, principalmente metano y dióxido de carbono, cuyo aprovechamiento se puede realizar en unidades de cogeneración (CHP) o mediante tratamiento posterior para conseguir mejorar su calidad hasta alcanzar niveles que permitan su valorización en usos equivalente al del gas natural.

La digestión anaerobia es una tecnología versátil que permite tratar una gran variedad de residuos orgánicos —p. ej.: desechos alimentarios, residuos de las urbes e industriales y materiales de desecho agrícola y animal—. La Tabla 1.1 recoge los rendimientos de biogás y metano obtenidos para diferentes residuos.

Tabla 1.1 Residuos orgánicos tratados por digestión anaerobia bajo condiciones mesofílicas y sus respectivas tasas de producción de biogás y metano

Residuos	**Tasa producción**	**Referencias**
Residuos de matadero	0,20-0,30 m^3 CH_4 kg SV^{-1}	Cuetos et al. 2017
Lactosuero	0,11-0,35 m^3 CH_4 kg DQO^{-1}	Martinez et al. 2018
Vinazas	0,08-0,18 m^3 CH_4 kg DQO^{-1}	Fernández Rodríguez et al. 2016
Lodo de depuradora	0,25-0,45 m^3 CH4 kg SV^{-1}	Martínez et al. 2017
Purín porcino	0,30-0,40 m^3 CH_4 kg SV^{-1}	Cuetos et al. 2011
Maíz	0,30-0,40 m^3 CH_4 kg SV^{-1}	
Gallinaza	0,03-0,10 m^3 CH_4 kg SV^{-1}	Fierro et al. 2014
Colza	0,25 m^3 CH_4 kg SV^{-1}	Galí et al. 2009
Girasol	0,20 m^3 CH_4 kg SV^{-1}	
Pulpa de naranja	0,25 m^3 CH_4 kg SV^{-1}	
Glicerol	0,30 m^3 CH_4 kg SV^{-1}	
Pulpa de pera	0,15 m^3 CH_4 kg SV^{-1}	
Pulpa de manzana	0,18 m^3 CH_4 kg SV^{-1}	
Pulpa y grano de café	0,65-0,73 m^3 CH_4 kg SV^{-1}	Pandey et al. 2000
Triticale	0,76 m^3 biogás kg SV^{-1}	Giuliano et al. 2013
Cebolla	0,92 m^3 biogás kg SV^{-1}	
Patata	0,83 m^3 biogás kg SV^{-1}	
Paja de arroz	0,24 m^3 biogás kg SV^{-1}	Lianhua et al. 2010
Paja de trigo	0,57 m^3 biogás kg SV^{-1}	Menardo & Balsari 2012
Pan seco	0,65 m^3 biogás kg SV^{-1}	
Pipas y piel de tomate	0,42 m^3 biogás kg SV^{-1}	Dinuccio et al. 2010
Tallo de uva	0,22 m^3 biogás kg SV^{-1}	
Col	0,26 m^3 CH_4 kg SV^{-1}	Labatut et al. 2011
Aceite vegetal usado	0,65 m^3 CH_4 kg SV^{-1}	
Residuos de destilerías	0,51 m^3 CH_4 kg SV^{-1}	Kafle et al. 2013
Residuos de almazaras	0,18-0,21 m^3 CH_4 kg DQO^{-1}	Fountoulakis et al. 2008

DQO: Demanda química de oxígeno, SV: Sólidos volátiles

Adicionalmente a la capacidad para tratar una gran variedad de residuos, cabe destacar la adaptabilidad de la digestión anaerobia a diferentes escalas, siendo ésta una cualidad que hace que la tecnología resulte atractiva en países con distintos grados de desarrollo económico.

En la Figura 1.1 se muestran ejemplos de la flexibilidad en la aplicación de la tecnología y un esquema representativo de las diferentes etapas enmarcadas en las instalaciones de tratamiento de residuos hasta la obtención de metano. Este gas puede ser usado como biocombustible o en aplicaciones equivalentes a las del gas natural. En dicha figura se muestra una imagen de la cocina de un hogar en la India en la que la generación de biogás se realiza de forma rudimentaria considerando los componentes básicos de digestor y almacenamiento de biogás. Esto permite la sobrepresión mínima necesaria para garantizar el suministro a una estufa de gas. Como contraste, en otra imagen se muestra la utilización del biogás como combustible principal de un autobús ecológico en Inglaterra. Su aplicación requiere de una tecnología avanzada que permita la eliminación del CO_2, agua y componentes traza del mismo para poder ser utilizado como carburante.

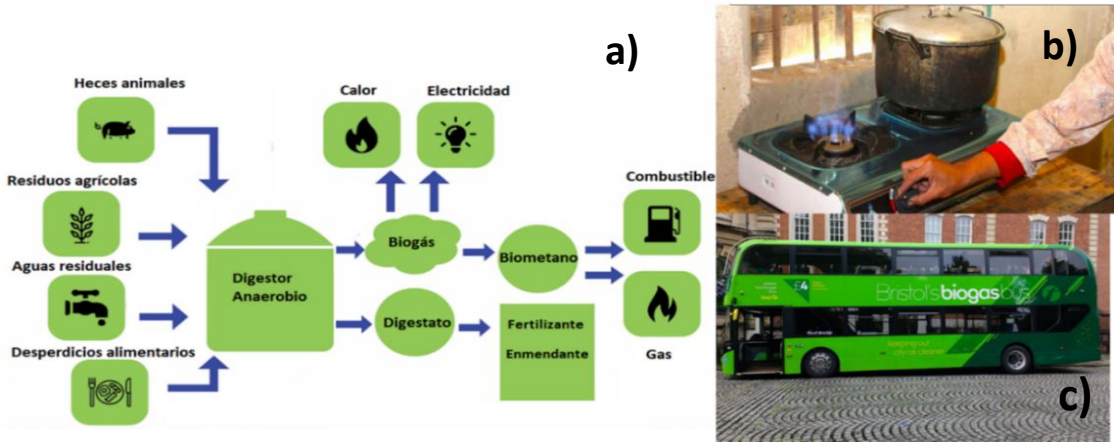


Figura 1.1 a) Materias primas de la digestión anaerobia y productos derivados, b) cocina de biogás en un hogar del sur de la India y c) primer autobús de biogás en Bristol (Reino Unido). [a](https://www.eesi.org 2017), [b](https://bioenergyinternational.com 2017) y [c](https://www.intelligenttransport.com 2017)

La aplicación de la tecnología de digestión anaerobia en general se hace de manera descentralizada en países en desarrollo utilizando digestores de pequeño tamaño. Por el contrario, en la UE se realiza en grandes plantas industriales centralizadas y monitorizadas. Según los últimos datos disponibles de 2017, existen 18 323 plantas de digestión anaerobia -17 783 productoras de biogás y 540 de biometano- (EBA Statistical Report 2018). En la Figura 1.2 se muestra la distribución y el número de plantas de biogás

y metano en los países de la UE. En ambos casos, Alemania presenta el mayor número de instalaciones contribuyendo al 50% de la producción de biogás. La mayoría son de pequeño tamaño y muy pocas superan los 150 kWe de potencia instalada (Scarlat et al. 2018; Theuerl et al. 2019).

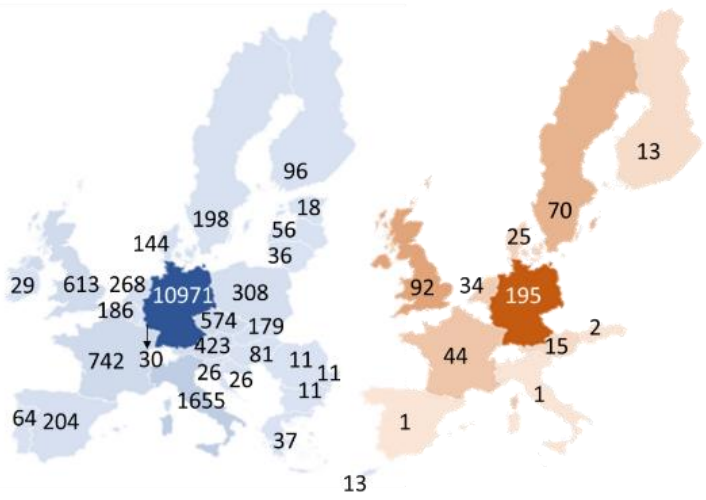


Figura 1.2 Número de plantas de digestión anaerobia productoras de biogás (en azul) y de biometano (en naranja) en la Unión Europea. Mapa coroplético de elaboración propia basado en los datos recogidos en (EBA Statistical Report 2019)

La construcción de plantas de digestión anaerobia en la Unión Europea ha sido muy notable: desde 2011 hasta 2017 ha habido un crecimiento del 30% en este tipo de instalaciones para la obtención de biogás y del 65% en plantas destinadas a la obtención de biometano —denominando así al biogás sometido a limpieza para conseguir un incremento de la riqueza hasta niveles similares al del gas natural—. Estas generaron un total de 195,68 y 19,4 GWh de biogás y biometano respectivamente. La mayoría de este gas procede de los residuos agrícolas y tratamientos de lodos de depuradora (Database - Eurostat 2020; EBA Statistical Report 2019).

1.1.2 Tecnologías de digestión anaerobia en función de la escala del proceso

Actualmente, la digestión anaerobia es una tecnología robusta ya que puede implementarse en multitud de escalas en función de las necesidades y de la cantidad de residuo que se necesite tratar o de las capacidades económicas y de tecnificación del

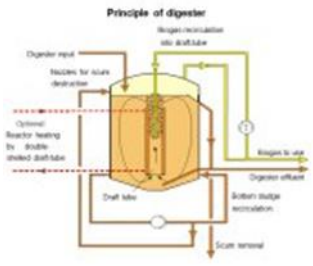
lugar donde se quiera implantar. Las diferentes escalas de las instalaciones de los digestores anaerobios se clasifican en micro, pequeña, e industrial. La producción energética de la escala micro no excede los 80 kW, mientras que la escala pequeña se sitúa entre los 100-300 kW y algunas de escala industrial supera los 1 000 kW (BiogasWorld 2019). En cuanto a la capacidad de tratamiento de residuos, la barrera sitúa a la escala micro y pequeña por debajo de las 1 000 toneladas de residuos anuales tratados (NNFCC 2017).

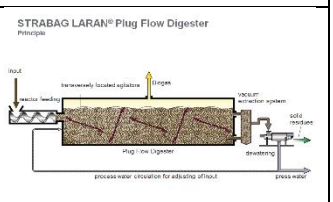
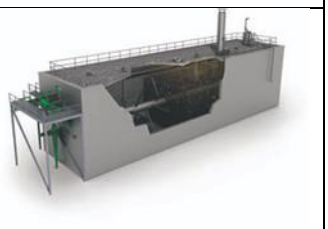
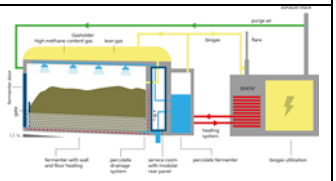
La implantación de digestores de escala micro o de pequeño tamaño se asocian tradicionalmente a países con economías de subsistencia o bajo nivel de tecnificación. Sin embargo, se considera que en países desarrollados este tipo de tecnología presentaría viabilidad en ambientes concretos del entorno rural con densidades bajas de población. Como ejemplo de tecnologías disponibles aplicables a pequeña escala se encuentra la compañía francesa HoSt con el sistema Microferm+ con un máximo capacidad de 7 400 t $año^{-1}$, utilizando como substrato principalmente estiércol de ganado y ensilado de maíz. Igualmente, CCI BioEnergy es otra compañía que ofrece soluciones mediante el sistema BioQUBE o el sistema lagoonQUBE con cubierta flotante destinada a la recogida de biogás de lagunaje. HomeBiogas es otra alternativa con capacidad de tratamiento inferior a 1 t $año^{-1}$ y ausencia de control.

1.1.3 Instalaciones comerciales de digestión anaerobia a escala industrial

Los procesos anaerobios se destinan al tratamiento de efluentes con una concentración de sólidos en el intervalo entre 4-10%. Son muy empleados para tratar la fracción orgánica de residuos sólidos urbanos, animales, agrícolas y los lodos producidos durante la depuración de aguas. En los últimos años ha aumentado en gran número las compañías que ofrecen diferentes tecnologías para el tratamiento anaerobio de residuos. En la Tabla 1.2 se detallan algunas de ellas y las características principales de su tecnología.

Tabla 1.2 Principales características de tecnologías de digestión anaerobia húmedas y seca aplicadas a plantas industriales

Compañía	**Características**	
Digestión anaerobia húmeda		
Arrow Ecology	https://www.arrowbio-global.com/ Proceso Arrow Bio Capacidad de tratamiento 90 000-180 000 t año^{-1} Riqueza de biogás 55-70% de metano Generación de electricidad y calor	
Citec	https://www.citec.com/offering/ Proceso Waasa Capacidad de tratamiento 3 000-230 000 t año^{-1} Riqueza de biogás 60% de metano Una sola etapa	
Ros Roca International	https://www.rosroca.es/ Proceso Biostab Capacidad de tratamiento 10 000-150 000 t año^{-1} Riqueza de biogás 45-60% de metano Pretratamiento mecánico por sistema de hidropulper	
Farmatic	https://farmatic.com/ Proceso Schwarting-Uhde Capacidad de tratamiento 18 000-220 000 t año^{-1} Sistema de dos etapas: mesofílica y termofílica Opera en flujo pistón	
Biogest	http://www.biogest.at/info/en/8/PowerRing.html Proceso Power Ring Capacidad de tratamiento ~200 000 t año^{-1} Opera en dos etapas	
Strabag (anteriormente Linde KCA/BRV)	http://www.strabag-umwelttechnik.com/ Digestores LARAN loop Capacidad de tratamiento 12 000 m^3 año^{-1} Requiere de un pretratamiento mecánico húmedo	

Compañías	**Características**	
Digestión anaerobia seca		
Strabag (anteriormente Linde KCA/BRV)	http://www.strabag-umwelttechnik.com/ Digestores LARAN de flujo pistón Capacidad de tratamiento 20 000-110 000 t año^{-1} Opera en semicontinuo	
MARTIN GmbH	https://www.martingmbh.de/en/dry-digestion-technology.html Operación continua Generación de electricidad y calor Uso de reactor flujo pistón THÖNI	
Hz-inova	http://www.hz-inova.com/ Proceso Kompogas Capacidad de tratamiento 5 000-100 000 t año^{-1} Riqueza de biogás ~66% de metano Sistema de una sola etapa	
BEKON	https://www.bekon.eu/en/ Operación continua Generación de electricidad y calor Riqueza de biogás 75% de metano	
IGW srl	https://www.igwsrl.com/en/biogas-biomethane/ Proceso Biocel Operación discontinua Capacidad de tratamiento 50 000 t año^{-1}	
Valorga	http://www.valorgainternational.fr/fr/ Proceso de una sola etapa Capacidad de tratamiento 10 000-497 000 t año^{-1} Riqueza de biogás 55-60% de metano	

Fuentes. (Durán et al. 2018; Syngellakis 2017)

A nivel industrial se han desarrollado varios sistemas de digestión anaerobia para tratar residuos de alta y baja concentración de sólidos. Los procesos que tratan residuos de alta concentración de solidos o denominados “procesos secos” sirven para tratar residuos con un contenido en sólidos entre el 20-40%. Mientras que aquellos que tratan residuos con baja concentración de sólidos o “procesos húmedos” para sistemas con un

contenido en sólidos menor al 14%. Entre los sistemas de digestión húmeda que se están utilizando en la actualidad destacan: Bigadan, BTA, HAASE y DRANCO los cuales se describen con mayor detalle a continuación.

El sistema **Bigadan** fue desarrollado en 1980 en Dinamarca por la empresa homónima y se expandió posteriormente a varios países europeos: Alemania, España, Reino Unido y Francia**.** Es un sistema de digestión húmeda de mezcla perfecta con una fuerte implantación en su país de origen donde hay más de 20 plantas operando. Se usa para la co-digestión de residuos ganaderos, industriales y urbanos a temperaturas medias y altas dependiendo del tipo de residuo. Inicialmente los residuos sólidos urbanos son triturados hasta obtener tamaños de 80 mm para luego mediante cinta transportadoras ser conducidos a una segunda etapa de trituración y esta vez alcanzar un tamaño medio de 10 mm. Posteriormente son mezclados con residuos ganaderos y lodos de depuradoras.

La mezcla se transporta al tanque de preparación donde se produce una agitación intensa y se garantiza la homogenización de modo que se forme una suspensión. Desde allí, se bombea a dos tanques de pasteurización que alcanzan una temperatura de 70 ˚C. El digestor opera a 38 ˚C con un tiempo de retención hidráulica de 20-24 días, obteniéndose rendimientos entre 60-70% de metano. Este gas es utilizado como combustible por un generador de energía o una caldera obteniéndose energía en forma de electricidad o calor (Noma et al. 2004). La cantidad diaria de biomasa digerida es de aproximadamente 200 toneladas produciendo entre 8 000 y 9 000 m^3 biogás d^{-1}.

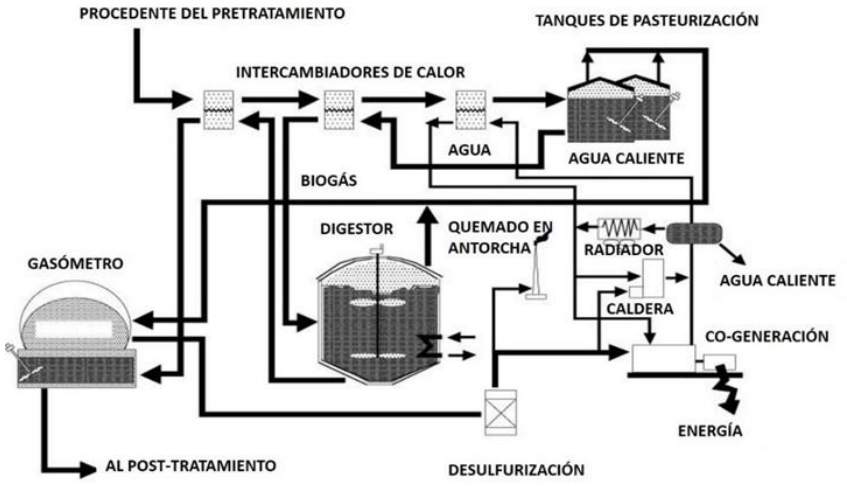


Figura 1.3 Esquema general del proceso de biogás de Bigadan (Noma et al. 2004)

El sistema **BTA** fue desarrollado en Alemania por BTA International para la digestión húmeda de FORSU. Su capacidad de tratamiento es de 1,2 millones de t año^{-1} por unidad instalada, aunque puede adaptarse a las necesidades del tratamiento durante la fase de diseño. El sistema BTA ha sido el pionero en el pretratamiento mecánico húmedo de residuos. Se basa en dos pasos: pretratamiento hidromecánico BTA y digestión anaerobia de la suspensión orgánica separada.

Durante el pretratamiento hidromecánico tiene lugar la eliminación de impurezas y la transferencia completa de los componentes orgánicos digeribles a una suspensión orgánica para su posterior digestión anaerobia. Este proceso es llevado a cabo por el pulper BTA y el sistema de desarenado BTA. El pulper es previamente llenado con agua y al añadir el material orgánico, ésta se va separando en fracciones aprovechando las propiedades naturales de flotación y sedimentación. Durante este proceso también se produce la destrucción en fibras de los materiales orgánicos no solubles por fuerzas de corte. En total, se generan tres fracciones: materia orgánica, fracción ligera —plásticos, textiles, etc.— y fracción pesada —piedras, huesos, etc.—. Sin embargo, todavía quedarían partículas finas de arena que se eliminan en el sistema de desarenado para evitar que su presencia dañe las piezas de la instalación y causen obstrucciones.

En este punto la fracción orgánica que ha sido limpiada pasa a ser digerida y se almacena temporalmente. En el fermentador, se digiere a temperaturas entre 35-38 ˚C pero, estas condiciones pueden modificarse en función de la composición de la fracción orgánica o de otras necesidades. Finalmente, la fracción orgánica se puede separar en una fracción líquida y otra sólida. La fracción líquida puede ser reutilizada en el mismo proceso para reducir el consumo de agua dulce. La fracción orgánica, una vez deshidratada, contiene aproximadamente un 30% de contenido en sólidos que si es estabilizada puede ser empleado como compost de calidad (http://www.bta-international.de/en/home.html 2019).

Figura 1.4 Sistema BTA. a) Agitación mecánica; b) Agitación mediante recirculación de biogás. (http://www.bta-international.de/en/home.html 2019)

El proceso **HAASE** se emplea para la co-digestión húmeda de diferentes residuos orgánicos —residuos ganaderos, industria alimentaria, etc.—. Emplea pretratamientos mecánicos y biológicos de bajo coste de operación. Destaca su presencia en San Román de la Vega (León) donde es la única planta de España que opera en dos etapas usando esta tecnología. Tiene la capacidad de tratar 200 000 t $año^{-1}$ de residuos sólidos urbanos cuya fracción orgánica —alrededor del 50 000 t $año^{-1}$— se transforma en biogás a temperaturas mesófilas (Martens 2005).

Figura 1.5 Unidades de la planta de digestión anaerobia de San Román de la Vega (León). a) mezclador; b) ósmosis inversa; c) unidad estacionaria de cogeneración CHP; d) antorcha de biogás tipo LTU (Martens 2005)

El proceso **DRANCO** es idóneo para la digestión seca de residuos urbanos. La digestión se lleva a cabo en reactores verticales de flujo pistón y sin mezcla mecánica. La materia prima se introduce por la parte superior del reactor y se reduce a un tamaño de 40 mm. Otros componentes como piezas textiles, papeles, plásticos son triturados hasta alcanzar ese tamaño. Materiales duros como vidrios, piedras o plásticos duros deben ser eliminados. El proceso DRANCO soporta altas concentraciones de contaminantes y de otros compuestos difícilmente digeribles, pero su consumo energético y abrasión será mayor. Por lo que cuanto más limpia sea la materia orgánica digerida, mejor será la eficiencia del mismo.

La fracción orgánica pasa a un digestor donde se mezcla con otro residuo previamente digerido en una proporción entre 1:6 y 1:8. Dicha etapa tiene lugar en el mezclador del tanque de alimentación. Posteriormente, se inyecta vapor para aumentar la temperatura del rango mesofílico al termofílico. Esta mezcla precalentada es bombeada desde el mezclador a lo largo de unos tubos de 1 m de diámetro interno y alcanzan una altura de 1 m de distancia con respecto al techo del digestor. Al digerido le lleva entre 2 y 4 días depositarse en el fondo cónico del digestor. En la etapa final, la mayor parte del digerido es reutilizado para la digestión y el restante se destina a su gestión final. El gas generado durante el tratamiento es almacenado para su valorización (De Baere 2013).

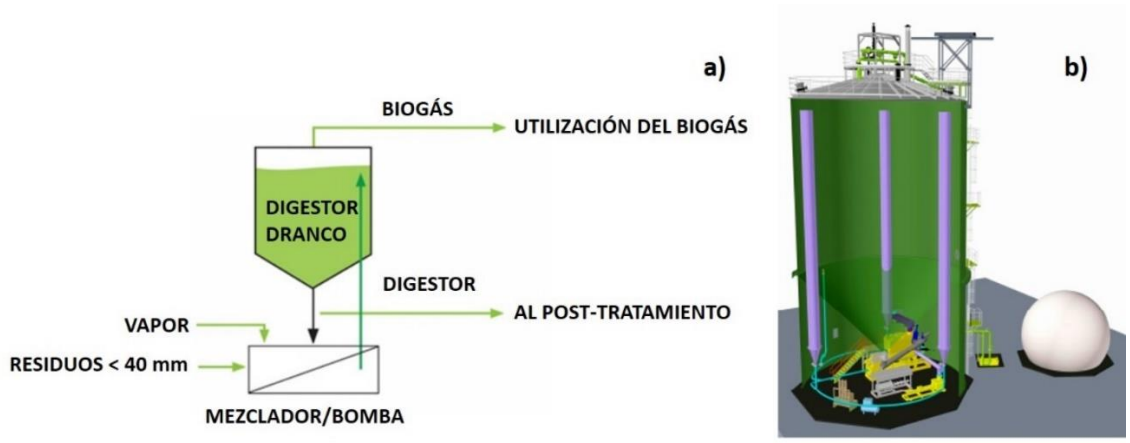


Figura 1.6 a) Proceso básico de digestor DRANCO y b) Digestor DRANCO con tubos internos de alimentación y bombeo debajo del tanque (De Baere 2013)

1.1.4 Digestión anaerobia de residuos de las estaciones depuradoras de aguas residuales: Lodos

Las estaciones depuradoras de aguas residuales (EDAR) son las encargadas de tratar las aguas procedentes de diversas actividades humanas. El proceso de depuración tiene dos líneas principales: línea de aguas y línea de lodos. En estas dos vías se engloban una serie de etapas donde se combinan procesos físicos, químicos y biológicos con el fin de obtener por un lado agua descontaminada y lodos estabilizados y por otro, energía que permita cierto autoabastecimiento.

1.1.4.1 Línea de aguas

Según se muestra en la Figura 1.7, el proceso se inicia con la introducción de aguas residuales en la estación depuradora. Antes iniciarse la depuración de las aguas como tal, se hace necesario la aplicación de una serie de pretratamientos. en ellos se engloban a un conjunto de operaciones —desbaste, desarenado, desengrasado y tamizado— para evitar el daño en los equipos que actúan en posteriores tratamientos (Metcalf & Eddy 1995).

El tratamiento de las aguas propiamente dicho empieza con la decantación primaria donde en algunos casos se aplican operaciones físico-químicas —coagulación, flotación y floculación— y de sedimentación. En este paso se elimina entre un 40-50% de los sólidos de las aguas residuales y se forma un sedimento grisáceo conocido como lodo primario que es conducido a la línea de lodos. A continuación, las aguas residuales pasan al tanque de tratamiento biológico o secundario donde, normalmente, se emplea el tratamiento de lodos activos para la eliminación de la materia orgánica presente por un cultivo bacteriano en suspensión. Dicha suspensión se consigue por la acción de aireadores mecánicos que sirven también para mantener la mezcla en constante agitación. Posteriormente el agua se dirige hacia un decantador secundario para su separación de la biomasa responsable del tratamiento biológico. La biomasa microbiana se separa en forma de lodo secundario, el cual una fracción del mismo se conduce a la línea de lodos y otra se recircula hacia la unidad de tratamiento biológico para mantener la carga microbiana constante. En algunos casos, antes del vertido de las aguas depuradas, se aplica un tratamiento terciario para desinfectar la corriente y eliminar N

y P, responsables directos de problemas medioambientales como la eutrofización (Elalami et al. 2019; Metcalf & Eddy 1995).

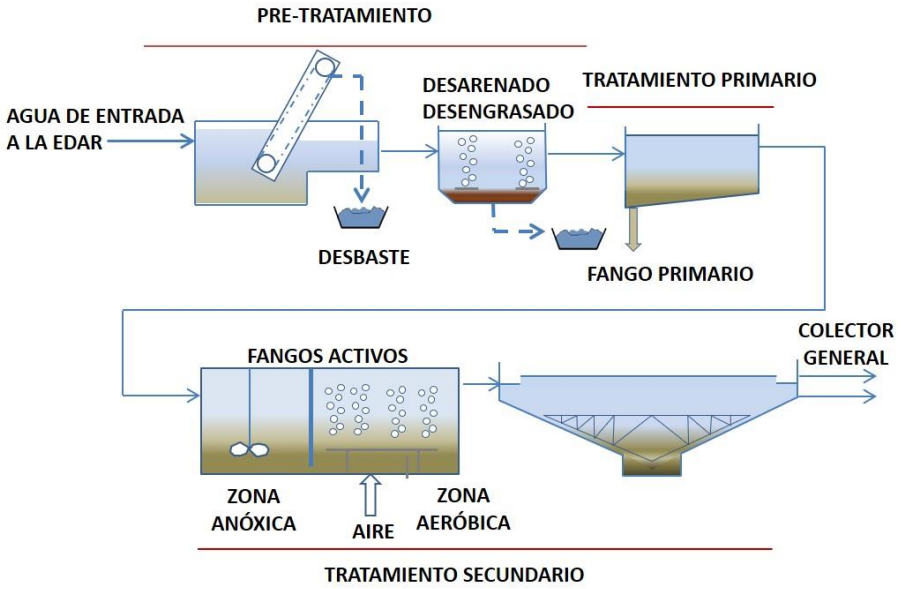


Figura 1.7 Línea de aguas dentro del tratamiento de aguas residuales (www.retema.es 2019)

1.1.4.2 Línea de lodos

En el tratamiento de las aguas residuales se producen dos tipos de lodos: lodos primarios y lodos secundarios. Los primeros se obtienen de decantadores primarios disponiendo de espesadores de gravedad para incrementar su contenido en sólidos. Los lodos secundarios se generan de la decantación de sólidos tras el paso de las aguas por el clarificador y son enviados a espesadores de flotación. Al igual que sucede en la línea de aguas, el proceso consta de varias etapas. El espesamiento permite incrementar la concentración de sólidos de los lodos y así eliminar una parte importante del agua que contienen hasta alcanzar un valor medio de sólidos del 5-6%.

En la etapa de estabilización de los lodos, normalmente interviene la digestión anaerobia en instalaciones de tratamiento de gran tamaño. Con este proceso se pretende reducir la fracción biodegradable y evitar su putrefacción incontrolada. La mezcla de lodos primarios y secundarios es estabilizada generando como resultado un lodo denominado digerido y biogás. Este lodo presenta, tras la digestión, un menor

contenido en materia volátil y un olor menos repulsivo además de un contenido inferior de gérmenes patógenos. Los digestores disponen de diferentes sistemas de agitación, pero el más ampliamente utilizado suele ser un sistema continuo de recirculación de lodos con el objetivo doble de lograr una buena homogeneización y calentar el lodo en el intercambiador de recirculación. La energía necesaria para mantener la temperatura del digestor normalmente suele obtenerse de la energía térmica resultante de la valorización del biogás mediante motogeneradores.

Actualmente, los rangos de producción de biogás en las plantas EDAR dependen de la concentración de sólidos volátiles de los lodos, produciendo entre 0,5-0,9 m^3 kg SV^{-1} de biogás y entre 0,24–0,34 m^3 CH_4 kg SV^{-1} (Bodík et al. 2011; Kiselev et al. 2019; Shen et al. 2015).

Finalmente, los lodos son deshidratados en decantadores centrífugos, transformándolos en un material con un contenido de humedad aproximado del 30% facilitando su manejo al área de secado-planta de compostaje. La Figura 1.8 muestra el diagrama de flujo simplificado de los elementos de la línea de lodos representados desde los decantadores primarios, secundarios hasta la deshidratación y área de secado- visualizando también el sistema de recirculación continua de los lodos en los digestores.

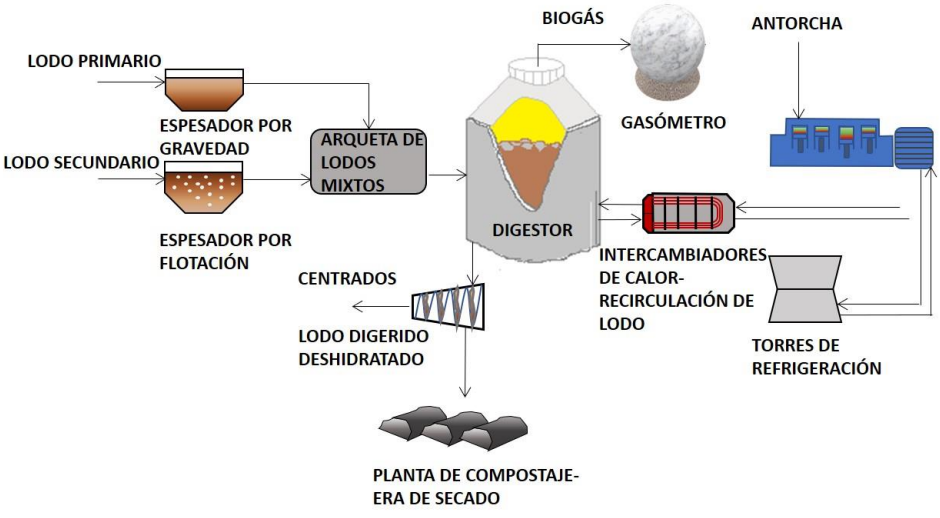


Figura 1.8 Esquema de tratamiento de la línea de lodos (www.retema.es 2019)

1.1.5 Digestión anaerobia de residuos procedentes de la industria vinícola

Dentro de la industria alimentaria, la producción de uva tiene un importante peso a nivel mundial. También, a nivel europeo ya que sólo tres países: Francia, Italia y España, producen el 50% del vino mundial (Da Ros et al. 2014). Se estima que de los 75 millones de toneladas de uva que se cultiva en todo el mundo, el 80% se destina a la producción de vino produciendo unos 9 millones de toneladas de residuos (Gómez-Brandón et al. 2019; Zhijing et al. 2018). Dichos restos contienen compuestos orgánicos e inorgánicos que requieren tratamiento (Zacharof 2017). Las distintas fracciones que conforman los restos de este tipo de industria comprenden: el raspón, el orujo y las vinazas, siendo ésta última un subproducto derivado de la fermentación del etanol (Bustamante et al. 2008). Las vinazas contienen un alto contenido en taninos, melanoidinas y polifenoles (Martinez et al. 2018; Zhijing et al. 2018). Estas macromoléculas tienen una mala digestibilidad por parte de los microorganismos y la gestión antiguamente aplicada consistente en el vertido al terreno de esta corriente ha demostrado tener un impacto negativo en los suelos (Sousa et al. 2019).

Una de las alternativas para valorizar las diferentes fracciones residuales de la industria vinícola ha sido el compostaje como método de estabilización de la materia orgánica. Se considera que esta metodología tiene propiedades beneficiosas ya que el compost es un reconocido material utilizado como enmienda de suelos, mejorando las propiedades de los mismos gracias a su efecto de liberación lenta de nutrientes y retención de humedad. Sin embargo, en el caso del compost procedente de los restos de la industria vinícola se han encontrado inconvenientes debido a su naturaleza ácida, su alto contenido en DQO (demanda química de oxígeno) y la emanación de compuestos orgánicos volátiles (Paradelo et al. 2013; Rondeau et al. 2013).

Otra alternativa de gestión estudiada en los últimos años comprende la digestión de vinazas o su co-digestión con otros materiales como pueden ser los lodos activados de depuradoras (Moletta 2005; Da Ros et al. 2017). Mediante esta tecnología ha sido posible neutralizar la acidez de las vinazas, reducir entre el 60-90% del contenido en DQO y producir entre 0,4-0,6 m^3 biogás kg DQO^{-1} con un contenido en metano del 60% (Moletta 2005). Sin embargo, el proceso no está exento de afrontar problemas, como

son una mayor inestabilidad operacional debido a los componentes orgánicos anteriormente citados y la presencia de inhibidores que se asocia a concentraciones perjudiciales de ciertos metales como el cobre (Melamane et al. 2007).

En la Figura 1.9 se muestra un ejemplo de un digestor anaerobio compacto para el tratamiento de vinazas a escala piloto. Según los autores consiguieron tener buenos resultados durante su digestión aplicando una extracción previa con agua en proporción 1:10 consiguiendo un sustrato con un contenido en polifenoles muy bajo (Eleutheria et al. 2016).

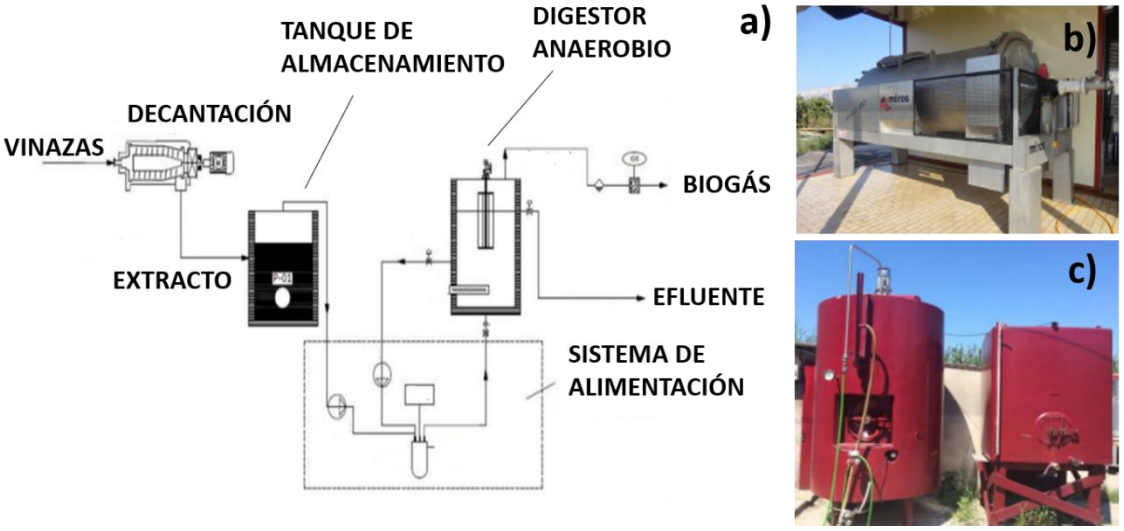


Figura 1.9 a) Flujo del proceso de digestión de las vinazas propuesto, b) Tanque de almacenamiento y c) Digestor anaerobio para vinazas (Eleutheria et al. 2016)

En la industria de producción de etanol se genera una gran cantidad de vinazas siendo, por lo general, entre 12-15 L de vinazas por cada litro de etanol (Christofoletti et al. 2013). Brasil, primera potencia mundial en producción de etanol, genera alrededor de $1{,}5x10^{11}$ L $año^{-1}$ de vinazas. Por lo general, las vinazas se depositan en los suelos para aprovechar su contenido mineral y favorecer el ahorro en irrigación pero no es una buena solución considerando su bajo pH y su alto contenido en material orgánico (Fuess & Garcia 2014).

Una posible solución para la gestión de estos residuos pasa por la incorporación de la digestión anaerobia a la industria del etanol. Se estima que mediante esta tecnología las

vinazas pueden llegar a producir 1,2x10^5 m^3 CH_4 $año^{-1}$, reducir la emisión de 466 g de CO_2 L $etanol^{-1}$ asociada a su habitual deposición y reducir la carga orgánica en alrededor de un 72% de DQO (Oliveira et al. 2015; Souza et al. 1992).

Con este propósito surgió la primera planta industrial construida en 1990 en Sao Martinho (Brasil) (Souza et al. 1992). En dicha planta se opera con un reactor de flujo ascendente con una capacidad de 5 000 m^3 y cuyo biogás se utiliza en su totalidad para el secado de levaduras durante la fermentación (Moraes et al. 2015). Esto sería insuficiente por lo que convendría aumentar el gasto en investigación en la digestión anaerobia de estos efluentes, planes nacionales para afrontar la generación de estos residuos y realizar más estudios sobre la viabilidad económica.

Bernal et al. (2017) señalan que tanto la producción de etanol como de biogás tienen viabilidad económica ya que podrían usarse como biocombustible y reducir los costes de transporte desde las cosechas a las destilerías pera aún es necesario avanzar en la optimización del proceso de digestión para lograr enriquecer el biogás a un coste menor. Otro estudio como el de Salomon et al. (2011) afirma que el digerido de vinazas es un fertilizante alternativo más rentable económicamente si es comparado con el fertilizante mineral basado en potasio. También concluye que el uso de biogás resulta más rentable si se utiliza para el secado de levaduras, tal y como se mencionó anteriormente y como combustible principal en motogeneradores. Cabría esperar que una mayor aplicación de la digestión de vinazas en plantas industriales, asociada a la generación eléctrica puedan ayudar a reducir los gastos energéticos asociados a la producción de etanol.

1.1.6 Estrategias para la mejora de la digestión anaerobia

La digestión anaerobia es una tecnología que, aunque robusta, puede no ser adecuada para ciertos tipos de substratos dadas las características químicas de los mismos y el carácter inhibitorio que puedan ejercer durante su degradación por esta vía. Algunos tipos de sustratos requieren la aplicación de tratamientos previos que faciliten la degradación anaerobia. En los apartados anteriores se ha descrito la relevancia del proceso de digestión en el tratamiento de residuos orgánicos, su vinculación esencial en los procesos de tratamiento de aguas residuales y por último la importancia que puede

llegar a tener si se vincula a la industria vinícola y de producción de bioetanol. Un aspecto crucial es su capacidad para generar energía que puede ser aprovechada in situ por los diferentes equipos que conforman la instalación.

En el caso de las EDAR resulta de gran relevancia el poder contar con una fuente extra de energía eléctrica dado el alto grado de demanda energética que requieren este tipo de instalaciones asociado al proceso de fangos activos. Por ejemplo, uno de los mayores gastos energéticos en una planta de tratamiento de lodos activos se destina a la aireación suponiendo entre un 50-60% de consumo eléctrico, seguido del tratamiento de lodos activos (15%) y la recirculación por bombeo (15%) (Gu et al. 2017). Por otra parte, el rendimiento de conversión de metano a electricidad es bajo, alrededor del 35% (Eastern Research Group Inc. and Resource Dynamics Corporation 2011; Logan 2004). Como las EDAR están lejos de autoabastecerse energéticamente de manera limpia y sostenible, es necesario que se busquen nuevas alternativas de baja demanda energética para que permiten agilizar la degradabilidad de los sustratos y, en consecuencia, la mejora de la producción de biogás en las depuradoras.

En plantas destinadas al tratamiento de aguas residuales y en industrias vinícolas que generan una cantidad considerable de efluentes de alta carga orgánica, el hecho de buscar nuevos métodos de tratamiento reduciría el coste del proceso industrial y favorecería el balance energético de la instalación. Es por ello que para aumentar la eficiencia o evitar la inhibición de la digestión, es necesario propiciar ambientes que promuevan tanto la ruptura de las células como de las macromoléculas. Para ello se han venido empleando diferentes pretratamientos: físicos, químicos, biológicos o en combinación en ellos para agilizar la cinética de las etapas de hidrólisis mediante la solubilización del material orgánico o de moléculas de menor tamaño generando una mejora sustancial en el proceso de digestión.

El mayor inconveniente que presentan los pre-tratamientos convencionales es que tienen un alto impacto sobre la energía consumida en el proceso. Por lo que es fundamental evaluar de forma adecuada las mejoras significativas que pueden conseguirse con la aplicación de los mismos o buscar nuevas alternativas que pudiesen coordinarse o reemplazar los pretratamientos convencionales (Barrios et al. 2017; Gil et al. 2018; Han et al. 2019; Liu et al. 2020; Lizama et al. 2017; Zhen et al. 2017). En la Tabla

1.3 se muestran algunos de los pretratamientos que se han empleado en lodos durante los últimos años y la mejora que han supuesto.

Tabla 1.3 Resumen de los pretratamientos empleados y su efecto producción de biogás y metano en la digestión anaerobia de los lodos

Tipo de pre-tratamiento	**Tecnología**	**Efecto: Rango de mejora en la producción de biogás comparadas con el control (%)**	**Referencias**
Físicos	Altas temperaturas	25-150	Bougrier et al. 2006a; Bougrier et al. 2006b; Chen et al. 2020; Ennouri et al. 2016; Gonzalez et al. 2018; Graja et al. 2005; Neumann et al. 2016; Pickworth et al. 2006; Xu et al. 2019
	Bajas temperaturas	10-984	Appels et al. 2010; Bougrier et al. 2008; Carvajal et al. 2013; Climent et al. 2007; Dwyer et al. 2008; Gonzalez et al. 2018; Kor-Bicakci & Eskicioglu 2019; Neumann et al. 2016; Ruffino et al. 2015; Xu et al. 2019
	Ultrasonidos	4-83	Gallipoli et al. 2014; Gonzalez et al. 2018; Lizama et al. 2017; Martín et al. 2015; Neumann et al. 2016; Şahinkaya & Sevimli 2013; Xu et al. 2019
	Microondas	16-50	Appels et al. 2013; Eskicioglu et al. 2007; Kuglarz et al. 2013; Neumann et al. 2016; Xu et al. 2019
	Homogenización por altas presiones	17-115	Gonzalez et al. 2018; Neumann et al. 2016; Wahidunnabi & Eskicioglu 2014; Xu et al. 2019; Zhang et al. 2012
	Pulso eléctrico	10-100	Lee & Rittmann 2011; Neumann et al. 2016; Salerno et al. 2009; Xu et al. 2019
	Congelación y descongelación	52	Cano et al. 2015; Gonzalez et al. 2018; Montusiewicz et al. 2010; Neumann et al. 2016; Xu et al. 2019; Yang et al. 2015
Químicos	Pretratamiento alcalino	13-120	Feng et al. 2014; Gonzalez et al. 2018; Kim et al. 2003; Li et al. 2008; Neumann et al. 2016; Shao et al. 2012; Xu et al. 2014; Xu et al. 2019
	Pretratamiento ácido	12-32	Appels et al. 2011; Devlin et al. 2011; Gonzalez et al. 2018; Neumann et al. 2016; Xu et al. 2018, 2019
	Ozonización	8-200	Bougrier et al. 2006a; Chen et al. 2020; Ennouri et al. 2016; Gonzalez et al. 2018; Neumann et al. 2016; Silvestre et

			al. 2015; Weemaes et al. 2000; Xu et al. 2019; Yeom et al. 2002
	Pretratamiento Fenton	19-30	Erden & Filibeli 2010, 2011; Gonzalez et al. 2018; Neumann et al. 2016; Patil et al. 2016; Xu et al. 2019; Zhen et al. 2017
Biológicos	Pretratamiento digestión aeróbica	10-79	Carrère et al. 2010; Dumas et al. 2010; Jang et al. 2014; Neumann et al. 2016; Xu et al. 2019
	Pretratamiento enzimático	12	Davidsson et al. 2006; Gonzalez et al. 2018; Neumann et al. 2016; Rashed et al. 2010; Xu et al. 2019; Yang et al. 2010
Integración de diferentes pretratamientos	Pretratamiento termoquímico	20-154	Cacho Rivero et al. 2006; Kim et al. 2003; Neumann et al. 2016; Takashima & Tanaka 2008; Valo et al. 2004; Xu et al. 2019
	Microondas + alcalino	44-228	Doğan & Sanin 2009; Jang & Ahn 2013; Neumann et al. 2016; Xu et al. 2019
	Homogenización a alta presión + alcalino	47	Fang et al. 2014; Neumann et al. 2016; Xu et al. 2019
	Alta presión + O_2	6% en semicontinuo 80% en discontinuo	Cheng & Hong 2013; Neumann et al. 2016; Xu et al. 2019
	Ultrasonidos + alcalino	38-55	Kim et al. 2010; Neumann et al. 2016; Xu et al. 2019
	Ultrasonido + O_2	26-36	Neumann et al. 2016; Tian et al. 2015; Xu et al. 2019
	Mecánico + alcalino	84	Cho et al. 2014; Neumann et al. 2016; Tian et al. 2015; Xu et al. 2019
	Pretratamiento electroquímico	63	Neumann et al. 2016; Xu et al. 2014; Xu et al. 2019

Otra forma de mejorar la eficiencia de los procesos de digestión anaerobia es mediante la incorporación o el acoplamiento de diferentes procesos biológicos. Este es el caso de la utilización de sistemas bioelectroquímicos los cuales se han probado de forma integrada con los procesos de digestión para el tratamiento de sustancias de baja biodegradabilidad (Cerrillo et al. 2016, 2017). Se ha comprobado que la introducción de este tipo de sistemas mejora la producción de biogás y también ejerce un efecto beneficioso que permite controlar los efectos de las sobrecargas orgánicas (Zhang & Angelidaki 2014). Estos sistemas bioelectroquímicos suelen acoplarse de dos maneras: sumergiendo los bioelectrodos en el interior del digestor o mediante la incorporación

de un circuito de recirculación entre el sistema bioelectroquímico y el digestor anaerobio (Inglesby & Fisher 2012).

Los sistemas bioelectroquímicos ofrecen ventajas a la digestión anaerobia dado que permiten reducir el estrés de la comunidad microbiana frente a sustancias inhibitorias. Además, en el caso concreto de las celdas de electrólisis microbiana son capaces de producir hidrógeno a partir de aplicación de un potencial gracias a la degradación de la materia orgánica.

La experimentación con sistemas bioelectroquímicos fuera de la escala de laboratorio es escasa o no ha sido exitosa. Heidrich et al. (2014) diseñaron una celda de electrolisis microbiana con una capacidad de 100 L que operó durante 12 meses en un rango de temperaturas de entre 1-22 ˚C alimentada con agua doméstica. La producción de hidrógeno de esta celda fue continua llegando a niveles de 0,6 L H_2 d^{-1} pero finalmente fue decreciendo. Se afirma que se recuperó algo menos del 50% de la energía eléctrica invertida en el proceso y se obtuvo una eficiencia culómbica del 41,2%. La eliminación de DQO fue insuficiente por limitaciones en el bombeo de la alimentación y el diseño de la celda.

Cusick et al. (2011) propuso el diseño de una celda de electrolisis microbiana a escala piloto (1 000 L) para tratar residuos procedentes de las bodegas. Dicha planta contaba con 144 pares de electrodos distribuidos en 24 módulos. El máximo de producción de gas alcanzada en el cátodo fue de 0,19 ± 0,04 L L^{-1} d^{-1} con un porcentaje de conversión a metano del 86 ± 0,6%. Se eliminaron de forma consistente el 62 ± 20% de la DQO soluble. Son buenos resultados, pero el periodo de preparación de los biofilms en este trabajo fue muy prolongado (2 meses).

Heidrich et al. (2013) emplearon una celda de electrolisis microbiana para tratar agua doméstica. Dicha celda tenía una capacidad de 120 L con el objetivo de producir hidrógeno puro en un periodo de tres meses a temperatura ambiente. El volumen de carga orgánica en este trabajo se situaba por debajo de lo habitual para cargas típicas de lodos activados (0,2-2 kg DQO m^{-3} d^{-1}) con un coste energético por debajo de 2,5-7,2 kJ g DQO^{-1}. El reactor generó 0,015 L H_2 L^{-1} d^{-1}, recuperando el 70% de la energía

consumida con una eficiencia culómbica del 55% aunque no se pudo alcanzar los objetivos prefijados.

El problema que han tenido varios investigadores es que al escalar la tecnología de los sistemas bioelectroquímico, el incrementar el tamaño de los componentes ha generado como resultado ensayos de prueba y error (Enzmann et al. 2019). La solución pasaría por estudios de modelización y optimización que permitan dar el salto entre escalas (Durst 2008). No obstante, son varios los retos que supone el escalado de estos sistemas cuando son testados fuera del laboratorio. Entre ellos están: i) aumento de la resistencia interna al aumentar la resistencia entre electrodos, ii) insuficiencia de contacto eléctrico entre bacteria-electrodo iii) competitividad entre microorganismos electrogénicos y no electrogénicos por los sustratos y iv) deterioro del cátodo con el tiempo (Ramírez-Vargas et al. 2018). Aunque se haya avanzado durante los últimos años en este campo, a día de hoy se trata de una tecnología joven con un gran margen de mejora y, por lo tanto, es necesario solucionar muchos retos técnicos.

Otro de los tratamientos utilizados en conjunción con la digestión anaerobia para incrementar su eficiencia comprende los procesos de oxidación avanzada (POA) y los procesos electroquímicos de oxidación avanzada (PEOA). Estos tratamientos han sido por mucho tiempo una de las pocas alternativas disponibles para la eliminación de contaminantes orgánicos no-degradables. Los PEOA son una alternativa eficiente para disminuir la cantidad de material orgánico presente. Sin embargo, el alto coste energético del tratamiento cuando se quiere lograr una mineralización completa de la materia orgánica hace que la viabilidad económica sea muy baja si se pretende una aplicación exclusiva del proceso electro-químico. Por lo que, la utilización conjunta con sistemas biológicos tradicionales incrementaría altamente la viabilidad económica del proceso global (De Vrieze et al. 2018). La integración de estos dos procesos permitirá aumentar la eficiencia de la digestión con relación a la producción específica de biogás y a la eliminación de materia orgánica (Cerrillo et al. 2016). En particular su aplicación como método para favorecer la degradación biológica y facilitar el ataque de los microorganismos a las moléculas compleja. Esto mejoraría los tiempos de digestión de sustratos complejos, reduciría su biotoxicidad y como consecuencia se incrementaría la

productividad del digestor en términos de la capacidad de carga orgánica capaz de tratar (Gonçalves et al. 2008; Gonçalves et al. 2012; Martinez et al. 2018; Yang et al. 2014).

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Capítulo/Chapter 2

Antecedentes y Objetivos

2.1 ANTECEDENTES

2.1.1 Descripción general de la digestión anaerobia

La digestión anaerobia es un proceso biológico a partir del cual se produce una degradación y estabilización de la materia orgánica en ausencia de oxígeno. Debido a la acción de un consorcio microbiano se obtiene como productos biogás y digestato. El biogás cuya composición depende del material digerido y del funcionamiento del proceso, es una mezcla de varios gases entre los que se encuentran el CH_4, CO_2, N_2 y H_2S y otros elementos traza siendo el metano el compuesto más abundante (60-70%) (Osorio & Torres 2009). Sus propiedades se indican en la Tabla 2.1.

Tabla 2.1 Características generales del biogás

Contenido energético	6,0-6,5 kW h m^{-3}
Equivalente de combustible	0,60-0,65 L petróleo m^{-3} biogás
Límite de explosión	6-12% de biogás en el aire
Temperatura de ignición	650-750 ˚C
Presión crítica	74-88 atm
Temperatura crítica	-82,5 ˚C
Masa molar	16 043 kg $kmol^{-1}$

Fuente. (Deublein & Steinhauser 2011)

La composición del digestato, depende en gran medida del tipo de tecnología y de las materias primas utilizadas como substrato durante la digestión. Su contenido en materia orgánica es menor que el del material originario dado que parte de éste se convierte en metano. El digestato contiene una fase sólida y una líquida que está compuesta en el primer caso por una serie de sustancias orgánicas de difícil degradación y sustancias minerales (N, P, Ca, K) (Möller 2012), mientras que la fracción líquida suele presentar un contenido relativamente elevado de amonio y sales disueltas justo con materia orgánica soluble o coloidal.

La digestión anaerobia es un proceso complejo que se caracteriza por una alta eficiencia en la estabilización de biorresiduos. Adicionalmente, este proceso suscita un gran

interés social ya que se enmarca en las tecnologías "verdes" y por tanto sigue manteniendo un carácter amigable con el entorno, guardando una relación positiva en el marco de una población cada vez más concienciada con el cuidado del medio ambiente.

Tradicionalmente la digestión anaerobia ha sido abordada como un sistema que se da en dos etapas: 1) Hidrólisis y fermentación de la materia orgánica compleja en ácidos simples e hidrógeno, y 2) Conversión de los ácidos orgánicos en metano, aceptando la existencia de dos grandes grupos de comunidades microbianas: las bacterias formadoras de ácidos o acidogénicas y las bacterias formadoras de metano o metanogénicas (McCarty et al. 1977). Sin embargo, en la actualidad es ampliamente reconocido que la digestión anaerobia es un proceso que se divide en cuatro etapas (Batstone et al. 2002; Gujer & Zehnder 1983).

La primera de estas etapas es la **hidrólisis** en la cual las bacterias hidrolíticas segregan enzimas extracelulares que son capaces de convertir carbohidratos, lípidos y proteínas en azúcares, ácidos grasos de cadena larga (AGCL) y aminoácidos, respectivamente (Li, et al. 2011). De este modo, los monómeros y/o dímeros resultantes de la ruptura enzimática difunden más fácilmente a través de las membranas celulares. No obstante, hay ciertas sustancias como la lignina, la celulosa y la hemicelulosa. La lignina posee una estructura muy compleja para poder ser degradada por estos microorganismos (Jimenez et al. 1990; Lin et al. 2010). Los polímeros de cadena larga de la celulosa y hemicelulosa deben ser hidrolizados a mono- y disacáridos por enzimas holocelulóticas extracelulares (Tong et al.1990). Tradicionalmente, se ha pensado que la hidrólisis es el paso limitante de la digestión anaerobia pero algunos estudios apuntan a que influye la proporción de microorganismos hidrolíticos frente a los metanogénicos (Luo et al. 2012; Ma et al. 2013). No obstante, hay otros parámetros como el pH, temperatura, tipo del sustrato o formación de determinados productos intermedios que pueden dificultar las etapas posteriores (Veeken et al. 2000).

La siguiente etapa es la **acidogénesis**, en esta etapa se produce la absorción de aquellos productos generados durante la hidrólisis a través de las membranas celulares. Debida a dicha absorción, los microorganismos acidogénicos son capaces de producir ácidos grasos volátiles (AGVs) y otros compuestos. Estos AGVs comprenden mezclas de ácidos

como el acético, propiónico y butírico. Sin embargo, también se pueden encontrar acompañados de pequeñas cantidades de lactato y etanol (Fierro 2014). Se trata de una etapa cinéticamente rápida y cuya producción de AGVs varía en función de las condiciones de los digestores. En esta misma etapa los lípidos experimentan una degradación del glicerol que se convierte en acetato y, también se da además la β-oxidación de los AGCL para dar también acetato (Cirne et al. 2007). En la Figura 2.1 se muestra de forma esquemática, las etapas de la digestión anaerobia.

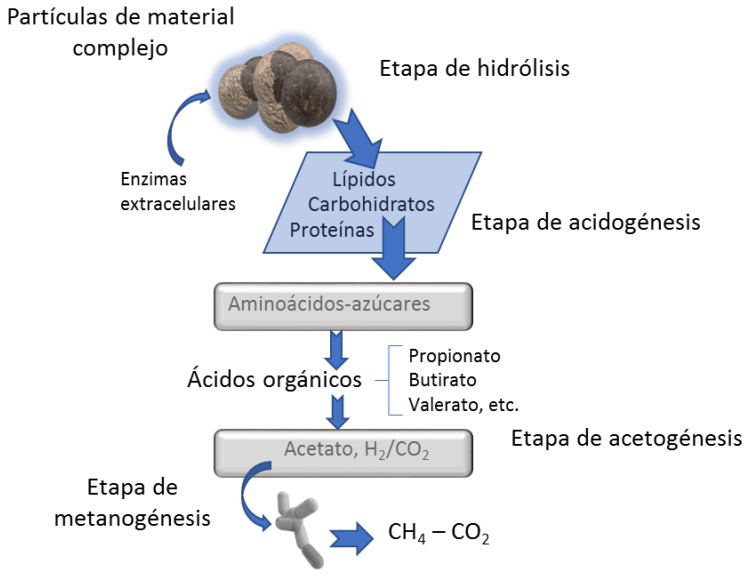

Figura 2.1 Diagrama de las etapas que comprenden la digestión anaerobia y los productos obtenidos en cada etapa (González et al., 2018)

En el paso de la **acetogénesis** se produce la degradación de los AGCL a ácido acético con la producción paralela de hidrógeno. Este paso es imprescindible para que los metanógenos acetoclásticos puedan reducir el ácido acético a metano. Esto es posible ya que en esta etapa se da una relación sintrópica interesante —la transferencia de hidrógeno entre comunidades microbianas—. La acetogénesis se lleva a cabo por bacterias homoacetogénicas y es productora de hidrógeno, pero si ésta es muy alta puede inhibir el metabolismo de los microrganismos. Sin embargo, gracias a la rápida acción de los metanógenos hidrogenotróficos se pueden mantener niveles de presión de hidrógeno muy bajos (10^{-3} atm) (Dinopoulou et al. 1988; Stams & Plugge 2009).

Al grupo de las bacterias homoacetogénicas pertenecen varios géneros de acetobacterias y clostridios que crecen bien por la vía glicolítica de fermentación de azúcares en piruvato, que es transformado en acetato con formación de ATP, liberando CO_2 e H_2. Esta producción concomitante de estos dos gases es vital para abastecer las necesidades de fuente de carbono y electrones del resto de los grupos (Kleerebezem & van Loosdrecht 2007).

La última etapa es la **metanogénesis** donde se consumen los productos accesibles para la síntesis de metano. Dependiendo de los sustratos consumidos para la producción de metano, en esta fase se puede dividir entre las que combinan CO_2 e H_2 para formar metano y agua **(metanógenas hidrogenotróficas)** y las que consumen grupos metilo del acetato para formar metano **(metanógenas acetoclásticas)**. La contribución de cada comunidad a la formación de metano suele ser 2/3 para las metanógenas acetoclásticas y 1/3 para las metanógenas hidrogenotróficas. Estas reacciones son llevadas a cabo por arqueas y bacterias metanogénicas (Belay et al. 1986; Brennan & Owende 2010; De Vrieze et al. 2012).

El proceso biológico de la digestión anaerobia puede representarse mediante la Ecuación 2.1 (Buswell & Mueller 1952):

$$C_nH_aO_b + \left(\mathrm{n} - \frac{a}{4} - \frac{b}{2}\right) H_2\mathrm{O} \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4 \qquad (2.1)$$

Esta expresión refleja la conversión de un sustrato complejo en metano y dióxido de carbono donde participan un número considerable de microorganismos en serie o en serie/paralelo (Baraza et al. 2003).

Los metanógenos son arqueas estrictamente anaerobias agrupadas en metanógenos acetoclásticos e hidrogenotróficos. Los sustratos comunes de los metanógenos hidrogenotróficos son H_2, CO_2 y formiato, mientras que el acetato es el sustrato principal para los metanógenos acetoclásticos. Estos últimos producen metano mediante acidogénesis y acetogénesis, aunque también pueden utilizar algunos otros compuestos como metanol, etanol y piruvato. Debido al metabolismo restringido de los metanógenos, los compuestos orgánicos se degradan en un ambiente anaeróbico por asociación con fermentación y bacterias acetogénicas. Esta relación sintrópica es sostenida por la transferencia de hidrógeno entre especies. El hidrógeno, que es

consumido por los metanógenos hidrogenotróficos, es por tanto un intermediario clave en dicha degradación (Martínez et al. 2019).

En las últimas décadas, se han aplicado varias técnicas de mejora de biogás a través de la introducción de H_2. Por ejemplo, el gas H_2 se inyectó en los digestores anaerobios para estimular el metabolismo de los metanógenos hidrogenotróficos, que utilizan CO_2 como única fuente de carbono e H_2 como fuente de electrones para producir metano (Weiland 2010).

Muchos estudios que examinan las comunidades microbianas se basan en los resultados obtenidos a partir de secuenciación a nivel de ADN. Este tipo de estudios permiten evaluar la diversidad general de una comunidad, incluidos los organismos vivos, los muertos y los inactivos, así como la ocurrencia de alteraciones en el funcionamiento in situ de los microorganismos por lo que son la mejor opción para caracterizar la estructura general de la comunidad microbiana (Zhao et al. 2019).

2.1.2 Parámetros que influyen en la digestión anaerobia

Las distintas etapas que tienen lugar en la digestión anaerobia deben producirse a una velocidad que permita un equilibrio en el proceso porque de lo contrario, provocaría desajustes en la sincronización global causando la inhibición de alguna de ellas. Es complicado alcanzar un consenso sobre cuáles han de ser las condiciones ideales para establecer un correcto funcionamiento en la digestión anaerobia ya que son varios los factores que influyen. A continuación, se mencionan algunos de los más importantes:

El **pH** tiene una fuerte influencia en la síntesis de diferentes tipos de AGVs. Un valor bajo de pH favorece la formación de ácido butírico mientras que a medida que el pH se mueve hacia valores alcalinos se favorece la formación de ácido acético (Strazzera et al. 2018). Dado que el consumo de los AGVs se lleva a cabo por las arqueas metanogénicas y estas operan en un rango que oscila entre 6.5-7.2, es recomendable mantener el sistema en este mismo rango para evitar inhibiciones (Rabii et al. 2019). El seguimiento del pH no sirve para anteponerse o predecir futuros fallos en el sistema, pero puede ayudar a determinar si la digestión se está produciendo de forma eficaz (Fisgativa et al. 2016).

El control de la **temperatura** es fundamental. La digestión anaerobia se clasifica en función del rango de temperatura en la que ésta tiene lugar. Estos son tres: procesos psicrofílicos los cuales operan a 25 ˚C, los mesofílicos operan a un rango de temperaturas entre 30-40 ˚C y los termofílicos entre 45-60 ˚C (Van Lier et al. 2001). Por lo general, los procesos termofílicos son los más eficaces en la eliminación de AGVs y presentan tiempos de retención más cortos que los mesofílicos (Ward et al. 2008). Sin embargo, son más difíciles de controlar reportándose que una variación de 1 ˚C diario puede generar grandes fluctuaciones en la producción de biogás (Appels et al. 2008).

La **proporción C/N** juega un papel muy importante en la digestión anaerobia Un desajuste en la composición de los nutrientes puede ser un factor de inhibición. La proporción C/N óptima oscila entre 15-35/1 (Khalid et al. 2011). Si la proporción es más baja, se trata de un medio con niveles altos de proteínas y, por lo tanto, se produce una concentración elevada de amonio libre en el medio. Niveles altos de este compuesto presentan efectos tóxicos para las baterías anaerobias y provocan variaciones en el pH del medio (Sialve et al. 2009).

Los AGVs son ácidos grasos de cadena corta que contienen entre dos y siete átomos de carbono como máximo. No son tóxicos por sí mismos pero su acumulación tiene propiedades inhibitorias en la digestión anaerobia. Si hay escasez de metanógenos acetoclásticos no se podrá asimilar los AGVs formados y dará lugar a un sistema sobrecargado produciéndose la inhibición de la digestión (Ward et al. 2008). Se ha reportado que proporciones de propiónico/acetato mayores a 1.4 y niveles superiores a 800 mg L^{-1} en ácido acético es síntoma de una digestión anaerobia fallida (Kwietniewska & Tys 2014).

La suplementación con **macronutrientes y micronutrientes** pueden tener efectos positivos ya que forman parte de la estructura de las enzimas que intervienen en el proceso. Por ejemplo, en el caso de las bacterias metanógenas, el azufre es un elemento muy demandado, pero en su forma de sulfito o sulfato puede ser también un inhibidor. Su reducción a H_2S hace que se difunda fácilmente a través de la pared celular y pueda formar entrecruzamientos con los polipéptidos mediante puentes de azufre, desnaturalizando las proteínas (Chen et al. 2008). Por otro lado, metales alcalinos y alcalinotérreos como calcio, magnesio, sodio y/o potasio son imprescindibles para el

buen funcionamiento celular, pero a la par pueden formar precipitados con fosfatos y carbonatos. Estos precipitados dificultan la operatividad del digestor y la concentración alcanzada a su vez determinará su biotoxicidad.

2.1.3 Estrategias para mejorar el proceso de digestión anaerobia

Debido a la complejidad en la composición de algunos sustratos para ser transformados durante el proceso de digestión, a menudo, es necesario aplicar estrategias diversas o pretratamientos para ayudar a estabilizar el proceso y aumentar los rendimientos en el biogás generado.

2.1.3.1 Pretratamientos

Como se mencionó anteriormente, durante la digestión anaerobia, la hidrólisis es una etapa limitante en el proceso. La acción de los pretratamientos se enfoca en la ruptura o degradación de biopolímeros tales como: proteínas, lípidos, ácidos nucleicos y carbohidratos para facilitar el proceso de digestión anaerobia. De esta forma, se acelera la hidrólisis a la vez que mejora la calidad del biogás.

Dependiendo del tipo de sustrato que se quiera digerir es imprescindible elegir el método de pretratamiento apropiado. Los pretratamientos que con más frecuencia se han explorado en la literatura se dividen en: mecánicos, térmicos, químicos, biológicos y combinaciones de éstos. No obstante, también se buscan nuevos tratamientos y estrategias alternativas para que la digestión anaerobia sea más eficiente y con un menor coste energético y económico.

Los pretratamientos mecánicos y térmicos suelen emplearse mayoritariamente en el tratamiento de los lodos generados en las EDAR mejorando las tasas de producción de biogás, incrementando la eficiencia de conversión a metano y disminuyendo los tiempos de retención (Li & Noike 1989; Nah et al. 2000). Los **pretratamientos mecánicos** como la sonicación, licuefacción o lisado por centrifugación permiten reducir el tamaño de partícula mejorando el contacto entre el sustrato y las bacterias anaerobias sin desprendimiento de malos olores, aunque resultan ineficaces para eliminar agentes patógenos.

Los **pretratamientos térmicos** reducen la cantidad de lodos generados, la viscosidad, favorecen la eliminación de patógenos y mejoran la producción de metano (Ariunbaatar et al. 2014). Sin embargo, resulta necesario controlar las condiciones del pretratamiento dado que se ha observado que a temperaturas entre 190-210 ˚C pueden darse fenómenos como la reacción de Maillard que genera productos de difícil degradación (Bougrier et al. 2006; Carrère et al. 2008; Climent et al. 2007).

Los **pretratamientos químicos** más empleados en la bibliografía son los tratamientos ácidos y alcalinos. Los pretratamientos con ácido clorhídrico, sulfúrico, málico, fórmico o acético son interesantes cuando hay un sustrato con una concentración alta en compuestos lignocelulósicos ya que permiten hidrolizar la hemicelulosa y precipitar la lignina, pero dan también lugar a la producción en paralelo de otras sustancias inhibitorias como los furfurales, 5-hidroximetilfurfural, ácidos fenólicos y aldehídos (Devlin et al. 2011; Hendriks & Zeeman 2009). Por lo general, si se opta por operaciones a temperatura ambiente los tratamientos alcalinos son una opción más atractiva. Estos producen modificaciones estructurales de lignina, hemicelulosa y celulosa mediante la ruptura de las cadenas laterales con enlaces tipo ésteres y glucosídicos. A temperatura ambiente, un pretratamiento alcalino optimizado puede eliminar hasta un 60 % de lignina y un 80% de hemicelulosa (Sun et al. 1995).

Los **pretratamientos biológicos** emplean las enzimas procedentes de microorganismos aerobios que son los únicos capaces de romper moléculas lignocelulósicas gracias a sus enzimas hidrolasas necesarias para la ruptura de polisacáridos y a su sistema lignolítico capaz de romper la lignina por la apertura de los anillos fenilos (Wagner et al. 2018). Después del pretratamiento fúngico, se han observado incrementos en la producción de metano entre un 5 y un 15% (Amirta et al. 2006).

2.1.3.2 Sistemas bioelectroquímicos

Los **sistemas bioelectroquímicos** conforman una familia de dispositivos que emplean microorganismo para generar corriente eléctrica a partir de la metabolización de materia orgánica (celdas de combustible microbiana) o para producir un producto final con un alto valor añadido como es el hidrógeno (celdas de electrólisis microbiana) conocidas ampliamente por sus siglas en inglés como MFC y MEC, respectivamente.

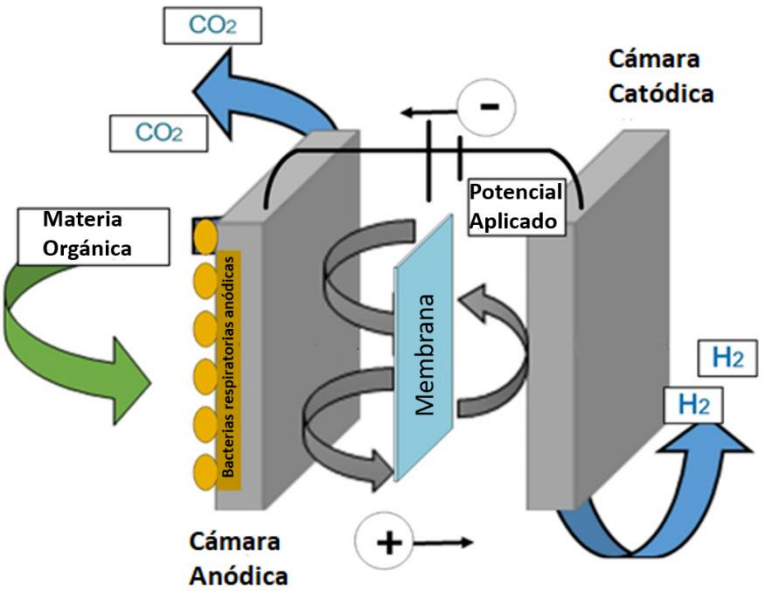

Figura 2.2 Representación esquemática del funcionamiento de una MEC

En los sistemas bioelectroquímicos, al menos una de las reacciones de los electrodos implica una interacción electroquímica con microorganismos. Con mayor frecuencia, es la reacción anódica la que requiere la presencia de ciertos microorganismos, generalmente conocidos como bacterias respiratorias anódicas (ARB), estas bacterias tienen la capacidad de transferir electrones de un sustrato biodegradable a un electrodo sólido (Escapa et al. 2016). La Figura 2.2 representa los principios de funcionamiento de MFC y MEC. Los electrones, uno de los principales subproductos del metabolismo de las bacterias, junto con los protones y el CO_2, se transfieren al ánodo y fluyen a través de un circuito externo al cátodo.

Dichos sistemas han sido propuestos para ser acoplados a la digestión anaerobia. Estos sistemas contribuyen a mejorar la digestión al tratarse de sistemas versátiles que presentan la capacidad de tratar sustratos de diversa índole a bajas concentraciones de DQO y a bajas temperaturas donde la digestión anaerobia presenta una cinética lenta (Pham et al. 2006). Por otro lado, la digestión en sí misma ofrece ventajas frente a los sistemas bioelectroquímicos, dado que la producción de metano tiene una mayor estabilidad operativa que aquellos sistemas que solo producen hidrógeno (Escapa et al. 2016). Esta sinergia entre los procesos de digestión y sistemas bioelectroquímicos

traduciéndose en resultados prometedores dando incrementos de enriquecimiento de biogás (Bo et al. 2014; Cerrillo et al. 2018; Feng et al. 2015).

Los sistemas bioelectroquímicos son una tecnología emergente de vanguardia. En particular, esta tecnología puede fijar eficientemente el CO_2 al convertirlo en metano a través de una vía termodinámicamente favorable en la cual los metanógenos electroactivos en el cátodo consumen electrones o causan una reducción equivalente, como el H_2, de acuerdo con las Fórmulas 2.2 y 2.3 (Noori et al. 2019):

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O, \text{ E}^\circ\text{= -0,24 V vs SHE} \quad (2.2)$$

$$2H^+ + 2e^- \rightarrow H_2, \text{ E}^\circ\text{= -0,42 V vs SHE} \quad (2.3)$$

donde SHE es un electrodo de hidrógeno estándar

Durante la última década, la producción bioelectroquímica de metano a partir de la fermentación específica de CO_2 ha sido de gran interés, con ella se busca maximizar la producción de biogás y acercar esta tecnología para su aplicación práctica (Beegle & Borole 2018; De Vrieze et al. 2018). Diversos autores han estudiado esta tecnología, pero uno de los problemas que atraviesan los sistemas bioelectroquímicos es el coste de los electrodos ya que constituyen hasta el 50% del precio de las celdas, siendo uno de los puntos desfavorables por los que estos sistemas biológicos no encuentran fácil su implantación a escala industrial (Li et al. 2014; Logan & Regan 2006). La utilización de materiales considerados como subproductos, tras la aplicación de un proceso de activación para convertirlos en materiales electroconductores en este tipo de sistemas podrían paliar los problemas relacionados con el alto coste de esta tecnología y otorgar un valor añadido. Los electrodos de procedencia de material lignocelulósico han sido estudiados anteriormente en distintos sistemas bioelectroquímicos consiguiéndose valores de producción energética de 532 ± 18 W m^{-2} comparables a otros que emplean carbono granular activado, pero con un coste de producción 23 veces más bajo (Huggins et al. 2014).

2.1.4 Procesos avanzados de oxidación electroquímica

La electrooxidación, también conocida como oxidación anódica, pertenece a los procesos electroquímicos de oxidación avanzada (PEOA). Dichos procesos tienen en común la capacidad de generar radicales hidroxilos para oxidar materia orgánica.

En el caso concreto de la electrooxidación, los radicales hidroxilos se generan en la superficie del ánodo cuando se establece una diferencia de potencial entre los electrodos de una celda. Los radicales hidroxilos son unos de los agentes con mayor poder oxidante (E=2,8 V). Es por ello que la electrooxidación junto a otras técnicas englobadas dentro de las PEOAs (p.ej.: electro-Fenton, foto-Fenton, Ozono/UV, etc.) han sido empleadas con éxito para tratar diversos efluentes. En los últimos años, se ha experimentado un creciente interés por los PEOA. Se ha visto como han sido empleados en la mineralización de la materia orgánica de diversa índole gracias a la capacidad de generar fuertes agentes oxidantes ($^{\cdot}OH$) in situ que oxidan compuestos biorefractarios de manera no selectiva y también se ha evaluado en el tratamiento de aguas residuales (Berenguer et al. 2019; Cai et al. 2020; García-Morales et al. 2013; Klidi et al. 2018; Martínez-Huitle & Panizza 2018; Oturan et al. 2018; Polcaro et al. 2009; Schmalz et al. 2009; Soriano et al. 2019; Ukundimana et al. 2018).

Debido a la heterogeneidad de los residuos en las aguas contaminadas es inviable emplear un método de descontaminación universal por lo que normalmente se emplean sistemas combinados. En la Figura 2.3 cada tratamiento tiene un rango de aplicabilidad óptimo. Los tratamientos biológicos como la digestión anaerobia son muy económicos, pero como se ha mostrado anteriormente que tienen dificultades para asimilar altas concentraciones de compuestos refractarios por lo que necesitarán de un método complementario. Los tratamientos térmicos a priori pueden aparecer como la opción más interesante ya que su versatilidad de aplicación es mayor al permitir la recuperación de energía, pero tienen como problema la emisión de gases y sólo pueden usarse a concentraciones altas de DQO. Otros tratamientos como la oxidación química pueden producir compuestos intermedios que son incluso más contaminantes que los contenidos en el residuo original y plantean el problema adicional de la disposición de un efluente de alta salinidad. Por estas razones es necesario la búsqueda de nuevas

alternativas que sirvan para reemplazar o simplificar los métodos destinados a mejorar la digestión.

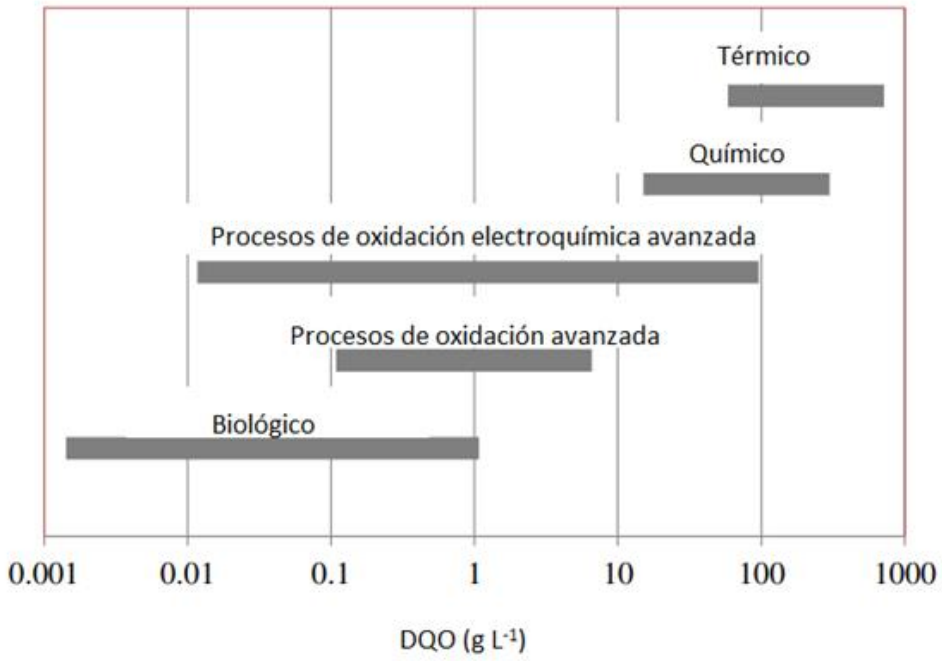

Figura 2.3 Rangos de aplicabilidad de pretratamientos en función de la concentración de DQO en sustratos. Adaptación de Fryda et al. (2003)

2.1.4.1 Mecanismos de electro oxidación

Existen dos vías usadas en la electrooxidación para degradar los contaminantes orgánicos: método directo y/o indirecto.

Oxidación directa: Se produce una transferencia electrónica desde el electrodo a la materia orgánica después de ser adsorbidos en la superficie del electrodo. Dicha vía presenta complicaciones de "biofouling", en otras palabras, presentan crecimientos de biopolímeros en la superficie del electrodo provocando, con el paso del tiempo, una disminución en el rendimiento de las operaciones.

Oxidación indirecta: La principal transferencia electrónica se produce en el seno de la disolución entre el mediador, que se trata de un agente oxidante generado in situ (Cl^-, $^{\cdot}OH$, O_3, $S_2O_8^{2-}$, entre otros) y la materia orgánica. Se produce una oxidación parcial de

los contaminantes (una conversión) o la oxidación total (la materia orgánica pasa a ser CO_2 y agua).

Debido a los problemas que comporta la oxidación directa de la materia orgánica en la superficie del electrodo, es preferible emplear electrodos o tratamientos que promuevan la oxidación indirecta. Generalmente la reacción involucrada en la formación de oxidación de la materia orgánica se explica a través de su interacción con el radical hidroxilo, siguiendo el mecanismo de Johnson et al. (1999) presente en la Reacción 2.4.

$$MOx + H_2O \rightarrow MO_x(OH^{\cdot}) + H^+ + e^- \quad (2.4)$$

El diferente grado de descomposición de la materia orgánica tiene que ver con la naturaleza del ánodo pudiéndose clasificar en dos tipos distintos dentro de este tipo de oxidación.

Electrodos activos: Se dan en los electrodos que tienen altos estados de oxidación por los que los radicales hidroxilos quedan adheridos a la superficie del metal y se conocen como "oxígeno activo quimiadsorbido" y debido a su selectividad producen oxidaciones parciales en la materia orgánica. En estos electrodos se dan las Reacciones 2.5 y 2.6.

$$MO_x(OH^{\cdot}) \rightarrow MO_{x+1} + H^+ + e^- \quad (2.5)$$

$$MO_{x+1} + R \rightarrow MO_x + RO \quad (2.6)$$

Electrodos no activos: Se dan en electrodos que no alcanzan altos estados de oxidación y favorecen la oxidación total de la materia orgánica ya que no se comportan de manera selectiva como se muestra en la Fórmula 2.7. Estos radicales hidroxilos se conocen como "oxígeno activo fisisorbido".

$$MO_x(OH^{\cdot}) + R \rightarrow MO_x + m\,CO_2 + n\,H_2O + H^+ + e^- \quad (2.7)$$

La tendencia de un electrodo a generar un tipo de oxidación u otra depende exclusivamente de su naturaleza intrínseca, pero como regla general aquellos que tienen un sobrepotencial de evolución de oxígeno (SEO) bajo se comportan como electrodos activos (p.ej.: grafito, carbono, platino) y aquellos que tiene un SEO alto se comportan como no activos (p. ej.: electrodos diamante dopados con boro (DDB), Ti-

PbO_2, Ti-SnO_2-Sb_2O_5). En reglas generales, los electrodos DDB se distinguen por ser inertes a la adsorción superficial, tener buena estabilidad electroquímica, un alto sobrepotencial de evolución de oxígeno, alta estabilidad a la corrosión y buena conductividad. Por estas razones los electrodos DDB pueden ser utilizados para la oxidación parcial o total de la materia orgánica contenida en las aguas residuales y de otros compuestos con una alta concentración en materiales biorefractarios o tóxicos.

2.1.4.2 ***Electrooxidación química de sustancias complejas con electrodos de diamante dopado con boro. Tratamientos para la mejora de la digestión anaerobia***

Los electrodos DDB han sido capaces de eliminar los contaminantes orgánicos en gran medida. En la Tabla 2.2 se recoge el empleo que han tenido en este campo durante los últimos años.

Tabla 2.2 Empleo de los electrodos de diamante dopado con boro para la eliminación de compuestos recalcitrantes

Tipo de contaminante	Actuación	Referencia
Amonio	Eliminación completa de amonio en presencia de cloruros	Kapałka et al. 2010
Cianuro	Reducción de entre el 70-80% de la concentración inicial de cianuros con un consumo energético comprendido entre 20-70 kWh m^{-3}	Cañizares et al. 2005
Fenoles	Eliminación del 95% de la DQO con un consumo 8,15 kWh m^{-3}	Sun et al. 2012
Anilina	Mineralización del 87,8% con un consumo de 270 kWh m^{-3}	Benito et al. 2017
Clorofenol	Entre 100-200 kJ kg^{-1} se alcanza el 100% de la eliminación	Wang & Li 2012
Melanoidinas	Se consumen 17 kWh por cada kg de COD eliminado alcanzándose el 60% de la eliminación de COD después de las 10 h	Martinez et al. 2018
Lignina	Eliminación del 100% de DQO a 10 kWh m^{-3} con adición de 2 g L^{-1} de NaCl	Klidi et al. 2018

La electrooxidación también ha sido evaluada en la literatura como un pretratamiento para acondicionar y mejorar la biodegradabilidad de los lodos antes de la digestión anaerobia (Xu et al. 2014). Barrios et al. (2017) estudiaron la electrooxidación parcial de los lodos activados reduciendo la DQO total y aumentando la DQO soluble. Esto facilitó la solubilización del sustrato y con ello, la degradación que se produce durante la etapa de hidrólisis consiguiéndose un incremento en la producción del biogás. Otra de las mejoras que aporta la electrooxidación en el post-tratamiento de los lodos es que mejora la estabilidad y deshidratabilidad gracias a la reducción de las sustancias poliméricas extracelulares (SPE) y la lisis microbiana (Yuan et al. 2011). Las SPE son responsables de la estabilidad estructural de los flóculos y su ruptura supone la liberación del agua intersticial e intracelular como se muestra en la Figura 2.4.

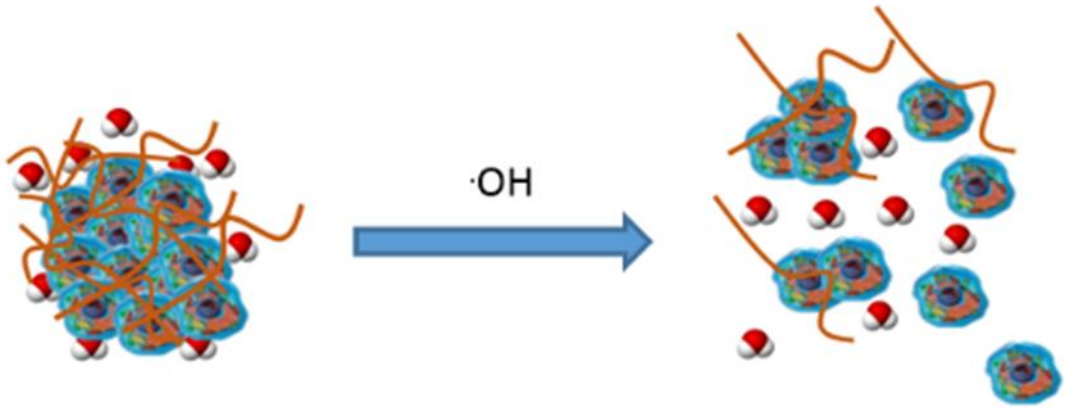

Figura 2.4 Ruptura por electrooxidación de los flóculos que componen los lodos. Las líneas en naranja representan los SPE y las esferas rojas y blancas representan las moléculas de agua retenidas

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2.3 OBJETIVOS

2.3.1 Objetivo principal

El objetivo general de este estudio es la implementación de diversas estrategias de optimización para mejorar el proceso de digestión anaerobia de sustratos complejos o con alta carga orgánica. Mediante la **adición de hidrogeno, la utilización de tratamientos bioelectroquímicos y de pretratamientos oxidativos avanzados** se pretende mantener en el reactor, las condiciones que permitan un funcionamiento estable. También se pretente conseguir un incremento en la riqueza y/o producción de biogás y unos efluentes con características adecuadas para su disposición final y/o reutilización.

2.3.2 Objetivos específicos

De manera específica, las actividades planteadas en este trabajo pretenden dar respuesta a los siguientes objetivos planteados:

- Incrementar la eficiencia de la digestión mediante la inyección de hidrógeno para promover la actuación de los microorganismos metanógenos hidrogenotróficos.
- Determinar la efectividad en la utilización de ánodos de bajo coste en sistemas bioelectroquímicos.
- Aplicación de la electrooxidación y evaluación como pretratamiento para la eliminación y/o degradación de sustancias complejas presentes en lodos de depuradora.
- Evaluación de alternativas destinadas a la mejora de la digestión como son la electrooxidación como pretratamiento y el suplemento de material de carbono conductor como el biochar.

2.3.3 Esquema de trabajo

El trabajo realizado se realizó conforme al siguiente esquema:

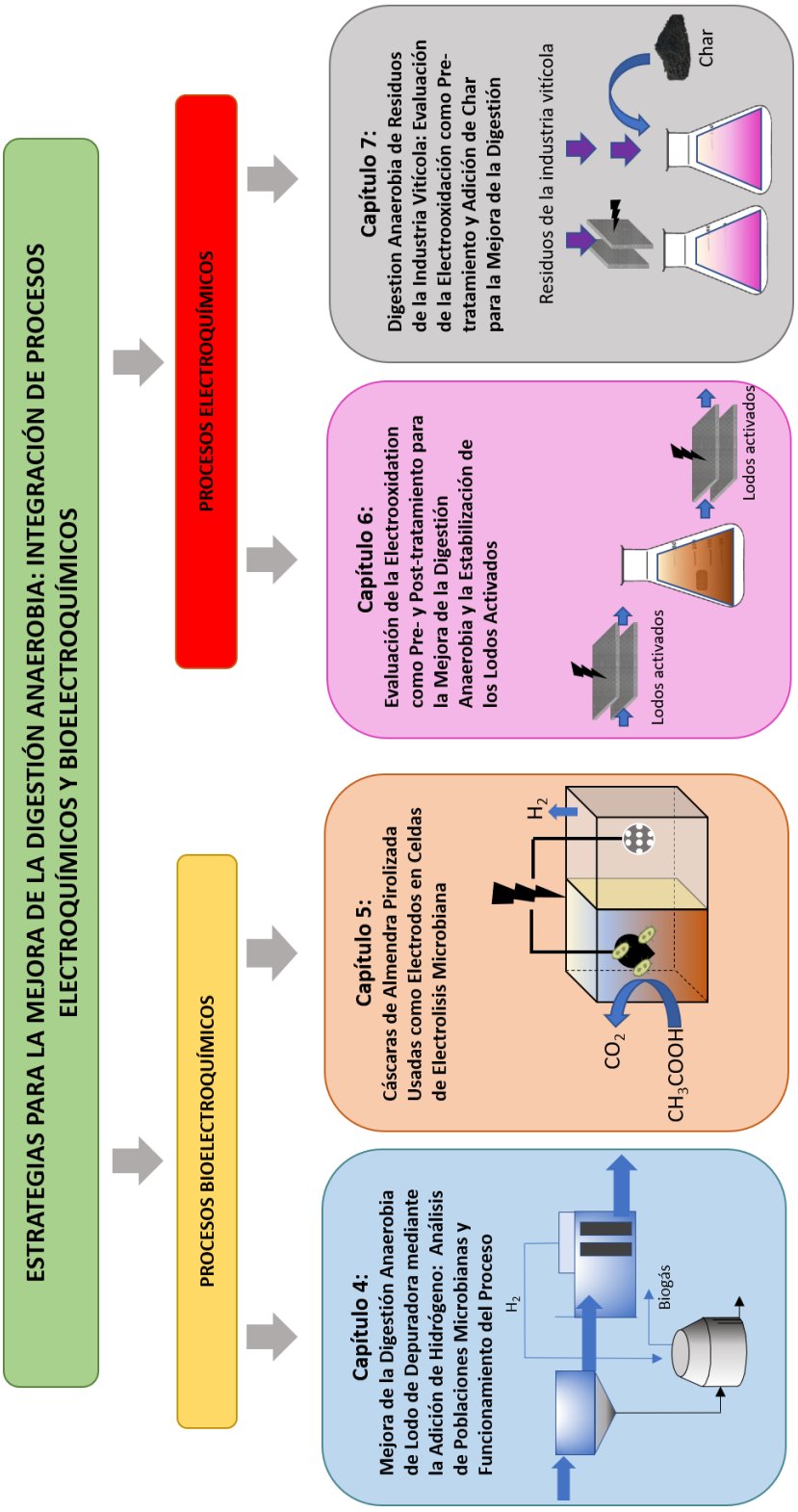

Figura 2.5 Esquema de trabajo

Capítulo/Chapter 3

Materiales y Métodos

3.1 MATERIALES Y SUSTRATOS EMPLEADOS

3.1.1 Inóculo

El inóculo utilizado en esta tesis procede de la Estación de Depuración de Aguas Residuales (EDAR) de Vilecha (León). Dicha instalación opera en condiciones mesofílicas y da servicio a León y a su Alfoz tratando un caudal diario de 107 mil m^3 ("SALEAL" 2020). El digestor anaerobio trata una mezcla de lodos primarios y secundarios, los cuales han sido previamente espesados en el primer caso por gravedad y en el segundo por flotación. Tras la digestión, el lodo digerido se transfiere a un tanque de estabilización final, de donde procede el inóculo utilizado.

Una vez recibido el inóculo, este material se almacena a temperatura ambiente con el fin de continuar con el proceso de estabilización y reducir su actividad microbiana hasta el punto que la evolución de biogás intrínseca del mismo no interfiera con las pruebas de degradabilidad realizada en los diferentes ensayos experimentales. El contenido de los sólidos totales de los inóculos empleados en esta tesis oscila entre 2,5-3,5% con un contenido en sólidos volátiles alrededor del 60% respecto al contenido de sólidos totales.

3.1.2 Sustratos

Lodo mixto conformado por la mezcla de lodos primarios y secundarios procedentes de la EDAR de León. La proporción volumétrica de mezcla de estos lodos es 30:70 (primario: secundario) siguiendo la misma proporción de generación en la depuradora. Este material fue utilizado como substrato en los **Capítulos 4 y 6**. En el capítulo 4 se evalúa el efecto de inyección de H_2 en la mejora de la digestión anaerobia. En la Tabla 3.1 se ilustran los parámetros que sirvieron para caracterizar los distintos tipos de lodos de la EDAR.

En el capítulo 6 se estudia la influencia de la electrooxidación en la mejora de la digestión anaerobia como pretratamiento y su capacidad de deshidratación de los lodos de depuradora en el post-tratamiento.

Tabla 3.1 Caracterización de los lodos procedentes de la EDAR de Vilecha

Parámetros químicos	Inóculo	Lodo Primario	Lodo activo	Mezcla
Sólidos totales (g kg^{-1})	30,8 ± 0,9	31,5 ± 0,9	27,0 ± 0,8	28,2 ± 0,7
Sólidos volátiles (g kg^{-1})	18,2 ± 0,5	26,4 ± 0,8	21,4 ± 0,6	22,9 ± 0,7
Carbono orgánico total soluble (g L^{-1})	2,97 ± 0,14	3,45 ± 0,17	1,06 ± 0,05	1,41 ± 0,08
Amonio (mg L^{-1})	742 ± 29	968 ± 38	487 ± 19	655 ± 21
pH	7,10 ± 0,01	5,20 ± 0,01	6,13 ± 0,01	5,62 ± 0,01
Demanda química de oxígeno (mg L^{-1})	2 178 ± 109	3 172 ± 159	2 569 ± 128	2 744 ± 137
Materia orgánica (%) *	42,43 ± 0,14	53,85 ± 1,06	59,21 ± 0,09	56,40 ± 0,49
Nitrógeno Kjeldahl (%) *	4,87 ± 0,19	5,10 ± 0,15	9,43 ± 0,28	7,86 ± 0,23
Proporción C/N	5,04	6,16	3,65	4,21
Ácido acético (mg L^{-1})	n.d.	2 279 ± 114	397 ± 16	1 007 ± 40
Ácido propiónico (mg L^{-1})	n.d.	1 581 ± 79	228 ± 14	682 ± 26
Ácido isobutírico (mg L^{-1})	n.d.	199 ± 10	49,42 ± 2,52	89,33 ± 4,46
Ácido butírico (mg L^{-1})	n.d.	1 471 ± 59	51,71 ± 1,55	517 ± 16
Ácido isovalérico (mg L^{-1})	n.d.	208 ± 26	51,23 ± 1,20	132 ± 21
Ácido valérico (mg L^{-1})	n.d.	449 ± 13	44,70 ± 1,10	209 ± 15
Ácidos grasos volátiles (mg L^{-1})	n.d.	5 083 ± 254	658 ± 18	2 639 ± 132
Cadmio (mg kg^{-1})	0,030 ± 0,001	0,020 ± 0,001	0,030 ± 0,001	0,020 ± 0,001
Cromo (mg kg^{-1})	1,10 ± 0,01	0,68 ± 0,01	0,73 ± 0,01	0,75 ± 0,01
Cobre (mg kg^{-1})	3,97 ± 0,20	2,67 ± 0,13	3,75 ± 0,15	3,32 ± 0,12
Fósforo (mg kg^{-1})	447 ± 22	336 ± 17	862 ± 43	681 ± 34
Níquel (mg kg^{-1})	0,66 ± 0,01	0,37 ± 0,01	0,34 ± 0,01	0,33 ± 0,01
Plomo (mg kg^{-1})	2,47 ± 0,12	1,87 ± 0,10	1,23 ± 0,10	1,39 ± 0,11

Nota. *% expresado en base a materia seca, n.d. no detectado

Acetato sódico en concentración 0,5 g L^{-1} utilizado en disolución a pH neutro en un medio mineral compuesto por las siguientes sales (por litro): 0,87 g de K_2HPO_4, 0,68 g de KH_2PO_4, 0,25 g de NH_4Cl, 0,453 g de $MgCl_2 \cdot 6H_2O$, 0,1 g de KCl, 0,04 g de $CaCl_2 \cdot 2H_2O$ y 10 mL de una disolución de elementos traza. Dicha disolución forma parte del analito de las celdas MEC del **Capítulo 5** y se encuentra en una proporción (80:20 en $V_{sustrato}$: $V_{inóculos}$), con una mezcla de inóculos procedente del lodo mixto y del efluente de una celda MEC (escala laboratorio) que estuvo en funcionamiento durante 6 meses para asegurar una cantidad alta de microorganismos exoelectrogénicos.

Residuos líquidos de la industria del vino procedentes de una explotación vinícola situada en La Morra (Cuneo, Italia). Se emplearon en el Capítulo 7. Debida a su elevada carga orgánica se hizo necesario someterlas a unos tratamientos adicionales a la digestión desarrollados en dicho capítulo.

3.1.3 Experimentos de biodegradabilidad

3.1.3.1 Reactores para operaciones en semicontinuo

Los reactores empleados en la Figura 3.1 se emplearon para los experimentos del **Capítulo 4**. Se tratan de reactores de metacrilato con un volumen de 3L con una camisa por la que circula agua termostatizada. Dependiendo de las necesidades del experimento estos pueden operar en semicontinuo o discontinuo. Los reactores se agitaron con un agitador de palas cuya velocidad era regulada con motor RZR2020. La medición del gas se realizó mediante medidores reversibles de contacto, teniendo un volumen de acumulación de gas en el rango de 42 – 50 mL. Cada vuelta es registrada por un contador electrónico de modo que la producción diaria de gas se corresponde al número de vueltas almacenadas por el contador y el volumen de almacenamiento del sistema determinado según calibración previa de cada unidad.

Figura 3.1 Reactores empleados en los experimentos del capítulo 4

3.1.3.2 Celdas de electrólisis microbiana

Para el experimento realizado en el **Capítulo 5**, se emplearon celdas bicamerales de policarbonato como las de la Figura 3.2 de dimensiones 76 x 81 x 76mm, separadas por una membrana de intercambio catiónico (International Membrane Inc.). A cada pieza del montaje de la celda se le adhirió una lámina de goma para prevenir el escape de gas. Las celdas operaban utilizando un sistema de dos electrodos. Dependiendo del tipo de ánodo, los electrodos de trabajo (ET) consistían en cáscara de almendra pirolizada o fieltro de carbono con áreas proyectadas de 2,8 y 4,0 cm^2, respectivamente. El contraelectrodo (CE) consistía en una malla de acero inoxidable (AISI304) de 5,1 cm^2 de área. Los electrodos estaban en contacto con la fuente de alimentación mediante un hilo de titanio (Goodfellow) que mantenía las celdas a un voltaje constante de 1,0 V y en condiciones normales. La adquisición de datos se hizo con un software Labview.

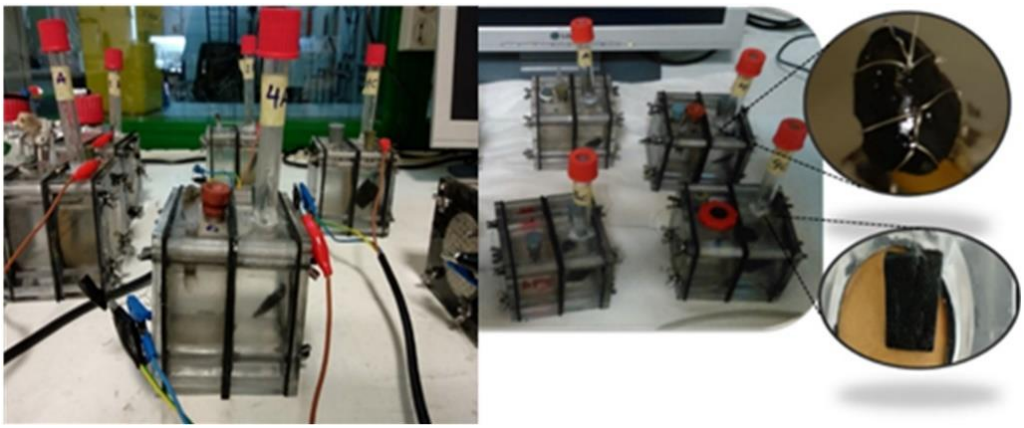

Figura 3.2 Celdas de electrolisis microbiana con los electrodos de cáscara de almendra pirolizada y fieltro de carbono

3.1.3.3 Matraces para operaciones en discontinuo

Se emplearon matraces Erlenmeyer de 250 mL de volumen para los experimentos en discontinuo realizado en los **Capítulos 6 y 7**. Los matraces operaron a temperatura controlada dentro de un baño termostatizado en condiciones mesofílicas (38,5 ± 1 ˚C). En el interior de los matraces se dispusieron barras magnéticas para su continua agitación. En la Figura 3.3, se puede observar que los matraces estaban conectados a matraces colectores de biogás. La cuantificación del volumen de biogás producido se

hizo por desplazamiento líquido. El líquido está compuesto por una disolución salina ácida y un indicador (naranja de metilo) a fin de evitar la posible disolución de CO_2 y ofrecer una lectura real de la composición del biogás producido.

Figura 3.3 Matraces Erlenmeyer empleados para el estudio de la biodegradabilidad de los lodos de depuradora y los residuos líquidos de la industria del vino

3.1.3.4 Celdas para electrooxidación

La electrooxidación de los sustratos se realizó en una celda BDD (Proaqua) como la que se ilustra en la Figura 3.4. Tiene un volumen de 70 mL y emplea dos electrodos de diamante dopado con boro de un área efectiva de 42 cm^2. El voltaje es proporcionado por una fuente de alimentación PSI 5000A–DC (Electro-Automatik) y el tratamiento de electrooxidación es monitorizado con el mismo software que controla la fuente de alimentación.

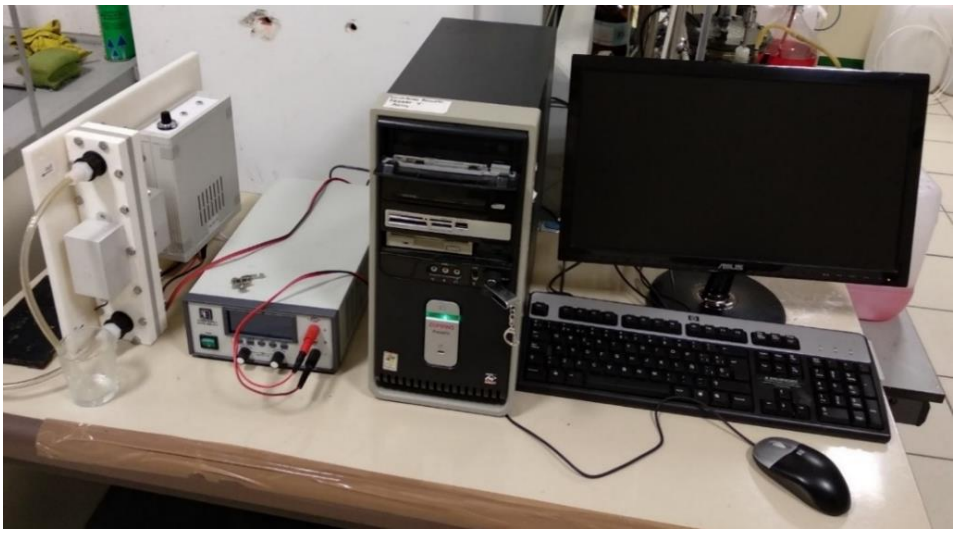

Figura 3.4 Celda con electrodos BDD conectada a una fuente de alimentación

3.2 MÉTODOS ANALÍTICOS

3.2.1 Sólidos totales y volátiles

Los sólidos totales (ST) y los sólidos volátiles (SV) fueron medidos siguiendo los métodos APHA (2005). Para la determinación de los sólidos se tomaron volúmenes de 10 mL de cada muestra homogeneizada y se depositaron en crisoles de porcelana resistentes a altas temperaturas. Los crisoles se introdujeron en una estufa (Memmert) a 105 ˚C durante 24 h hasta evaporación total del agua contenida en las muestras. El cálculo de los ST se obtuvo mediante la diferencia de peso entre la muestra original y la resultante. Posteriormente, dichos crisoles se introdujeron en una mufla (Hobersal 12PR/300 8B) a 550 ˚C durante 2 h. El peso de la muestra calcinada permitió determinar los SV por diferencia de pesada.

3.2.2 pH y conductividad

Las muestras fueron medidas de manera directa con un pH-metro (GLP22, Crison Instruments S.A.) previamente calibrado en los rangos de 4,0 y 9,0 unidades. La conductividad fue medida con un conductivímetro (sesion^{TM} 156 Multiparameter Hach Co.).

3.2.3 Materia orgánica

La materia orgánica fue medida siguiendo el método Walkley-Black (Walkley & Black 1934). Este método consiste en la oxidación de la materia orgánica mediante la digestión de la muestra en presencia de una mezcla de K_2MnO_4 y H_2SO_4 1N durante 30 min. Posteriormente, la muestra se valora con sulfato de hierro (II), amonio hexahidratado (sal de Mohr) 0,5 N y difenilamina como indicador. La materia orgánica se calcula considerando que el 58% de la muestra contiene carbono orgánico (Kerven et al. 2000).

3.2.4 Demanda química de oxígeno

La demanda química de oxígeno (DQO) se determinó mediante la adición de 0,5 mL de muestra a unos tubos de kit comercial (LCK 014 1000-10000 mg L^{-1} de O_2, Hach Lange). Para ello se adiciona la muestra al tubo que contiene una disolución ácida de $KMnO_4$. Después la muestra es digerida a 150 ˚C durante 2 h y se deja enfriar hasta alcanzar temperatura ambiente. La cuantificación se hace mediante fotometría con la ayuda de un espectrofotómetro (DR3900, Hach).

3.2.5 Carbono orgánico total

En la Figura 3.5 se muestra el aparato con el que se cuantificó el carbono orgánico total (COT). Es un analizador de alto rendimiento de multi N/C 3100 (AnalytikJena). Tiene un horno integrado que alcanza temperaturas de 950 ˚C. Para calcular la cantidad de carbono orgánico que contiene la muestra se acidifica con H_3PO_4 al 10% liberando el carbono inorgánico en forma de CO_2. El COT se determina la diferencia entre el carbono total y el inorgánico. Los valores se expresan en porcentajes.

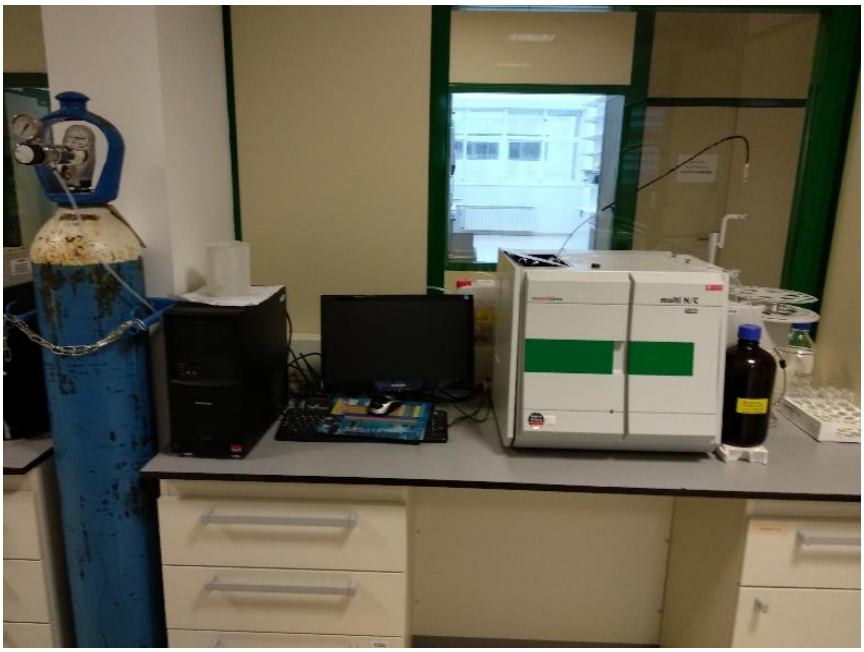

Figura 3.5 Multi N/C 3100 de AnalytikJena

3.2.6 Nitrógeno Kjeldahl

La determinación de nitrógeno procedente de la materia orgánica se realizó empleando el método Kjeldahl (Kjeldahl 1883). El método consta de tres etapas. En la digestión se acidifica la muestra y el nitrógeno contenido en las proteínas se convierte en amonio en presencia de un catalizador a 400 ˚C. Posteriormente se realiza una destilación donde los iones amonio se transforman en amoníaco mediante la adición de NaOH. Finalmente, se realiza una valoración ácido-base determinándose la cantidad de nitrógeno en función del ácido neutralizado.

3.2.7 Amonio

La cuantificación del amonio en las muestras se llevó a cabo con un medidor pH Metron 781 al que se acopla un sensor electro selectivo (No133/1e). Inicialmente las muestras se centrifugaron a 6461 x g durante 12 min en una centrifuga 2-16P (Sigma). Por otro lado, se prepararon patrones para establecer una recta de calibrado. Al sobrenadante de la muestra centrifugada y a los patrones preparados se les adiciona 1 mL de NaOH 1 N para convertir los iones amonio a amoniaco y determinando la concentración de amonio en función de la respuesta analítica.

3.2.8 Análisis elemental

El análisis elemental de P, K, Na, S, Ca y Mg se hizo mediante la digestión de 5 g de muestra en 10 mL de ácido nítrico al 65% en un digestor a presión atmosférica con reflujo programado con las siguientes condiciones de temperatura y tiempos: De 20 a 45 ˚C en 30 min, cuya temperatura final se mantuvo durante 60 s. Después, se elevó a 60 ˚C en 25 min donde se mantuvo la temperatura 5 min. De 65 a 100 ˚C en 15 min, manteniéndose a 100 ˚C durante 120 min. Finalmente, se dejaron enfriar y se aforaron los tubos digestores a 20 mL con agua MilliQ. Posteriormente, se realizaron los patrones oportunos y se determinaron los elementos con un espectrómetro de emisión con fuente de ionización (ICP-OES 5110 SVDV, Agilent).

3.2.9 Polifenoles totales y color

Los polifenoles se midieron por colorimetría en una longitud de onda a 760 nm con un espectrofotómetro tipo DU640 (Beckman) usando el reactivo Folin-Ciocalteau. Para ello se mezcló 650 µL de agua desionizada, 50 µL de muestra, 600 µL de Na_2CO_3 al 7,5% y 200 µL de reactivo Folin-Ciocalteau. Se empleó ácido gálico para como patrón para establecer la curva de calibración. Los resultados obtenidos se expresan en equivalentes de ácido gálico (EAG) (Wiseman et al. 2001; Waterhouse 2002).

La absorbancia fue medida en el mismo espectrofotómetro. Dicho rango se eligió teniendo en cuenta que las melanoidinas presentes en el vino absorben a longitudes de onda entre 314-325 nm (Jiranuntipon et al. 2008; Dwyer et al. 2009). La reducción del color está relacionada con la eliminación de las melanoidinas presentes en la muestra.

3.2.10 Cromatografía de gas

3.2.10.1 Caracterización del biogás obtenido

El análisis del biogás se hizo mediante el empleo de un cromatógrafo de gas (CP 3800 GC, Varian) como el de la Figura 3.6 equipado con un detector termoconductor. Para separar el CH_4, CO_2, H_2, O_2 y N_2 se acopló una columna de 4 m rellenada de HayeSep seguida de otra columna de 1 m. El gas portador fue helio y las columnas operaban a 50 ˚C con una presión de 331 kPa.

Figura 3.6 Cromatógrafo de análisis del biogás

3.2.10.2 Determinación de los ácidos grasos volátiles

Los ácidos grasos volátiles se midieron en un cromatógrafo de gas 450 GC (Bruker) como el mostrado en la Figura 3.7. Tiene incorporado un autosampler CP-8400 (Bruker) y, una columna capilar Nukol (30 m X 0,25 mm X 0,25 µm) de Supelco y un detector tipo FID. El helio es el gas portador. La temperatura del detector y el inyector fueron 220 y 250 ˚C, respectivamente. La temperatura del horno se fijó a 150 ˚C durante 3 minutos y luego se aumentó hasta 180 ˚C. El límite de detección de AGVs del equipo son 5,0 mg L^{-1} de AGVs. El cromatógrafo se calibró usando una mezcla de ácidos grasos volátiles con longitudes de cadena entre 2 y 7 átomos de carbono (Supelco) y fenol 50 mM como patrón interno. Las muestras se ajustaron a pH 3,0-4,0 y se centrifugaron a 6461 x g durante 12 min. Finalmente se filtraron mediante un filtro de celulosa con tamaño de poro de 0,45 µm.

Figura 3.7 Cromatógrafo de análisis de ácidos grasos volátiles

3.2.10.3 Determinación de alcoholes

Los alcoholes fueron elucidados con un cromatógrafo de gas 7890B GC System (Agilente Technologies) como el que se ilustra en la Figura 3.8, equipado con un detector FID. El cromatógrafo tenía una columna CP-Wax 57 CB (25mx 0,25 mm x 0,2 µm) de Agilent J&W GC Columns. El gas portador es hidrógeno cuyo flujo era 30 mL min^{-1}. Se usó un método para programar la temperatura del horno: se incrementa la temperatura hasta 50 ˚C donde se mantiene durante 30 s. y posteriormente se incrementa hasta los 150 ˚C a una velocidad de 10 ˚C min^{-1}. La detección y cuantificación de los alcoholes de la muestra se hace con la preparación de una disolución patrón para establecer una recta de calibración y que contiene los siguientes alcoholes: 1-butanol, 2-butanol, 1-propanol, 2-propanol, metanol y etanol.

Figura 3.8 Cromatógrafo para la determinación de alcoholes

3.2.11 Determinación de los parámetros de deshidratabilidad

Estos experimentos se realizaron para estudiar las propiedades físicas relacionadas con la deshidratación de lodos y se emplearon en el capítulo 6 de esta tesis.

3.2.11.1 Resistencia específica a la filtración

Se trata de la resistencia que opone un lodo a ser filtrado en un área determinada y sirve para estudiar los requerimientos energéticos y pretratamientos asociados a los procesos de extracción de agua mediante filtración, prensado o centrifugación. En la Figura 3.9 se adjunta un dibujo y una imagen del montaje.

En el laboratorio, se filtra un determinado volumen (V) con ayuda de una bomba de vacío hasta la formación de una "torta". El experimento se considera finalizado una vez

la torta empieza a resquebrajarse por una caída en la presión. La resistencia específica a la filtración se calcula con la Fórmula 3.1:

$$r = \frac{2PA^2b}{\mu c}, \qquad (3.1)$$

siendo: **r**=resistencia específica a la filtración (cm g^{-1}), **P**=presión de vacío aplicado (dinas cm^{-2} = g cm^{-1} s^{-2}), **A**=Área del filtro (cm^2), **b**=pendiente (s cm^{-6}), **μ**=viscosidad del filtrado (poise = g cm^{-1} s^{-1}), **c**=concentración de sólidos totales por volumen de filtrado (g cm^{-3}) (Christensen & Dick 1985; Sorensen et al. 1995).

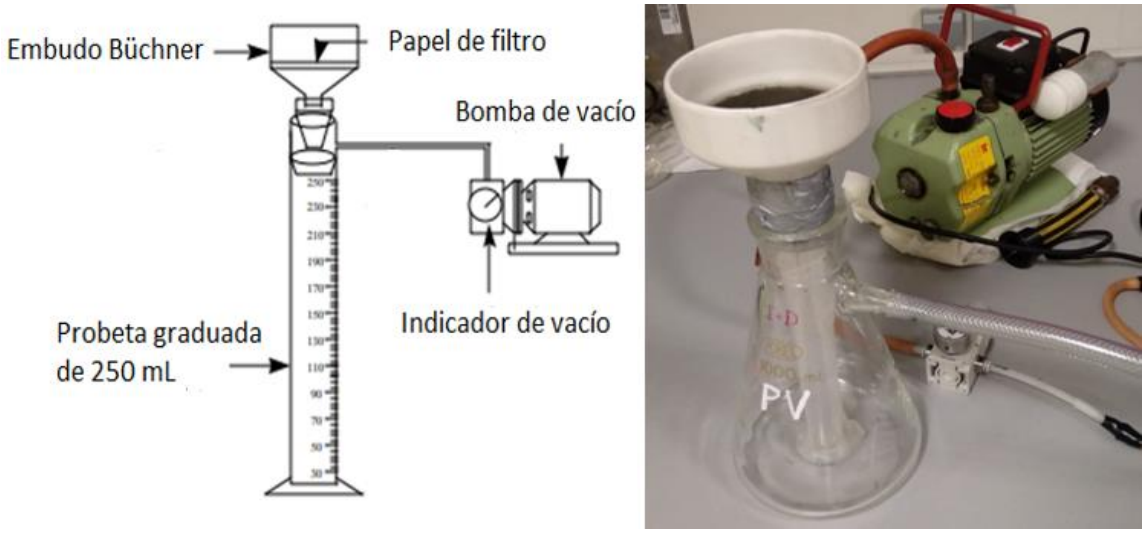

Figura 3.9 Esquema del montaje del test de resistencia específica de filtración e instrumental empleado

3.2.11.2 Tiempo de succión capilar

Es un test rápido y sencillo que permite observar la efectividad de un pretratamiento para deshidratar un lodo. Se adiciona un volumen de lodo a través de un tubo en posición vertical. Debajo del tubo hay papel de filtro grueso que irá succionando el agua contenida en el lodo. El tiempo y la amplitud de la marca de agua en las direcciones XY sobre el papel de filtro determinarán la efectividad del tratamiento.

El procedimiento consistió en la adición de 2 mL de lodo pretratado contenido en un tubo de acero inoxidable de 0,535 cm de diámetro interno. El filtro de papel utilizado es Whatman nº17 al que se dibujaron dos círculos concéntricos de radios 0,5 y 2 cm. Para hallar el valor de TSC se empleó la Fórmula 3.2

$$TSCm = \frac{\mathrm{TSC}-\mathrm{TSCf}}{\mathrm{C}}, \qquad (3.2)$$

Siendo: **TSCm** = tiempo de succión capilar modificado, **TSC** = tiempo de succión capilar de la muestra original(s), **TSCf** = tiempo de succión capilar de filtrado (aprox. 8s), **C** = concentración de sólidos en suspensión (mg L^{-1}) (Yin et al. 2004; Vesilind & Örmeci 2000).

3.2.11.3 Agua libre ligada

Para hacer un seguimiento de la eliminación del agua libre y ligada del lodo se emplea un método termogravimétrico. Se centrifugó una porción de lodo a 6461 x g durante 12 min y se tomó la fase sólida. Posteriormente, una muestra 40 mg de lodo se sometió a secado en una termobalanza Q600 (TA Instruments) como se representa en la Figura 3.10 con un flujo de aire de 300 mL min^{-1} hasta alcanzar los 105 ˚C. Los resultados se presentan como el porcentaje de masa perdida frente al tiempo (Kopp & Dichtl 2001).

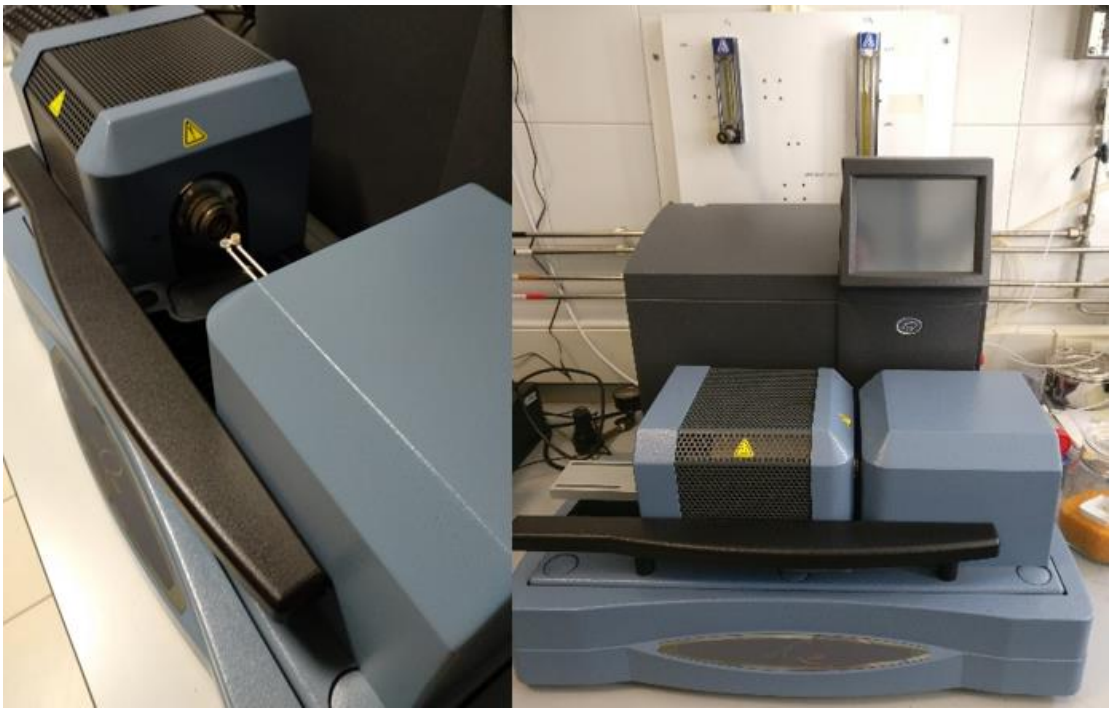

Figura 3.10 Termobalanza Q600 de TA Instruments

3.2.12 Análisis del tamaño de partícula

El tamaño de partícula está íntimamente ligado a algunas propiedades que presenta la materia orgánica como son la viscosidad, la filtrabilidad y la deshidratabilidad (Karr &

Keinath 1978). El criterio para determinar cuál es el tamaño de una partícula depende de la forma de la partícula en sí. El tamaño de las partículas esféricas esta descrito por su diámetro, pero en el caso de las que tienen formas "no esféricas" o irregulares se debe estimar una longitud característica de una partícula.

En el análisis de distribución de tamaño de partícula (DTP) por difracción de láser se aportan los resultados en percentiles conocidos como d10, d50 y d90. Entre d10 y d90 son dos puntos que incluyen a las partículas más finas y más gruesas. Al igual que d50, d90 describe que el 90% de las partículas de la distribución de un determinado valor son más pequeñas y el 10% son más grandes. Análogo para d10, el 10% de las partículas son más pequeñas y el 90% más grandes.

Por último, el área de superficie específica (ASE) es un valor de utilidad ya que describe con más precisión una partícula que tiene porosidad como es el caso de las partículas de los lodos (Sørensen & Wakeman 1996).

La DTP y la ASE se realizaron en un dispositivo como el mostrado en la Figura 3.11 se realizó el análisis de tamaño de partícula. Se trata de un difractor de rayos láser LS 13 320 (Beckman Coulter) que permite medir tamaño partículas con entre 0,045-2000 µm. Dicho equipo cuenta con dos fuentes: una que emite a 750 nm que permite la medición de tamaños de partícula ≥ 0,4 µm (Buurman et al. 1997). Para tamaños de partículas ≤0,4 µm, el difractor de rayos láser emplea una fuente secundaria llamado sistema PIDS (por sus siglas en inglés, *polarization intensity differential of scattered light*) que emplea haces de luz polarizada a longitudes de onda 450-,600-,900- nm. El sistema PIDS tiene la capacidad de medir entre 0,1-0,6 veces el tamaño de partícula la longitud de onda del haz alcanzando el límite de 0,045 µm ("COULTER LS SERIES Product Manual," 2011). La medición de cada muestra se hizo en dilución 50:1 con agua del grifo antes de ser sometida a 10 barridos

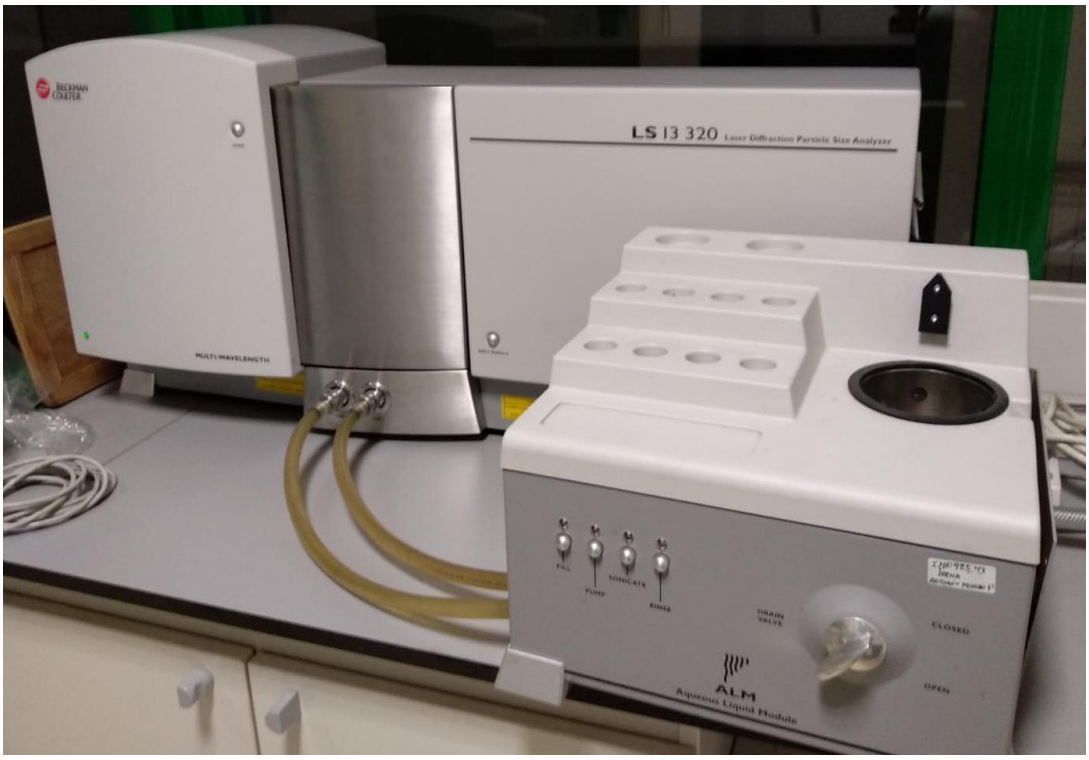

Figura 3.11 Analizador de tamaño de partícula por difracción laser utilizado para el análisis

3.2.13 Espectro de infrarrojos con transformada de Fourier

La espectroscopía de infrarrojos es una técnica muy versátil ya que puede emplearse para diversos tipos de muestras: sólidos, líquidos, gases, etc. Esta técnica se ha empleado con éxito por varios grupos de investigación para caracterizar lodos o para hacer un seguimiento de los cambios que pueden experimentar los lodos al aplicarse distintos tipos de tratamientos (Amir et al. 2004; Provenzano et al. 2014; Fels et al. 2013).

El fundamento de dicha técnica reside en que la absorción de la radiación infrarroja a una determinada frecuencia provoca cambios en la energía vibracional de las moléculas. Expresándose en el espectro de infrarrojo mediante un pico. La radiación infrarroja es lo suficientemente energética para producir cambios a nivel rotacional y vibracional en las moléculas. Pero esto no se produce en todo tipo de moléculas. Para que haya una absorción de la radiación infrarroja es necesario que dichas moléculas tengan un momento dipolar. Las moléculas polares tienen una distribución de carga electrónica irregular y, al vibrar, pueden interaccionar con la radiación infrarroja. Si la frecuencia de

radiación iguala a la frecuencia de vibración natural de la molécula, tiene lugar una transferencia neta de energía produciéndose un cambio en la amplitud de la vibración molecular.

Las moléculas vibran fundamentalmente de dos maneras: tensión y flexión. La tensión implica un cambio en la distancia interatómica entre dos o tres átomos a lo largo del eje de enlace que los conecta y la flexión genera un cambio en el ángulo entre dos enlaces siendo de cuatro tipos: de tijereteo, de balanceo, de aleteo y de torsión. La interpretación de los espectros de moléculas poliatómicas resulta complicada ya que implican la vibración de varios centros y la interacción entre ellos (Skoog 2009).

Para el análisis de muestra, se tomó una pequeña fracción del material seco previamente molido con un mortero de porcelana. La muestra se depositó en el ATR de un espectrofotómetro FT/IR-4600 (JASCO). Se hicieron 25 barridos en un rango de longitudes de onda entre 4000-400 cm^{-1}.

3.2.14 Secuenciación masiva de alto rendimiento del gen 16S rARN basado en bibliotecas génicas

El ADN se extrajo con el kit de aislamiento de ADN PowerSoil® DNA (MoBio Laboratories Inc.) Todas las PCR se llevaron a cabo con un Mastercycler (Eppendorf), y el tamaño de las muestras de PCR se comprobó en gel de agarosa al 1%. El extracto de ADN fue sometido a secuenciación masiva de alto rendimiento del gen 16S rARN basado en bibliotecas génicas para poblaciones totales de eubacterias y arqueas. Cada muestra fue amplificada con el primer set 27F mod (5'-AGRGTTTGATCMTGGCTCAG-3') con 519R modBio (5'-GTNTTACNGCGGCKGCTG-3')(Callaway et al. 2009) y Arch 349F (5'-GYGCASCAGKCGMGAAW-3') con Arch 806R (5'-GGACTACVSGGGTATCTAAT-3')(Takai & Horikoshi 2000) para la población de eubacterias y arquea respectivamente. Las lecturas del ADN obtenido fueron recogidas en los archivos FASTq para más información siguiendo las instrucciones de MR DNA Research Lab (http://www.mrdnalab.com/). Las unidades taxonómicas operacionales (UTOs) fueron taxonómicamente clasificadas usando la base de datos ribosómica (https://rdp.cme.msu.edu/).

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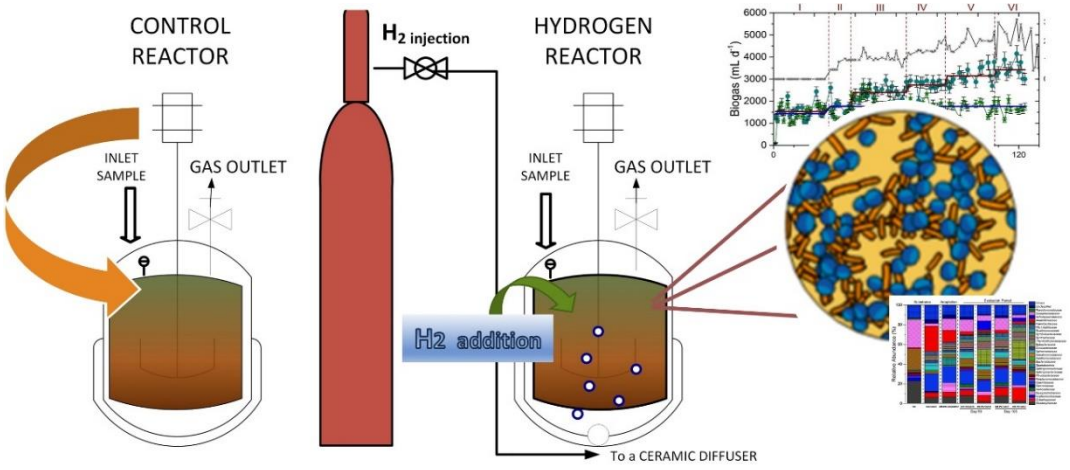
Capítulo/Chapter 4

Improving Anaerobic Digestion of Sewage Sludge by Hydrogen Addition: Analysis of Microbial Populations and Process Performance



Martínez, E.J., Sotres, A., **Arenas, C.B**., Blanco, D., Martínez, O., & Gómez, X. Improving Anaerobic Digestion of Sewage Sludge by Hydrogen Addition: Analysis of Microbial Populations and Process Performance. *Energies* **2019**, *12*, 1228

Abstract

The effect of hydrogen pulse addition on digestion performance of sewage sludge was evaluated as a means for studying the increase in efficiency of methane production. Microbial communities were also evaluated to get an insight of the changes caused by the operational modifications of the digester. An energy evaluation of this alternative was performed considering the theoretical process of coupling bioelectrochemical systems (BES) for the treatment of wastewater along with hydrogen production and the subsequent anaerobic digestion. The addition of hydrogen to sewage sludge digestion resulted in an increase of 12% in biogas production over the control (1353 mL CH_4 d^{-1} at an injection flow rate of 1938 mL H_2 d^{-1}). The liquid phase of the sludge reactor and the H_2 supplemented one did not show significant differences, thus indicating that the application of hydrogen as the co-substrate was not detrimental. High-throughput sequencing analysis showed slight changes in archaeal relative abundance after hydrogen addition, whereas eubacterial community structure and composition revealed noteworthy shifts. The mass and energy balance indicated that the amount of hydrogen obtained from a hypothetical BES can be assimilated in the sludge digester, improving biogas production, but this configuration was not capable of covering all energy needs under the proposed scenario.

Keywords: sludge digestion; hydrogen addition; microbial community analysis; energy performance

4.1 INTRODUCTION

The most widely used technology for the treatment of municipal wastewaters is based on an activated-sludge process in spite of its high energy cost (up to ~75% of the total energy costs of a plant) (Gude 2016). The number of wastewater plants has increased due to rapid urbanisation and it has brought along a significant increase of the volume of sludge needing adequate management (Wacławek et al. 2018).

This sewage sludge is characterised by high organic content needing to be properly disposed of. However stringent regulations and pollution problems associated with uncontrolled decomposition of organic matter and nutrient run off make it imperative to search for feasible treatment alternatives. Traditionally, sludge is treated by extended aeration when the size of a wastewater treatment plant (WWTP) is too small, thus making it unfeasible to install an anaerobic digester. For large plants, the stabilisation via anaerobic digestion is the preferred choice. The production of biogas and subsequent conversion into electrical energy by means of a combined heat and power (CHP) unit makes this process a desirable option for reducing the external energy demand of a WWTP. Nevertheless, some disadvantages are associated with the digestion of waste activated sludge (WAS) such as low methane production due to its low biodegradability. Therefore, different pre-treatment technologies have been developed with the aim of increasing the biogas yield, accelerating the degradation rate, and producing a biosolid that is safer and easier to handle and dispose of (Hosseini Koupaie et al. 2018; Martínez et al. 2015; Xia et al. 2018). However, the application of pre-treatments may bring along an increase in the energy demand of the global process (González et al. 2018).

The enhancement of anaerobic digestion (AD) can also be achieved by the addition of a co-substrate. Several co-substrates have been evaluated in an attempt to increase the organic loading of a reactor and to balance nutrients (Keucken et al. 2018; Martínez et al. 2016; Al bkoor Alrawashdeh et al. 2017). AD is a complex process where the conversion of organic matter takes place by means of microorganisms in the absence of oxygen. The breakdown of organic matter can be summarised in four major stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis, which are carried out by a

consortium of mutually dependent microorganisms including hydrolytic-fermentative bacteria, proton-reducing acetogenic bacteria, hydrogenotrophic methanogens and acetoclastic methanogens (Zinder 1984). The development of AD is strongly related to the structure of the microbial communities present in the reactor in addition to the operating conditions applied (Demirel & Scherer 2008). Therefore, introduction of hydrogen gas as a co-substrate into an anaerobic digester may cause changes in microbial communities, and these changes may in turn result in modifications of the main degradation pathways.

Methanogens are strictly anaerobic archaea grouped into acetoclastic and hydrogenotrophic methanogens. The common substrates of hydrogenotrophic methanogens are H_2, CO_2 and formate, whereas acetate is the main substrate for acetoclastic methanogens producing methane via acidogenesis and acetogenesis although a few other compounds like methanol, ethanol and pyruvate can also be utilized (Thauer et al. 2008; Holmes & Smith 2016). Due to the restricted metabolism of methanogens, the organic compounds present in sewage sludge (SS) are degraded in an anaerobic environment by association with fermenting and acetogenic bacteria. This syntrophic relationship is sustained by interspecies hydrogen transfer. Hydrogen, which is consumed by hydrogenotrophic methanogens, is a key intermediary in the anaerobic degradation of organic matter. Hydrogen levels and interspecies hydrogen transfer optimize the metabolism of the entire microbial community present in biogas-producing consortia (Cayol et al. 2002; Bagi et al. 2007).

There exist several studies on the application of hydrogen with the aim of improving biogas production. One approach is to promote the production of hydrogen and its transfer between syntrophic methanogenic partners via bioaugmentation by the introduction of a H_2-producing bacterium into the anaerobic consortia (Bagi et al. 2007; Nzila 2017), and the other approach involves injection of hydrogen gas into the reactor. Although utilisation of hydrogen by microorganisms is hindered by mass transfer limitations, several studies have shown that its application in pulses significantly increases methane production (~30%) by the action of hydrogenotrophic methanogens (Luo et al. 2012; Luo & Angelidaki 2012, 2013a, 2013b).

The replacement of the activated-sludge process by a bioelectrochemical system (BES) has been proposed by several authors as a means for reducing the high energy demand of wastewater treatment systems (Escapa et al. 2016; McCarty et al. 2011). The use of microbial fuel cells allows for the net production of energy that comes from the conversion of the organic material contained in wastewater. However, there is a limited understanding about the capacity of these systems for converting organic matter contained in wastewater and turn it into energy to attain a net positive energy balance (Stoll et al. 2018). On the other hand, in microbial electrolysis cells (MECs), organic matter of the liquid stream is transformed by microorganisms with application of small voltage resulting in production of hydrogen at the cathode (Pant et al. 2012). Due to the urgent need for reducing the large amount of energy necessary for the treatment of municipal wastewater, the key for attaining energy self-sufficiency seems to be closely related to reducing aeration requirements and increasing the biogas yield.

In the present study, a sludge digester supplemented with hydrogen was assessed to evaluate performance of the reactor and the effect on the energy balance of a WWTP when a BES serves as an alternative for the treatment of wastewaters. The aim of the present work was to study the microbiological changes taking place during digestion of sewage sludge along with the effect on biogas productivity, digestion performance and energy demand of the whole combined configuration.

4.2 MATERIALS AND METHODS

4.2.1 Substrates

Primary sludge (PS), waste activated sludge (WAS) and digested sludge from the anaerobic reactor were obtained from the WWTP of León (Spain). The plant has an anaerobic digester operating under a mesophilic regimen. The digested sludge was used as inoculum. Sewage sludge used as substrate was a mixture of PS and WAS at a 30:70 (v/v) ratio. The selection of this ratio was based on the volumetric proportions of sludge flow produced in the WWTP. The chemical characteristics of these materials are presented in Table 3.1 (see *Chapter 3: Materials and Methods*).

4.2.2 Experimental set-up: semi-continuous digestion

Semi-continuous reactors were evaluated using sewage sludge as substrate. An initial adaptation period of 28 d was established with a hydraulic retention time (HRT) of 25 d. The operating performance of the reactor was subsequently evaluated at an HRT of 21 d for a 106 d period. The experiments were carried out in completely mixed reactors (working volume of 3 L under mesophilic conditions: 37 ± 1 ˚C). Reactors were manually fed once a day and were equipped with mechanical agitators and outer-jackets to circulate heating water and sampling ports for the withdrawal of liquid samples and gas collection. Feeding was manually performed once a day and the samples were taken three times a week before feeding the reactors and after complete homogenisation. Daily gas production was measured using a reversible liquid displacement device with a wet-tip counter.

The reactors were labelled as control reactor (CR) treated sludge as a sole substrate and the reactor supplemented with hydrogen pulses was designated as HR and also utilised sludge as a carbon source. The addition of hydrogen was carried out daily through a ceramic diffuser at the bottom of the reactor for 6 h. The flow rate was adjusted in the range between 0.5 and 2 L H_2 L $_{Reactor}^{-1}$ d $^{-1}$ using a flow meter model FR2A12BVBN from KI instruments.

The utilisation of hydrogen by microorganisms was calculated based on the difference between the volume of biogas measured from the HR and the measurements of methane and hydrogen gas from the CR and HR. It was assumed that all hydrogen measured in the exit gas stream corresponds to the excess amount injected that was not transferred into the liquid phase. The amount of hydrogen derived from the metabolism of sludge was disregarded because of the large injected amount as hydrogen flow.

Microbial analysis was performed on samples taken from these reactors on day 28, representing the end of the adaptation period, and on days 59 and 105, with these two latter samples being representative of the performance of the digester when hydrogen supplementation was fully implemented.

4.2.3 Analytical techniques

Total and volatile solids (TS, VS), pH, ammonia and alkalinity were measured in accordance with APHA Standard Methods (Rice et al. 2012). Nitrogen concentration was measured by the Kjeldahl method. Organic matter was analysed in accordance with the Walkley–Black method (Walkley & Black 1934). An Analytik Jena Multi N/C_3100 systems by thermocatalytic oxidation was used for the quantification of total organic carbon (TOC) and total nitrogen (TN). The analysis of metals was carried out on a PerkinElmer Optima 2000 DV inductively coupled plasma atomic emission spectrometer as described by Fierro et al.(2016).

Biogas composition was daily monitored by means of a gas chromatograph (Varian CP3800 GC) equipped with a thermal conductivity detector. A packed column (HayeSep Q 80/100; 4 m) along with a molecular-sieve column (1 m) was used to separate CH_4, CO_2, N_2, H_2 and O_2. The carrier gas was helium, and the columns were operated under a pressure of 331 kPa and a temperature of 50 ˚C. Volatile fatty acids (VFAs) were measured using a gas chromatograph and a flame ionisation detector (FID) equipped with a Nukol capillary column (30 m × 0.25 mm × 0.25 μm) from Supelco. The carrier gas was helium. The injector and detector temperatures were 220 and 250 ˚C, respectively. The oven temperature was set to 150 ˚C for 3 min and was increased to 180 ˚C with a ramp of 10 ˚C min^{-1}. The detection limit for VFA analysis was 5.0 mg L^{-1}. The system was calibrated with a mixture of standard volatile acids from Supelco (for the analysis of fatty acids C2 to C7). Samples were pre-centrifuged (10 min, 3500 × g) and the supernatant was passed through 0.45 μm cellulose filters.

4.2.4 High-throughput sequencing of massive 16S rRNA gene libraries

DNA was extracted with the PowerSoil® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) from substrates (SS), inoculum, and digested sludge obtained from reactors as described in the experimental set-up sub-section. All PCRs were carried out on a Mastercycler (Eppendorf, Hamburg, Germany), and PCR samples were checked for size of the product on a 1% agarose gel. The entire DNA extract was subjected to high-throughput sequencing of the 16S rRNA gene based massive libraries for total eubacterial and archaeal populations. Each sample was amplified with the primer set

27F mod (5'-AGRGTTTGATCMTGGCTCAG-3') with 519R modBio (5'-GTNTTACNGCGGCKGCTG-3') (Callaway et al. 2009) and Arch 349F (5'-GYGCASCAGKCGMGAAW-3') with Arch 806R (5'-GGACTACVSGGGTATCTAAT-3') (Takai & Horikoshi 2000) for the eubacterial and archaeal population, respectively. The obtained DNA reads were compiled into FASTq files for further bioinformatics processing following the MR DNA Research Lab instructions (http://www.mrdnalab.com/). Operational taxonomic units (OTUs) were then taxonomically classified using the Ribosomal Database Project (https://rdp.cme.msu.edu/).

4.2.5 Energy balance

Based on results obtained from the digester working under different regimens of hydrogen injection, a mass and energy balance was determined to evaluate the benefits of integrating a MEC system instead of a conventional WAS process. The hydrogen produced by the BES was assumed to be injected into the anaerobic digester. Two scenarios were evaluated. The first one takes into consideration a conventional WWTP for 150,000 equivalent inhabitants (Eq. Inh.), while the second scenario assumes installation of a BES for the treatment of wastewater. Under this latter scenario, the hydrogen produced from the water line was transformed into methane in the digester. Calculation of energy production was based on hydrogen conversion values obtained from the experimental set-up.

The base scenario regarded the water line as composed of a conventional WAS system and an air flotation unit for concentrating the secondary sludge. The sludge line consisted of the primary settler and the gravity thickener where the primary sludge was concentrated and posteriorly mixed with the secondary sludge. The anaerobic digester treated this mixture at an HRT of 20 d.

4.3 RESULTS AND DISCUSSION

4.3.1 Analysis of methane production

The parameters obtained from the anaerobic digestion of SS are summarised in Table 4.1. A remarkable increase in biogas production and a slight increase in methane concentration were achieved in the HR compared with the CR. Figure 4.1 shows the

biogas production and composition of both systems under the semi-continuous regimen. An increase in the average biogas production was observed with the increase in the injection of hydrogen, indicating the capacity of the consortium for hydrogen utilisation. The maximum gas production of 1353 mL CH_4 d^{-1} was reached at an injection rate of 1938 mL H_2 d^{-1} with injection velocity 5.38 mL min^{-1} (period V). This value represents an improvement of 12% over the control. The utilisation of hydrogen by microorganisms at the different injection flow rates tested was not greater than 75%, and the excess injected hydrogen became a part of the exiting biogas from the reactor (see biogas composition in Figure 4.1 d, e).

Despite the significant improvement achieved in biogas production, this was not directly related to a significant increase in methane concentration (methane content in biogas was ~60–68%) for the HR system. Studies by Luo and Angelidaki (2012) of gas upgrading (in a thermophilic anaerobic reactor treating sludge) revealed greater content of CH_4 (~90%) in biogas when digested manure served as an inoculum among others and gas injection rates were between 3 and 24 L $L_{reactor}^{-1}$ d^{-1}.

Table 4.1 Parameters of the digestion process

Periods	Control Reactor (CR)		Hydrogen Reactor (HR)					
	Adaptation	Evaluation	Adaptation	Evaluation				
	I	(II-VI)	I	II	III	IV	V	VI
Days evaluated	28	105	28	11	27	20	24	23
HRT (d)	25	20	25	20	20	20	20	20
OLR ([kg VS] L^{-1} d^{-1})	0.99	1.08	0.99	1.14	1.04	1.1	0.97	1.17
H_2 injection flow (mL d^{-1})	n.a.	n.a.	0	780 ± 269	1125 ± 207	1588 ± 260	1938 ± 330	2004 ± 350
H_2 Transported (mL d^{-1})	n.a.	n.a.	0	617 ± 291	683±286	709 ± 166	768 ± 196	750 ± 206
Volatile solids (g L^{-1})	13.2±0.1	10.52	13.6 ± 0.1	12.5 ± 0.1	8.2 ± 0.1	8.0 ± 0.1	8.1 ± 0.1	8.3 ± 0.1
Volatile solids removal (%)	46.8	49.46	45.1	49.5	59.7	55.4	57.1	60.6
Biogas (mL d^{-1})	1434±436	1762 ± 235	1524 ± 338	1779 ± 368	2398 ± 273	2736 ± 249	3146 ± 364	3417 ± 390
CH_4 (mL d^{-1})	857±25	1113.23 ± 55	927 ± 15	1030 ± 20	1318 ± 26	1266 ± 25	1353 ± 27	1222 ± 24
Biogas without H_2 (mL d^{-1})	--	--	1524 ± 338	1523 ± 237	1955 ± 258	1904 ± 270	1976 ± 271	1842 ± 450
%CH_4	59.79 ± 1.43	63.18	60.86 ± 1.71	59.39 ± 3.19	55.81 ± 6.91	44.41 ± 8.07	41.61 ± 6.50	41.78 ± 6.5
%CH_4 normalised*	--	--	60.86 ± 1.71	67.67 ± 1.63	67.42 ± 5.52	66.51 ± 3.60	68.51 ± 2.70	66.39 ± 4.5

Note. *Normalisation without hydrogen (methane + carbon dioxide), HRT: hydraulic retention time, OLR: organic loading rate, SMP: specific methane production.

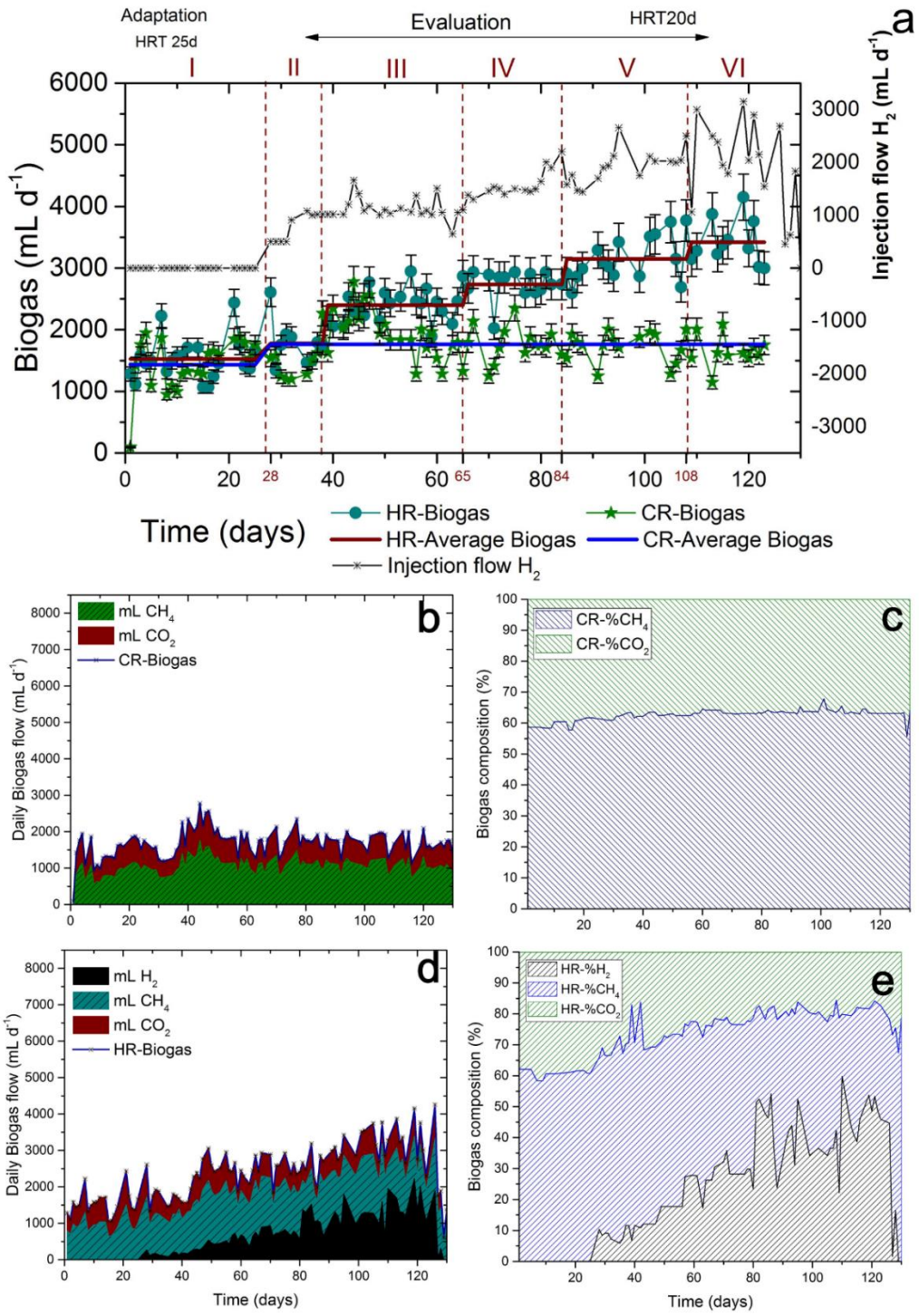


Figure 4.1 Biogas production and composition in the hydrogen reactor (HR) and control reactor (CR) systems when digesting sewage sludge (SS) under semi-continuous conditions. (a) Biogas production and hydrogen flow injection in the HR system, (b) daily volumetric flow of CH_4 and CO_2 in the CR, (c) biogas composition in the CR, (d) daily volumetric flow of H_2, CH_4 and CO_2 in the HR, (e) biogas composition in the HR

In the present study, hydrogen flow was added during a single cycle of 6 h d^{-1}. The injection velocity for the 6 h cycle was gradually increased during the evaluation period but did not affect the utilisation of this gas by microorganisms which does not depend on injection velocity. Figure 4.2 shows that hydrogen output and transfer were proportional to the volume of hydrogen injected. The transfer of hydrogen depends only on the properties of the liquid, diffusivity of the gas and biological characteristics of the process (Bensmann et al. 2014).

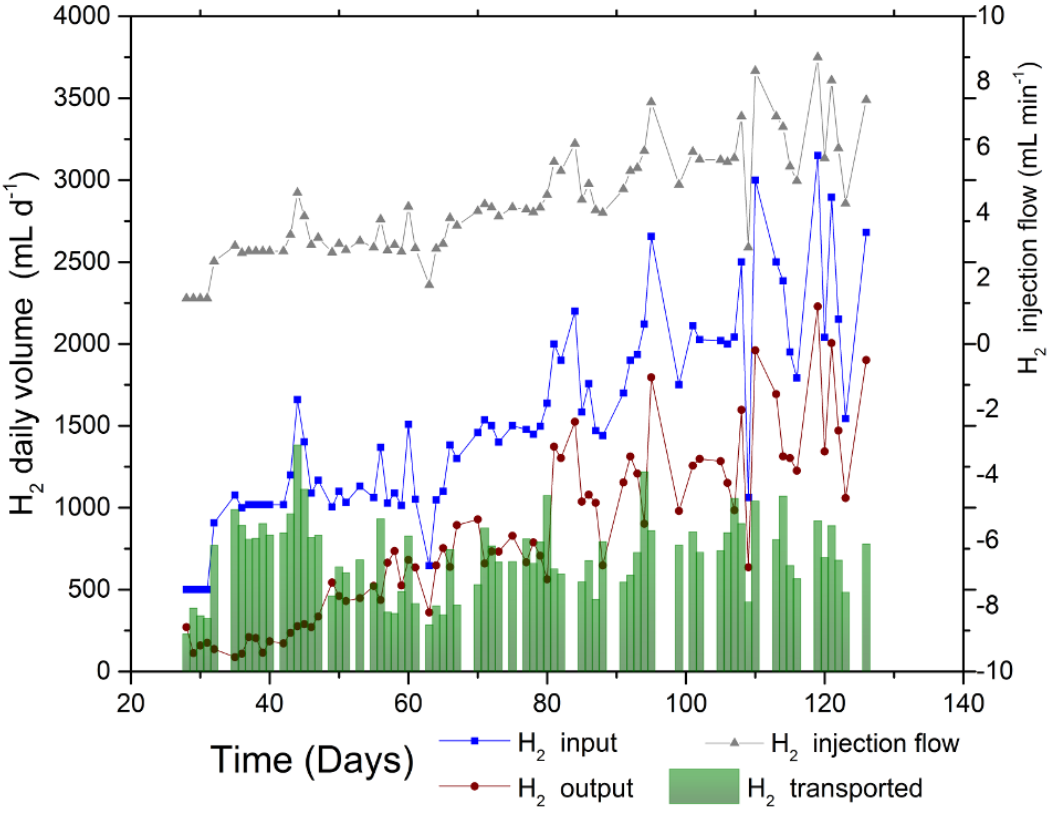


Figure 4.2 The relation between velocity of hydrogen injection and hydrogen uptake in the HR system

The results on process performance are shown in Figure 4.3. The HR showed an increase in total VFA amounts on day 70 onwards (Figure 4.3a), coinciding with the increase in the average hydrogen injection flow rate from 1125 to 1588 mL d^{-1} and reaching a maximum of ~90 mg L^{-1} on day 80. Figure 4.3b indicates that this difference is mainly due to the increase in acetic acid concentration in this reactor. This effect may be

associated with changes in microbial populations owing to the greater availability of hydrogen gas. Higher production of acetic acid derives from the conversion of hydrogen in this reactor. This fact would cause an acetic acid build-up unless this event was accompanied by an increase in microbial populations capable of the conversion of this compound into methane. The later decrease in the acetic acid amount may then be associated with the proliferation of acetoclastic methanogens, which prevented a progressive build-up.

The results of monitoring the digestion process did not reveal significant differences in the parameters measured (Figure 4.3d–f). These data revealed similar values (pH, alkalinity and ammonium content) between the two reactors (CR and HR), indicating that the addition of hydrogen did not interfere with assimilation of the carbon source. The removal of VS was on average 54.6% ± 6.1% for the HR: only slightly higher than that of CR (see Table 4.1 and Figure 4.3f, where lower values of VS and TS were detected in the HR system). Therefore, the enhancement of biogas production by this method does not represent any detriment to the digestion performance, nor does it interfere with the stabilisation of the sludge; these properties are a disadvantage observed when readily degradable co-substrates are added for boosting biogas production (Fierro et al. 2016).

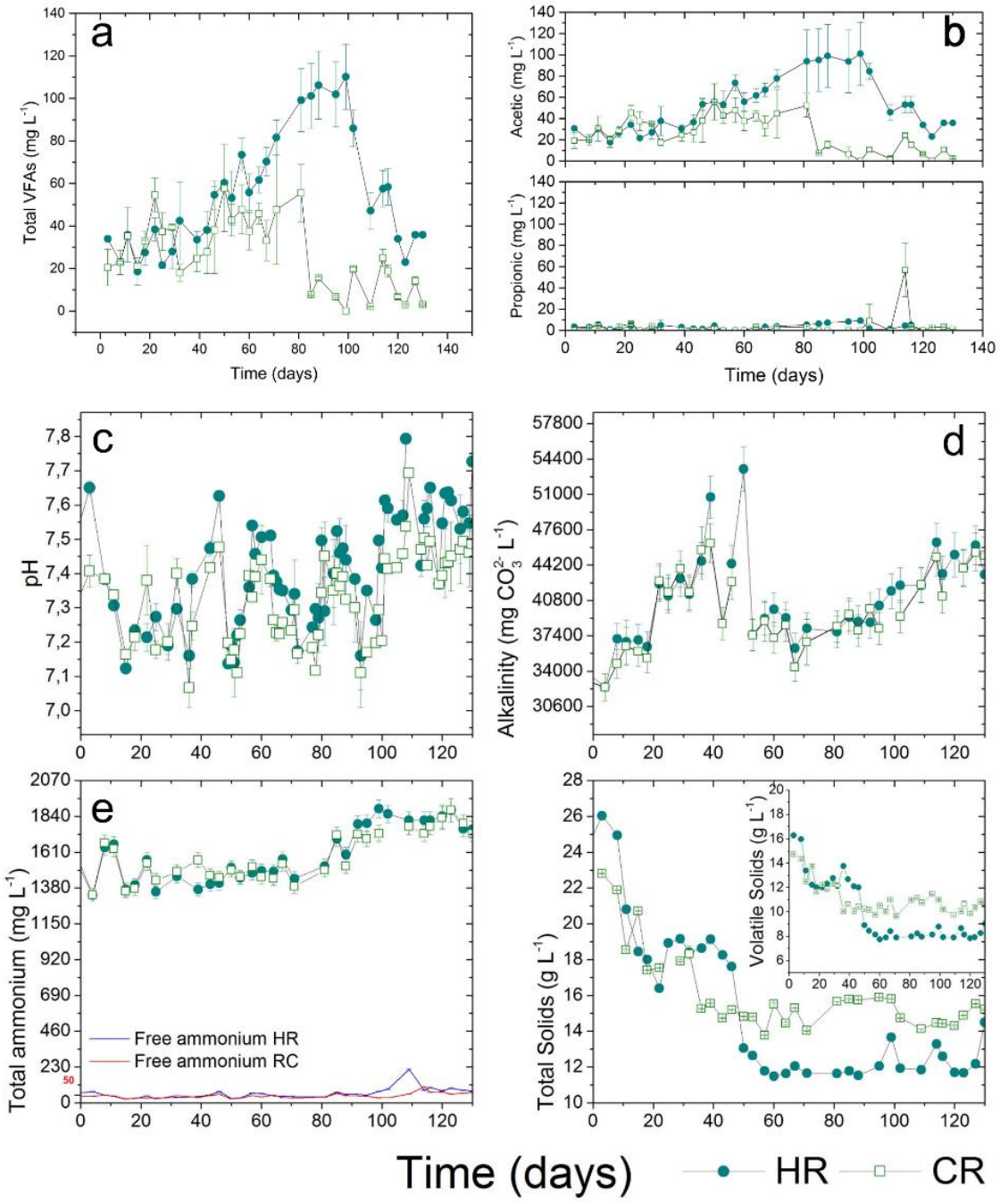


Figure 4.3 Chemical parameters evaluating the performance of the digestion of SS for the CR and HR. (a) total volatile fatty acids (VFAs), (b) acetic and propionic acids, (c) pH, (d) alkalinity, (e) ammonium and free ammonia, and (f) total solids (TS) and volatile solids (VS)

4.3.2 Effect of hydrogen addition on microbial communities

Archaeal and Eubacterial community analysis of the samples at the family and genus level are presented in Figure 4.4 a, b and Figure 4.5 a, b respectively.

The family level of Archaeal community analysis (Figure 4.4a) indicated that the three predominant groups were *Methanoregulaceae* and *Methanobacteriaceae* (both hydrogenotrophic archaea) and *Methanosaetaceae*, which follows the acetoclastic pathway. These families accounted for 94% and 88% of the entire community for SS and inoculum samples, respectively. In SS samples, it is more common to find *Methanosaetaceae* (45%) relative to *Methanosarcinaceae* (2.5%). *Methanosarcinaceae* is able to maintain both the acetoclastic and hydrogenotrophic pathway and is also easier to find in other substrates like pig slurry.

With respect to the samples obtained from the reactors, no changes were observed in the dominant groups during the adaptation period; however, shifts in the relative abundance of these families emerged due to changes in the dynamics of the reactor (lower HRT) and greater availability of hydrogen for the HR. Figure 4.4 shows that contrary to what would have been expected (Luo & Angelidaki 2013a), acetoclastic methanogens also comprise the majority of methanogens in HR, with *Methanosaetaceae* being the dominant family. Nonetheless, some hydrogenotrophic methanogens, such as *Methanospirillum* (Demirel 2014) (belonging to *Methanospirillaceae* family), increased in abundance during hydrogen addition periods (samples from the HR).

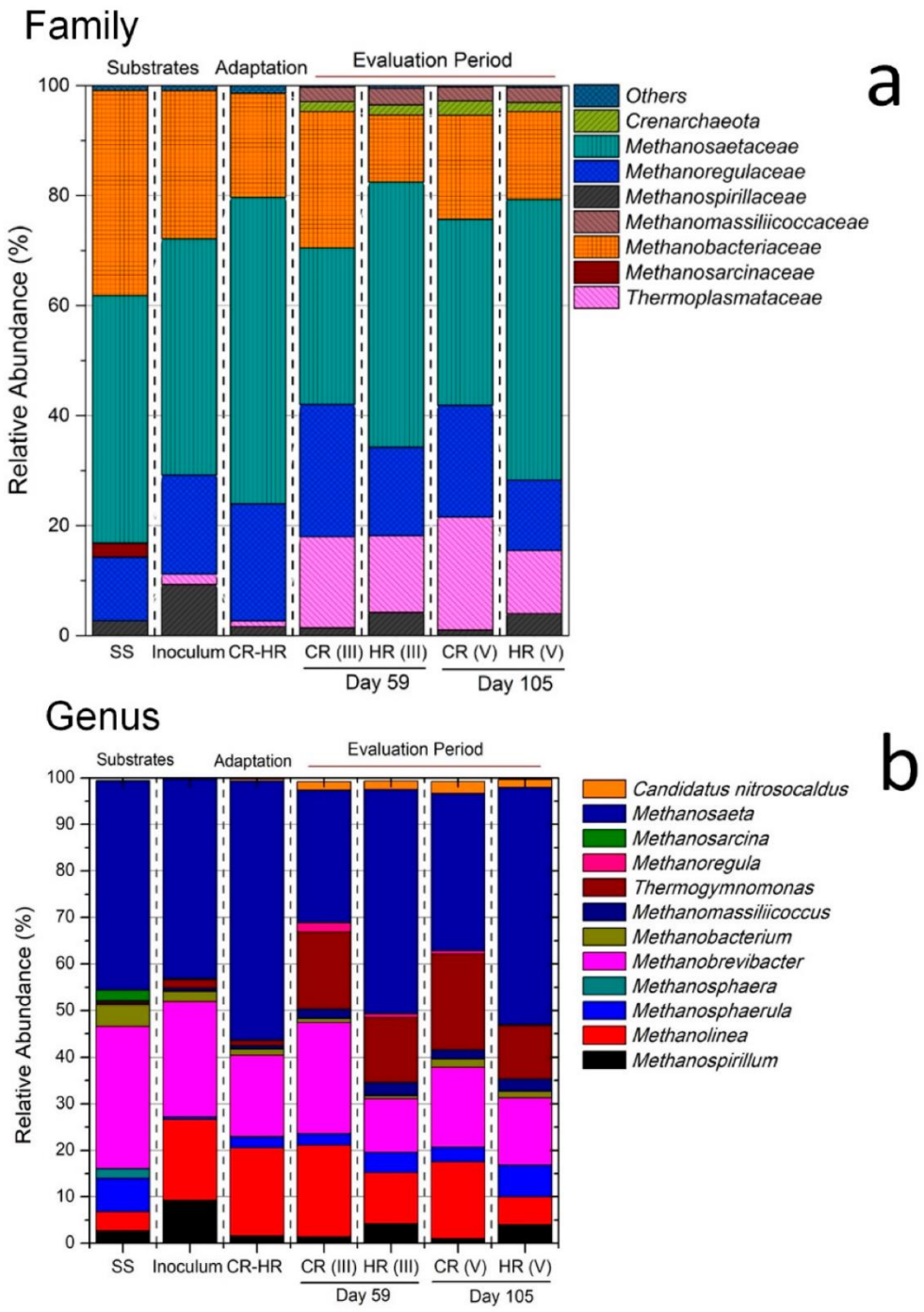


Figure 4.4 Taxonomic classification of high-throughput sequencing data on the 16S rRNA gene from archaeal communities at a family (a) and genus (b) level. For groups family making up less than 1% of the total number of sequence reads were classified as 'others'. The genera belonging to the families were identified above 1%

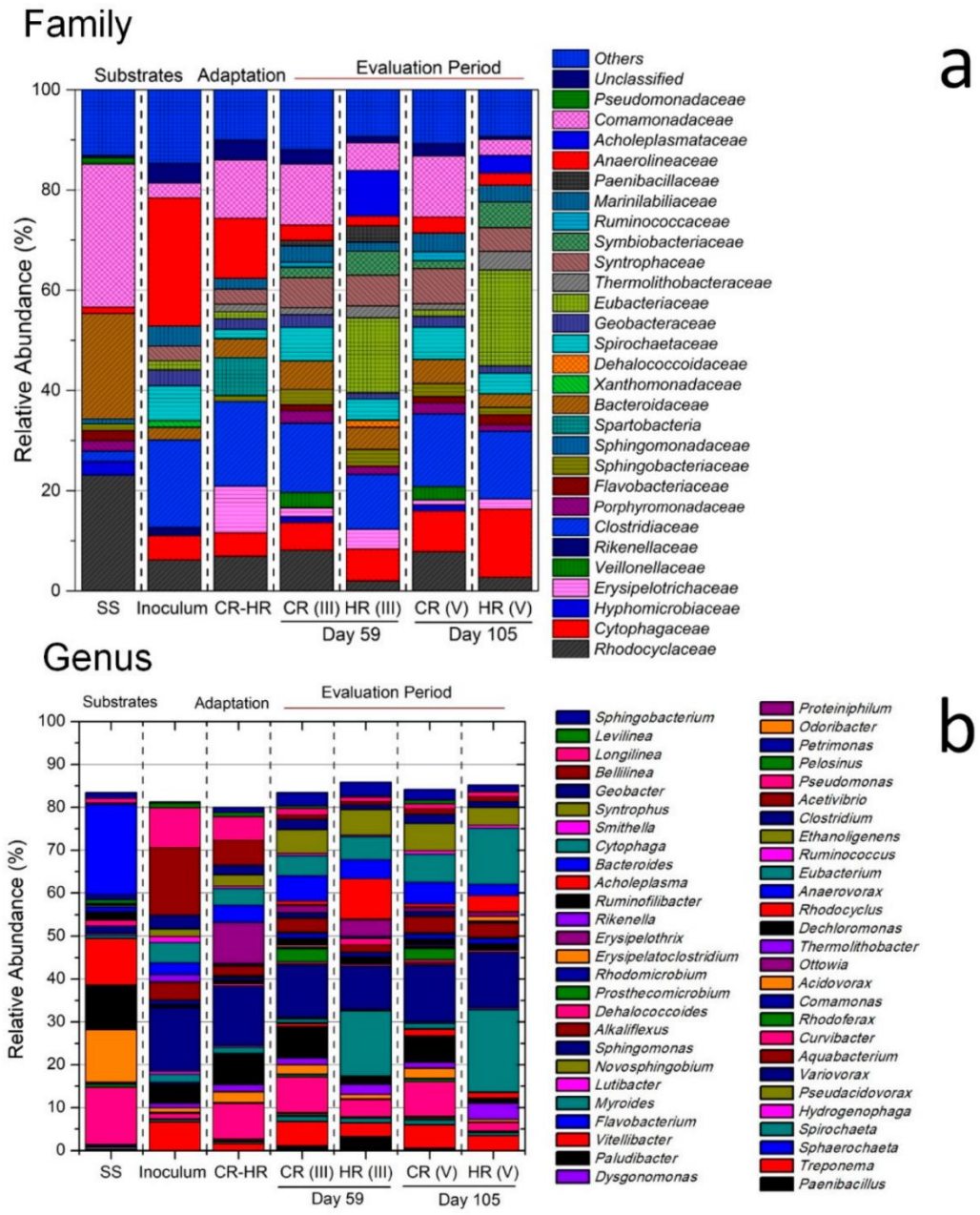


Figure 4.5 Taxonomic classification of high-throughput sequencing data on 16S rRNA gene from eubacterial communities at family (a) and genus (b) level. For groups family making up less than 1% of the total number of sequence reads were classified as 'others'. The genera belonging to the families were identified above 1%

Regarding eubacterial populations, the diversity was much greater for the eubacterial than for archaeal community. Besides, dramatic shifts were observed in the different evaluated samples from HR and CR, compared to the samples of substrates and

adaptation period (Figure 4.5). Clear enrichment in homoacetogenic families such as *Clostridiaceae* and *Eubacteriaceae* was detected. Figure 4.5 suggests that *Eubacteriaceae* was identified in the inoculum at very low relative abundance (1.8%), but it reached ~15% and 19% in the HR for the samples taken on days 59 and 105, respectively. *Clostridiaceae* was also abundant in the inoculum and remained one of the most abundant families throughout the experiment. The sum of the total homoacetogenic bacteria increased from 18% in the adaptation period to 26% and 33% in the HR on days 59 and 106, respectively. These increments stayed related to the different hydrogen flow rates of 1095 and 1870 mL d^{-1} applied in each case. These eubacterial populations can grow in a CO_2/H_2 atmosphere, and therefore had optimal conditions for their growth.

The enrichment of these homoacetogenic groups along with other acetogenic microorganisms increased the acetate production in HR. This is because these bacteria utilised the hydrogen (transferred from the gas phase) and CO_2 to produce acetate, which is subsequently consumed by acetoclastic methanogens (Figure 4.4). Accordingly, the proliferation of homoacetogenic bacteria in this reactor (HR) could be favouring the proliferation of the acetoclastic Methanosaeta, which follow the acetoclastic route (Karakashev et al. 2006). Microbial analysis supported the hypothesis advanced in the previous section regarding the course of acetic acid content in the HR reactor and explains the higher levels of acetic acid obtained for this system and its posterior decrease as illustrated in Figure 4.3b.

Hence, our results allowed us to hypothesise that due to the conditions in the reactors, the pulses of hydrogen chiefly stimulated the enrichment of certain homoacetogenic microbes above the hydrogenotrophic route. Bioaugmentation by H_2-producing bacteria has been previously studied for improvement of biogas production and promotion of the hydrogenotrophic pathway (Ács et al. 2015). Nevertheless, and contrary to promoting the utilisation of hydrogen and its conversion into methane as expected, the H_2-producing bacteria present in the CR such as *Rhodocyclaceae*, *Syntrophaceae*, and *Ruminococcaceae* decreased in abundance in the HR when hydrogen pulses were added to the reactor. The increase in acetate content in this reactor (as seen in Figure 4.3b) and results from microbial analysis indicated that this behaviour may probably be

associated with the conversion of hydrogen by homoacetogenic bacteria (producing acetate) thus favouring the acetoclastic pathway.

4.3.3 Energy balance

The main characteristics for the base scenario are presented in Table 4.2. The volumetric proportion of PS and WAS 30:70 based on the data reported by the WWTP of the city of León. The energy needs of the WAS system account for ~60% of the total energy demand of the plant (Gu et al. 2017). The chemical oxygen demand (COD) of the wastewater treated in the WAS system was assumed to be 310 mg L^{-1}.

An activated-sludge process requires aeration, which accounts for up to 75% of the WWTP energy costs, while the treatment and disposal of sludge may be responsible for up to 60% of the total operational costs (Gude 2016). The substitution of the conventional WAS system with a BES leads to a significant decrease in the energy demand. Gil-Carrera et al. (2013) reported an energy demand of 0.11 kW h kg^{-1} COD removed when treating domestic wastewater using a semi-pilot MEC. Heidrich et al. (2013) have reported a value of 0.64 kW h kg^{-1} COD removed when using a semi-pilot scale microbial cell. These values lead to hypothetical average consumption of ~22% of the average energy demand reported for conventional WWTPs.

The energy demand of the conventional WWTP was estimated at 938 kW, assuming that 60% of this value corresponds to the energy needs of the WAS (Gu et al. 2017), whereas this value decreased to 585 kW for the alternative proposed. The substitution of the conventional WAS system with a MEC also brings as an advantage the production of hydrogen from conversion of organic matter contained in wastewater. With the hydrogen yield reported by Selembo et al. (2009) of 1.87 m^3 H_2 $m^3_{reactor}{}^{-1}$ d^{-1}, this assumption would translate into an estimate of 185.5 m^3 H_2 h^{-1} from the treatment of wastewater by the BES. This gas is assumed to be subsequently injected into the anaerobic digester. The hydrogen transfer rate calculated from experimental results was 40.4 mL H_2 L^{-1} h^{-1}. Therefore, this figure may lead to the capacity for hydrogen utilisation in a large-scale digester of 178 m^3 H_2 h^{-1}, assuming a 10% enhancement in the gas transfer rate owing to pressure effects in the large-scale system. This value is slightly lower than that of the estimated production of hydrogen by the hypothetical MEC;

therefore, a small fraction of hydrogen will not be transformed into methane in this configuration. In addition, a conservative assumption was made, estimating that only 80% of the hydrogen flow transferred was assimilated by microorganisms (Luo et al. 2012).

Table 4.2 Main characteristics of the conventional WWTP considered (base scenario) and the alternative system (BES unit and H_2 conversion)

Parameter	Value	Unit
WWTP capacity (Houillon & Jolliet 2005)	150 000	Eq. Inh.
Wastewater quantity	350	L (Eq. Inh.)$^{-1}$ d^{-1}
Characteristics of conventional WWTP (base scenario)		
Energy demand WAS (Mizuta & Shimada 2010)	1.1	kW h m^{-3}
WWTP energy consumption (Hernández-Sancho et al.2011)	1.68	kW h (kg COD removed)$^{-1}$
Sludge flow	8.0	m^3 h^{-1}
TS	53.0	g L^{-1}
%VS	73	%
Digester volume	4000	m^{-3}
Electric efficiency CHP unit (Pöschl et al. 2010)	33	%
Energy production from biogas valorisation by CHP	342	kW
BES and H_2 conversion during anaerobic digestion		
Energy consumption	0.38	kW h (kg COD removed)$^{-1}$
Secondary sludge production	2.7	m^3 h^{-1}
Sludge flow	3.8	m^3 h^{-1}
Biogas production from H_2 conversion (from experimental results)	0.33	(m^3 biogas) (m^3 H_2)$^{-1}$
Energy production from biogas valorisation by CHP	318	kW
Addition of co-substrate: Total energy production from biogas valorisation by CHP	554	kW

The sludge production of the BES configuration was considered to be 25% of that of the conventional WAS system according to the data reported by Brown et al. (2015). Therefore, the anaerobic digester does not work at full load when the BES is introduced into an existing plant. The energy production thus calculated for the digester was not enough for covering the whole energy demand of the WWTP with the BES configuration; this drawback is due to the lower sludge flow generated by the BES system. Operating the anaerobic digester at full capacity by the addition of a co-substrate with characteristics similar to those of sludge may result in an extra amount of energy, 237 kW, which is still not enough for making this scenario energy self-sufficient. A co-substrate with specific methane production of ~400 mL CH_4 g VS^{-1} would be necessary (considering similar VS content) for covering all the energy needs of the WWTP when BES is chosen as an alternative. Suitable co-substrates may be those with high organic content of readily degradable materials as glycerol, cheese whey, food wastes (Menon et al. 2016; Escalante et al. 2018; González et al. 2019) or those with high lipid and protein contents (Angelidaki & Ellegaard 2003).

This study only takes into account energy facts, to make an initial approximation to the feasibility of integrating a BES as alternative to the conventional activated sludge systems. However, it must be borne in mind that at the current state of the art the capital investment required is too high to materialise this concept. Escapa et al. (2012) speculated that if large-scale BES implementation is to become a reality, the feasibility of using this technology is closely dependent on the improvement attained in cathode performance coupled to the use of low-cost cathode materials, approximating the threshold price for investment cost to 1200 € m^{-3} of anodic chamber.

4.4 CONCLUSIONS

The benefit of hydrogen addition to the digestion of sludge was associated with the increase in biogas production owing to the conversion of hydrogen into acetate and subsequent transformation into methane. This process did not lead to a high enrichment in the composition of biogas produced, but on the contrary, significantly increased the productivity of the digester in terms of the volumetric gas production rate. This finding is explained by the fact that the hydrogenotrophic pathway was not favoured in spite of

the injection of hydrogen. The uptake of hydrogen was carried out to a great extent by the eubacterial community, thereby promoting the enrichment of acetoclastic methanogens. The addition of hydrogen did not worsen the stabilisation of sludge, with the reactor yielding values of VS removal similar to those of the CR, which was intended to evaluate the digestion of sludge as a sole substrate.

The presence of hydrogen gas promoted a shift in eubacterial populations, specifically towards homoacetogenic bacteria, which increased the production of acetate. This change in turn favoured the enrichment of the acetoclastic methanogen family Methanosaetaceae, which was already identified in high abundance in the inoculum and fresh sludge, therefore avoiding an acetic acid build-up. The substitution of a conventional WAS system by a BES leads to a significant reduction in energy demands, but this approach itself does not guarantee the energy self-sufficient status. The lower availability of organic matter to the anaerobic digester results in lower energy production becoming another challenge for this type of technology in addition to the well-known price constraint. Unless a reduction in energy consumption is attempted via an increase in the efficiency of various pieces of equipment at the WWTP or via an increase in biogas production by means of an extra co-substrate, energy self-sufficiency may not be possible with the proposed approach.

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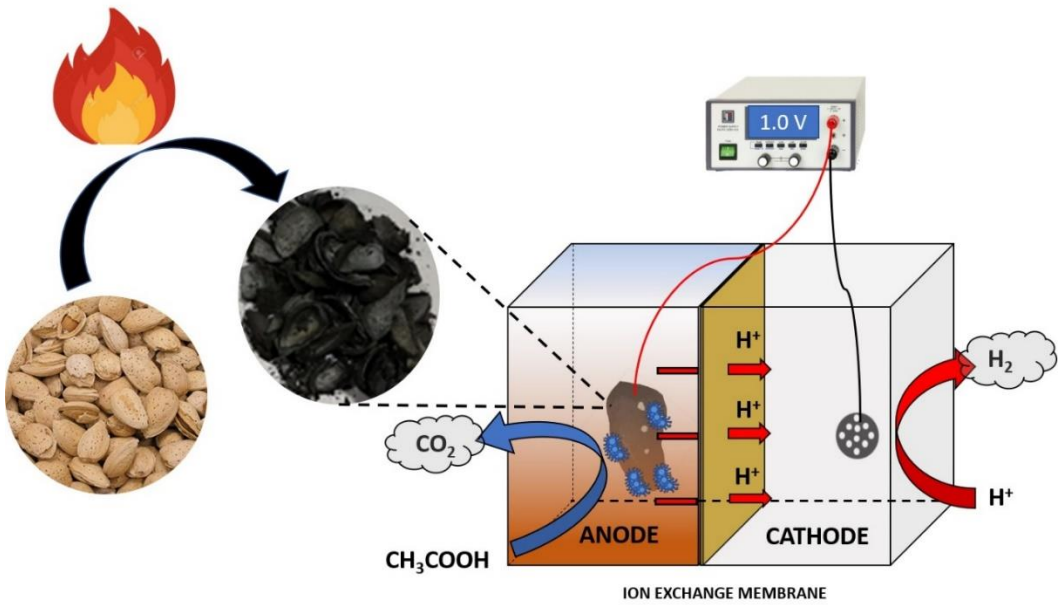
Capítulo/Chapter 5

Pyrolysed Almond Shells Used as Electrodes in Microbial Electrolysis Cell



Arenas, C. B, Sotres, A., Alonso, R.M., González, J., Morán, A., & Gómez, *X.* Pyrolysed almond shells used as electrodes in microbial electrolysis cell. *Biomass Conv. Bioref.* (2020). https://doi.org/10.1007/s13399-020-00664-7

Abstract

The large cost of components used in microbial electrolysis cell (MEC) reactors represents an important limitation that is delaying the commercial implementation of this technology. In this work, we explore the feasibility of using pyrolysed almond shells (PAS) as a material for producing low-cost anodes for use in MEC systems. This was done by comparing the microbial populations that developed on the surface of PAS bioanodes with those present on the carbon felt (CF) bioanodes traditionally used in MECs. Raw almond shells were pyrolysed at three different temperatures, obtaining the best conductive material at the highest temperature (1000 ˚C). The behaviour of this material was then verified using a single-chamber cell. Subsequently, the main test was carried out using two-chamber cells and the microbial populations extant on each of the bioanodes were analysed. High-throughput sequencing of the 16S rRNA gene for eubacterial populations was carried out in order to compare the microbial communities attached to each type of electrode. The microbial populations on each electrode were also quantified by real-time polymerase chain reaction (real-time PCR) to determine the amount of bacteria capable of growing on the electrodes' surface. The results indicated that the newly developed PAS bioanodes possess a biofilm.

Keywords: microbial electrolysis cells, bioanodes, biomass pyrolysis, electrodes

5.1 INTRODUCTION

The ever-increasing demand for energy, as well as associated environmental problems (such as water pollution and depletion of resources), have led to an intensive search for renewable energy sources and processes capable of generating green materials. The advances achieved in these research fields have important applications in the efficient management of wastewater treatment plants, leading to the development of a variety of novel treatment strategies. Bioelectrochemical systems (BES), more specifically microbial electrolysis cells (MECs), represent promising technologies with applications in wastewater treatment and in the recovery of resources (Escapa et al. 2016). MEC operation is based on the oxidation of organic compounds, a process catalyzed by microorganisms capable of transferring electrons to a solid element, the anode. The electric current thus generated has an important role in reduction reactions taking place in the cathode, which is the counter electrode. The cathodic reaction allows the production of valuable chemicals like methane, ethanol and hydrogen, as long as a certain potential is applied. This potential is necessary for pumping the electrons from the anode to the cathode. One key element that contributes to both the efficiency and cost of MEC systems is the anode, due to its relatively high cost and its influence on the current density. This fact has led to numerous studies focused on the development of new and cheaper anode materials (Hegab et al. 2018; Sonawane et al. 2017; Zhang et al. 2014).

Ideal electrodes for use in BES should possess high conductivity, good biocompatibility, good chemical stability, a large surface area, reasonable pore distribution and should be low-cost (Thambidurai et al. 2014). The use of carbonaceous materials as anodes has been extensively explored, as they meet most of these requirements. These carbon-based materials include graphite rods, carbon brushes, carbon cloth, carbon paper and carbon felt (CF). All of these materials possess good conductivity and biocompatibility (Mohanakrishna et al. 2017). While the cost of electrodes is not often an issue in small-scale MECs, it represents a barrier that can limit the large-scale implementation of this technology. It is estimated that the cost of the anode can be as high as 20–50% of the total BES cost (Rozendal et al. 2008). Reducing the cost while maintaining the properties

that make these materials useful in BES is crucial in order to ensure that industrial applications are cost-effective (Maksimova 2019).

Recently, biomass-derived products have been evaluated as an alternative to traditional electrodes. They are low-cost, possess an inherent porous architecture, good biocompatibility and eco-friendly properties (Pandey 2019), making them ideal for applications such as electrochemical storage systems (Sonawane et al. 2017) and BES (Chen et al. 2017; Momodu et al. 2017). A number of successful examples of using these materials for these applications exist. Chen et al. (2018) tested the use of chestnut shell and reported that this material increased the power output 2.3-fold as compared with carbon cloth anodes in microbial fuel cells (MFCs). Huggins et al. (2014) evaluated the effect of using wood-derived biochar as cathode and anode material in an MFC on the treatment of wastewater and the recovery of nutrients. Their results showed that chemical oxygen demand (COD) removal was 95%, with an average reduction rate of 0.53 kg $COD \cdot m^{-3} \cdot d^{-1}$ in batch mode. Next, Chen et al. (2012) evaluated the use of a three-dimensional macroporous kenaf anode. This material showed higher electrochemical activity and better performance than that of graphite rods. Zhang et al. (2014) also compared the performance of a tubular bamboo charcoal with graphite rods and found that this biomass-derived material improved the power output by approximately 50%. Finally, Chen et al. (2018) prepared an anode from carbonized waste tire and obtained good results in an MFC achieving higher current density than when using a carbon felt electrode. Taken together, these findings clearly suggest that the carbonisation of biomass is an effective method for producing conductive materials for use in low-cost electrodes.

The almond (*Prunus dulcis L.*) shell is an abundant by-product from the almond processing industry and represents an abundant renewable resource in almond-producing countries such as Spain (Queirós et al. 2019) and the US (Aktas et al. 2015). The worldwide annual mean production of almonds is estimated at 1.97 million tons (MT), while Spain alone produces approximately 196,700 tons. This translates to between 0.68 and 1.47 MT of almond shells annually. As such, this material is an accessible and low-cost biomass that can be converted into conductive material via an activation process.

To our knowledge, the evolution of active and non-active microbial populations in this type of low-cost electrodes and the influence on the functioning of an MEC has not been studied yet. Therefore, the main objective of this work was to evaluate the feasibility of using pyrolysed almond shell (PAS) as a low-cost anode in an MEC. The experimental work proposed also intends an evaluation of bacterial populations growing in biofilms formed in anodes based on low-cost materials and those formed in commonly used anodes in bioelectrochemical systems as it is carbon felt. The abundance of total bacteria and active bacteria present in the biofilm, as well as their taxonomic compositions, were compared between the PAS and CF bioanodes.

5.2 MATERIALS AND METHODOLOGY

5.2.1 Working electrode preparation

Pyrolysis of raw almond shells was conducted in a semi-pilot scale rotatory tubular reactor (Nabertherm P300, Germany). Prior to the pyrolysis process, three holes were made in each half shell in order to allow electrical connection when used as an electrode. The temperature selected for the pyrolysis process was based on results reported in other works (Feng et al. 2018; Malika et al. 2016) that showed that a temperature near 1000 ˚C reduces the oxygen/carbon (O/C) ratio and favours the presence of carbon bonds necessary for free electron movement. This fact was proven by preparing two other samples with a similar method but raising the samples to a lower final temperature (Gupta et al. 2019a). The pyrolysis process applied to the almond shells was composed of three stages. In the first stage, a heating ramp of 5 ˚C min^{-1} was applied until reaching the final temperature (400, 600 and 1000 ˚C). Once this temperature was reached, the heat was held for 75 min (second stage), before cooling to room temperature (third stage). The PAS were subjected to a post-treatment in order to remove impurities and improve their hydrophilicity. The post-treatment consisted of consecutive immersion in a nitric acid solution (1 M, 30 min), acetone solution (1 M, 10 min) and ethanol solution (1 M, 10 min). Afterwards, the shells were immersed in deionised water and sonicated using an ultrasonic processor UP200S (Hielscher, Germany), which applied 57 W for 15 min (Fig. 5.1-a).

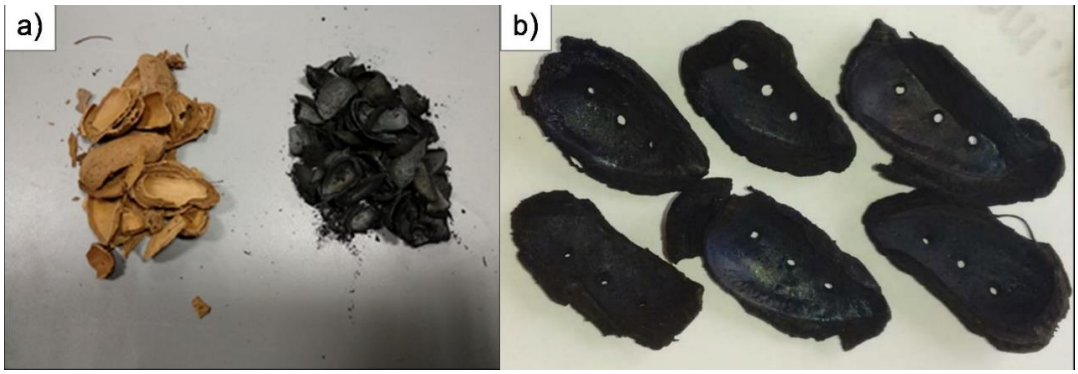


Figure 5.1 a) Conversion of raw almond shell to pyrolysed almond shell, b) PAS ready to use as electrodes

5.2.2 MEC set-up and operation

A preliminary test was conducted for studying the suitability of PAS as electrode material for a bioanode. Once the PAS anode was operative, a second test was performed to compare its treatment capacity with that of a contrasting MEC system using a CF electrode as an anode.

The experiments were carried out using polycarbonate single-chambered MEC with dimensions of 50 x 55 x 55 mm and an approximate volume per chamber of 80 mL the cells were operated in a single chamber disposition, using a stainless-steel mesh (AISI 304) as the counter and reference electrode and the corresponding anode (PAS or CF) as the working electrode (two electrodes mode). The projected area of the anode in these cells was 2.5 cm^2, while the cathode was a stainless-steel mesh (AISI 304) with an area of 5.1 cm^2. Titanium wire (Goodfellow, UK) was passed through the established holes of all electrodes (Fig. 5.1-b). A power source applied a voltage of 1.0 V between the anode and cathode. The MECs were inoculated with a mixture of a wastewater-activated sludge (from the wastewater treatment plant located in León, Spain) and an effluent from other MECs that were operating in the lab for 6 months, to ensure the presence of an exoelectrogen microbiome. In each cycle, the feed was a synthetic solution (190 mL) that had the following composition (per liter): 0.87 g of K_2HPO_4, 0.68 g of KH_2PO_4, 0.25 g of NH_4Cl, 0.453 g of $MgCl_2 \cdot 6H_2O$, 0.1 g of KCl, 0.04 g of $CaCl_2 \cdot 2H_2O$, 10 mL of trace elements solution (Moreno et al. 2016) and sodium acetate as a carbon source at a concentration of 683 $mg \cdot L^{-1}$. The evolution of organic load during the

experiments was followed through the total organic carbon (TOC) analysis, where the initial TOC was 200 $mg \cdot L^{-1}$. Once the reactor was charged, it was then purged with N_2 for 20 min in order to remove the oxygen from the solution. The electrical conductivity of the initial anolyte solution was 4.32 $mS \cdot cm^{-1}$ and the pH was adjusted to 7.0. A phosphate buffered saline solution (PBS; 0.1 M, pH 7.8) was used as the catholyte solution. The two initial MECs were operated for 2 months after the start-up period, which meant 17 regular cycles in batch mode.

In a subsequent test of MECs, two different types of electrodes were employed. Two cells used PAS and another used CF as the anode. The PAS anodes had the same characteristics previously described, while the latter had a projected area of 2.7 cm^2. The cells were denoted as PAS-MEC and CF-MEC based on the anode material used. The tests were conducted using two replicates of PAS-MEC. The cells used in this experiment are shown in Figure 5.2. The experiments were carried out using polycarbonate double-chambered MECs with dimensions of 76 x 81 x 76 mm and an approximate volume per chamber of 50 mL the cells were operated in a two-electrode arrangement, using a stainless-steel mesh (AISI304) as the counter and reference electrode and the anode (PAS or CF) as the working electrode. A cation-exchange membrane (CMI-7000; Membranes International Inc., USA) was used for chamber separation. The cathodes of these cells and their connections were similar to those described for the preliminary experiment. MECs were inoculated and operated under fed-batch conditions using an autoclaved synthetic solution, similar to previous tests. PAS-MEC and CF-MEC replicates were working for 94 days after start-up, providing 13 fed-batch cycles. The samples for microbiological analysis were taken from two anodes of PAS-MEC and CF-MEC at the end of the experiment.

Figure 5.2 Experimental design with pyrolysed almond shell (PAS) and carbon felt (CF) as electrodes

5.2.3 Measurements and analytical determinations

Chemical structure and functional groups were elucidated by FTIR spectroscopy at three pyrolysis temperatures. A Thermo IS5 Nicolet (USA) spectrophotometer was used for obtaining FTIR spectra and acquired from 400 to 4000 cm^{-1} at room temperature (16 scans and spectral resolution of 4 cm^{-1}); the peak positions were determined using Origin 2015 software.

For the XRD analysis, the equipment used was a Scanning Electron Microscope JSM-6480LV, JEOL (Japan), with an X-ray microanalysis detector: ULTIM MAX, Oxford Instruments (UK). The software that allows one to take the photos and identify the elements is AZtec 4.0 SP2, Oxford Instruments, (UK).

The voltage of the cells was maintained using an adjustable direct current (DC) power unit. The current data was acquired by a computer using an analogical output board (PCI-6713; National Instruments, Austin, TX). Current data was recorded at 12 min intervals. The effluents of the MECs were analysed by measuring the TOC content. This parameter was determined using a TOC analyser multi N/C 3100 (AnalytikJena, Germany). Coulombic efficiency (CE, %) is calculated as the ratio of electrons evacuated from the anode relative to the total electrons available from organic carbon oxidation.

5.2.4 Microbial community analyses

Once the second test was ended (two with PAS anodes and one with CF), the study of the microbial communities attached to the electrodes was performed. Pyrosequencing was utilised to sequence the 16S rRNA eubacterial gene for each anode type (PAS and CF), and this information was used to compare and contrast their microbial populations. The PAS electrode sample contained the populations from both PAS anodes. Genomic DNA and RNA were extracted from 150–250 mg of each sample to be analysed. These samples included the inoculum used, as well as those obtained from the PAS and CF electrodes after 2 months of operation. A physical–chemical extraction protocol using bead-beating was performed using the PowerSoil® DNA isolation kit (MoBio Laboratories Inc., Carlsbad, CA, USA). The quality of the DNA extracts was tested using a 1% agarose gel dyed with GelRed™ nucleic acid gel stain. Extracted RNA was transformed to cDNA (complementary DNA synthesised from the messenger RNA (mRNA), by reverse transcription polymerase chain reaction (RT-PCR), using the PrimeScript™ kit (Takara Bio Inc., Japan). The reaction was performed in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, USA). With both DNA and cDNA extracts obtained, massive sequencing was performed on these libraries. The 16S rRNA eubacterial gene was used to characterise the entire microbial community present in each sample. Illumina sequencing was carried out using a GS-FLX 454 sequencer (Roche, Switzerland) at the Molecular Research DNA Laboratory (Shallowater, Texas, USA). Each sample was amplified with the 27Fmod primer (5`-AGRGTTTTGATCMTGCTCAG-3`) and 519R modBio primer (5`-GTNTTACNGCGGGKGCTG-3`). The PCR reaction was carried out in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, USA). The DNA readings obtained were compiled into FASTq files for computer processing. Later, the operational taxonomic units (OTUs) were taxonomically classified using the MOTHUR software (version 1.33.3) and the Ribosomal Database Project (https://rdp.cme.msu.edu/). The wealth and diversity indices were calculated using the MOTHUR (version 1.35.1) software and samples were normalised to the lowest number of sequences.

An RT-PCR analysis of the 16S rRNA gene was also performed to quantify the total populations of eubacteria attached to both electrodes at the end of the experiments.

Quantitative PCR (qPCR) analyses were performed on both DNA and cDNA samples. The set of primers used was 314F qPCR (5′-CCTACGGGGAGGCAGCAGCAG-3) and 518R qPCR (5′-ATTACCGGGGCTGGCTGGG-3′). The thermocycler used was StepOne Plus Real-Time PCR System (Applied Biosystems, USA) and the results were processed with the same software as this equipment.

5.3 RESULTS

5.3.1 Material preparation

The results of the XRD show, as expected, a greater oxygen loss as the final pyrolysis temperature increased, and at the same time, an enrichment of carbon that results in a graphitisation of the lignocellulosic material. At 400 ˚C, the O:C ratio is 32.5:65.5, at 600 ˚C it is 14.8:79.5 and at 1000 °C it is 10.5:85.0. After pyrolysis, other elements such as Ca, K, Mg, Si, Al, P, and Fe were detected in the biochar samples which is in accordance with results found in literature (Gupta et al. 2019b).

The chemical structure and functional groups were elucidated by FTIR spectroscopy at three pyrolysis temperatures (Fig. 5.3). A peak at 3600 cm^{-1} is ascribed to O-H stretching vibration with no hydrogen bond and is easier to find at higher temperatures, while the other O-H vibrations (3500–3200 cm^{-1}) appear in the biochar at 400 ˚C and disappear at higher temperatures. Signals between 3000 and 2800 cm^{-1} correspond to the C-H stretching band, and between 1475 and 1350 cm^{-1} to C-H deformation bands.

Peaks between 1580 and 1675 cm^{-1} are related to C=C stretching vibrations and at 1300–1000 cm^{-1} they correspond to C-O stretching vibrations. The band around 1045 cm^{-1} is associated with the vibration of C–O–C links that is clear at the highest temperatures and is related to the decrease in the O/C ratio and the loss of oxygenated functional groups.

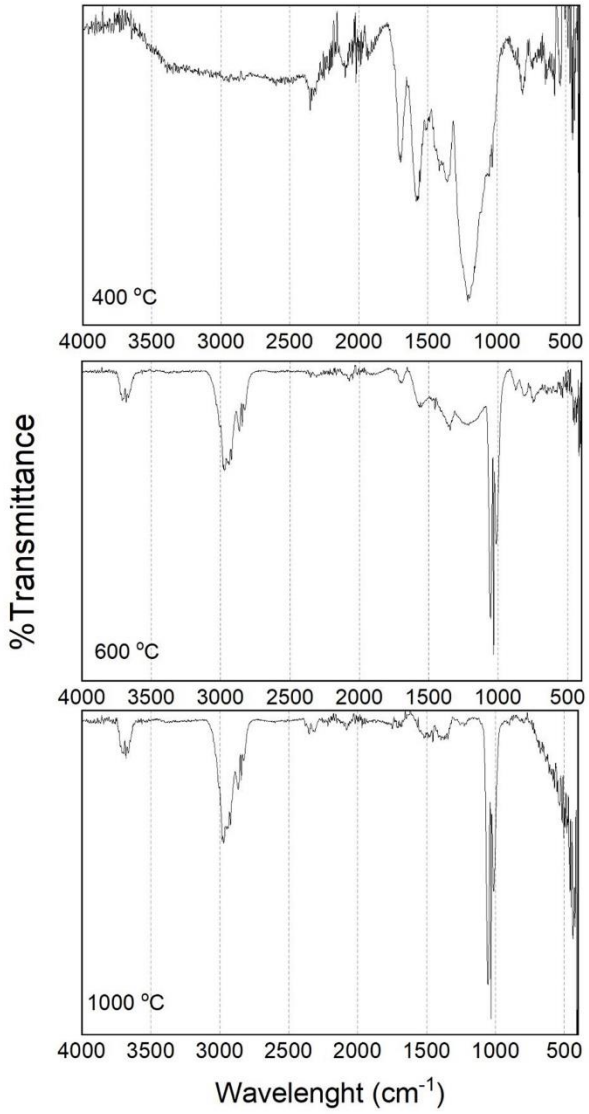


Figure 5.3 FTIR spectra (4000–400 cm^{-1}) of biochars produced at three different temperatures

The resistivity of PAS was measured with the voltage probes. This value was found to be between $1.5x10^{-3}$ and $2x10^{-3}$ Ω·m of resistivity for PAS at 1000 ˚C, which is about the same order of magnitude as the CF resistivity, at $3x10^{-3}$ Ω·m. The resistivity of PAS produced at 400 and 600 ˚C was greater than 10 Ω·m. These results confirm that the development of electrogenic biofilms in materials produced at 1000 ˚C could be enhanced because of the greater capacity of this material to evacuate the electric current.

5.3.2 Preliminary experiments

In each cycle, the current produced was recorded and the acetate degradation was evaluated in terms of TOC removal. The first cycle (Fig. 5.4-a) shows the typical lag phase (~30 h) followed by the exponential growth phase. This first cycle includes the initial development of an anodic biofilm, which stabilises its growth during the beginning of the second cycle and can be observed in the following cycles (Fig. 5.4-b).

This fact can be deduced from the mean slope at the beginning of each cycle, which is very sharp (step response) from the third cycle onwards. The coulombic efficiencies were between 66% and 82%, and the average current obtained was 0.74 ± 0.09 mA. The maximum current density was 1.7 $A \cdot m^{-2}$ with respect to the surface of the projected area of the anode, a value that could be considered as within a normal range for anodic mixed biofilms dominated by Geobacter species. After 30 days, at the end of each cycle, the organic matter degradation was complete in the two cells, yielding an average TOC removal rate of 50 ± 10 $mg \cdot L^{-1} \cdot d^{-1}$. The results of these preliminary tests confirmed the viability of PAS as the working electrode in BES. The current densities and the ohmic resistances obtained indicate that the performance of these materials is comparable to the typical carbon materials used in BES, such as CF.

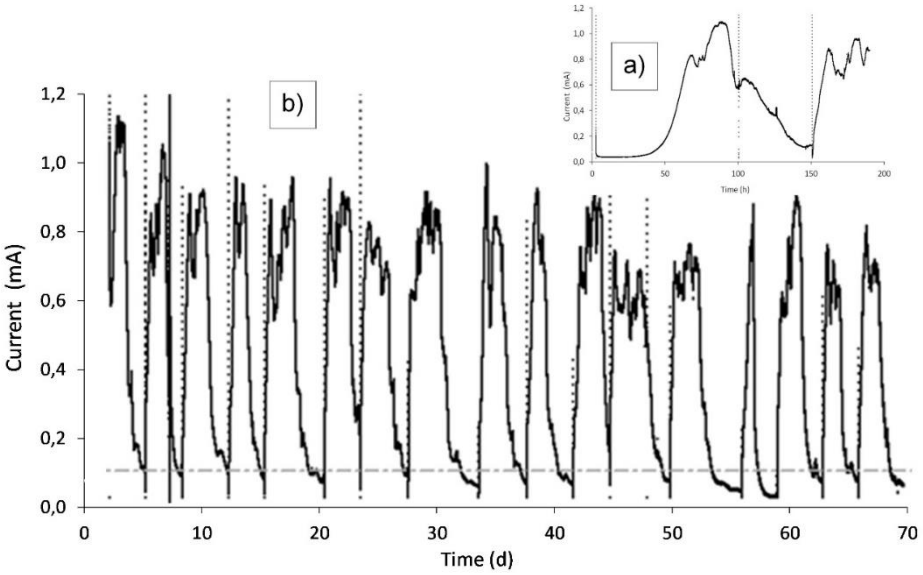


Figure 5.4 a) Current during the first cycle, b) current during the preliminary experiment

5.3.3 PAS-MEC and CF-MEC performance

Once the possibility of using PAS as a BES electrode had been demonstrated, the subsequent stage involved the comparison between the PAS and a conventional carbon-based electrode. The surface characteristics of the PAS (Aktas et al. 2015) make the CF a suitable benchmark material. The PAS-MEC and the CF-MEC were run for 13 fed-batch cycles. Overall, the behaviour of the two cells was similar except with regard to the establishment of system stability in the initial cycles. This was due to differences in the placement of the membranes. On average, each fed-batch cycle took 5 days to complete. It should be noted that in the intermediate cycles, there were some problems due to power outages in the lab (Fig. 5.5).

For this reason, cycles 6, 8 and 9 were not taken into account in the calculation of efficiencies. The highest density peak was reached by PAS-MEC with a value near 3.5 $A \cdot m^{-2}$ and an average current density close to 2 $A \cdot m^{-2}$. Figure 5.5 shows that in the start-up (first 2 cycles), there were differences between the cells. In the following cycles, a very similar trend and behaviour is shown. The coulombic efficiencies were between 62% and 85% for PAS-MEC and 64% and 79% for CF-MEC (Table 5.1). These values indicate a high efficiency in the organic matter conversion through exoelectrogenic bacteria. The organic matter removal in each cycle is near 90% (Table 5.1) for both types of electrodes. These results demonstrate that using PAS electrodes as bioanodes results in similar values to those obtained from the CF-MEC bioanodes.

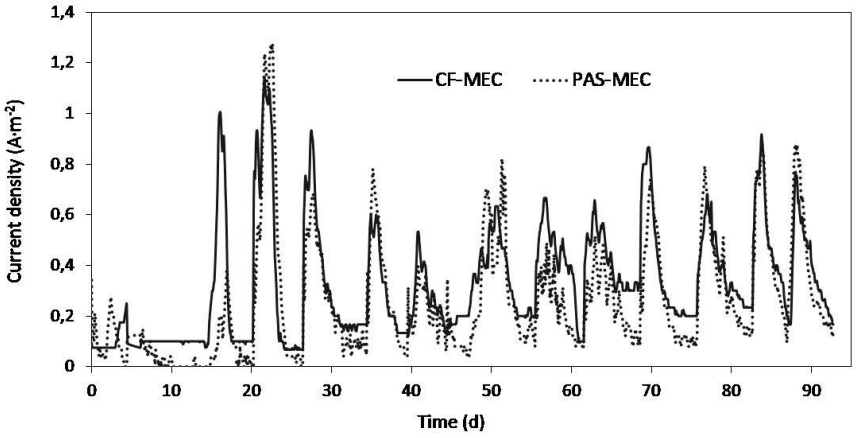


Figure 5.5 Current density profiles of PAS-MEC and CF-MEC in experiments using polycarbonate double-chambered MECs

Table 5.1 Parameters of PAS-MEC and CF-MEC performance experiments

Parameter	Pyrolysed almond shell (PAS)	Carbon felt (CF)
Organic matter removal (%)	91.5±6.2	93.0±4.9
Coulombic efficiency (%)	62-85	64-79
Average current density ($A \cdot m^{-2}$)	1.8±0.4	1.9±0.3

5.3.4 Microbial community analysis

At the end of the last cycle, samples were taken from the bioanodes to characterise the microbial populations attached to the electrodes. The numbers of total bacteria (DNA) and active bacteria (cDNA) were quantified by qPCR of the 16S rRNA gene (Fig. 5.6) to determine the amount of bacteria present in the biofilm. The amount of bacteria present in the sewage sludge used as inoculum was also quantified, showing the increase in the total number of gene copies per gram in the MECs. Comparisons were made between the bioanodes from PAS-MEC and CF-MEC. The number of gene copies was higher in the sample from CF-MEC than PAS-MEC, which was supported by a very close ratio between cDNA and DNA. The ratios between active and total bacteria were approximately 15% and 10% for the PAS and CF samples, respectively.

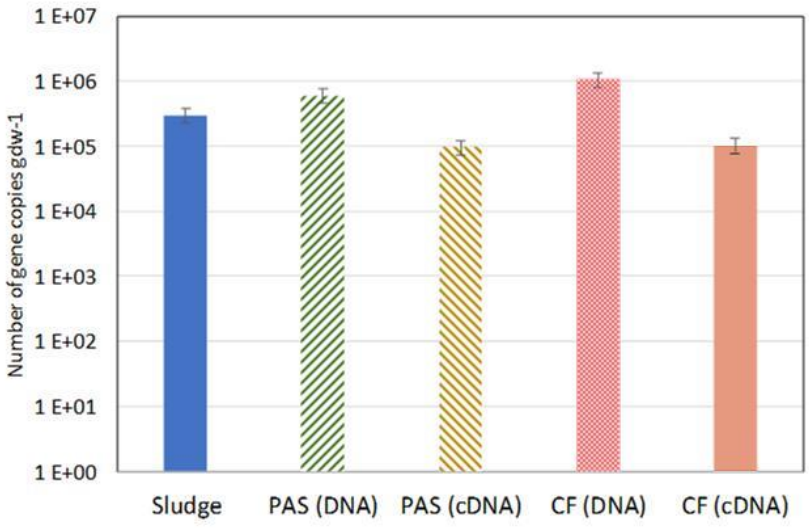


Figure 5.6 Number of 16S rRNA gene copies obtained from DNA and cDNA samples

There are differences in the amount of total bacteria present in each of the biofilms. However, the active populations are of the same order of magnitude for both electrodes (Fig. 5.6), which explains why the current densities reached at the end of the experiment are very similar for both reactors. While the initial bacterial growth is faster in the CF-MEC sample, the populations appear to equalise over time (Fig. 5.5).

Table 5.2 contains data on the number of reads and the mean length of the sequenced fragments (base pairs) of each sample. The number of successful reads was between 41,750 and 74,124, with an average length of 492 to 517 base pairs. Based on these results, the sequencing data is of good quality and is suitable for further analysis.

Table 5.2 Characteristics of the sequences obtained for the analysis of microbial populations

Sample	Number of reads	Mean length (bp)
Inoculum (sludge)	74,124	492.5
PAS (DNA)	41,750	500.4
PAS (cDNA)	49,681	516.4
CF (DVA)	63,096	492.3
CF (cDNA)	47,341	517.0

The indices of richness and diversity were calculated to compare the microbial population structure in both electrodes with respect to those initially present in the inoculum sample, as well as to be able to compare results between electrodes at the end of the experiment. The diversity indices calculated were Shannon, Inv Simpson and Fisher Alpha, while the richness estimators were the observed OTUs (OBS), Chao1 and ACE.

The results showed that the sewage sludge has higher indices of both richness and diversity than the electrode samples (Table 5.3), as would be expected. Richness and diversity were reduced in all bioanode samples due to specialization of the microbial populations. Likewise, it was observed that the diversity was lower in the active populations (cDNA) compared to those from the total populations (DNA). Finally, there are no large differences in the richness or diversity of the electrode samples, but there is a greater DNA richness in CF than PAS, in line with a greater number of reads. The

richness in cDNA is similar for PAS and CF, indicating the similar behavior of both types of electrodes.

Table 5.3 Richness and diversity index at the genus level

Sample	Shannon	Inv Simpson	Fisher Alpha	OBS	Chao1	ACE
Inoculum (sludge)	3.84	19.2	63.0	446	560.7	556.9
PAS (DNA)	3.06	10.9	29.8	216	279.2	280.9
PAS (cDNA)	2.98	6.7	38.7	277	326.5	326.8
CF (DNA)	3.28	10.7	42.9	313	403.2	385.1
CF (cDNA)	2.47	4.8	32.5	237	314.4	333.8

The composition of the eubacterial community in the sludge was analysed in depth in order to acquire information about the microbiological relationships related to the functioning of the reactors. At the phylum level (Fig. 5.7), the Proteobacteria group is the most abundant in the electrodes and is the dominant phylum in all samples. Compared to a 24.4% abundance in the inoculum, this group accounts for 37.0% to 71.0% of the anodic samples. This phylum, one of the most relevant in the BES, (San-Martín et al. 2019) has several exoelectrogenic bacteria. Another phylum enriched in the electrodes that was not identified in the inoculum was Acidobacteria, with an abundance of 7% to 18%. This group has some functional connections with Acidobacteria and Actinobacteria in MFCs (Li et al. 2018).

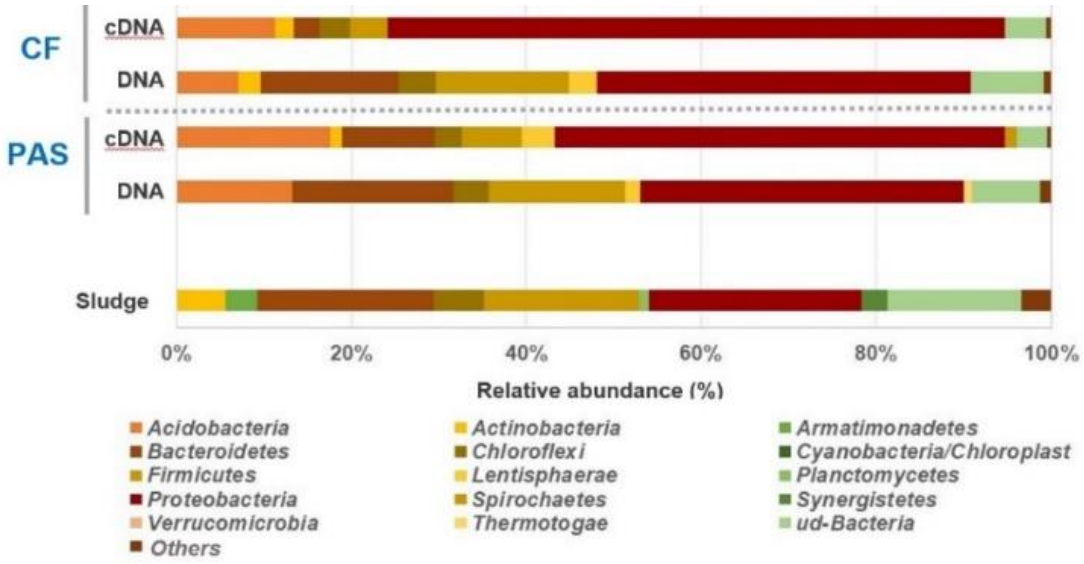


Figure 5.7 Taxonomic classification at the phylum level obtained by massive sequencing of the 16S rRNA gene. Groups accounting for less than 1% of the total number of sequences per sample are classified as "Others"

Figure 5.8 depicts the main genus identified in the PAS and CF electrodes in terms of relative abundance. As seen in Figure 5.6, the abundance of the gene copy number of the 16S rRNA gene in the active populations (cDNA) was an order of magnitude below that of the total populations (DNA). However, the relative abundance of the two main genera was above the value for the active microbial community (cDNA).

These two genera are Geobacter and Geothrix, which were identified in both DNA and cDNA samples, increasing their abundance from 36% to 38% in the total community to 54% to 55% in the active community (Fig. 5.8). Both genera are well-known electroactive microorganisms that are mainly responsible for organic matter oxidation, with acetate being one of its preferable substrates. The presence of these genera in the PAS-MECs is indicative of the suitability of this material for bioelectrochemistry applications. Therefore, there are no major differences in the amount of bacteria capable of growing on the surface of either electrode, nor in the genera of dominant bacteria. These results agree with those obtained in terms of reactor yields, where the organic matter degradations, coulombic efficiencies and current densities recorded at the end of the experiments were similar for both CF-MEC and PAS-MEC conditions. They also agree

with the results reported by Martínez et al., (2018) who evaluated the microbial communities in an anaerobic digester supplemented with biochar. These authors reported that the addition of this carbon-conductive material favoured the development of electroactive microorganisms, which resulted in an improvement of the digestion performance. These results support the production of low-cost biochar-based electrodes, as proposed by Mian et al., (2019) for reducing the capital investment required for bioelectrochemical systems and illustrate an example of the actual effect of biochar on the environment (Wang & Wang 2019).

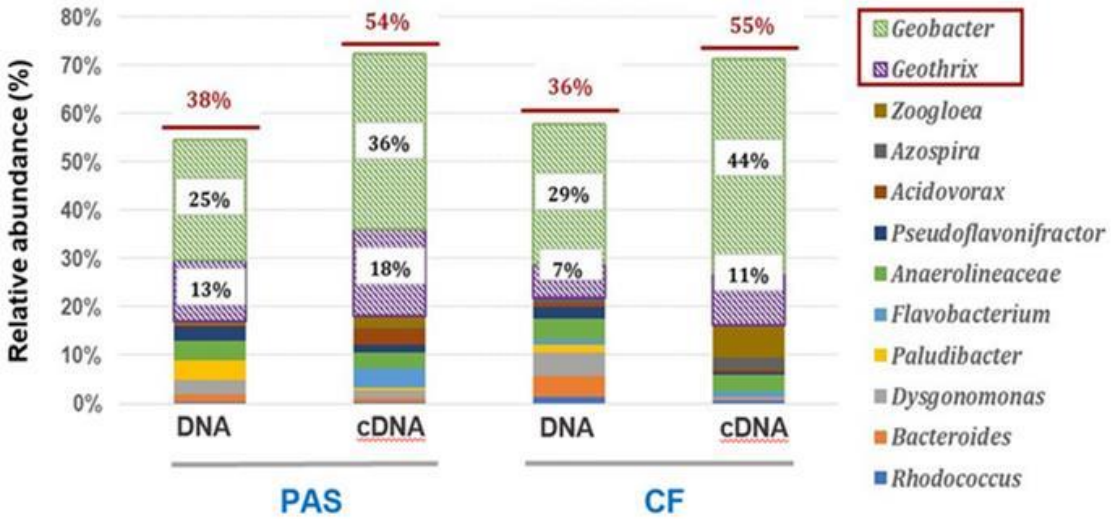


Figure 5.8 Taxonomic classification at genus level obtained by massive sequencing of the 16S rRNA gene. The number over the columns are the sum of the genus in boxed

5.4 CONCLUSIONS

The results of this research demonstrate that the use of pyrolysed almond shells in the production of sustainable and cost-effective electrodes for bioelectrochemical systems is feasible, as they perform comparably to carbon felt electrodes. The results of the microbiological study revealed a similar structure and taxonomic composition between the PAS electrode and the CF electrode, confirming the good electrical connection bacteria-electrode and increasing the electron transfer efficiency between

microorganisms and the low-cost material. These results demonstrate the suitability of using PAS as anode material in MECs.

Acknowledgements

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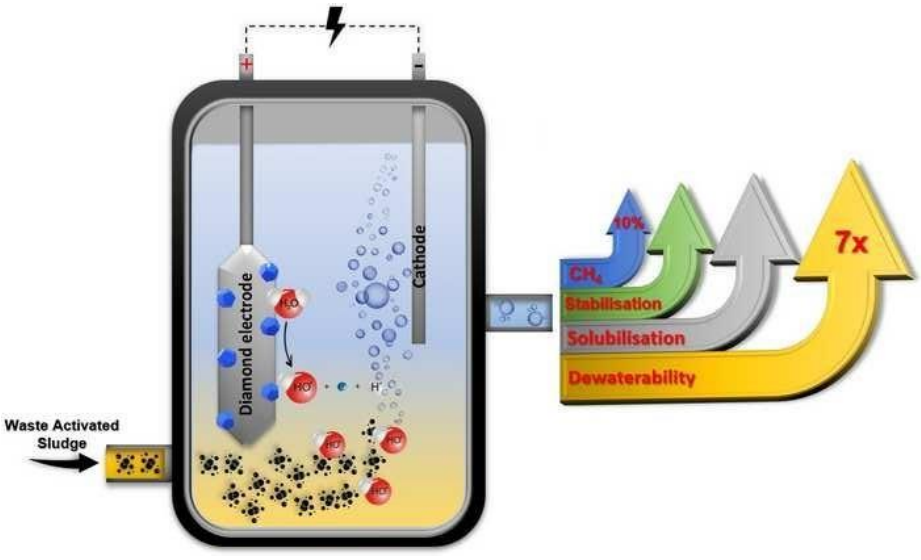
Capítulo/Chapter 6

Assessment of Electrooxidation as Pre and Post-treatment to Improve Anaerobic Digestion and Stabilisation of Waste Activated Sludge



Arenas. C. B., González, R., González, J., Cara, J. et al. Assessment of electrooxidation as pre and post-treatment to improve anaerobic digestion and stabilisation of waste activated sludge. Sent to: Journal of Environmental Management

Abstract

This study evaluates the effects of electrooxidation to promote sludge stabilisation. Boron-doped diamond electrodes were used to treat waste activated sludge before and after anaerobic digestion under different experimental conditions (current density, conductivity, pH, time) to evaluate their influence on the organic matter solubilisation to enhance anaerobic digestion and to improve main dewaterability characteristics. All electrooxidation treatments showed an augmentation of those values due to solubilisation of sludge components. The efficiency of electrooxidation as pre and post-treatment was enhanced by the conditions applied (increasing conductivity, higher current density and extended time of treatment). The improvement in the solubilisation of the organic matter might have improved the hydrolysis rate by the increase in the amount of readily available compounds (Volatile Fatty Acids). Three types of short-chain volatile fatty acids were mainly observed (acetic acid, propionic acid and butyric acid). An increase in methane yield was observed with the application of electrooxidation pre-treatment. The adjustment of pH to 7 by the addition of an alkaline solution (T5) improved the methane yield about 18%.

Keywords

Electrochemical oxidation, digestion, dewaterability, FTIR

6.1 INTRODUCTION

Wastewater treatment based on activated sludge process is the most widely applied technology to prevent water pollution. Although the efficiency of the process is very high, the great energy demand associated with this type of biological treatment is a great concern. Another relevant feature associated with wastewater treatment plants (WWTPs) is the management of sewage sludge. The treatment and disposal of biosolids may represent up to 50% of plant operating costs and it is estimated that the disposal of sludge is responsible for 40% of the total greenhouse gas emissions from WWTPs (Appels et al., 2008; Gherghel et al., 2019). Sustainable technologies for sludge management involve its reduction and the production of better-quality sludge, promoting valorisation options instead of final disposal hence reducing the impact on the environment (Canziani & Spinosa, 2019).

Anaerobic digestion is a biochemical process, which includes the conversion of organic matter into methane and CO_2. It is a well-known and effective process for sludge stabilisation and valorisation. Anaerobic digesters in WWTPs usually treat a mixture of primary and secondary (waste activated) sludge. In general terms, waste activated sludge (WAS) is more difficult to digest than primary sludge (Lafitte-Trouqué & Forster, 2002) due its structure, WAS is formed by flocs of filamentous microorganisms, ionic components, colloids, mineral particles and extracellular polymeric substances (EPS) (Dai et al., 2013; Shao et al., 2010). These substances are secreted by microbes and are a constituent of microbial cell membranes. The recalcitrant character of these substances restrains the rate of the hydrolysis step in anaerobic digestion which limits the biological process (Li et al., 2019).

Even though the organic content of sludge, measured as total chemical oxygen demand (COD), is high, the soluble fraction of COD is usually low, with this later being the organic material accessible to microorganisms responsible of the acidogenesis phase. Thus pre-treatment of sludge is required to rupture the cell walls and facilitate the release of intracellular material into the aqueous phase to accelerate biodegradation and enhance anaerobic digestion (Kim et al., 2003). Several technologies, including mechanical, thermal, ultrasonic, microwave, enzymatic, chemical and electrochemical oxidation pre-

treatments have been proposed to improve sludge digestibility (Barjenbruch & Kopplow, 2003; Li et al., 2012; Mainardis et al., 2019; Ruffino et al., 2015; Saha et al., 2011; Tedesco et al., 2013; Villamil et al., 2019; Yu et al., 2014).

Anaerobic digestion transforms the organic matter into biogas and a solid-liquid by-product called digestate, which contains a high level of nutrients and a stabilised organic matter with lower putrescible degree (Möller, 2012). Digestate is a versatile by-product which can be applied as fertiliser directly or by valorising its two conforming fractions separately. In this later case the two fractions are separated into a liquid phase rich in ammonium and the remaining solid fraction which is rich in organic components and fulvic acids can be used as organic amendment (Nuchdang et al., 2018). However, the application of digestates as fertilisers is still under debate since the fate of digestion affects the characteristics and stability of the organic material obtained. The high pH values and ammonia content of digestates may increase the risk of emitting ammonia and nitrous oxide into the atmosphere, this fact along with their content in some metals (such as Cu and Zn), salinity, degree of biodegradability, phytotoxicity and hygiene characteristics and other relevant factors should also be considered when evaluating the agronomic valorisation of digestates (Alburquerque et al., 2012; Nkoa, 2014).

Digestate management is a key factor in order to circumvent pollution problems associated with the release of high-risk contaminants into the environment. The adequate disposal of digestates is a challenging task, due to their high water content making difficult handling, transport and storage operations thus increasing final disposal costs (Bauer et al., 2009). These shortcomings have set the focus of research on different pre-treatment strategies that may aid in the stabilisation of the organic material and dewatering (Li et al., 2017; Martínez et al., 2015; Mo et al., 2015). The different pre-treatment techniques are not always capable of attaining all needs associated with enhancing the dryness of sludge, reducing odours, toxicity and removal of pathogens (Bureau et al., 2012). Among these, electrolysis has arisen recently as a dewatering and stabilising technique with the capacity of solubilising the organic matter contained in sludge to a great extent with a negligible environmental impact and thus reducing costs regarding sludge handling and final disposal (Rahmani et al., 2015; Tuan et al., 2012; Xiao et al., 2019).

Electrochemical oxidation or electrooxidation (EO) is an attractive technology due to its ability to treat under moderate conditions, ambient temperature and pressure, complex organic pollutants (Martinez et al., 2018). Electrooxidation includes photoelectro-Fenton, eletro-Fenton and anodic oxidation. The later are considered as advanced oxidation processes with the common feature of in situ generating hydroxyl radicals (·OH) (Markou et al., 2017). Advanced oxidation interacts with pollutants by direct electron transfer to the anode surface (M) or through heterogeneous reactive oxygen species produced as intermediates from the oxidation of water to oxygen, including the powerful physiosorbed hydroxyl radical (·OH) at the anode surface, denoted M(·OH), generated via Eq.(6.1), and weaker oxidants like H_2O_2 produced from M(·OH) dimerization by Eq.(2) (Panizza et al., 2008). However, both oxidation mechanisms may coexist during electrooxidation processes (Yu et al., 2014). The ·OH radical can promote the breakdown of sludge and dissociation of EPS via chemical oxidation at very low concentration.

$$M + H_2O \rightarrow M(\cdot OH) + H^+ + e^- \quad (6.1)$$

$$2\,M(\cdot OH) \rightarrow 2MO + H_2O_2 \quad (6.2)$$

When cells contained in WAS are exposed to and external electric field, the electrical charges inside and outside of the cell membrane build-up and the membrane potential increases. When the elastic resistance of the membrane reaches a limit value, the natural structure of the cell is destroyed, thus organic substances within the cell will be released thus favouring the hydrolysis stage in anaerobic digestion (Salerno et al., 2012).

The objective of this study was to evaluate the effect of electrooxidation for aiding in the stabilisation of sewage sludge. The electrooxidation procedure was applied as a pre-treatment to improve the solubilisation of organic material contained in WAS for enhancing anaerobic digestion and as a post-treatment, once sewage sludge was submitted to anaerobic stabilisation. The treatment of sewage sludge consisted in the use of a boron-doped diamond (BDD) anode. The efficiency of the pre-treatment was assessed in terms of the quantity and quality of biogas produced whereas in the case of post–treatment, the efficiency was evaluated using digestate dewatering characteristics,

particle size distribution, solubilisation and changes in main functional groups by the use of FTIR analysis.

6.2 MATERIAL AND METHODS

6.2.1 Electrochemical oxidation experiments

Pre-treatment experiments were carried out using a 150 mL chamber. BBD electrodes were used as anodes and stainless steel was used as cathode. Experiments were carried out at an applied current density of 6.6 mA cm^{-2} (5 V for 1 hour). Post-treatment tests were performed by employing 75 mL cell supplied by Proaqua. This cell contains BDD anode and cathode. Current density inputs were in the range of 5.5 to 18.8 mA cm^{-2} (15 – 25 V for a treatment time ranging from 5 min to 1 h). The experiments were conducted under batch conditions at room temperature (25 ± 1 ˚C).

Pre- and post-treatment tests were labelled according to the conditions set for the experiments presented in Table 6.1. WAS used for pre-treatment experiments was collected from the dissolved air flotation unit of the WWTP of the city of León. This sludge had a total solid (TS) content of 21.8 g L^{-1} and a volatile solid (VS) concentration of 14.4 g L^{-1}. The assessment of the efficiency of electrooxidation as a post-treatment technology was performed using digested sludge. This sludge was obtained from a laboratory batch reactor treating WAS without the application of any type of pre-treatment. The digestion was carried out for 40 days. After this period, the digested contained a TS content of 22.0 g L^{-1} and a VS concentration of 10.1 g L^{-1}. The solubilisation attained was examined along with the effect of electrooxidation post-treatment on dewaterability characteristics at different conditions. Na_2SO_4 was added as supporting electrolyte to adjust conductivity (Jiang et al., 2010). Alkaline solution was prepared adding 40 g of $NaHCO_3$, 60 g of KOH and 40 g of KH_2PO_4 to 1 L of distillate water.

Table 6.1 Pre- and post-treatment conditions set for the experiments

Nomenclature	Experiment conditions	Characteristics
WAS	System used as control. No pre-treatment was applied	
Digestate		
Application of electrooxidation as pre-treatment Current density of 6.6 mA cm^{-2} (5 V for 1 h).	T1	Without conductivity adjustment or pH controls
	T2	Addition of Na_2SO_4 at 3 g L^{-1}
	T3	Addition of Na_2SO_4 at 6 g L^{-1}
	T4	pH 4 adjusted with H_2SO_4
	T5	pH 10 adjusted with alkaline solution
Application of electrooxidation as post-treatment At fixed electric potential of 15 V and 25 V (variable current density for 5 min and 1 h)	P1	15 V for 5 min (5.5 mA.cm^{-2})
	P2	15 V for 5 min (10.7 mA cm^{-2}) at pH 10 (adjusted with alkaline solution)
	P3	25 V for 5 min (11.4 mA cm^{-2})
	P4	25 V for 5 min (18.8 mA cm^{-2}) at pH 10 (adjusted with alkaline solution)
	P5	25 V for 1 h (6.8 mA cm^{-2})

6.2.2 Anaerobic digestion experiments

Batch digestion experiments were performed to evaluate the evolution on methane production of WAS after electrooxidation pre-treatment. Digested sludge was used as inoculum. The digestate was obtained from the sludge digester of the WWTP of the city of León (Spain). This sludge had a TS content of 19.3 g L^{-1} and a VS concentration of 11.4 g L^{-1}.

Experiments were carried out in triplicate using 250 mL Erlenmeyer flasks. Flasks were filled with inoculum and substrate at a ratio of 1:1 (substrate: inoculum, expressed in VS). Tap water was added to complete the volume. Gas production of the reactors and its composition was periodically measured. Gas volumes were measured using bottle gasometers and corrected to standard temperature and pressure (STP, 0 ˚C and 100 kPa). A blank reactor containing only inoculum was used to subtract the background gas production. The temperature was controlled by a water bath set at 37 ± 1 °C. Agitation was provided by means of magnetic stirrers. Digestion systems were labelled as shown in Table 6.1 where the characteristics of the different batch test were described.

Methane production was fitted to the modified Gompertz equation:

$$P_{(t)} = P_{max} \cdot \exp[-\exp(R_{max} \cdot e/P_{max})(\lambda - t) + 1] \quad (6.3)$$

Where $P_{(t)}$ is the cumulative methane yield (L kg VS^{-1}); P_{max} is the maximum methane yield (L kg VS^{-1}), R_{max} is the maximum methane production rate (L kg VS^{-1} d^{-1}), λ is the time of the lag-phase (d) and e is Euler's number (approx. 2.718).

6.2.3 Analytical techniques

Total Solid (TS), Volatile solid (VS) and pH were measured in accordance American Public Health Association, (1998). Total organic carbon (TOC) was analysed using a high-performance analyser multi N/C® by combustion of the sample at 980 ˚C , whereas inorganic carbon was measured by acidification of the sample using a solution of 10% phosphoric acid (H_3PO_4) according to (Shimadzu, 2017). Organic carbon was calculated by the difference between total carbon and inorganic carbon. COD was measured by the use of commercial kit tubes LCK 514 in the range of 1000 - 10000 mg L^{-1} and, subsequently, measured by a spectrophotometer DR 3900. Kit tubes and spectrophotometer were supplied by Hach Lange.

Biogas was measured by gas chromatography using a Varian CP-3800 GC model, equipped with a thermal conductivity detector (TCD). Measurement of H_2, CH_4, CO_2, N_2 y O_2 was made was made simultaneously using a HayeSep Q 80/100 column of 4 m length followed by a molecular sieve (1.0 m x 1/8''x 2.0 m) column. The carrier gas was helium and the columns operated at 331 kPa and at 50 ˚C.

Volatile fatty acids (VFAs) were analysed using a Bruker 450-GC chromatograph with a flame ionisation detector (FID) equipped with a Nukol capillary column (30 m x 0.25 mm x 0.25 mm) from Supelco. The carrier gas was helium. The injector and detector temperatures were 220 °C and 250 °C, respectively. The oven temperature was set to 150 °C for 3 min and increased to 180 °C with a ramp of 10 °C min^{-1}. Methods for measuring biogas composition and VFAs concentration were described in detail elsewhere (Martínez et al., 2019)

Particle size analysis was carried out using a Beckmann Coulter LS 13 320 laser diffraction particle size analyser. The LS 13 320 was equipped with an optical bench and a universal liquid module to measure the size distribution of particles. The scatter generated by

particles is estimated based on the Fraunhofer optical model. Samples were previously diluted in tap water for analysis. Ten measurements were performed for each sample.

After anaerobic digestion of WAS, dewaterability characteristics of sludge were measured, Solid removal expressed as Percentage of volatile solids removal (%) was calculated according to the following:

$$(\%)\ VS_{removal} = VS_{digestate} - VS_{digestate\ after\ EO}\ /VS_{digestate} \quad (6.4)$$

$VS_{digestate}$ is the volatile solid content of the raw digestate and $VS_{digestate\ after\ EO}$ is the volatile solids of the digestate after each EO treatment (g kg^{-1}).

Capillary suction time (CST) was measured using 10 mL of digestate and samples obtained from post-treatment tests. The sample was poured into a stainless-steel tube (1.0 cm inner diameter) in contact with Whatman Nº. 17 chromatography-grade paper. CST was defined as the time required for the wetting front to pass from the first radius located at 1.0 cm of the cylindrical reservoir to the second radius placed at 3.0 cm. Three replicates were used. The CST results were normalised to CST_s (s g L^{-1}) by dividing the value obtained by the amount of total suspended solids concentration (TSS) and subtracted by Capillary suction time of sludge water (CST_w) according to the equation at (Yin et al., 2004) (CST_w = 8 s).

$$\mathrm{CST} = CST_s = \mathrm{CST} - CST_w \quad (6.5)$$

Specific resistance to filtration (SRF) was measured using a 9 cm standard Buchner funnel (fitted to a constant vacuum pressure) into which the sludge sample was poured. Filtrate volume and filtration time were recorded. SRF was calculated as the slope of the linear plot of volume vs. time/volume (Lo et al., 2001). The water content of the sludge cake trapped by the filter paper was measured in accordance with standard methods (American Public Health Association, 1998). Three replicates were evaluated.

Free and bound water in sludge was measured using a thickened sludge sample that was centrifuged at 7600 x g for 20 min. A subsample was collected for drying at a constant airflow of 100 mL min^{-1} at 105 °C (Kopp & Dichtl, 2001) using a STD Q600 TA Instruments thermobalance. The water distribution was derived from the curve of drying time vs.

water content (mass of water/mass of solids) of the sample. Three replicates were used for obtaining this curve.

Digested samples were evaluated by spectroscopy techniques using Fourier-transform infrared spectroscopy (FTIR). This technique was applied to evaluate the transformation attained by the organic material after the digestion of substrates. FTIR spectra were obtained in the 4000 - 500 cm^{-1} wavelength range by a JASCO FT/IR-4000 spectrometer. It was equipped with a DLaTGS detector stabilised by a Peltier system, 2 mg of each sample powder were put on the spectrometer lens and, subsequently, they were trapped by the diamond crystal. Results were analysed using OriginPro 2015 software.

6.3 RESULTS AND DISCUSSION

6.3.1 Results of pre-treatment of sludge by electrooxidation

Figure 6.1 shows the enhancement on the solubilisation of the organic matter compared with the control sample denoted WAS. Initial values of soluble COD obtained from the control sample were about 1073 ± 53 mg L^{-1} which accounted for a value of 385 ± 95 mg L^{-1} TOC. All EO treatments showed an augmentation of those values due to solubilisation of the component in WAS. The increase in TOC and COD values were obtained from the treatment presenting the maximum value tested of initial conductivity (treatment T5). The results reflect the effectiveness of cell lysis and release of intracellular organic materials.

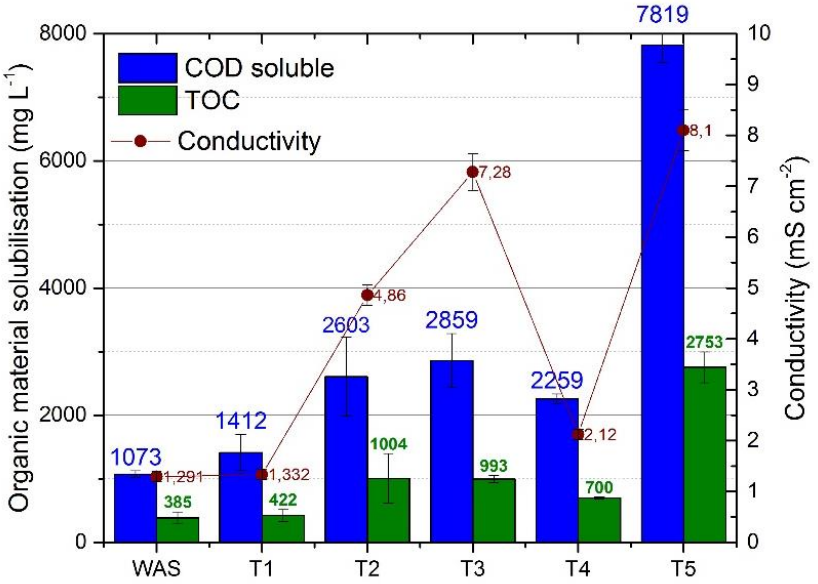


Figure 6.1 Measurements of soluble COD and TOC obtained before and after different pre-treatments of sludge electrooxidation

The efficiency of electrooxidation as pre-treatment was greatly enhanced by the conditions applied. The treatment T1 where the conductivity of the system was not modified reports higher soluble COD values but not significant differences for TOC values between those measured for this sample and the control (WAS). The second and third treatment (T2 and T3) which evaluated the effect of increasing conductivity by the addition of Na_2SO_4 reported a 2-fold increased when comparison with the control is made. However, the increment in the concentration of the sulphate salt did not report a great increase in the solubilisation of the organic material.

The performance of the electrooxidation pre-treatment was evaluated under acidic (T4) and alkaline (T5) conditions. This later pre-treatment test reported the best values of solubilisation obtained as previously commented, probably associated with the intrinsic enhancement of conductivity provided by the addition of the alkaline solution to increase the pH of the reactor to 10 units.

Several authors observed the effectiveness of electrooxidation treatment for waste activated sludge solubilisation and digestion improvement. The electrooxidation experiments of Feki et al. (2015) improved the solubilisation of COD in 28% where the

experiments were conducted at pH 7 under a current density of 2.5 A/dm^2 for 2 h. The studies of Barrios et al., (2017) showed a reduction of the total COD and an increase in soluble COD for the best pre-treatment conditions applied (28.6 mA cm^{-2}) leading to higher methane production (up to76 - 80%) after anaerobic digestion. In contrast, the studies of Pérez-Rodríguez et al. (2019) with different operating conditions for sludge electrooxidation (current density around 28.6 mA cm^{-2} for 30 min), resulted in a maximum degree of solubilisation of just 1.78%.

The difference in results obtained in the present work (with values being lower) compared to those reported by other authors might be explained by the lower current density and shorter treatment time applied. Although, current density which is directly associated with the amount of hydroxyl radicals generated (to react with the organic matter) and the time of pre-treatment applied are factors known to improve solubilisation, any increase in these two parameters increase the energy demand of the treatment and therefore reduces the technical feasibility of this process.

Electrooxidation pre-treatment could contribute to the release of soluble organic matter from sludge, while not reducing the total content of organic compounds under the test conditions. Complex organic molecules in wastes are converted into simpler forms that become easily accessible for acetogenic bacterias to generate VFAs. Figure 6.2 shows the increase obtained in VFA concentration after pre-treatment which is also in accordance with increase of TOC and soluble COD values observed. Three types of short-chain volatile fatty acids were mainly measured (acetic acid, propionic acid and butyric acid) with the highest concentration corresponding to acetic acid. The disintegration of EPS and membrane cell exposed the inner organic material (carbohydrates and proteins) which were released into the liquid phase and contributed to higher content of short-chain fatty from the pre-treatment of sludge (Li et al., 2019).

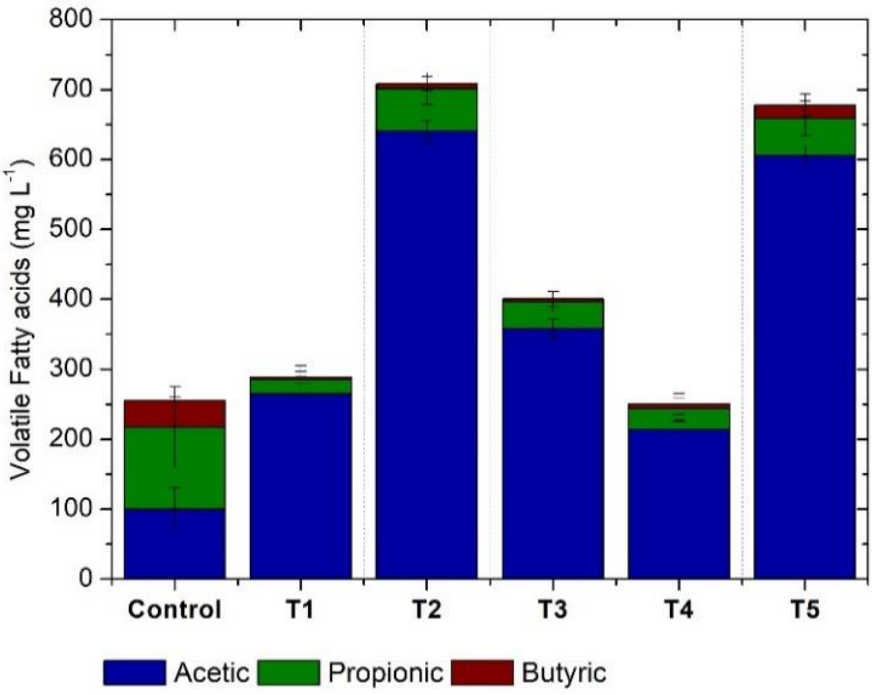


Figure 6.2 Volatile fatty acids concentration before and after electrooxidation experiments

6.3.2 Anaerobic digestion optimisation

Cumulative methane production curves obtained from the different batch assays are shown in Figure 6.3. An increase in methane yield was observed with the application of the pre-treatment (T5, T1, T4) and a decrease with the application of pre-treatment T2 and T3 (addition of Na_2SO_4). The adjustment of pH to 7 by the addition of an alkaline solution (T5) improved the methane yield about 18%.

Sludge hydrolysis has been regarded as the rate-limiting step of anaerobic digestion of particulate substrates but in this case, the improvement in the solubilisation of the organic matter might have improved the hydrolysis rate by the increase in the amount of readily available compounds (VFA). Acetic acid is the direct substrate for methanogens which is transformed into methane through methanogenesis, thus, the enhancement on the production of acetic acid observed is directly associated with the increase in methane production.

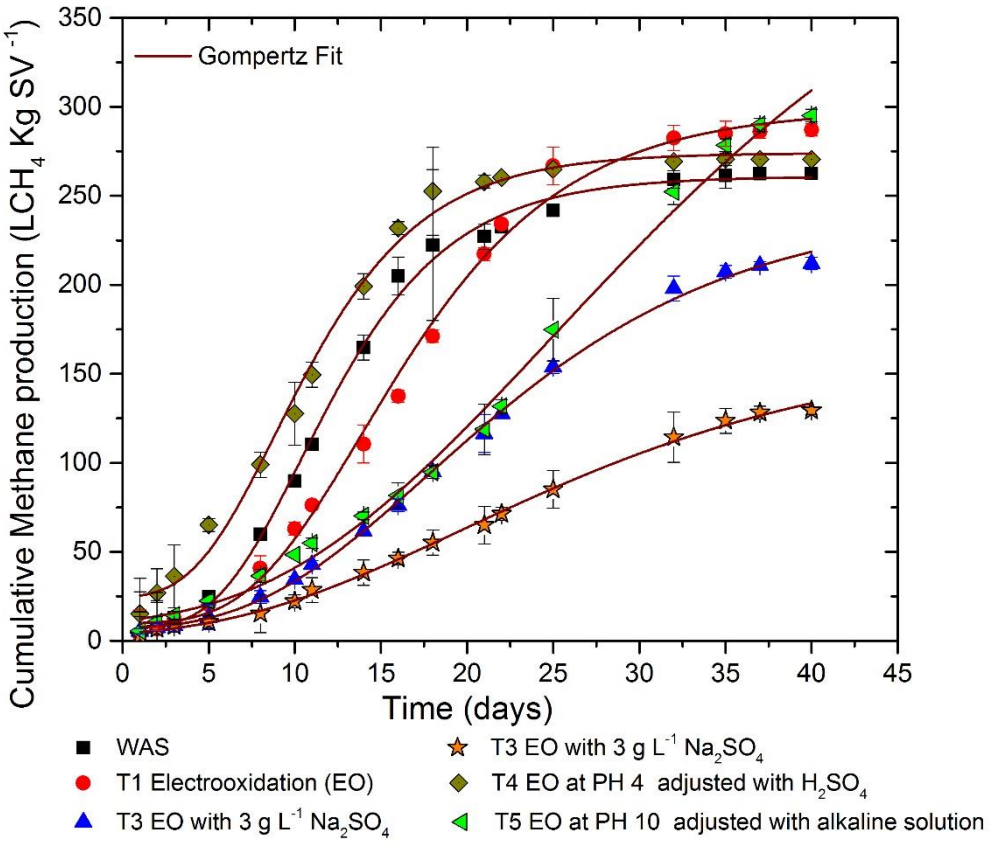


Figure 6.3 Cumulative methane production and fitted curves of the sample after EO pre-treatment

The fitted curves of the Gompertz model are also represented in Figure 6.3, model parameters are summarised in Table 6.2. The curves of methane production showed a sigmoid profile with an initial lag phase that may be associated with the complexity in structure of the substrate (i.e. extracellular polymeric substance and cell wall). Although the application of the pre-treatment to the sludge resulted in an improvement in the specific methane potential (T1, T4, T5), the lag-phase obtained from the pre-treated experiments is greater than the one obtained from the control systems. This behaviour may be explained as an indication of the microbial system needing time to adapt to the new acidic conditions or to the higher levels of salinity caused by the different conditions applied to the electrooxidation tests.

Table 6.2 Kinetic parameters of Gompertz adjustment

Sample	Pmax ($L_{methane}$ kg VS^{-1})	Rmax (mL d^{-1})	λ (days)
WAS	253±15	19.21±1.23	5.44±0.21
T1	288±12	16.15±1.42	7.30±0.23
T2	237±10	8.65±1.33	7.63±0.24
T3	162±6	4.52±0.89	6.40±0.15
T4	249±15	19.35±2.65	6.11±0.13
T5	298±13	10.36±1.89	9.25±0.28

6.3.3 Effect of electrooxidation post-treatment on sludge dewaterability and their stability

The effect of electrooxidaton applied as post-treatment was tested applying different conditions and using parameters such as particle size reduction and dewaterability characteristics to evaluate the effectiveness of the process. Figure 6.4 shows the enhancement obtained on the solubilisation of the organic matter compared with raw digestate. The values of organic content of the digestate sample were 417±12 mg L^{-1} TOC (measurement of the soluble fraction) and 1158±57 mg L^{-1} for soluble COD. The application of electrooxidation to the digestate sample led to a slight increment of TOC and COD measurements for the soluble fraction which was more noticeable after 1 h of post-treatment (P5). This enhancement is explained by the extended time of treatment applied (despite the lower value of current density (6.8 $mAcm^{-2}$) or conductivity associated with this test). The results obtained after 5 min of treatment did not shows significant differences between them having all of them a similar conductivity and value of current density, P1 (5.5 mA cm^{-2}), P2 (10.7 mA cm^{-2}) and P3 (11.7 mA cm^{-2}). Nevertheless, P4 treatment showed higher solubilisation compared with the previous treatments (P1 to P3) which is due to much higher values of current density 18.8 mA cm^{-2} and conductivity 16.6 mS cm^{-1} being applied for this test.

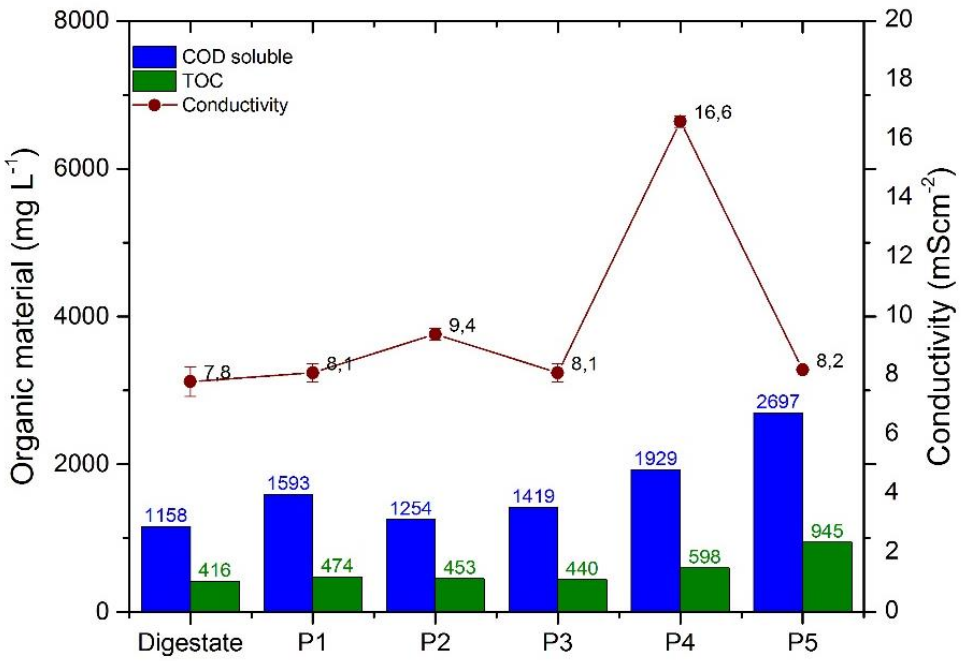


Figure 6.4 Organic material content before and after EO post-treatment of sludge digestate

Drying curves for evaluating water distribution are shown in Figure 6.5. Three zones are observed, for free water associated with solid particles, interstitial water (trapped inside interstitial spaces of flocs and microorganisms), and chemically bound water. The graph shows results obtained from digestate and the five electrooxidation conditions applied to digestate when using the electrooxidation process as post-treatment. Interpretation of these curves was adapted from Kopp and Dichtl (2001). Post-treatment P5 showed the faster loss of interstitial water compared with the other experiments. This result is to be expected since this treatment option had an excessive treatment time with a duration of 1 h whereas the other tests were evaluated for just 5 min. This higher duration also improved the release of interstitial and bound water requiring a much shorter time for the total elimination of water. However, the excessive energy demand that would suppose this post-treatment prevents from selecting it as the preferable option. Thus, treatment P4 with a treatment time of only 5 min is considered to show interesting results regarding the release rate of water and the time required for the drying of the sample.

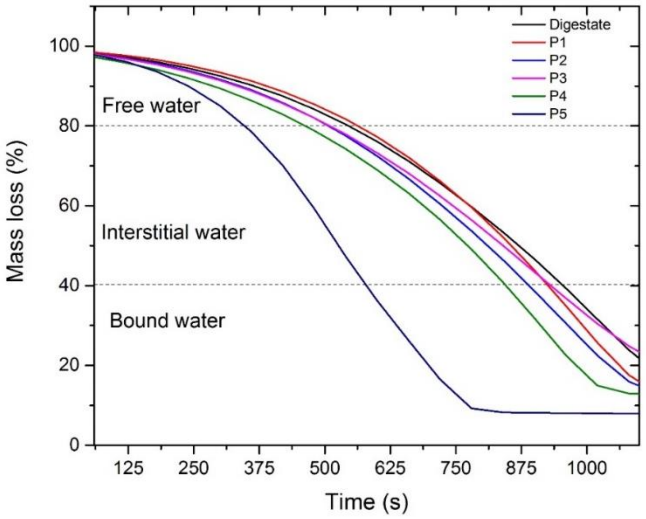


Figure 6.5 Effect of electrooxidation post-treatment on drying curves; zone interpretation at curve

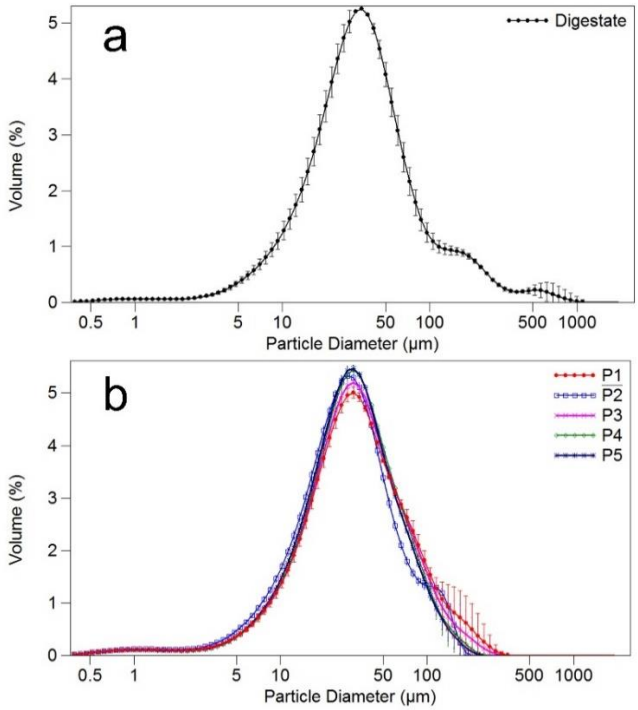


Figure 6.6 Effect of electrooxidation on particle size distribution for digestate (a) and treated samples (b)

Results from the particle size analysis are presented in Figure 6.6 a and b. Digested sample presented a main peak centered at around 30 µm and a dispersion of particles

of greater size with two lower peaks centered at around 200 µm and 500 µm, which may be explain by the presence of aggregates (Fig 6.6a). The disruption of bigger size flocs was successfully attained for any of the post-treatment as can be observed in Figure 6.6b. The particle size distribution profiles of the different samples obtained after treatments did not show any longer the peak centered at 500 µm and all of them presented a similar distribution with a small shifts to the left in the location of the main peak (regarding the scale of particle diameter). The post-treatment P4 (having higher current density) and P5 (maximum duration of electrooxidation treatment) produced particles of smaller sizes. This is observed by the lower values of the mean particle size parameter obtained (as shown in Table 6.3). In addition to the mean particle size, Table 6.3 also shows others indicators such as median particle size and specific surface area (SSA). The application of the post-treatment resulted in a significant increase in the value of SSA obtained for any of the conditions tested along with a decreased in particle size. No significant differences were found with the variance at pH or conductivity of the samples.

Table 6.3 Dewatering parameters after electrooxidation as post-treatment

Parameter	Current density (mA cm^{-2})	Mean (µm)	Median (µm)	SSA (cm^2mL^{-1})	VS removal (%)	TS (g kg^{-1})	CST_s (s)	SRF x 10^{12} (m kg^{-1})
Digestate	n/a	61.5±1.23	35.0±1.06	2874±57	n/a	21.8±0.65	>2000	4.17±0.05
P1	5.5±0.01	38.8±0.77	30.8±0.99	3539±65	8.8±0.01	19.4±0.50	914±30	3.75±0.06
P2	10.7±0.01	37.8±0.66	29.1±1.02	3758±45	9.2±0.03	19.0±0.56	873±25	3.24±0.04
P3	11.4±0.01	36.7±0.65	29.9±1.01	3752±68	9.8±0.02	18.8±0.68	753±28	3.00±0.08
P4	18.8±0.01	35.6±0.58	29.6±0.95	3803±65	9.7±0.03	18.5±0.63	625±26	2.89±0.05
P5	6.8±0.01	34.6±0.54	28.7±1.02	3934±50	22.2±0.02	12.0±0.59	348±35	2.02±0.03

Zeng et al. (2019) also observed a decreased and a reduction in the peak representing the volumetric percentage of particle size with increasing voltage applied (from 0 to 15V). This modification was explained by the fragmentation of sludge flocs and destruction of microbial cells leading to a decrease on aggregation and as consequence a lower mean particle size was attained for sludge samples.

After sludge stabilization, dewatering is required for the disposal of WAS indistinctly of its final use since is a way of reducing handling costs. The main parameters related with

sludge dewaterability are summarised also in Table 6.3. The removal of VS was higher with the increase of electrooxidation time from 5 to 60 min (P5). The post-treated sample exhibited a VS removal from 8.8 to 22.2 percent (calculated according equation 4), with the highest value corresponding to the P5 post-treatment. The electrooxidation treatment of the digestate have also impacted on CST and SRF showing a decrease in these values when comparing with those obtained for raw digestate. The highest values were derived from digestate sample thus indicating lower dewaterability (or filterability) of the sludge. Results elucidate a visible trend; the higher is the current density applied the higher is the dewaterability of the post-treated sample. Post-treatment P4 shows successful results with a duration time of just 5 min, thanks to the combined effect of pH increase (to 10 units) and higher current density. Post-treatment P5 shows the highest dewatering since the duration of the treatment had a significant impact on dewaterability parameters. However, the excessive duration of the post-treatment makes of this option an unfeasible strategy due the great demand of energy necessary. Different authors have studied the enhancement on dewaterability after the application of several types of treatments. Yu et al. (2009) used microwave irradiation to improve dewatering characteristics applying different energy ranges (by varying power and time); obtaining a reduction of the SRF from 5.39 x 10^9 m/kg for untreated sludge to 1.84 x 10^9 m/kg for the sample treated at 900 W. In a different study, Erdincler and Vesilind, (2000) carried out a pre-treatment employing thermal and ultrasound techniques among others. At these studies were able of disrupting flocs and disintegrate aggregates thus reducing the CST from 19.7 s to 15.2 and 17.1 s when thermal and ultrasound treatments were tested, respectively. In addition, advanced chemical oxidant reaction as Fenton treatment was also studied by Buyukkamaci (2004) as a technique for dewatering reporting a reduction of 44% in CST. In the present research, this particular parameter was improved reducing the CST from >2000 s to 625.8 for P4 and to 348.9 s for P5 post-treatment.

Raynaud et al. (2012) found that pH is also an important factor when evaluating particle size distribution of sludge and dewaterability characteristics. High pH values favored breakdown of EPS releasing fine particles, which are responsible of clogging problems in filtration media, whereas the decrease of pH promotes aggregation of flocs improving

filterability. Apparently, the filterability depends on the amount of supracolloidal substances and not directly on the particle size of the sample, as it was corroborated by the work of Karr and Keinath (1978). However, the modification of pH at large scale have a significant effect on operating costs and sets a new challenge to final disposal options. In the present study post-treatment P4 where the pH was set to 10 units presented good performance regarding dewaterability parameters even though the high value of pH tested.

6.3.4 Results of FTIR analysis

FTIR analysis were carried out to assess the effect of digested sludge stability after the application of electrooxidation as post-treatment. Figure 6.7 presents the spectra obtained from inoculum, digestate and those samples obtained when digested sludge was submitted to the different post-treatments (P1 – P5). Main absorbance bands and assignments of FTIR spectra has been performed using previously reported data for different digestates available in literature (Martínez et al., 2016, 2012; Provenzano et al., 2011).

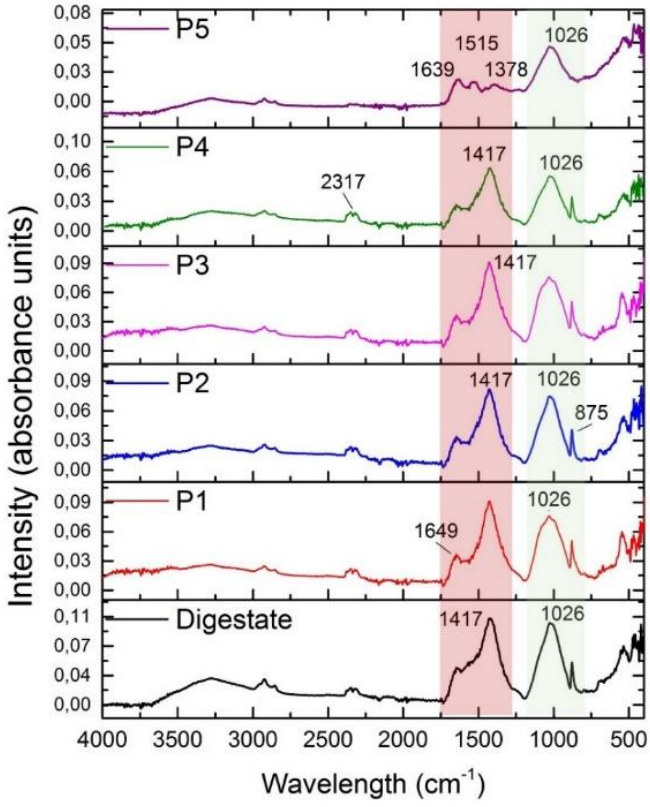


Figure 6.7 FTIR spectra of sludge samples before and after EO post-treatment

The digested sludge was characterised by a broad and mid-intense band in the region between 3920-2890 cm^{-1}. This band was attributed to O-H groups due to the stretching vibration of carboxylic, phenolic or alcoholic groups. Apparently, the O-H stretching vibration is due to intramolecular hydrogen bonds between cellulose. Also, it is characteristic of N-H vibration of amine and aromatic amine from proteins and peptides (Kataki et al., 2017; Kowalski et al., 2018; Provenzano et al., 2011). Visible bands at 2925 cm^{-1} and 2851 cm^{-1} were attributed to asymmetrical –C-H stretching vibration of aliphatic bonds in fats and lipids (Fels et al., 2013; Cuetos et al., 2010). Doublet band at 2345 cm^{-1} was ascribed to unavoidable presence of CO_2 in FTIR chamber (Hong et al., 1995). Visible bands were observed at 1633 cm^{-1} and 1648 cm^{-1} which is related to aromatic C=C bonds, C=O in primary amides, ketone and quinone groups (Martínez et al., 2015; Provenzano et al., 2011; Kataki et al., 2017).

The high-intense bands at 1416 cm^{-1} in Figure 6.6 and at 1435 cm^{-1} were attributable to C-H stretching in aliphatic structure and the intense band at 1029 cm^{-1} and 1027 cm^{-1} may be addressed to C=O stretching in polysaccharides (Ramamurthy & Kannan, 2007; Naumann et al., 2010). The band at 875-876 cm^{-1} was assigned to the presence of inorganic carbonates and the region between 600-500 cm^{-1} which is characteristic of the absorption of phosphates (Smidt et al., 2002).

The differences between the spectra obtained from the digestate and post-treated digestate samples were associated with the lower intensity recorded for the main bands for post-treated samples. Electrooxidation enhanced the stability of digested sludge, this is especially true for those treatments having more severe conditions, as it is P4 (higher current density) and P5 (extended time of treatment). A diminish of signal bands were observed at 1639 cm^{-1} (related to aromatic C=C bonds or C=O in primary amides) and 1026 cm^{-1} (addressed to C=O stretching in polysaccharides). The peaks at 1417 cm^{-1} (attributable to aliphatic structure) and 875 cm^{-1} assigned to the presence of inorganic carbonates were no longer visible for the sample obtained from P5 post-treatment.

6.4 CONCLUSIONS

Electrooxidation was studied as pretreatment and post-treatment alternative for improving digestion performance and sludge handling. In the first case, electrooxidation

proved to be a suitable alternative for enhancing hydrolysis of sewage sludge by favouring the solubilisation of organic matter and hence aiding in anaerobic degradation of complex compounds. The application of electrooxidation on WAS previous to anaerobic digestion caused cell destruction releasing cell inner materials making them accessible to anaerobic microflora and facilitating degradation. Methane yield was increased 18% as result of the pretreatment in alkaline condition (T5).

On the other hand, the use of electrooxidation as post-treatment of digested WAS proved to have a significant effect on sludge rheological behaviour, particle size reduction and dewaterability properties, increasing the specific surface area and improving parameters such as capillary suction time (CST) and specific resistance to filtration (SRF). Future work will aim to evaluate the benefits of these later characteristics and the effect on the energy demand on a WWTP.

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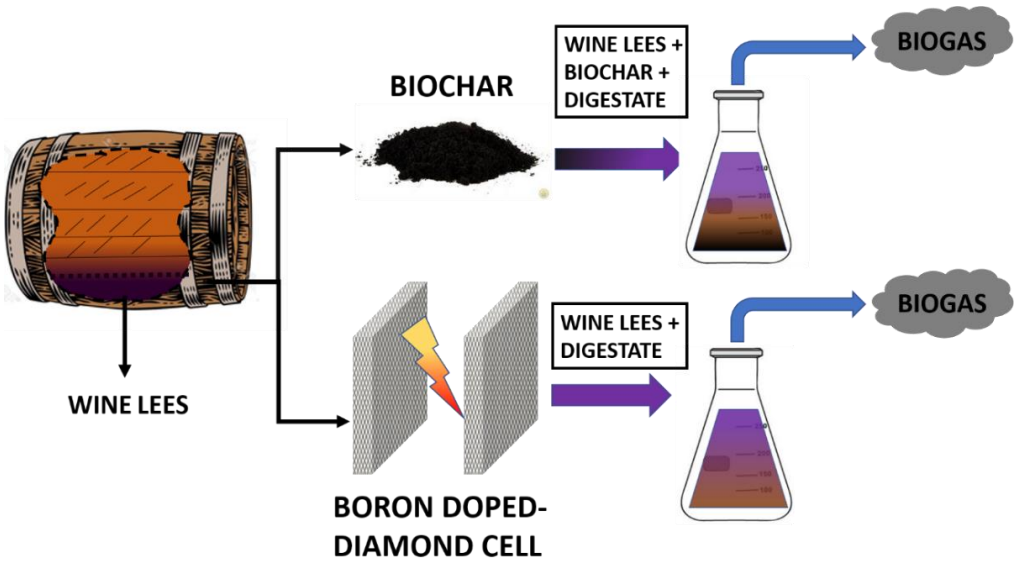
Capítulo/Chapter 7

Anaerobic Digestion of Wastes from the Wine Industry: Evaluation of Electrooxidation as Pre-treatment and Char Addition for Enhancing Digestion



Abstract

Wine-making industry has an important in the agro-industrial sector in southern European countries. Couple to the process of wine making is the production of a large amount of wine lees that that present a difficult management. The treatment of this material by anaerobic digestion shows malfunctioning due to its acidity, high content of chemical oxygen demand (COD) and the presence of refractory compounds such as polyphenols and melanoidins. It is widely contrasted that addition of biochar and electrooxidation process are methods that reduce the bio-toxic compounds that may be present in some wastes. In this work, a boron-doped diamond cell worked at current densities between 11-18 mA cm^{-2} in the range of 0.08-1.50 h and the use of soft wood pyrolised at 550 ˚C has been studied to enhance anaerobic digestion. After 47 days, electrooxidation achieved an improvement of biogas production between 28.8-50% and a reduction of COD up to 20% for an extended period. In contrast, biochar addition did not show any effect on biogas production after 25 days of experiment. The presence of Biochar in the digestion reactor did not provide any aid in the degradation.

Keywords: Wine lees, complex substrate, advanced oxidation process**,**

7.1 INTRODUCTION

Circular economy can be defined as the type of economy that maintains the value of products, materials and resources along with the minimisation in the generation of wasted material. All these characteristics suppose an essential task to develop a sustainable economy. This economy should be characterised by using resources efficiently thus achieving the status of low carbon economy but still being competitive. Food processing industries are one of the areas where the tools and strategies of the circular economy are implemented.

In Mediterranean countries, millions of tons of wastes from wine industries are produced every year. The wine sector involves numerous stages of production and processing which causes many impacts on the environment (Ruggieri et al. 2009). Wine industry generates a number of wastes and by-products. These materials include wine pruning, grape stalks, grape pomace and grape seeds, lees and wastewater. The different waste streams or by-product can find valorisation as fertilizer, animal feed, or fuel (Arvanitoyannis et al. 2006). Liquids streams of wine industry such as vinasse or lees are obtained after distillation of ethanol fermentation broth. Wine lees (WL) are the deposit of dead yeast and other particles that precipitate to the bottom of the vat after fermentation and aging. The dissemination of liquids streams of wine industry in soil and water bodies is no longer possible due to the negative effect on the environment (Janke et al. 2015). Among the different treatment technologies, biological processes such as anaerobic digestion are commonly the preferred option based on the environmental benefits offered by this treatment.

Anaerobic digestion is a mature technology widely used for energy recovery from waste streams with high organic content (Martínez et al. 2018a). However, this technology has two important limitations. On the one hand, the sensitivity of methanogenic microorganisms to environmental factors such as organic overloading or steep pH changes. These factors usually lead to volatile fatty acids (VFAs) accumulation and threatens the stability of the process. On the other hand, the recalcitrance or low degradability of certain organic compounds which restrict the use of AD only to those wastes easily degradable.

Chemical composition and the main characteristics of liquids streams of wine industry may differ depending on the feedstock and conditions of ethanol production process. Stillage is an acidic effluent (pH around 3.5–5) which has a dark brown colour and high organic content (chemical oxygen demand – COD range between 10 and 80 g L^{-1}) (España-Gamboa et al. 2011; García García et al. 1997). Some organic and inorganic compounds present in lees and vinasses are acetic acid, glycerol, lactic acid, ethanol, potassium, nitrogen, phosphates, calcium and sulphates. In addition, recalcitrant substances like phenolic compounds and melanoidins are also contained in this waste stream.

Some of these compounds may be toxic to many microorganisms involved in the anaerobic processes. Despite of this, anaerobic digestion has been studied as a feasible alternative for the treatment of lees and vinasses. Some authors have reported on the toxic effects on the anaerobic microbial consortium when digesting effluents from wine industry (Pandey et al. 2003). An interesting pre-treatment option for the treatment this type of effluent may be the use of advanced oxidation processes to attain a partial conversion of inhibitory or recalcitrant compounds previous to the biological stage. The electrochemical oxidation is expected to decrease the toxicity of some organic compounds therefore favouring subsequent degradation of the substrate by the anaerobic consortia.

Advanced oxidation processes (AOPs) are characterised by the formation and utilisation of radical species (typically hydroxyl radical) to cause the chemical destruction of recalcitrant and toxic contaminants. This process has been used as an effective and alternative treatments for winery wastes and complex wastewater, especially when non-biodegradable compounds are present (Candia-Onfray et al. 2018; Serna-Galvis et al. 2019).

AOPs include electrochemical oxidation, Fenton's oxidation, photo-Fenton, ozonation and photolysis/photocatalysis (Deng & Zhao 2015; Martínez-Huitle & Panizza 2018). However, some of these treatments shows significant drawbacks such as high operational costs, generation of iron sludges, ozone losses to atmosphere and difficult removal/ recovery of heterogeneous catalysts.

Electrochemical oxidation, also known as electrooxidation or anodic oxidation is an attractive technology due to its ability to treat under moderate conditions (ambient temperature and pressure) toxic and/or complex organic pollutants without the need for chemicals, leaving therefore no toxic residues in the effluent stream. The electrochemical oxidation of organic compounds in wastewater treatment can be achieved in different ways, by direct electrooxidation (EO), where electron transfer occurs at the electrode surface without participation of other substances and indirect EO, where organic pollutants are oxidized through the mediation of some electroactive species generated at the anode surface (Figure 7.1):

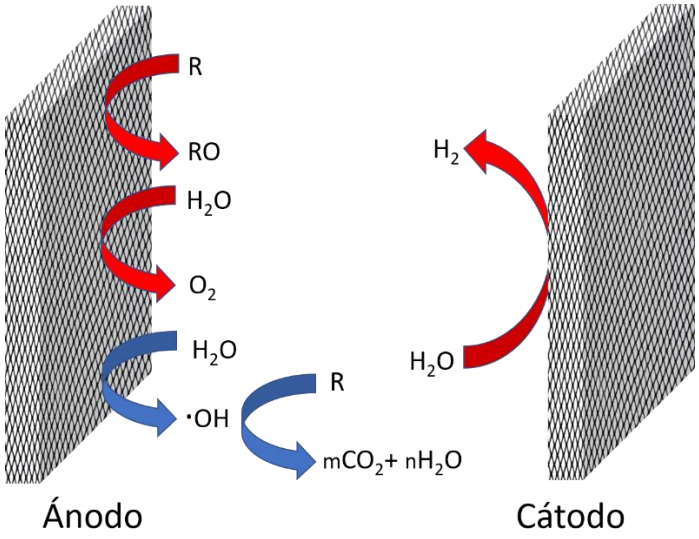


Figure 7.1 Oxidation direct reactions (red arrows) and indirect reactions (blue arrows) on BDD electrodes during oxidation of organic compounds (R)

Anodic oxidation is theoretically possible at low potentials, before oxygen evolution is observed, but under these conditions, the surface is rapidly deactivated due to the deposition of a polymeric layer on the anode surface, that is commonly called the poisoning effect (Martínez-Huitle & Panizza 2018). This deactivation depends on the adsorption properties of the anode surface and the concentration and the nature of the organic compounds. The poisoning effect can be avoided by performing the oxidation in the potential region of water discharge due to the participation of intermediates of oxygen evolution as it is represented by reactions 7.1 and 7.2:

$$M + H_2O \rightarrow M(\bullet \mathrm{OH}) + H^+ + e^- \quad (7.1)$$

$$R + M(\bullet \mathrm{OH}) \rightarrow \mathrm{M} + \mathrm{C}O_2 + H_2O \quad (7.2)$$

The boron-doped diamond (BDD) electrodes are preferred because the weak BDD-OH interaction and higher O_2-overpotential, leading to the generation of higher amounts of reactive physisorbed BDD(OH) radicals, which mineralises aromatics and aliphatic contaminants more effectively than other anodes such as Pt and PbO_2 (Farinos & Ruotolo 2017; Peralta-Hernández et al. 2016; Vasconcelos et al. 2016).

On the other hand, new strategies such as biochar addition, are being analysed in order to avoid inhibitory conditions in anaerobic digestion. Different authors observed that biochar addition to anaerobic digestion may reduce the negative effects of toxic compounds, promotes the immobilization of microbial biomass and reduces the lag-phase, due to the microporous structure and electro-donating capacity (González et al. 2018; Liu et al. 2014; Luo et al. 2015; Martínez et al. 2018b; Qin et al. 2020).

Sun et al. (2019) evaluated the addition of biochar in the anaerobic digestion in beer lees revealing that biochar addition led a huge improvement of 82.9 and 82.6% in the cumulative methane production and yield, respectively in mesophilic conditions. Zhang et al. (2020) assessed the impact of biochar in the anaerobic digestion operating in semicontinuous at mesophilic and thermophilic conditions with digesters being fed by a mixture of a 25% of food waste and 75% of algal biomass. Mesophilic conditions provided higher methane yields and an improvement between 12-54% compared to the control system was observed. Wei et al. (2020) employed corn stover biochar to maximize the energy recovery and reduce the solid in primary sludge. The biochar addition improved methane content increasing from 67.5 to 87.3%, and improved methane production by 8.6-17.8%. Indren et al. (2020) evaluated the efficiency of biochar in the anaerobic digestion of poultry manure. The addition of woody biochar provided an augmentation of 32% in the methane yield compared to control digesters obtaining an 44% increase in the peak daily methane yield and a reduction of 33% in the lag-phase compared to controls.

This works aims to integrate AOPs into anaerobic digestion for the treatment of winery wastes to close the loop under the Circular Economy framework. This works attempts to the reduction of waste streams and their valorisation to produce clean energy necessary in the process to achieve the status of energy-efficient and zero waste industry.

The experiments evaluate the digestion of wine lees, using BDD anodes as electrochemical oxidation pre-treatment prior to anaerobic digestion. The addition of biochar is also evaluated in a second experimental stage with the aim of enhancing biogas yields. This study was carried out in a collaboration between University of León and Polytechnic University of Turin.

7.2 MATERIALS AND METHODS

7.2.1 Substrate characterisation

Wine lees (WL) were sampling from a wine exploitation situated in Cuneo, in the Italian region of Piedmont. Wine lees characterisation is shown in Table 7.1.

Table 7.1 Characterisation of inoculums and substrate

Parameters	**WL**
pH	3.71 ± 0.02
Conductivity (mS cm^{-1})	2.37 ± 0.03
NH^{+4}-N (mg L^{-1})	17.84 ± 0.89
Total organic carbon (mg L^{-1})	67135 ± 3356
Chemical oxygen demand (g L^{-1})	287 ± 14
Nitrates (g L^{-1})	3.22 ± 0.16
Organic matter (%)	8.87 ± 0.44
Kjeldahl nitrogen (%)	0.09 ± 0.01

7.2.2 Electrooxidation pre-treatment

Electrooxidation (EO) pre-treatment of wine lees were conducted in batch mode using a 70 mL cell pro aqua Diamond Electrodes, Niklasdorf, Austria which contained two

boron-doped diamond (BDD) electrodes used as anode and cathode. These BDD electrodes had 42 cm^{-2} of effective surface and were situated at a distance of 2 mm (Figure 7.2).

Figure 7.2 Proaqua cell coupled to a power source and controlled by software for wine lees treatment

Electrooxidation experiments were carried out at room temperature (25 ± 1 ˚C) with a voltage applied of 25 V (equivalent to current densities in the range of 11 to 18 mA cm^{-2}) the experimental conditions were chosen according to previous experiments for high organic load substances (data not shown).

Electrooxidation experiments were labelled according with the sample (Wine Lees *WL* for control sample) and for treated samples, adding the time of electrooxidation applied. The treatment time was in the range from 0.08 to 1.5 h (5 to 90 min). Therefore, assay denoted as WL_0.08 h, refers to wine lees treated for 0.08 h.

7.2.3 Biochar addition

In order to study the effect of materials addition on anaerobic digestion a soft wood biochar was used. The biochar (SWP550) was obtained after biomass pyrolysis at 550 ˚C. This material was provided by UK Biochar Research Centre, Edinburgh, United Kingdom. Aside from physical properties, biochar also presents relevant chemical characteristics.

Chemical contribution of biochar to improve the anaerobic digestion was compared to a chemically inert material such as pumice stone. Granular pumice stone was purchased in Bonsai Shopping. Both materials were powdered with an agate mortar and were added separately to digesters at a concentration of 10 g L^{-1}.

Digesters were labelled as WL for raw substrate, Bio_WL regarding to wine lees supplemented with 10 g L^{-1} of soft wood biochar and as PS_WL for reactor treating wine lees supplemented with 10 g L^{-1} of pumice stone.

7.2.4 Anaerobic digestion

Digested sludge used as inoculum was obtained from wastewater treatment plant (WWTP) of Biella (Italy). The inoculum had a total solid (TS) content of 31.10 ± 0.05 g L^{-1} with volatile solid (VS) content of 43.1% (expressed as percentage based on TS content). Digestion systems were labelled according with EO conditions and additives employed.

Each experiment was carried out in 250 mL vessels in triplicate. These were connected to Drechsel bottles that contained an acid saline solution with methyl orange as displacement solution. Each Drechsel bottle was also connected to a 2 L sampling inert-foil gas-bag (Supelco, Pennsylvania, USA). Vessels were filled with inoculum and substrate at a ratio of 1:1 (substrate: inoculum, expressed in volatile solids (VS)) and were buffered with $NaHCO_3$ against pH changes. Tap water was added to complete the set volume. Gas production and composition were periodically measured. The temperature was controlled by a water bath set at 38 ± 1 ˚C. Agitation was provided by means of magnetic stirrers between 250-300 rpm.

7.2.5 Analytical techniques

Total solids (TS), volatile solids (VS), pH, conductivity and ammonium content were measured in accordance with American Public Health Association Standard Methods (APHA 2005). Nitrogen concentration was measured using the Kjeldahl method (Ministerio de Agricultura, Pesca y Alimentación 1994). Organic matter was analysed in accordance with the Walkley-Black method (Walkley & Black 1934).

Total organic carbon (TOC) was determined performed by a high-performance analyser multi N/C® by sample combustion at 980 ˚C, while inorganic carbon (IC). TOC was

calculated by the difference between total carbon and inorganic carbon. Chemical Oxygen Demand was obtained by using a commercial kit tubes LCK 514 in the range of 1000-10000 mg L^{-1} and, subsequently, measured by a high-performance spectrophotometer DR 3900. Kit tubes and spectrophotometer were supplied by Hach Lange.

Volatile fatty acids (VFAs) were measured using the same gas chromatograph and a flame ionisation detector (FID) equipped with a Nukol capillary column (30 m x 0.25 mm x 0.25 mm) from Supelco. The carrier gas was helium. The injector and detector temperatures were 220 ˚C and 250 ˚C, respectively. The oven temperature was set to 150 ˚C for 3 min and increased to 180 ˚C with a ramp of 10 ˚C min^{-1}. The detection limit for VFA analysis was 5.0 mg L^{-1}. The system was calibrated with a mixture of standard volatile acids from Supelco (for the analysis of fatty acids C2 to C7). Samples were previously centrifuged (10 min, 3500 x g) and the supernatant filtered through 0.22 and 0.45-mm cellulose filters, both methods: biogas composition and VFAs determination are described by (Martínez et al. 2018a).

Alcohols content was acquired by gas chromatography by Agilent Technologies 7890B GC System equipped by an Agilent CP97713 column (25m x 0.25 mm x 0.2 μm) and an FID detector. The carrier gas is hydrogen that flows at 30 mL min^{-1}.

Changes in the absorbance were recorded to assess the extent of decolourisation using as parameter the pigment. Reduction. Absorbance was scanned in the range of 400–800 nm on a Beckman DU640 spectrophotometer based on the colour associated with melanoidins which is recorded at 475 nm (Dwyer et al. 2009; Jiranuntipon et al. 2008). Total polyphenols (TP) were measured by colourimetry at 760 nm on a Beckman DU640 spectrophotometer using the Folin–Ciocalteau reagent as described in Martinez et al., (2018b).

Biogas was measured by gas chromatography by a Varian CP-3800 GC model, equipped with a thermal conductivity detector (TCD). It determined the H_2, CH_4, CO_2, N_2 y O_2 content. The chromatograph relies on HayeSep Q 80/100 4 m length column and Molecular Sieve (1.0m x 1/8’’x 2.0 m) molecular sieve column. The carrier gas was helium and it operated at 331 kPa and at 50 ˚C.

7.3 RESULTS AND DISCUSSION

7.3.1 Electrooxidation of wine lees

Wine lees were pretreated at BDD anodes from 5 min to 90 min (0.08h to 1.5h). The evolution of parameters measured is presented in Table 7.2. The removal attained of organic matter and decolourisation are shown in Figure 7.3a-d. A rapid decay in the content of the different parameters measured was observed. VFA concentration is as an exception to this trend, experiencing an increasing behaviour with time.

Table 7.2 Influence of EO in the anaerobic digestion

Parameters	**WL**	**WL_0.08 h**	**WL_0.5 h**	**WL_1.0 h**	**WL_1.5 h**
pH	3.68 ± 0.02	3.76 ± 0.02	3.57 ± 0.02	3.33 ± 0.02	3.43 ± 0.02
Conductivity (mS cm^{-1})	2.34 ± 0.03	2.53 ± 0.03	2.61 ± 0.03	2.06 ± 0.03	2.08 ± 0.03
Total organic carbon (g L^{-1})	68.11 ± 2.89	62.57 ± 3.13	59.94 ± 2.99	52.49 ± 2.62	50.79 ± 2.83
Inorganic carbon (mg L^{-1})	700 ± 21	917 ± 45	903 ± 46	929 ± 45	910 ± 47
Chemical oxygen demand (g L^{-1})	284 ± 9	268 ± 10	263 ± 12	252 ± 7	212 ± 8
Volatile fatty acids (mg L^{-1})	1982 ± 99	2129 ± 106	2456 ± 122	2535 ± 126	3221 ± 161
Acetic acid (mg L^{-1})	1090 ± 55	1172 ± 57	1357 ± 68	1395 ± 70	1772 ± 89
Colour at 475 nm	0.79 ± 0.04	0.65 ± 0.03	0.63 ± 0.01	0.61 ± 0.03	0.60 ± 0.04
Ethanol (g L^{-1})	39.22 ± 1.96	39.93 ± 1.99	33.35 ± 1.67	24.06 ± 1.22	27.11 ± 1.35
Polyphenols (mg GAE L^{-1})	58.02 ± 2.90	39.31 ± 3.11	27.69 ± 2.84	23.03 ± 3.51	35.49 ± 4.01

For all cases studied, TOC and COD decreased progressively under prolonged electrolysis time achieving 20% of organic material removal (Figure 7.3a). Nevertheless these results are lower comparing with other studies where electrooxidation treatment of industrial food wastes is evaluated (Alvarez-Pugliese et al. 2016; Vilar et al. 2018).

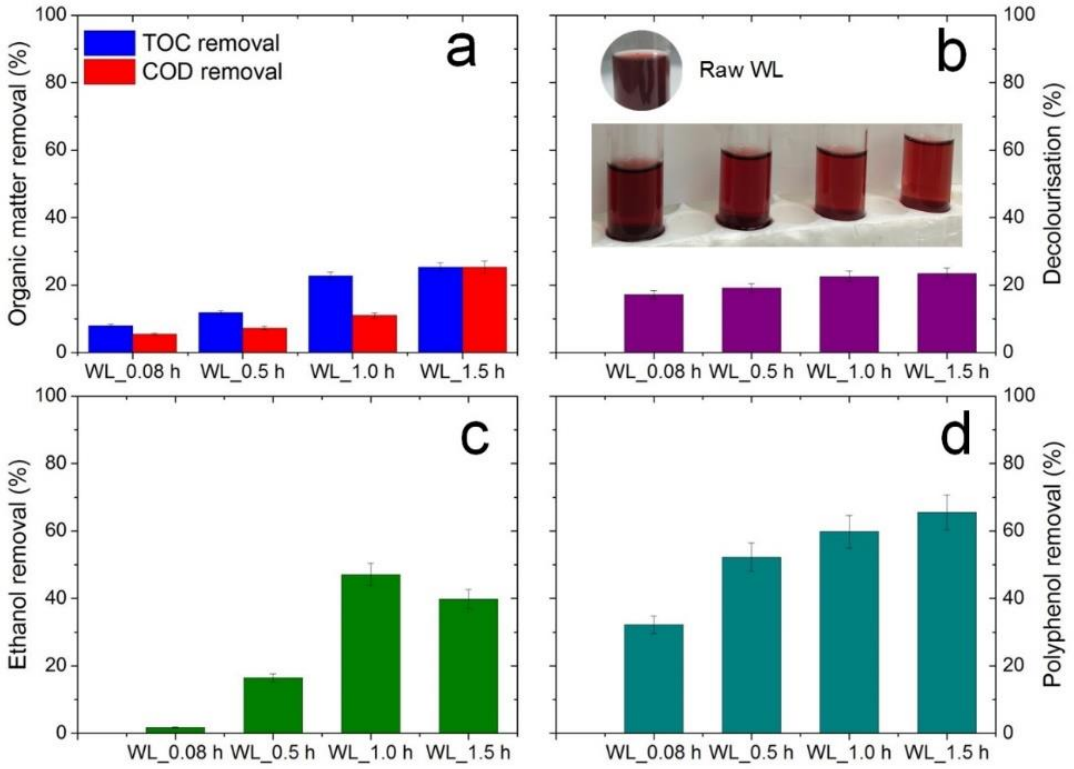


Figure 7.3 Organic matter and colour removal achieved after electrooxidation experiments compared with untreated wine lees: Chemical oxygen demand – COD and Total organic carbon – TOC (a), Decolourisation recorded at 475 nm (b), Ethanol (c) and Total polyphenols (d)

The experiments of Candia-Onfray et al., (2018) treating winery wastewater using BDD electrodes reach an almost complete mineralization of organic matter when applying 20, 40 and 60 mA cm^{-2} at 0.1 L of aqueous solutions containing 3490 mg L^{-1} of COD. In the present study, current density was lower (18.8 mA cm^{-2}) and wine lees sample showed higher content of organic matter and lower conductivity (see Table 7.2) which reduced the EO performance when using BDD cell.

The characteristic dark colour of wine lees is provided by the presences of melanoidins, which are a recalcitrant pigment produced by the Maillard reaction between amino and carbonyl groups present in the organic matter and can be measured using a spectrophotometer (Jiranuntipon et al. 2008; Sánchez-Galván et al. 2015). A decolourisation of the sample was achieved thanks to the oxidation of these molecules as it is shown in Figure 7.3b.

Ethanol and polyphenols content decrease for subsequent EO times achieving higher removal (Figure 7.3c-d), this is due to its oxidation and volatilisation of these compounds in the sample.

Figure 7.4 shows the increase experienced in VFA concentration after pretreatment. Three types of short-chain volatile fatty acids were mainly measured (acetic acid, propionic acid and butyric acid) with the highest concentration corresponding to acetic acid. Electrooxidation pretreatment could contribute to the conversion of complex organic molecules in wastes into simpler forms that become easily degradable for bacteria.

Acetic acid is the direct substrate for methanogens which is transformed into methane through methanogenesis, thus, the enhancement on the production of acetic acid observed is directly associated with the increase in methane production (see concentration in Table 7.2). Nevertheless, the concentration of this acid must be within values that do not cause inhibition and deterioration of the anaerobic digestion process.

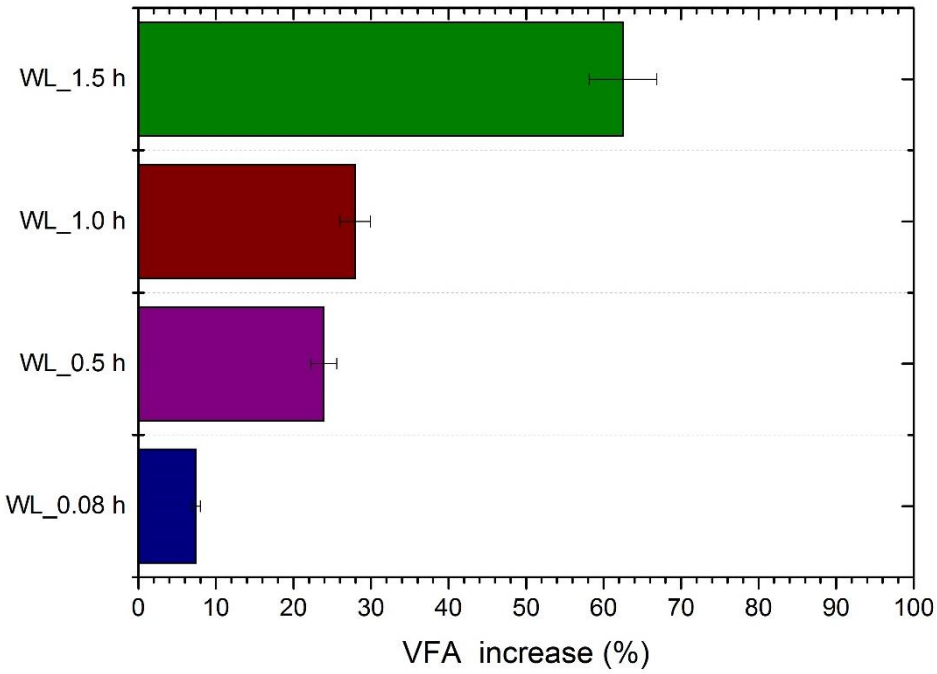


Figure 7.4 Increase at volatile fatty acid concentration of the treated samples obtained from electrooxidation experiments compared with untreated wine lees

7.3.2 Effect of EO pre-treatment on biogas production

Different authors studied the degradation of complex wastewaters (e.g. textile dye wastewaters, paper mill waste, toxic pesticide pollutants) and investigated the adequacy of coupling electrooxidation pretreatments and biological treatments.

The results obtained from batch digestion tests are presented in Figure 7.5 for the digestion of wine lees (WL) and treated wine lees using the BDD electrode. The cumulative biogas production curve makes evident differences for the pretreated systems when compared to the raw digestion system. Better performance was observed with regard to the final volume obtained and also when comparing the gas production rate during the whole experiment.

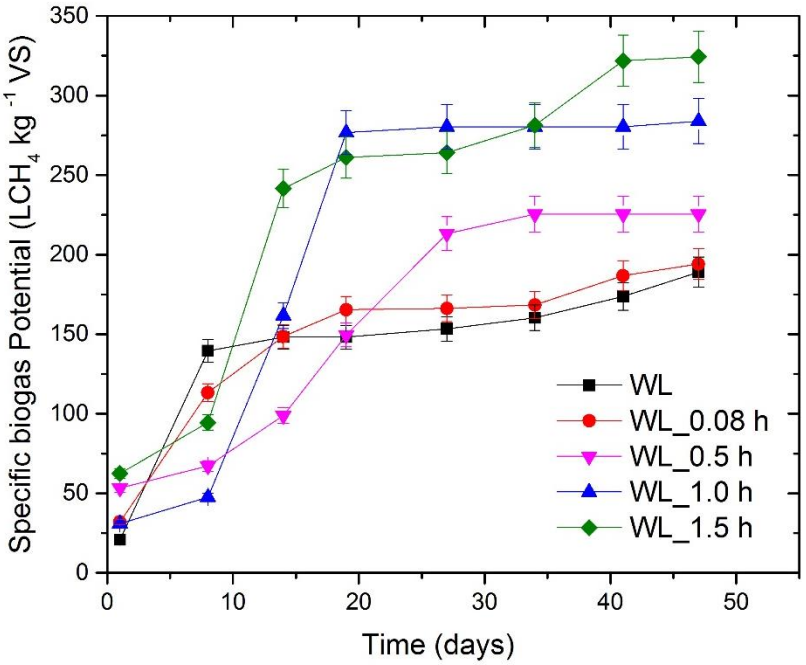


Figure 7.5 Cumulative specific biogas potential of the treated samples obtained from electrooxidation experiments compared with untreated wine lees

Volatile fatty acids are inhibitors for AD processes in concentrations above 50-250 mg L^{-1} (Khanal 2008). Nevertheless the inhibition of the acidogenic process is not only related to the VFA concentration but also to the molecular form of the acid, dissociated or undissociated which depends on pK, pH is a key fact for inhibition phenomena; undissociated forms can freely enter the cytoplasm through the membrane and be metabolized to proceed to further AD process phase, while the cell membrane remains impermeable to the dissociated ion (Infantes et al. 2012). Although the AD tests in this study started with a relevant concentration of VFAs (Figure 7.4), pH was adjusted to 7.0 and no inhibition was observed in biogas production (Figure 7.5).

The inhibitory range of polyphenols has been also studied, for recalcitrant materials and other complex organic wastes, an extra disintegration process is necessary, either as a prior process or even during fermentation (Patinvoh et al. 2017).

Apparently, the substrate employed in this study contains a high content of polyphenols that may hinder the anaerobic digestion. However, the electro oxidation pretreatment

achieved a reduction between 25 to 60% of elimination of this substance and no inhibition of the anaerobic system was observed (see Table 7.2 and Figures 7.3 and 7.5).

7.3.3 Effect of biochar addition for biogas production

Valorisation of winemaking by-products has been recommended and understudy (Devesa-Rey et al. 2011). Valorisation of winery wastes through anaerobic digestion has been carried out in mesophilic and thermophilic conditions (Rodríguez et al. 2007; Da Ros et al. 2016). Amongst winery wastes (grape marc, stalks or grape pomace) wine lees have shown a high potential for valorisation regarding its high nutritional value and biodegradability (~53%) (Hungría-Estévez et al. 2020). Hungría-Estévez et al. (2020) demonstrated good results in the anaerobic digestion of wine lees providing a mean of CH_4 production of 332 ± 37 mL STP CH_4 g VS^{-1}. However, further research is required to stabilise the process and controlling the such abundant and polluting waste generated.

The use of low cost adsorbents such as biochar may become a feasible solution to avoid inhibitory conditions in anaerobic digestion. In addition, it should be borne in mind that biochar is frequently produced from agro-industrial wastes; as such, this approach for preventing inhibition in AD results in a holistic valorisation, thus allowing the integration of biological and thermal processes.

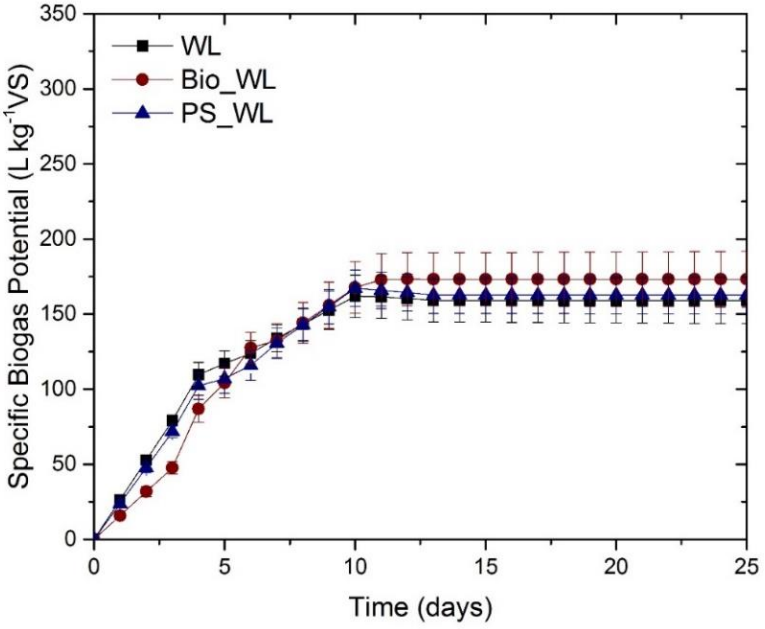


Figure 7.6 Evolution of specific biogas production of WL, Bio_WL and PS_WL for 25 days

Physicochemical properties of biochar such as large surface area and porosity favours strengthen metabolism of microbes, increases the contact between enzymes and substrates and reducing the phytotoxicity of organic substrates (Pan et al. 2019; Shen et al. 2020; Wang et al. 2017). Nevertheless, a first approach to an evaluation of biochar addition to the anaerobic digestion of wine lees did not show significant differences for biogas production. After 25 days of digestion the SBP obtained was around 150 L kg SV^{-1} for all the system (see SBP in Figure 7.6). The evaluation time was not enough to observe changes in the process.

7.4 CONCLUSIONS

Considering the complex nature of wine lees, a strong oxidation pre-treatment appears crucial to improve biogas production and support methanogenesis. The combined configuration of electrooxidation followed by anaerobic digestion showed an effective mineralisation of the organic matter contained in the wine lees sample. The partial oxidation of recalcitrant organic substances enhanced the methane production during anaerobic digestion. Nevertheless, further research is necessary to investigate in details the operative conditions that could assure a methane production that allows the achievement of energy self-sufficiency for the digestion system. Secondly, the experimental approach explored in this research regarding biochar addition did not show positive effects on AD under mesophilic conditions as it is reported in literature. The investigation on the role of biochar as a mere physical support for AD biomass did not show any results for the time analysed. Wine lees are a critical substrate, highly biodegradable but full of inhibitors and strongly acidic. It is necessary to explore operative conditions that could prevent overloading of digestion systems and effectively support methanogenesis.

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Capítulo/Chapter 8

Conclusiones Generales/ General Conclusions

De este estudio se han obtenido las siguientes conclusiones generales:

Referidas a la mejora de la digestión anaerobia de lodo de depuradora mediante la adición de hidrógeno

El beneficio de adicionar hidrógeno se asoció con el aumento de la producción de biogás debido a su conversión en acetato y posterior transformación en metano. Este proceso no condujo a un alto enriquecimiento en la composición del biogás, pero aumentó la productividad del digestor en términos de la producción volumétrica de gas. Este hallazgo se explica porque la vía hidrogenotrófica no se vio favorecida con la inyección de hidrógeno. La captación de hidrógeno fue llevada a cabo en gran medida por la comunidad eubacteriana, promoviendo el enriquecimiento de metanógenos acetoclásticos.

La sustitución de un sistema convencional de lodos activos por un sistema bioelectroquímico condujo a una reducción significativa de la demanda de energía, pero este enfoque no garantizó por sí mismo el estado de autosuficiencia energética. La menor disponibilidad de materia orgánica en el digestor anaeróbico se tradujo en una menor recuperación de energía afectando negativamente dicho balance.

Referidas al uso de cáscara de almendra pirolizada como electrodo en celdas de electrolisis microbiana

Los resultados ofrecen una alternativa a la producción de electrodos para sistemas bioelectroquímicos a partir de materiales de bajo coste, ya que el rendimiento evaluado fue comparable al de los electrodos de fieltro de carbono. Los resultados del estudio microbiológico revelaron una estructura y composición taxonómica similar entre el electrodo de cáscara de almendra pirolizada y el electrodo de fieltro de carbono. Se confirmó la buena conexión eléctrica bacteria-electrodo que permite aumentar la eficiencia en la transferencia de electrones entre microorganismos y dicho material.

Referidas a la evaluación de la electrooxidacion como pre- y post-tratamiento para la mejora de la digestión anaerobia y la estabilización de lodos activados.

Se estudió la electrooxidación como pre-tratamiento y post-tratamiento para mejorar el rendimiento de la digestión y la gestión de lodos. En el primer caso, la electrooxidación

demostró mejorar la hidrólisis al favorecer la solubilización de la materia orgánica y, por lo tanto, ayudar en la degradación de los compuestos complejos. La aplicación de la electrooxidación como pretratamiento causó la destrucción de las células liberando su material interno, haciéndolo accesible a la microflora anaeróbica. El rendimiento de metano aumentó un 18% como resultado del pretratamiento bajo condiciones alcalinas.

Por otra parte, la utilización de la electrooxidación como postratamiento del digerido demostró tener un efecto significativo en el comportamiento reológico de los lodos, la reducción del tamaño de partícula y las propiedades de deshidratación, aumentando la superficie específica y mejorando parámetros como el tiempo de succión capilar (CST) y la resistencia específica a la filtración (SRF). La labor futura tendrá por objeto evaluar los beneficios de estas últimas características y el efecto en la demanda de energía en una EDAR.

Referidas a la digestión anaerobia de subproductos derivados de la industria vinícola: Evaluación de electrooxidación como pre-tratamiento y adición de char para mejorar la digestión

Teniendo en cuenta la compleja naturaleza de las lías del vino, un fuerte pretratamiento de oxidación parece ser crucial para mejorar la producción de biogás y apoyar la metanogénesis. La configuración combinada de electrooxidación seguida de digestión mostró una efectiva mineralización de la materia orgánica contenida en la muestra de lías de vino. La oxidación parcial de las sustancias orgánicas recalcitrantes aumentó la producción de metano.

El enfoque experimental explorado en esta investigación en relación con la adición de biochar no mostró efectos positivos en la digestión contrario a los resultados encontrados en la literatura. La investigación sobre el papel del biochar como mero soporte físico de la biomasa anaerobia no mostró resultados favorables. Las lías del vino son un sustrato crítico, altamente biodegradable pero lleno de inhibidores y fuertemente ácido. Es necesario explorar las condiciones operativas que podrían prevenir la sobrecarga de los sistemas de digestión y apoyar eficazmente la metanogénesis.

The following conclusions can be drawn from the present study:

Regarding to improving anaerobic digestion of sewage sludge by hydrogen addition: analysis of microbial populations and process performance

The benefit of adding hydrogen to sludge digestion was associated with an increase in biogas production due to the conversion of hydrogen into acetate and its subsequent transformation into methane. This process did not lead to a high enrichment in the composition of biogas but increased the productivity of the digester in terms of the volumetric production of gas. This finding was explained by the hydrogenotrophic route which was not favoured for the injection of hydrogen. Hydrogen uptake was largely carried out by the Eubacterial community, promoting the enrichment of acetoclastic methanogens.

Replacing a conventional active sludge system with a bioelectrochemical system led to a significant reduction in energy demand, but this approach did not in itself guarantee the state of energy self-sufficiency. The lower availability of organic matter in the anaerobic digester resulted in less energy recovery thus negatively affecting the energy balance.

Regarding to pyrolysed almond shells used as electrodes in microbial electrolysis cell

The results offer an alternative to the production of electrodes for bioelectrochemical systems from low-cost materials, since the evaluated performance was comparable to that of carbon felt electrodes. The results of the microbiological study revealed a similar structure and taxonomic composition between the pyrolysed almond shell electrode and the carbon felt electrode. The good bacterial-electrode electrical connection was confirmed, which allows for increasing the efficiency in the transfer of electrons between microorganisms and this material.

Regarding to assessment of electrooxidation as pre and post-treatment to improve anaerobic digestion and stabilisation of waste activated sludge

Electrooxidation was studied as pretreatment and post-treatment alternative for improving digestion performance and sludge handling. In the first case, electrooxidation proved to be a suitable alternative for enhancing hydrolysis of sewage sludge by

favouring the solubilisation of organic matter and hence aiding in anaerobic degradation of complex compounds. The application of electrooxidation on WAS previous to anaerobic digestion caused cell destruction releasing cell inner materials making them accessible to anaerobic microflora and facilitating degradation. Methane yield was increased 18% as result of the pretreatment under alkaline conditions.

On the other hand, the use of electrooxidation as post-treatment of digested WAS proved to have a significant effect on sludge rheological behaviour, particle size reduction and dewaterability properties, increasing the specific surface area and improving parameters such as capillary suction time (CST) and specific resistance to filtration (SRF). Future work will aim to evaluate the benefits of these later characteristics and the effect on the energy demand on a WWTP.

Regarding to anaerobic digestion of wastes from the wine industry: Evaluation of electrooxidation as pre-treatment and char addition for enhancing digestion

Considering the complex nature of wine lees, a strong oxidation pre-treatment appears crucial to improve biogas production and support methanogenesis. The combined configuration of electrooxidation followed by anaerobic digestion showed an effective mineralisation of the organic matter contained in the wine lees sample. The partial oxidation of recalcitrant organic substances enhanced the methane production during anaerobic digestion. Nevertheless, further research is necessary to investigate in details the operative conditions that could assure a methane production that allows the achievement of energy self-sufficiency for the digestion system. Secondly, the experimental approach explored in this research regarding biochar addition did not show positive effects on anaerobic digestion under mesophilic conditions as it is reported in literature. The investigation on the role of biochar as a mere physical support for anaerobic biomass did not show any results for the time analysed. Wine lees are a critical substrate, highly biodegradable but full of inhibitors and strongly acidic. It is necessary to explore operative conditions that could prevent overloading of digestion systems and effectively support methanogenesis.