EVALUACIÓN DE LA INFLUENCIA DE VARIABLES DE INCIDENCIA SOBRE EL PROCESO DE DIGESTIÓN ANAEROBIA DE RESIDUOS DE ALIMENTOS SEPARADOS EN LA FUENTE Y CON RECOLECCIÓN SELECTIVA

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TRABAJO DE INVESTIGACIÓN PRESENTADO COMO REQUISITO PARCIAL PARA OPTAR POR EL TÍTULO DE MAGISTER EN INGENIERÍA - ÉNFASIS INGENIERÍA SANITARIA Y AMBIENTAL

UNIVERSIDAD DEL VALLE
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2020
SEDE MELÉNDEZ

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LISTA DE ACRÓNIMOS

AGVs	Ácidos Grasos Volátiles
AB	Alcalinidad Bicarbonática
AME	Actividad Metanogénica Específica
ASTM	Aerican Society for Testing and Materials
AT	Alcalinidad Total
BOM	Biorresiduos de Origen Municipal
CH_4	Metano
C/N	Carbono/Nitrógeno
CO_2	Dióxido de Carbono
DQO	Demanda Química de Oxígeno
DA	Digestión Anaerobia
DF	Disposición Final
g	Gramos
GEI	Gases Efecto Invernadero
H_2	Hidrógeno
kg	Kilogramos
L	Litros
mg	Miligramos
mL	Mililitros
MSR	Metodología de Superficie de Respuesta
MVCT	Ministerio de Vivienda Ciudad y Territorio
NT	Nitrógeno Total
NTC	Norma técnica Colombiana
ODS	Objetivos de Desarrollo Sostenible
OPS	Organización Panamericana de Salud
PBM	Potencial Bioquímico de Metano
PeD	Países en Desarrollo
PMRS	Plantas de Manejo de Residuos Sólidos
PNUD	Programa de las Naciones Unidas para el Desarrollo
RA	Residuos de Alimentos
RS	Relleno Sanitario
RSM	Residuos Sólidos Municipales
SSPD	Superintendencia de Servicios Públicos Domiciliarios
ST	Sólidos Totales
SV	Sólidos Totales
t	Toneladas
UPME	Unidad de Planeación Minero Energética

Resumen

La gestión de los Residuos Sólidos Municipales (RSM) es una de las problemáticas con más relevancia en las sociedades modernas tanto a nivel nacional como internacional, debido a su crecimiento y a los problemas derivados del manejo tradicional como la disposición final en rellenos sanitarios (RS), por aspectos como la ocupación de mayores cantidades de terreno y la generación de lixiviados y gases de efecto invernadero (GEI). Sin embargo, dada la composición predominantemente orgánica de los RSM (50-60% Biorresiduos - BOM y dentro de éstos, los residuos de alimentos – RA, que representan del orden de 50-70%), existe un alto potencial de valorización y aprovechamiento a través de procesos biológicos como la Digestión Anaerobia (DA), mediante la cual se pueden obtener subproductos de alto valor agregado como el biogás (principalmente metano, que es fuente de energía renovable). La forma tradicional de analizar el potencial de producción de metano de un sustrato es mediante pruebas a escala de laboratorio, denominadas de Potencial Bioquímico de Metano (PBM); sin embargo, aunque muchos investigadores alrededor del mundo han hecho esfuerzos para estandarizar este ensayo, esto aún no se ha logrado, por lo cual existen diferentes configuraciones y protocolos experimentales, lo que hace la comparación de resultados compleja. Este trabajo de investigación se centró en identificar los diferentes factores y variables que inciden sobre el proceso de DA de BOM y RA y que contribuyen al incremento y/o descenso en la producción de metano, identificándose variables asociadas al sustrato, al inóculo, ambientales y de operación. Aunque variables como el *head space* y la temperatura tienen gran influencia, aún faltan estudios al respecto; por esta razón, en este estudio se evaluó experimentalmente, por medio de un diseño central compuesto, la incidencia simultánea de estas dos variables (*head space* - X_1: 20, 35 y 50% y temperatura - X_2: 20, 35 y 55°C); así mismo, mediante la aplicación de los modelos cinéticos de primer orden y de Gompertz Modificado, se hallaron la constante de hidrólisis y la fase de latencia respectivamente. Se encontró que los puntos óptimos fueron 20% para el *head space* y 48.9°C para la temperatura, siendo esta última la variable de mayor incidencia del proceso, además se identificó que valores superiores al valor hallado no incrementarían la producción, pero si representaría un gasto energético innecesario; en cuanto al *head space* se encontró que valores superiores (>20%), desencadenan una inhibición del proceso debida a la producción de ácidos grasos volátiles (AGVs) que no alcanzan a ser neutralizados por el CO_2 disuelto en el medio. Se evidenció que la configuración experimental (20% *head space* y 55°C) que obtuvo una mayor constante de hidrólisis (0.03) hallada con el modelo de Primer Orden, fue la más cercana a la encontrada como óptima (20% *head space* y 48.9°C), lo cual ratificó lo hallado experimentalmente. El modelo cinético que mejor describió el proceso fue el de Gompertz Modificado, debido a su ajuste y producción teórica de metano ligeramente similar a la experimental.

Palabras clave: Digestión Anaerobia, *Head Space*, Potencial Bioquímico de Metano – PBM, Residuos de Alimentos, Temperatura.

Abstract

The management of Municipal Solid Waste (MSW) is one of the most relevant problems in modern societies, both nationally and internationally, due to its growth and the problems derived from traditional management such as final disposal in sanitary landfills (MS), for aspects such as the occupation of greater amounts of land and the generation of leachates and greenhouse gases (GHG). However, given the predominantly organic composition of MSW (50-60% Bio-waste - BOM and within protocols, food waste -RA, representation of the order of 50-70%), there is a high potential for recovery and use through biological processes such as anaerobic digestion (DA), through which two high added value by-products can be obtained such as biogas (mainly methane, which is a renewable energy source) and digestate (with potential for agricultural use as a source of nutrients). The traditional way of analyzing the methane production potential of a substrate is through laboratory scale tests, called Methane Biochemical Potential (PBM); however, although many researchers around the world have made efforts to standardize this trial, this has not yet been achieved, so there are different controls and experimental protocols, which makes the comparison of complex results. This research work focused on identifying the different factors and variables that affect the BOM and RA AD process and that affect the increase and / or decrease in methane production, identifying variables associated with the substrate, the inoculum, environmental and of operation. Although variables such as headspace (X1: 20, 35 and 50%) and temperature (X2: 20, 35 and 55 ° C) have great influence, studies are still lacking in this regard; For this reason, in this study, the simultaneous incidence of these two variables was experimentally evaluated by means of a composite central design; Likewise, the first order and Modified Gompertz kinetic models were used to find the hydrolysis constant and latency phase, respectively. It was found that the optimal points were 20% for the head space and 48.9°C for the temperature, the latter being the variable with the highest incidence of the process, and it was also identified that values higher than the value found would not increase production, but would represent a unnecessary energy expenditure; Regarding head space, it was found that higher values (> 20%), trigger an inhibition of the process due to the production of volatile fatty acids (VFAs) that cannot be neutralized by the CO2 dissolved in the medium. It was evident that the experimental configuration (20% head space and 55°C) that obtained the highest hydrolysis constant (0.03) found with the First Order model was the closest to that found as optimal (20% head space and 48.9°C), which confirmed the experimental results. According to the kinetic modelling, it was identified that the model that best described the process was the Modified Gompertz model, due to its adjustment and theoretical methane production slightly similar to the experimental results.

Key Words: Anaerobic Digestion, Head Space, Biochemical Methane Potential - BMP, Food Waste, Temperature

PRODUCTOS DEL TRABAJO DE INVESTIGACIÓN

Artículos

- ✓ *Identification of Factors and Variables that Influence the Anaerobic Digestion of Municipal Biowaste and Food Waste*. Sometido en: *Waste Biomass and Valorization* (Q2).

- ✓ *Evaluation of Simultaneous Incidence of Head Space and Temperature on Biochemical Methane Potential in Food Waste*. Publicado en: *Cogent Engineering* (Q2) 2020. (7) 1-15. DOI: 10.1080/23311916.2020.1729514.

Eventos Académicos

Lugar/Fecha	Evento	Título
Pereira, Colombia 13 al 15 de noviembre de 2019	V SEMINARIO INTERNACIONAL DE COOPERACIÓN UNIVERSITARIA PARA EL DESARROLLO SOSTENIBLE 1er ENCUENTRO INTERNACIONAL DE DOCTORADOS EN CIENCIAS AMBIENTALES COLOMBIA 2019	Incidencia del *Head Space* Sobre el Potencial Bioquímico de Metano en la Digestión Anaerobia de Residuos de Alimentos

Dirección Trabajos de Grado en Curso (Propuestas Aprobadas)

- ✓ Evaluación de la Influencia del *Headspace* sobre la Producción de Metano en la Digestión Anaerobia de Residuos de Alimentos. Juan David Gómez y Juan David Jojoa. Programa Académico de Ingeniería Sanitaria y Ambiental, Universidad del Valle, 2020.

- ✓ Influencia de la Temperatura sobre la Generación de Gas Metano a partir de la Digestión Anaerobia de Residuos de Alimentos. Ana Yulieth García y Jackeline Diaz. Programa Académico de Ingeniería Sanitaria y Ambiental, Universidad del Valle, 2020.

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INTRODUCCIÓN

Una de las problemáticas más importantes y que pone en riesgo la protección y preservación del ambiente, es la creciente generación de Residuos Sólidos Municipales (RSM), cuya cantidad y composición son variables, dependiendo de aspectos como el nivel de ingresos, estilo de vida, hábitos, cultura, ubicación geográfica, así como de las condiciones meteorológicas (Khalid, 2011). Para el año 2014, se estimó una generación mundial de 1.3 billones de toneladas, con posibilidad de aumentar a 2.2 billones a 2025 (Hoornweg y Bhada-Tata, 2012) y Silpa et al., (2018) estiman que para el 2050 se generarán 3.4 billones de toneladas.

En la mayoría de los Países en Desarrollo (PeD), es común el inadecuado manejo de los RSM, siendo la forma tradicional de manejo (90% de los RSM), la disposición final (DF) en sitios que incluso rara vez alcanzan a ser rellenos sanitarios (RS) ()y tan solo el 10% del total de RSM generados, son recuperados o aprovechados (Hoornweg y Bhada-Tata, 2012); estas prácticas de disposición inadecuada de los RSM, son los causantes del 5% del total de los Gases de Efecto Invernadero (GEI) a nivel mundial, estos contribuyen al deterioro de la capa de ozono y la salud de las personas cuando existen exposiciones a altas concentraciones y por tiempos prolongados (Christensen, 2011; Silpa et al., 2018), además de la producción lixiviados que son responsables de algunos de los problemas de contaminación de aguas subterráneas y superficiales, suelos, alteración de flora y fauna y de impactos a la salud humana (Arrieta, 2015). Williams (1998) estimó que 1 tonelada de residuos dispuestos produce aproximadamente 276L de lixiviado en un periodo de 30 años en condiciones normales, lo que representa una problemática que podría agravarse si se tiene en cuenta que la generación de RSM aumentará debido al incremento en los hábitos de consumo (Lin et al., 2014).

La fracción orgánica y de fácil degradación de los RSM, denominada FORSU o Biorresiduos de Origen Municipal (BOM), posee una gran cantidad de Residuos de Alimentos (RA), representando la facción predominante de los RSM (50 y 70 %) en países en desarrollo (PeD) (Oviedo et al., 2012; Thi et al., 2015), los cuales son considerados un flujo de residuos heterogéneo, a diferencia de residuos como los industriales o agrícolas (Wang y Nie, 2001), lo que se debe principalmente a aspectos como la variación de región a región debido a los hábitos, clima y cultura, además de no ser separados en la fuente (De Gioannis et al., 2008).

En Colombia, de acuerdo con la Superintendencia de Servicios Públicos Domiciliarios (SSPD, 2015), la ciudad y departamentos con mayor producción de RSM son Bogotá, Antioquia, Valle del Cauca, Atlántico y Cundinamarca, cada uno con una producción superior a 1000 ton/día, lo que representa el 53% del total de los residuos generados en el país; así mismo, el informe de Disposición Final de Residuos Sólidos – 2017, emitido por la misma entidad, señala que la disposición de residuos en Rellenos Sanitarios (RS), sigue siendo la principal opción de manejo (97%), (a pesar de que existen rellenos sanitarios como el del Huila donde existe una planta de aprovechamiento gracias a la separación en la fuente de los residuos (SSPD, 2018)), a pesar de ello los sitios de tratamiento de residuos no emergen; así mismo, sitios como botaderos a cielo abierto y celdas transitorias aún persisten. El informe también destaca que el 35% (55) de los 158 sitios de DF del país, tienen una vida útil vencida o próxima a vencerse (0-3 años), poniendo en riesgo la prestación del servicio en términos de continuidad y calidad.

Por otro lado, la Política Nacional para la Gestión Integral de Residuos Sólidos (CONPES 2016), indica que de continuar con la generación actual de residuos y sin la toma de medidas para el aprovechamiento y tratamiento, para el año 2030 se presentarán emergencias sanitarias en la mayoría de las ciudades de Colombia y un aumento en la generación de GEI; además, se proyecta que para 2030 la generación de residuos sea de 18.74 millones de toneladas anuales, de los cuales 14,2 millones tendrán que ser dispuestos en RS que no cuentan con la capacidad para almacenar tal cifra, ya que el déficit para ese año será de 10.28 millones de toneladas.

La DF no es la estrategia más adecuada para la gestión de los RA y BOM, lo cual es soportado por investigaciones sobre el análisis de ciclo de vida, GEI producidos por la disposición final de esta clase de residuos y costos respecto a otras alternativas de aprovechamiento y valorización como la digestión anaerobia (DA) (Schott et al., 2016) y el compostaje (Saer et al., 2013); sin embargo, en países en desarrollo (PeD) como Colombia, aún predomina la DF, en sitios que rara vez llegan a cumplir con los requerimientos técnicos para poder denominarse rellenos sanitarios (RS); adicionalmente, esta práctica se encuentra amparada por normativas vigentes como el Decreto 1077 (2015) formulado por el Ministerio de Vivienda, Ciudad y Territorio (MVCT, 2015).

De acuerdo con autores como Mirmohamadsadeghi et al. (2019) y Ohemeng-Ntiamoah y Datta (2019), la DA es una alternativa para el tratamiento y aprovechamiento de residuos como los BOM y los RA, por el alto potencial para la producción de biogás (principalmente metano - CH_4) que puede emplearse como combustible o transformarse en energía eléctrica; además, es posible obtener otro subproducto semi-sólido, conocido como digestato o biol, el cual puede emplearse como mejorador de suelos por su aporte de nutrientes. El proceso bioquímico de DA ocurre en varias etapas en las que intervienen diferentes microorganismos y se desarrolla en condiciones de ausencia de oxígeno molecular, transformando compuestos complejos como carbohidratos, proteínas y lípidos, en productos más simples como biogás (Lin et al., 2018). Se ha identificado que en la DA de sustratos sólidos como RA o BOM, la fase limitante es la hidrólisis debido a la alta producción de ácidos grasos volátiles (AGVs) por lo cual el uso de modelos cinéticos contribuye a identificar la incidencia de distintas variables en el proceso (Parra-Orobio et al., 2018).

Teniendo en cuenta el significativo contenido de RA en los RSM y los impactos generados por un inadecuado manejo, aunado al potencial energético mediante DA de estos residuos y su aporte a la mitigación de las deficiencias de energía, principalmente en zonas no interconectadas y en periodos secos asociados al fenómeno del Niño, que provoca la reducción de los embalses (por la alta dependencia de energía hídrica del país que compromete el suministro de energía) (UPME, 2013), es importante optimizar el proceso de DA de estos residuos, lo que además contribuirá a prolongar la vida útil de los rellenos sanitarios que en Colombia están por colmatarse y cuya oferta de sitios disponibles para tales efectos es limitada (Manyoma et al., 2015).

Los ensayos de Potencial Bioquímico de Metano (PBM) son una forma de evaluar la DA a escala de laboratorio. El PBM cuantifica la máxima producción de gas metano (CH_4) que alcanza un sustrato ya sea líquido o sólido de origen orgánico bajo condiciones previamente establecidas de nutrientes, relación sustrato/ inóculo (S/I), concentración de sólidos volátiles, temperatura, *head space*, pH, alcalinidad total y bicarbonática, entre otras. Uno de los sustratos que han sido evaluados con este ensayo son los RA, los cuales han sido empleados como fuente de energía alternativa alrededor del mundo (Banks et al., 2018)

Una de las principales dificultades que presenta el PBM es la comparación de resultados debido a que no existe un protocolo estandarizado para su desarrollo, alrededor del mundo se encuentran distintos protocolos para su desarrollo y puesta en marcha los cuales se encuentran en función principalmente de los recursos económicos (Cárdenas-Cleves et al., 2016; Ohemeng-Ntiamoah y Data, 2019), lo cual hace que la comparación de hallazgos sea compleja o no se pueda hacer aun cuando existan características similares entre los ensayos. Por lo anterior se requieren estudios que contribuyan a la estandarización del proceso y que identifiquen puntos óptimos, es decir, valores de las distintas variables que hagan que la producción se maximice, obteniendo así la mayor cantidad de gas metano posible, de manera que se viabilice cada vez más este tipo de tratamiento biológico que contribuye a la obtención de energía limpia y disminución de impactos en las tres matrices (agua, aire y suelo)

Este proyecto, se encuentra enmarcado en los lineamientos de los Objetivos de Desarrollo Sostenible – ODS emitidos por el Programa de las Naciones Unidas para el Desarrollo (PNUD, 2016) como son la acción por el clima, la energía asequible y no contaminante y la salud y el ambiente. De acuerdo con lo anterior, el objetivo general del trabajo de investigación fue *Evaluar la influencia de variables de incidencia sobre el Proceso de Digestión Anaerobia de Residuos de Alimentos (RA) separados en la fuente y con recolección selectiva*.

La parte experimental de esta investigación se realizó a escala de laboratorio en las instalaciones de la Facultad de Ingeniería de la Universidad del Valle, empleando como sustrato RA reconstituidos a partir de los residuos de una cafetería universitaria y de plazas de mercado de la ciudad de Cali. Los objetivos específicos planteados fueron:

- ✓ Identificar, a través de herramientas de vigilancia tecnológica, los factores de mayor incidencia sobre el proceso de DA de los RA.

- ✓ Evaluar de manera simultánea, mediante ensayos de Potencial Bioquímico de Metano (PBM), al menos dos de los factores de mayor incidencia en el proceso de DA de los RA, para la producción de energía en forma de metano.

- ✓ Evaluar mediante modelos cinéticos, la influencia de los factores evaluados experimentalmente, sobre la hidrólisis del proceso.

Este documento se presenta bajo la modalidad de artículos, la cual se encuentra establecida en la Resolución del Consejo Académico de la Universidad del Valle No. 142, del 29 de octubre de 2015. Los artículos producto de este trabajo, así como su relación con los objetivos específicos planteados se presentan en la Tabla 1.

Tabla 1 Relación de artículos producto del trabajo con los objetivos planeados

Objetivos	**Artículo**	**Estado**
Identificar, a través de herramientas de vigilancia tecnológica, los factores de mayor incidencia sobre el proceso de DA de los RA.	*Identification of factors and variables that influence the anaerobic digestion of municipal biowaste and food waste*	Sometido a *Waste Biomass and Valorizarion*
Evaluar de manera simultánea, mediante ensayos de Potencial Bioquímico de Metano (PBM), al menos dos de los factores de mayor incidencia en el proceso de DA de los RA, para la producción de energía en forma de metano. Evaluar mediante modelos cinéticos, la influencia de los factores evaluados experimentalmente sobre la fase de hidrólisis del proceso.	*Evaluation of Simultaneous Incidence of Head Space and Temperature on Biochemical Methane Potential in Food Waste*	Publicado en el *journal Cogent Engineering*. 2020 (7) 1-15. DOI: 10.1080/23311916.2020.1729514

La Figura 1 presenta la estructura general del presente documento, el cual consta de cuatro capítulos. El primero corresponde al marco conceptual y referencial del trabajo de maestría. El capítulo 2 da cumplimiento al primer objetivo específico, el cual se desarrolló a través de una vigilancia tecnológica para los últimos 10 años, en torno a la identificación de los factores y variables de interés en el proceso de DA de BOM y RA; en este capítulo se resalta, entre otras cosas, la importancia de cada variable identificada y los rangos recomendados para cada una y además se identifican las variables que han sido menos investigadas. Estos resultados se recopilan en el artículo "*Identification of Factors and Variables that Influence the Anaerobic Digestion of Municipal Biowaste and Food Waste*" que se encuentra sometido en Waste Biomass And Valorization.

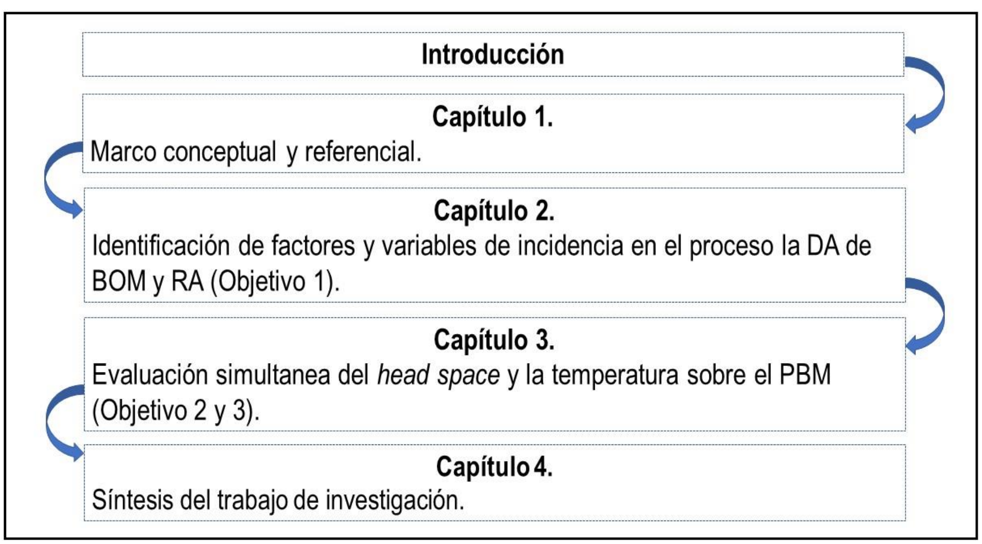

Figura 1. Estructura del documento de Maestría

El tercer capítulo da cumplimiento a los objetivos específicos 2 y 3, los cuales corresponden al desarrollo de la fase experimental del trabajo y el análisis cinético del proceso. De acuerdo con lo encontrado en el capítulo anterior sobre las variables que requieren más estudios y la escasa información sobre la interacción de las mismas, se seleccionaron el *head space* y la temperatura como variables a evaluar experimentalmente.

La evaluación de la incidencia simultánea de las variables evaluadas sobre el proceso de DA, se llevó a cabo a través de la metodología de Superficie de Respuesta (MSR) seleccionado puntos mínimos, intermedios y máximos para cada variable de acuerdo con la literatura; se halló para cada caso el punto donde la producción de metano se maximiza. Una vez finalizada la etapa experimental, los datos de producción de metano se emplearon para hallar la incidencia de dichas variables sobre la hidrólisis del proceso y la fase de latencia, lo cual se analizó a través de los modelos cinéticos de Primer orden y de Gompertz Modificado. Estos resultados están recopilados en el artículo *"Evaluation of Simultaneous Incidence of Head Space and Temperature on Biochemical Methane Potential in Food Waste"* publicado en el *Journal Cogent Engineering.*

Finalmente, el capítulo cuatro presenta la síntesis del trabajo en la cual se presentan además las principales conclusiones y recomendaciones de la investigación.

CAPÍTULO 1. MARCO CONCEPTUAL Y REFERENCIAL

Este capítulo se centra en la conceptualización de aspectos clave del tema de estudio como los Residuos Sólidos, los Biorresiduos de Origen Municipal (BOM), los Residuos de Alimentos (RA) y sus clasificaciones, economía circular, jerarquía de los residuos, haciendo énfasis especial en el proceso de Digestión Anaerobia (DA), la cual es una tecnología promisoria de tratamiento y valorización que además de producir biogás, también produce digestato que tiene potencial de ser empleado como mejorador de suelos. En este tipo, el proceso se evaluó a través de ensayos de Potencial Bioquímico de Metano (PBM) que permiten determinar el máximo potencial que tiene un residuo para producir biogás; así mismo, se presentan algunos estudios donde se resalta la ausencia de un protocolo estandarizado dada la variedad de configuraciones y configuraciones experimentales encontradas; sin embargo, el ensayo es ampliamente empleado dada su aplicabilidad y bajo costo, aunque no existe información suficiente sobre sus variables de incidencia y tampoco sobre la interacción que puede existir entre ellas.

Otro aspecto importante que se presenta en este capítulo son algunos de los modelos cinéticos que han sido desarrollados para esta clase de procesos biológicos y que usualmente se usan para la descripción del proceso y la identificación de posibles falencias, ya sea sobre la fase de latencia o la hidrólisis del proceso, que es la etapa limitante del proceso de DA (Luo et al., 2019).

1.1. Residuos Sólidos

Los Residuos Sólidos (RS) tiene distintas connotaciones alrededor del mundo, debido a que éste se encuentra asociado a distintos factores como la normatividad y las tecnologías para el tratamiento y/o aprovechamiento. La Unión Europea los define como el objeto en cualquier estado (líquido, solido o gaseoso) procedente de un proceso extractivo de transformación o utilización, el cual no tiene o no representa valor para el generador (CE, 1975). En Estados Unidos fueron definidos por la ley "*Resource Conservation and Recovery*" como: aquella basura, desperdicio, lodos de plantas de tratamiento de residuos o para agua potable o instalaciones de control de calidad de aire y otros materiales de desecho, dentro de los cuales se incluyen semisólidos, sólidos, líquidos o material con contenido gaseoso; lo anterior como producto de la industria, comercio, minería, agricultura.

En Colombia, los Residuos Sólidos son definidos por el Ministerio de Vivienda, Ciudad y Territorio (MVCT, 2015), por medio del Decreto 1077 de 2015 "como aquellos objetos, materiales, sustancias o elementos, principalmente sólidos resultantes del consumo o uso de un bien en actividades domésticas, industriales, comerciales, institucionales o de servicios, que el generador presenta para su recolección por parte de la persona prestadora del servicio público de aseo. Igualmente, se considera como residuo sólido, aquel proveniente del barrido y limpieza de áreas y vías públicas, corte de césped y poda de árboles"; además, son clasificados por este mismo decreto en aprovechables y no aprovechables, donde los primeros corresponden a aquellos que son susceptibles de ser incorporados a un proceso productivo, mientras que los segundos generan un costo de disposición, ya que no generan valor comercial alguno y requieren un tipo de tratamiento.

Así mismo, el Reglamento Técnico para el Sector Agua y Saneamiento Básico (RAS, 2012), definió los residuos sólidos como "cualquier objeto, material, sustancia o elemento sólido que se abandona, bota o rechaza después de haber sido consumido o usado en actividades domésticas, industriales, comerciales, institucionales, de servicios e instituciones de salud y que es susceptible de aprovechamiento o transformación en un nuevo bien, con valor económico" y los dividió en aprovechables y no aprovechables.

Existen diversas clasificaciones que pueden ser hechas a los residuos, aunque no existe uniformidad en los distintos países; por ejemplo, la Organización Panamericana de la Salud (OPS) tiene clasificaciones de fermentabilidad, inflamabilidad, procedencia y volumen, dividiéndolos en: orgánicos e inorgánicos, combustibles y no combustibles domésticos, jardinería, de barrido y en convencionales y especiales respectivamente (Bustos, 2009).

1.1.1 Residuos Sólidos Municipales (RSM)

De acuerdo con el informe "*What a waste. A Global Review of Solid Waste Management*" los RSM pueden ser definidos como aquellos elementos en estado sólido o líquido que son generados en centros poblados y que incluyen desechos domésticos (principalmente desperdicios de alimentos), de comercio, algunas instituciones, pequeñas empresas, jardines y barrido de calles (Hoornweg Y Bhada-Tata, 2012). En la Tabla 2 se puede observar la clasificación hecha por Tchobanoglous, (2009) a los RSM.

Tabla 2. Clasificación de los RSM

Tipo de Residuo		Actividad, instalación o locación de origen
Municipal	Residencial	Hogar o establecimiento similar
	Comercial	Almacenes, depósitos, hoteles, restaurantes, plazas de mercado, entre otros.
	Institucional	Hospitales, escuelas o colegios, universidades y entes gubernamentales

Fuente: Tchobanoglous, (2009)

Themelis et al., (2013) estimaron que aproximadamente el 80% de los RSM a nivel mundial, terminan en rellenos sanitarios; de esa cantidad, tan solo un 20% es dispuesta en vertederos que han sido construidos y manejados de manera tal que las condiciones de degradación son propicias, ejemplo la impermeabilización de las celdas.

La generación de residuos varia de un país a otro y es muy diferente entre países desarrollados (PD) y en desarrollo (PeD), ya que de acuerdo con autores como Hoornweg y Bhada-Tata, (2012) y Oviedo-Ocaña et al., (2012), los primeros tienen una menor presencia de materia orgánica (RA) que los segundos, aproximadamente 28 y 70% respectivamente.

1.1.2. Biorresiduos de Origen Municipal (BOM) y Residuos de Alimentos (RA)

Para dar un adecuado aprovechamiento a los residuos orgánicos, es indispensable conocer que existen tres clasificaciones importantes: putrescibles, fermentables y no fermentables; los putrescibles tienden a producir olores desagradables si no son controlados, debido a que su degradación es rápida; los fermentables tienden a descomponerse rápidamente pero sin la expedición de olores desagradables, mientras que los no fermentables se degradan lentamente, ya que son resistentes a este proceso (Comisión Europea, 2008). Los BOM se constituyen fundamentalmente de RA, los cuales están compuestos principalmente por residuos putrescibles, que son aquellos generados en hogares, restaurantes, servicios de restauración colectiva y establecimientos de consumo al por menor y residuos comparables procedentes de plantas de transformación de alimentos y en general establecimientos que lleven a cabo procesos con éstos (Comisión Europea, 2008). No se incluyen lodos de depuración ni otros biodegradables como los textiles y tampoco los residuos agrícolas o estiércol de animales (CCE, 2008).

Dada la alta biodegradabilidad de los RA y los BOM, éstos son un tipo de residuos con alto potencial para ser aprovechados, pudiendo ser objeto de transformación a través de procesos biológicos como la DA, de modo que se contribuya al cumplimiento de la Jerarquía de los residuos que dicta el Departamento Nacional de Planeación (DNP, 2016) y que también se encuentra enmarcada en principios de la Economía Circular; sin embargo, estos sustratos presentan características desfavorables como deficiencias en cuanto a algunos nutrientes como el nitrógeno, bajo pH y composición heterogénea; es decir, presencia de materiales de rápida y lenta biodegradación, así como la presencia de fracciones recalcitrantes (Campuzano y González-Martínez, 2016; Götze et al., 2016)

1.1.3. Jerarquía de los Residuos

De acuerdo con el DNP, (2016) las medidas para reducir y gestionar los residuos, es conocida como Jerarquía de los Residuos, la cual contempla el manejo integral desde la generación, pasando por la etapas de separación en la fuente, recolección, transferencia y transporte, aprovechamiento, tratamiento y disposición final; en ese sentido, la jerarquía es presentada como una pirámide invertida (ver Figura 2), indicando que la prioridad la tiene la prevención y la menos deseable sería la disposición final (Manyoma et al., 2015).

Figura 2 Jerarquía de los residuos
Fuente: DNP, (2016)

2. Digestión Anaerobia

La DA es un proceso biológico que degrada la materia orgánica a través de microorganismos, en condiciones de ausencia de oxígeno molecular, obteniéndose como principales subproductos la generación de biogás (con mayor proporción de gas metano (CH_4) con respecto a otros gases como el dióxido de carbono (CO_2), sulfuro de hidrógeno (H_2S), monóxido de carbono (CO), entre otros) y de otro subproducto semisólido conocido como digestato o biol, que puede ser empleado como mejorador de suelos el cual, dependiendo de sus características fisicoquímicas, microbiológicas y parasitológicas, generalmente debe recibir un tratamiento previo antes de la aplicación (Zhang y Banks, 2013; Lin et al., 2018).

La DA transforma distintos compuestos complejos como carbohidratos, proteínas y lípidos presentes en los distintos sustratos (BOM, RA, estiércol de animales y lodos, entre otros) en productos más simples (Foresti et al., 1999). En el proceso de transformación intervienen distintos grupos bacterianos que actúan mediante reacciones específicas y en diferentes etapas de manera secuencial (hidrólisis, acidogénesis, acetogénesis y metanogénesis), lo que se muestra en la Figura 3.

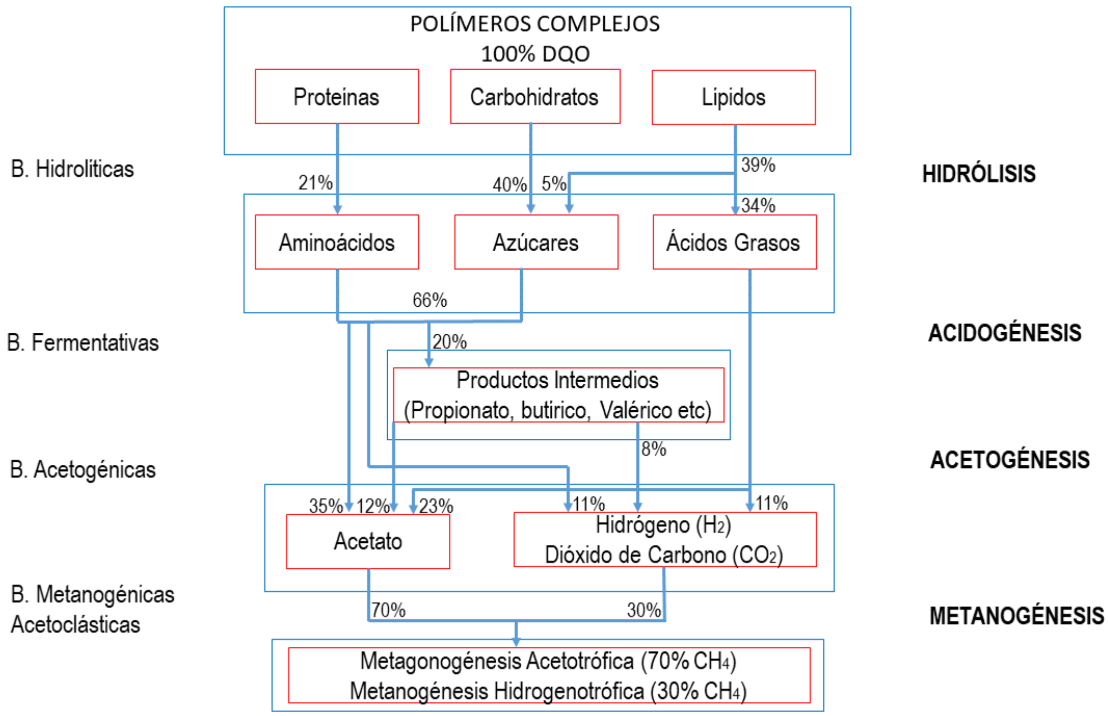

Figura 3. Fases de la Digestión Anaerobia. B: Bacterias
Fuente: Adaptado de Mata (2003) y Nayono (2010)

La *hidrólisis* es la primera fase de la DA, durante la cual enzimas extracelulares son excretadas por bacterias anaerobias facultativas y estrictas, con el fin de transformar materiales orgánicos insolubles y compuestos de alta masa molecular en materiales orgánicos solubles para ser empleados como fuente de energía (Schnurer y Jarvis, 2010; Adekunle y Okolie 2015). Es de resaltar que ésta es la fase considerada como limitante en la degradación de RA por medio de DA, ya que la descomposición de celulosa y hemicelulosa es un proceso lento y por tanto limita la digestión global del proceso (Luo et al., 2019). La *acidogénesis* da lugar a las bacterias fermentativas, que son anaerobias facultativas encargadas de absorber los monómeros producidos en la fase previa. Como resultado, se obtienen dióxido de carbono (CO_2), hidrógeno (H_2), alcoholes y ácidos como el propiónico, butírico, entre otros, siendo el más importante el ácido acético, ya que es el principal precursor del metano (Foresti et al., 1999). La producción de ácidos hace que el pH baje hasta valores entre 4.5 y 5.5 unidades (Gerardi, 2003; Merlin Christy et al., 2014)

La *acetogénesis* es la fase en que los AGVs son transformados en acetato, hidrógeno y dióxido de carbono por las bacterias acetogénicas que son anaerobias estrictas. En esta etapa es muy importante que la presión parcial de hidrógeno no sea baja, debido a que esto dificultaría la conversión de acetato a metano (Blanco, 2011). La *metanogénesis* es la última etapa en la cual, por acción de las *Arqueas metanogénicas* los sustratos monocarbonados o con dos átomos de carbono se transforman predominantemente en metano, ya que aproximadamente el 70% del gas metano producido es por medio de la conversión del acetato (Madigan et al., 1997). Dentro de las potencialidades y limitaciones de la DA se destacan las presentadas en la Tabla 3.

***Tabla 3.** Potencialidades y limitaciones de la DA*

Potencialidades	Limitaciones	Referencia
-Reducción de problemas de localización de RS -Menor requerimiento de área -Contribuye a prolongar la vida útil de los RS -No requiere aireación -Reducción de olores -Producción de energía (Potencialidad de producción de hidrógeno y metano) -Mejor manejo de residuos húmedos -Control y reducción de emisiones de GEI comparados con los producidos en los RS -Recuperación de nutrientes	-Arranque lento cuando el inóculo no ha sido adaptado -Degradación lenta	Lin et al., (2014); Manyoma et al., (2015); Cárdenas-Cleves et al., (2016); Ji et al., (2017); Parr-Orobio et al., (2018); Lee et al., (2018); Satchwell et al., (2018)

2.1. Potencial Bioquímico de Metano (PBM)

Una de las formas de mayor aplicación para evaluar la viabilidad de la DA de un sustrato, es a través de montajes a escala de laboratorio denominados ensayos PBM, que son un montaje relativamente sencillo que se emplea para cuantificar la máxima producción y la velocidad con la que se produce el biogás obtenido a partir de un sustrato al cual se le suministra un inóculo y algunos nutrientes, bajo ciertas condiciones, en términos de la concentración de sólidos volátiles (SV) y volumen (expresado en mililitros - mL). Este ensayo, realizado en modo *batch,* evalúa rápidamente si un sustrato (que puede ser líquido o sólido) de tipo orgánico puede ser degradado y la máxima biodegradación del mismo, bajo condiciones anaerobias (Wagland y Tyrrel, 2009; Esposito et al., 2012; Cárdenas-Cleves et al., 2016; Sanchez-Reyes et al., 2016).

El PBM contribuye a la búsqueda de sustratos que son vistos desde otros puntos de vista como rechazos, dentro de los cuales están los RA, lo cual es de gran importancia dado que sustratos como éstos están siendo ampliamente usados como fuente de energía en países de Oceanía (Australia), Norte América (Canadá), Asia (China, Indonesia y Vietnam) y Europa (Reino Unido), entre otros (Banks et al., 2018). Dentro de las aplicaciones del ensayo PBM se encuentran: *i.* identificar el potencial de producción energético de un sustrato, *ii.* determinar la cinética de degradación, lo que permitiría simular procesos de digestión y poder predecir el comportamiento de un digestor a escala, *iii.* identificar y desarrollar indicadores para evaluar nuevos sustratos (Esposito et al., 2012, Lisboa et al., 2013 y Cárdenas-Cleves et al., 2016) para lo cual se ha trabajado entre otras cosas en los modelos cinéticos, los cuales pueden identificar problemas en el arranque del ensayo o en la fase de hidrólisis.

Para el desarrollo de los montajes PBM, se debe tener en cuenta el origen y la caracterización tanto del sustrato como del inóculo, en términos de variables como el pH, AGVs, alcalinidad total y bicarbonática, sólidos totales y volátiles y nutrientes, con el fin de evidenciar alguna falencia que pueda alterar o inhibir el proceso. De otro lado, para estimar la generación de biogás existen diferentes métodos de cuantificación (volumétrico o manométrico), cada uno con ventajas y limitaciones (Angelidaki at al., 2009; Torres y Pérez, 2010; Cárdenas-Cleves et al., 2016).

Una limitación importante del PBM, es que el procedimiento no se encuentra estandarizado, por tanto, se hallan distintas configuraciones y protocolos experimentales, así como gran diversidad de potenciales sustratos e inóculos empleados, lo cual dificulta la comparación de los resultados, aun cuando haya condiciones similares (Raposo et al., 2012; Cárdenas-Cleves et al., 2016; Ohemeng-Ntiamoah y Data, 2019), lo que puede llevar a resultados inconsistentes e incluso irreproducibles, dados los distintos contextos de desarrollo. La Tabla 4 sintetiza las características de diferentes configuraciones experimentales del ensayo PBM empleando como sustrato fracción orgánica de los residuos o RA.

En ella se evidencian las diferencias existentes entre los ensayos dentro de los cuales se destacan variables como la adición de nutrientes, el *head space* (espacio libre, aplicable al método manométrico) y la temperatura, autores como Cárdenas-Cleves et al., (2016); Komilis et al., (2017); Ohemeng-Ntiamoah y Data, (2019) mencionan que estas variables, en la mayoría de los casos controladas, son de gran importancia e inciden fuertemente sobre la producción de metano.

Tabla 4. Configuraciones experimentales de distintos ensayos de tipo PBM

Sustrato	Inóculo	Relación S:I (gSV/gSV)	Reactor	Volumen de reactor (L)	Rango de temperatura (°C)	Tiempo (d)	Espacio libre (%)	pH (Unidades)	Nutrientes	Fuente
RA	Lodo digestor anaerobio de AR agroindustrial	-	Semicontinuo	0.50	Mesofílico (37)	368	50%	7.5 - 8	I(SN), II(Co), III(Mo), IV(Ni), V(Co, Mo y Ni), VI(Co, Mo, Ni y Fe)	Zhang y Jahng, (2012)
Fracción orgánica RSM	Lodo digestor mesofílico	1:4.5	Semicontinuo	5.00	Mesofílico (36)	430	21%	-	-	Zhang y Banks, (2013)
RA	Lodo digestor anaerobio de PTAR	-	Semicontinuo	6.00	Mesofílico (37)	462	25%	6.8 – 7.5	I ($FeCl_24H_2O$, $CoCl_26H_2O$ y $NiCl_26H_2O$) II ($CoCl_26H_2O$ y $NiCl_26H_2O$) III ($FeCl_24H_2O$)	Wei et al., (2014)
RA crudos y preparados	Lodo digestor anaerobio de PTAR	1	Batch	0.25	Mesofílico (30)	40	20%	5.5, 6, 6.6, 7 y 8	SoluciónTorres y Pérez (2010)	Parra et al., (2014)
RA crudos y preparados	Lodo digestor anaerobio de PTAR	0.25, 0.5, 1, 2, 4 y 9	Batch	0.25	Mesofílico (30)	40	20%	7	Solución Torres y Pérez (2010)	Parra et al., (2015)
Fracción orgánica de RSM	Lodo digestor anaerobio termofílico	0.5	Batch	1.00	Termofílico (50)	30	50%	-	-	Li et al., (2016)
RA	Lodo de planta de DA de RA (mesofílico y termofílico)	-	CSTR	10.0	Mesofílico (37) Termofílico (55)	152	40%	7.7 – 8.0	-	Zamanzadeh et al., (2016)
RA crudos y preparados	Lodos UASB y digestor anaerobio de PTAR	0.5, 1, 2, 4, 9 y 12	Batch	0.25	Mesofílico (35)	21	20%	7.0	Solución Torres y Pérez (2010)	Parra et al., (2016)
RA procesados y sin procesar	Lodo de anaerobio de ARD y planta de beneficio animal	1.0	Batch	0.25	Mesofílico (35)	35	20%	7.0	Solución Torres y Pérez (2010)	Parra-Orobio et al., (2018)
RA	Lodo de anaerobio reactor UASB	1.0	Batch	0.25	Mesofílico (35)	30	20%	7.0	-	Hobbs et al. (2018)
RA	Estiercol porciono	0.5 y 1.0	Batch	5.00	Mesófilo (20)	84	-	7.5	-	Muñoz (2019)

2.2. Parámetros de Influencia en el ensayo PBM

Los procesos de DA desarrollados con RA son propensos a la inhibición, no solo por la acumulación de los AGV que son propios del proceso de degradación (Parra-Orobio et al., 2018; Panigrahi y Dubey et al., 2019), pues una de las características de los RA es el pH ácido (Götze et al., 2016; Soto-Paz et al., 2017), por lo cual se debe hacer control y monitoreo del parámetro, además de otras variables como la alcalinidad total y bicarbonática, que se encuentran estrechamente relacionadas con el pH; a su vez, éste también depende de otros aspectos o estrategias de optimización del proceso como el pretratamiento del sustrato y las concentraciones de nutrientes (Ohemeng-Ntiamoah y Datta et al., 2019).

Existen distintas clases de pretratamientos del sustrato evaluados con el fin de incrementar la producción de metano. Entre ellos se encuentran los de tipo físico, térmicos, químicos y biológicos (Kondusamy et al., 2014). Entre los primeros se encuentran aquellos cuyo objetivo es separar los RA de otro flujo de residuos como los plásticos (Bernstad et al., 2013), la adecuación o disminución del tamaño de partícula (trituración) para aumentar el área superficial, de tal modo que se facilite y acelere la degradación, que se encuentra altamente relacionado con la producción de AGVs y con los tiempos de retención, lo que finalmente se traduce en menores costos de operación (Parra-Orobio et al., 2017). El ultrasonido es otro tipo de pretratamiento físico con el que investigadores como Rasapoor et al., (2019), encontraron que permite incrementar la producción de biogás hasta en un 80% comparada con el sustrato sin aplicación del pretratamiento, tanto en reactores a escala de laboratorio como piloto, empleando una potencia de 0.2 W/mL.

Los nutrientes son esenciales para el buen desarrollo del proceso; algunos elementos como el nitrógeno, fósforo, potasio y micronutrientes tales como selenio, níquel, zinc, entre otros (Banks, et al., 2012; Walker et al., 2017) aportan a la actividad de las bacterias (Xu et al., 2018); sin embargo, se debe estudiar si el inóculo carece o no de los mismos y además si la adición representa una producción de biogás significativa de modo que se evite incurrir en gastos innecesarios en el arranque y operación de los reactores anaerobios (Raposo et al., 2012; Parra-Orobio et al., 2018; Ohemeng-Ntiamoah y Data, 2019). En cuanto al *head space*, se puede afirmar que la presión parcial del CO_2 interna puede llegar a interferir en la medición del PBM, arrojando datos erróneos debido a la sobreestimación o subestimación de la producción del biogás (Angelidaki et al., 2009; Valero et al., 2016; Ohemeng-Ntiamoah y Data, 2019); así mismo, Valero et al., (2016) afirman que el pH y el crecimiento bacteriano, pueden verse afectados por altas presiones de CO_2, consecuencia de la solubilización de los gases obtenidos a partir del proceso de DA.

Con relación a la temperatura, ésta puede ocurrir en rangos diferentes (psicrofílico, mesofílico y termofílico), lo que influye sobre las diferentes variables de respuesta del proceso como sobre la producción de metano y la higienización del digestato final; además, de ésta depende la fase de latencia y las comunidades microbianas, entre otros (Kim et al., 2002; Ferrer et al., 2010; Komilis et al., 2017; Duan et al., 2019; Giwa et al., 2019). Los rangos más estudiados han sido los mesofílico y termofílico, siendo el primero mucho más estable que el segundo, reportándose temperaturas óptimas en 35 y 55°C respectivamente (Connaughton et al., 2006; Kothari et al., 2014). De otro lado, se ha reportado que si bien las temperaturas mesofílicas son más estables, las termofílicas presentan ventajas como mayor degradación (alrededor del 50%) comparado con la mesofílica y además se obtiene un digestato con menor cantidad de patógenos; su mayor desventaja es el alto requerimiento energético para mantener la temperatura, además es más propensa a la acumulación de ácidos como el propiónico (Mao et al., 2015; Panigrahi y Dubey, 2019).

La alta diversidad de condiciones experimentales identificadas (ver Tabla 4), evidenció la necesidad de realizar una vigilancia tecnológica en torno a los factores y variables de incidencia del proceso con el fin de identificar y evaluar aquellas que no hayan sido ampliamente investigadas y por tanto existan vacíos de información, lo que además permitió identificar las condiciones en las cuales las variables son usualmente medidas y las diferentes estrategias que han sido empleadas para potencializar la producción de gas metano.

2.3. Modelos Cinéticos

Los modelos cinéticos son herramientas que permiten el diseño, operación y control de sistemas biológicos (Lübken et al., 2007). Dentro de estos modelos existen algunos como el de primer orden, que permiten considerar factores de influencia, tanto ambientales como operacionales. Teniendo en cuenta que en la DA de residuos sólidos en general, la etapa limitante del proceso es la hidrólisis, los modelos cinéticos permiten establecer la incidencia de los diferentes factores y variables sobe la misma (Donoso-Bravo et al., 2009; Parra-Orobio et al., 2017). En ese sentido, se han desarrollado otros modelos que permiten predecir la relación biomasa-subproductos, priorizando la producción de metano (Zhao et al., 2019).

Dentro de estos modelos se hallan algunos que permiten evaluar variables como la fase de latencia, que a su vez se relaciona con dos etapas de la degradación anaerobia (acidogénesis y acetogenesis) (Aldin et al., 2011); entre estos modelos se encuentra el modelo de Gompertz Modificado (Ecuación 1) el cual supone que la producción obtenida es proporcional a la actividad microbiana, pero que disminuye con el tiempo de retención de sólidos (Donoso-Bravo et al., 2010; Li et al., 2012). Cabe resaltar que éste es el más utilizado en procesos de DA empleando como sustrato RA, aún cuando algunos autores afirman que en ocasiones puede llegar a sobreestimar la producción (Fang et al., 2012). Por su parte el modelo de Primer orden permite (Ecuación 2) hallar la constante de hidrólisis la cual es la etapa limitante del proceso de DA con RA y de acuerdo a ello se puede establacer la incidencia de la variable que se está estudiando (Parra et al., 2018). La Tabla 5 presenta las ecuaciones y variables del modelo de primer orden y de Gompertz Modificado.

Tabla 5. Modelos Cinéticos

Modelo	Ecuación	Definición de Variables	Supuestos	Fuente
Gompertz Modificado	**1.** $PBM = PBM_0\, e\left\{-e\left[\frac{R_{max}\, e}{PBM_0}(\lambda - t) + 1\right]\right\}$	**PBM:** Potencial Bioquímico de CH_4 acumulado (mL CH_4 gSV^{-1}) **PBM_0:** PBM teórico del sustrato (RA) (mL CH_4 gSV^{-1}) **k:** Constante de hidrólisis de primer orden (d^{-1}) **t:** Tiempo ensayo (d) **R_{max}:** Tasa máxima de producción de CH_4 (mL CH_4 gSV^{-1} d^{-1}) **λ:** Fase de Latencia (d) ***e*** (Valor exponencial de 1) = 2.7183	La producción de biogás es proporcional a la actividad microbiana y disminuye con el tiempo	Lay et al., (1997)
Primer Orden	**2.** $PBM = PBM_0(1 - e^{-k\,t})$		La hidrólisis de material particulado sigue una cinética de primer orden	Nielfa et al., (2015)

Otro aspecto que llama la atención es que en general, son pocos los estudios en esta área que investigan la incidencia simultánea de varios factores; por ello y de acuerdo con lo antes descrito, se hace necesario continuar investigando la DA de RA a través de ensayos PBM. En este sentido, metodologías como la Superficie de Respuesta (MSR) permiten hallar puntos óptimos, además del análisis de más de una variable de manera simultánea; por su parte los modelos cinéticos contribuyen al análisis de parámetros como la constante de hidrólisis y la fase de latencia; estas herramientas que ha sido empleadas en otros estudios como los de Wang et al., (2012), Parra-Orobio et al., (2018) y Kim et al., (2019).

CAPÍTULO 2. IDENTIFICACIÓN DE FACTORES Y VARIABLES DE INCIDENCIA EN EL PROCESO DE DIGESTIÓN ANAEROBIA DE BIORRESIDUOS DE ORIGEN MUNICIPAL Y RESIDUOS DE ALIMENTOS

2.1. Síntesis del capítulo

El objetivo de este capítulo fue realizar una vigilancia tecnológica que permitiera identificar los factores y variables de incidencia en el proceso de DA de BOM y RA; para tal fin, se tuvieron en cuenta metodologías como las de Aleixandre-Benavent at al., (2011) y Gómez-Luna *et al.,* (2014). La búsqueda se realizó en las bases de datos Scopus y Scielo, con el fin de obtener una perspectiva tanto internacional como latinoamericana. Se empleó una ventana de observación de 10 años comprendidos entre los años 2009-2019, para ello se elaboraron ecuaciones de búsqueda con palabras clave como digestión anaerobia, residuos de alimentos, parámetros de incidencia, entre otras en idioma español e inglés, así mismo se emplearon operadores boleanos los cuales permiten restringir la búsqueda al tópico de interés.

Los artículos encontrados, fueron recopilados y procesados a través del software Refviz®, el cual agrupó los artículos en galaxias a través de las cuales se identificaron las distintas variables que posteriormente fueron agrupadas en factores. De las variables logró identificarse los valores reportados como óptimos y que son empleados usualmente, así mismo, se identificó el grado de investigación que ha tenido cada una, y la baja evaluación de dos o más variables de manera simultánea.

Como resultado de la vigilancia tecnológica, se encontró que los países con mayor producción intelectual alrededor de esta temática, son en su orden: China, Estados Unidos, Reino Unido, Italia e India, mientras que a nivel de América Latina y el Caribe se destacan Brasil, Chile, Bolivia y Colombia, además se identificó que el número de artículos al respecto ha venido incrementado en los últimos años. Se identificó que los factores más influyentes en el proceso son: *i.* el sustrato y factores ambientales, *iii.* inóculo y iii. configuración experimental.

Entre las variables más estudiadas se encuentran el pH, nutrientes y temperatura; así mismo logró identificarse que, aunque el *head space* es una de las variables de incidencia en el proceso de DA, su estudio con RA o BOM aún es incipiente. Igualmente, se encontró que si bien la temperatura ha sido una variable de amplio estudio, la atención se ha centrado más en el estudio del proceso en condiciones mesofílicas y termofílicas y se ha dejado de lado el sicrofílico, en el cual el desarrollo con este tipo de sustratos ha sido menos estudiada si se compara con otras y resulta en una oportunidad de estudio sobre todo en sitios donde los recursos son escasos para poder suministrar energía con el fin de aumentar la temperatura del proceso, por lo cual cual vale la pena continuar investigando sobre todo pensando en zonas de clima frío, de forma que se pueda viabilizar el proceso. Así mismo logró identificarse que los estudios que evalúan variables de manera simultánea son escasos (18%) y por tanto la información de las interacciones entre ellas también es escasa.

Los resultados de la vigilancia tecnológica (objetivo 1) se encuentran recopilados en el artículo titulado "*Identification of Factors and Variables that Influence the Anaerobic Digestion of Municipal Biowaste and Food Waste*" en la revista *Waste Biomass and Valorization* categorizada como Q2 de acuerdo a Scimago.

Anexo 1. Identification of Factors and Variables that Influence the Anaerobic Digestion of Municipal Biowaste and Food Waste

Identification of factors and variables that influence the anaerobic digestion of municipal biowaste and food waste

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Abstract

The inadequate management of Municipal Solid Waste (MSW) can cause environmental and health issues, however the predominance of Municipal Biowaste (BW) and Food Waste (FW) make anaerobic digestion (AD) an attractive option for the treatment and recovery of organic matter. From AD two by-products with high potential use are obtained, such as methane gas (present in biogas) and the digestate, whilst the process contributes to a mitigation of the problems generated by final disposal. Nevertheless, the AD process is affected by different factors associated with environmental conditions, the substrate, inoculum, and experimental configuration. This paper presents an analysis and reflection on the effect of the variables associated with these factors from a technological surveillance over the period 2009-2019. Among the most studied variables, are temperature (in the thermophilic and mesophilic ranges), pH, and nutrients, while evaluation of psychrophilic temperature conditions and the head space effect are less studied. In addition, few studies have evaluated the simultaneous performance of different variables, which is of particular interest, when considered that their interaction can elucidate elements that lead to a greater approximation between the theoretical and experimental production of methane.

Keywords: Anaerobic digestion, biowaste, circular economy, food waste, green economy, incidence parameters.

1. Introduction

Aspects such as population growth, consumption habits, lifestyle and culture, have led to an increase in the production of Municipal Solid Waste (MSW), a trend predicted to continue, with total production between the period 2014 to 2050 projected to rise from 1.3 to 3.4 billion tons. Worldwide, final disposal is the predominant form of MSW management, being landfill responsible for approximately 5% of all greenhouse gas (GHG) emissions [1,2] and carrying to the generation of large quantities of leachates. Considering both the environmental and health problems associated with final disposal, the treatment of MSW is a problematic situation requiring urgent attention.

In Economically Developed Countries (DCs), Biowaste (BW) accounts for approximately 25-28% of total MSW, and in the Least Economically Developed Countries (LEDCs) 50-70% [3, 4], of which Food Waste (FW) represents 20-50% of BW worldwide [5] and 50-70% in LEDCs [6]. Furthermore, Slorach et al. [7] estimated that FW generates 6.7% of annual, global, anthropogenic GHG emissions. BW and FW come from domestic household activities, food processing industries, the hospitality sector, and public places such as cafeterias and restaurants. According to the Food and Agricultural Organization (FAO), approximately one-third of all food (1,300 million tons) finishes up as waste each year. In DCs, this wastage mainly occurs at the consumption stage, whilst in LEDCs, FW results from economic and administrative limitations, inappropriate collection and storage techniques [8].

The National Planning Department [9] estimates that in Colombia, from the total food supply of 28.5 million tons per year, 9.76 million tons are wasted, with 64% of these losses attributable to the stages of production, post-harvest, storage and industrial processing. As for FW production per inhabitant, authors such as Magnet [10] point out that it is of the order of 361, 278, 157, 154, 74.7, 63, 51, and 44 Kg / year in Australia, the United States, Japan, Germany, the United Kingdom, Colombia, India and China, respectively.

Given its predominantly organic composition, Anaerobic Digestion (AD) provides a promising treatment for BW and FW. AD is a multistage biological process developed in the absence of molecular oxygen [11, 12] that transforms organic substrates (*i.e.* carbohydrates, proteins and lipids) into digestate (with high potential for agricultural use [13]), and mainly biogas, with methane as the predominant gas [14]. With global dependence on fossil fuels estimated to exceed 80% of total requirement, the biogas produced from AD offers a versatile, renewable energy source which can be used for both electric power generation and as vehicular fuel [15, 16], thereby contributing to a reduction in the unsustainable reliance upon fossil fuels; particularly important given the depletion of reserves and atmospheric impacts [17, 18]. This is an aspect of great importance in the framework for concepts of the circular and green economies, which are aimed at reducing negative impacts, risks to the environment, GHG emissions and reintroducing waste into productive processes [5].

Hydrolysis is the first stage of the AD process, where extracellular enzymes are excreted by facultative and strict anaerobic microorganisms, in order to transform insoluble organic materials and compounds of high molecular mass into soluble organic materials [19, 20]. This is considered the limiting stage in the degradation of solid waste such as BW and FW, since the decomposition of long-chain carbohydrates such as cellulose, hemicellulose and lignine, predominantly present in these substrates, is slow. This limits the process because methane production does not develop rapidly [14]. Authors such as Li et al., [21], Parra-Orobio et al., [22], Morales-Polo et al., [23], Ohemeng-Ntiamoah and Datta [24] and Mirmohamadsadeghi et al., [5], indicate that variables such as temperature, pH, substrate composition and structure, particle size, substrate/inoculum (S/I) ratio, and the number of stages, among others, are of great influence at this stage.

One of the ways most widely used to evaluate the viability of AD substrates, is through a laboratory scale test known as Biochemical Methane Potential (BMP), which allows quantification of maximum biogas production. The process and the test are both based on aspects specific to the substrate, the inoculum, the method of measurement, biogas composition, and the environmental and operational factors [25, 26, 27, 28, 29].

Researchers such as Donoso-Bravo et al. [30], Ariunbaatar et al. [31], Komilis et al. [32] and Tabatabaei et al. [33] have identified the need to further investigate aspects such as: *i*. the improvement of substrate conditions, i.e. pH and nutrients; *ii*. inoculum quality improvement; *iii*. process kinetics; *iv*. reactor configuration, and *v*. digestate quality, among others. Although there is no methodological consensus regarding the BMP test, papers by Angelidakii et al. [25], Raposo et al. [34], Holliger et al. [28] and Cárdenas-Cleves et al. [29] should be highlighted, because they contribute to establishing the main aspects for consideration, however in most cases only one variable is evaluated. The aim of this article is therefore to use technological surveillance to identify the main incident variables and those which have been the most and least studied, in order so that future work can be orientated in this regard.

2. Materials and Methods

Considering methodologies such as those used by Aleixandre-Benavent et al. [35] and Gómez-Luna et al. [36] a technological surveillance process consistent with the stages shown in Figure 1 was carried out.

Figure 1 Stages of technological surveillance

The observation window established was between 2009 - 2019 and bibliometric tools were used to identify the required information, to process the data by carrying out systematic searches in English and Spanish languages in order to consider both the international (Scopus) and Latin American (SciELO) contexts, and to identify both research, reflection and review articles on anaerobic digestion (AD) studies of BW and FW. The search equations were developed using a combination of keywords (Anaerobic Digestion, Biochemical Methane Potential, Kitchen Waste, Food Waste, Influential Operating Parameters, Influencing Factors, among others) and Boolean operators that allowed the search to be restricted to the topic of interest.

The information found was collected, filtered and processed using the free software Refviz® which also eliminates duplication. Clusters that grouped themes associated with AD and that presented proximity or similarity between them were formed . This methodology has been applied in other works such as Soto-Paz et al., [37] and Parra-Orobio et al., [38], demonstrating its validity. Due to the number of publications found, articles that had 20 or more citations were selected, and in the case of recent documents, the sections corresponding to the title, summary, keywords and conclusions were reviewed. In this way, 15 articles were selected for each cluster and in the case of not achieving the required number, existing articles were read and a complete mandatory reading was done to extract key aspects for the object of study.

Using the aforementioned methodology, the trends and countries which have done the greatest research around the AD substrates of interest (BW and FW) were identified. Once the conformation of the galaxies was completed, a grouping was carried out around the main factors involved in AD, which permitted the identification of thematic groups so that the different variables involved in each factor were established more clearly, their optimal or most commonly used values, and the main treatments applied to the substrate and inoculum to increase methane production, the results of which will be presented in sections 3.1, 3.2 and 3.3.

Finally, a reflection was made with relation to the variables of greatest study and those that have not yet been widely investigated and therefore represent an opportunity for future work, in order to contribute not only to the progress and strengthening of this type of technology, but to also improve AD viability and to present opportunities to scale it for different contexts. Finally, some recommendations are made, with the objective of contributing to the identification of possible interactions between the incidence variables, and comparing the results with theoretical productions, with the aim of contributing to the standardization of the BMP test on which there are currently different experimental variations.

3. Results and Discussion

3.1. Trends in Research and Current Status

AD is an alternative treatment for the recovery of substrates that seeks to transform waste, mainly into energy in the form of methane biogas. Figure 2 shows the geographical distribution of worldwide intellectual production in the 2009 -2019 period, associated with the AD of BW and FW.

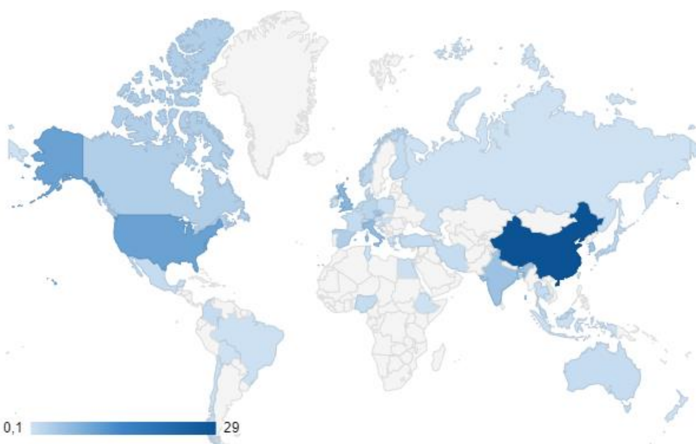

Figure 2 Geographical distribution of the Intellectual Production on Anaerobic Digestion of BW and FW

Figure 2 shows that DCs have developed and published more studies on the subject, related to the fact that these countries have subsidy policies and incentives which promote the development and acquisition of energy from renewable sources such as BW and FW [39]. It is therefore not surprising that these countries have biogas production plants [39]; in Europe for example, there are more than 120 full-scale plants to treat this type of organic waste and produce energy, with annual capacities exceeding 4 million tons [39]. Globally, the five countries with the highest rates of intellectual production on this subject are China (28.9%), the United States

(9.8%), the United Kingdom (6.8%), Italy (5.8%) and India (5.0%). Whilst, in Latin America and the Caribbean, Brazil, Chile, Bolivia and Colombia stand out.

Figure 3 shows the temporal distribution in the percentage of intellectual production between 2009 and 2019, which in accordance with the results obtained by Lin et al. [40] shows that the study of AD with different substrates has been increasing in recent years, probably as a result of the possibility of obtaining not only biogas in the form of methane used as fuel or energy, but other forms of energy such as hydrogen. Furthermore, hydrogen production is also sometimes used as an indicator of reactor stability [41, 42] and the digestate, which is the semi-solid by-product can be used in agriculture to improve soil properties [43].

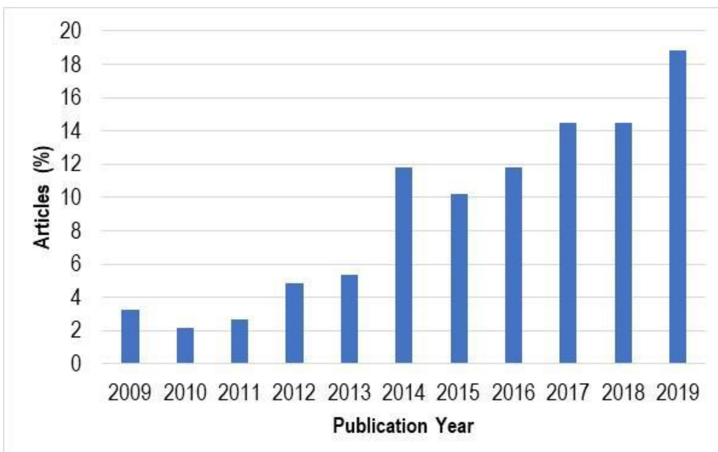

Figure 3 Temporal distribution percentage of articles published per year (2009 - 2019)

Another possible explanation for the increase in intellectual production related to AD, is the inclusion of concepts such as the circular economy and green economy, which are closely related to what is intended with this type of technology, specifically the inclusion of waste in value chains, thereby reducing generation and final disposal, contributing to people's well-being and reducing negative environmental impacts [5]. AD thus minimizes the negative effects of traditional BW management through final disposal; traditional BW management generates environmental and health problems, in addition to depleting and shortening the life of sanitary landfills [44].

It was also found that although there have been efforts to standardize methodologies such as the Methane Biochemical Methane Potential (BMP) is the most widely used test to evaluate the potential production of methane energy from any substrate, but these has not yet been standardized. It therefore presents different experimental and operational configurations, conditions that are subject in most cases to the availability of the economic resources and technological capacity of the research groups or centres where the study is carried out [25, 28, 29, 34].

Authors such as Mirmohamadsadeghi et al., [5], | Esposito et al., [26], Lisboa et al., [27], Kondusamy and Kalamdhad [45], and Gallipoli at al., [46] highlight that applications of the BMP test allow: *i*. to identify the potential for energy production of a substrate; *ii*. determine the degradation kinetics, which would allow simulation of the digestion processes and prediction of the behaviour of a scale digester; and *iii*. to identify and develop indicators to evaluate new substrates and inoculums. Among its limitations, slow degradation stands out, a problem which can be solved by performing strategies such as particle size reduction and substrate pre-treatment [21, 45, 47], in addition to the issue of slow start-up, which may be associated with conditions of lack of adaptation or acclimatization of the inoculum to the substrates [34, 48].

In the following section, the factors and variables of incidence in the AD process are highlighted.

3.2. Factors and Variables Influencing the AD Process

Different factors affect biogas production and the removal efficiency of the AD process. According to classifications made by authors such as Angelidaki et al. [25], Cárdenas-Cleves et al. [29] and, Raposo et al. [34] who categorized the variables for each of the principal factors affecting the AD process, the main factors to include are *i:* substrate and environmental, *ii.* inoculum and *iii.* reactor design and operation factors.

To make AD viable, and a consequence of the sensitivity of the biochemical methane production process, it is necessary to identify the points at which the different variables affect the process, either positively or negatively. Figure 4 shows the different clusters identified and the respective number of articles found for each variable; the clusters are grouped by factor in Figure 5.

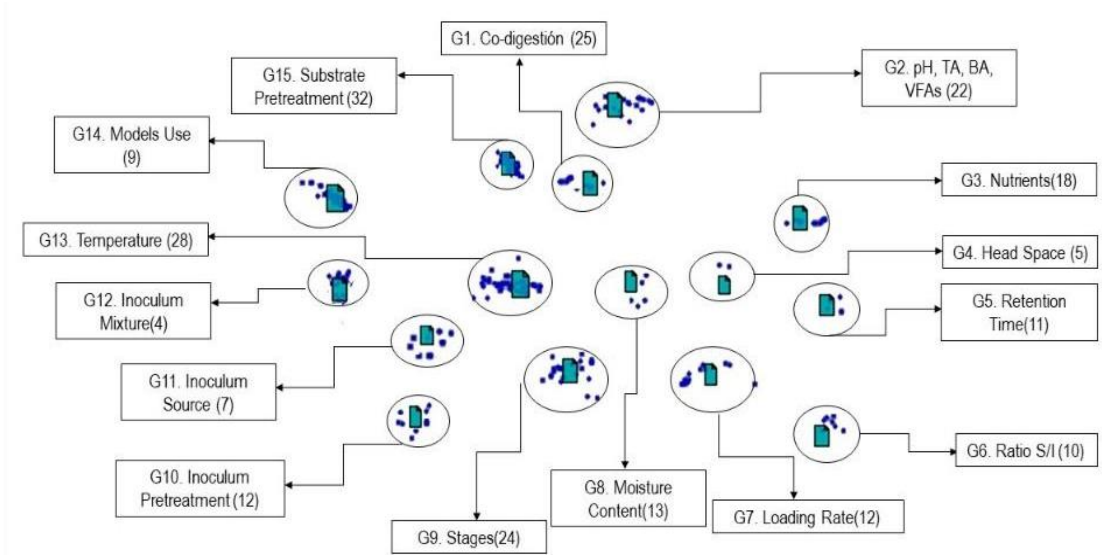

Figure 4 Incidence variables of the AD process with FW and BW

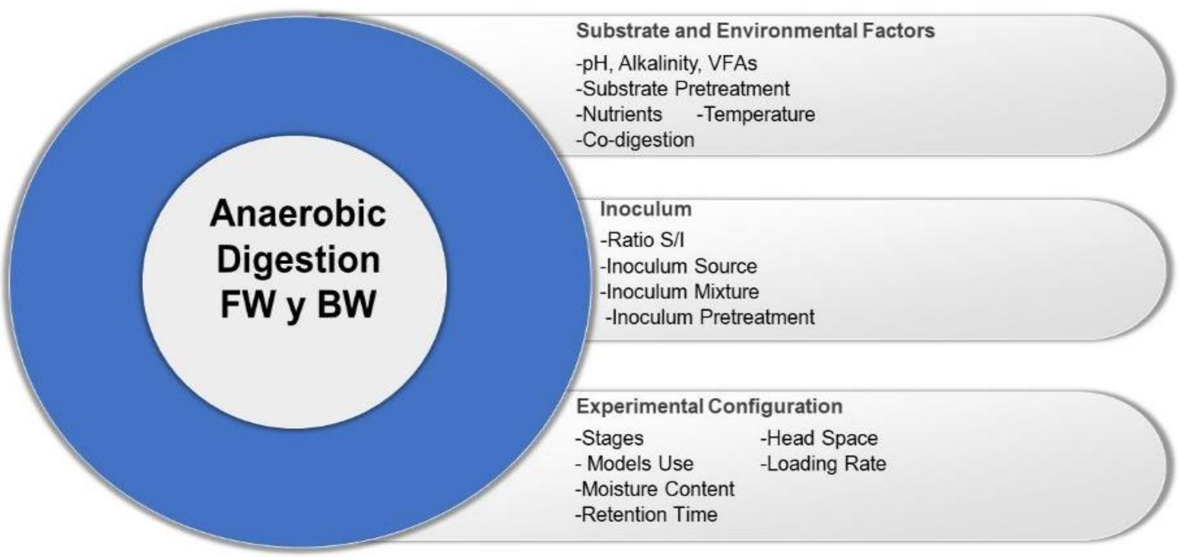

Figure 5 Factors and Incidence variables of the AD process with FW and BW

The most evaluated factor is related to experimental configuration, because it has the greatest number of associated variables, although most have not been widely investigated. The most studied aspects include the Organic Load Rate (OLR) and the number of stages involved in the process. Next was substrate and environmental factors, with pre-treatment being the most studied variable. Last is inoculum, which is mainly associated with variables such as the inoculum mixture, substrate inoculum ratio (S/I) and pre-treatment, with the latter being the most studied.

In contrast, among the aspects least studied are head space (2.16%) and retention time (3.03%); similarly, although temperature is a widely studied variable, most studies mainly reference to mesophilic and thermophilic regimes. Additionally, after reviewing the studies and the variables analysed in them, it was found that only 18% of the total studies simultaneously evaluated two or more variables, an analysis that is useful for establishing whether or not there are simultaneous interactions between the different variables [49, 50], determining the optimal points of each and the region in which the variable of interest is maximized. Given the information that can be extracted from them, studies of this type are scarce but nevertheless important.

Next, each of the AD influencing variables with FW is described, and some optimal process ranges are also mentioned. The current state of research on the variables with the highest influence in each of the factors is also analysed.

3.2.1. Substrate

AD processes developed with BW and FW are prone to inhibition due to the accumulation of volatile fatty acids (VFAs) [18; 51] because one of the characteristics of BW and FW is acidic pH [37, 52]. This parameter, in addition to other variables such as total and bicarbonate alkalinity, which are closely related to pH, must be controlled and monitored, and in turn, this process depends on other aspects or strategies for optimizing the process, such as substrate pre-treatment and nutrient concentration [24]. In the following section, the main variables and aspects associated with this factor are presented.

pH, alkalinity and volatile fatty acids (VFAs)

These variables are of great importance because they can inhibit the AD process due to VFA accumulation [53]. Hydrolysis and acidogenesis usually develop under pH conditions between 5.5 and 6.5 units [54], while microbial methanogenesis consortia (methanogenic *Archaea*) require neutral conditions; otherwise, inhibitory processes can occur, generating a low methane yield [55]. Authors such as Mirmohamadsadeghi et al. [5], Cárdenas-Cleves et al. [29] and Yang et al. [53] claim that the optimal operating pH range for the AD process is between 6.0 and 8.0 units.

Alkalinity is closely related to pH and is one of the main control and monitoring variables that can indicate process state [29]. Therefore, Total Alkalinity (TA) and Bicarbonate Alkalinity (BA), and alkalinity indices such as α (BA/TA) and IA/PA (intermediate alkalinity/partial alkalinity) [56], must be adjusted according to the inoculum source and substrate characteristics. According to authors such as Sharma et al. [57], the recommended value for alkalinity is between 2000 - 4000 mg $CaCO_3$/L for plant-based waste. In addition, the predominance of BA is important, because it neutralizes VFAs, which can inhibit processes such as hydrolysis [58] as a consequence of the high content of slow-degradation organic matter, thereby causing a decrease in pH [59].

VFAs such as acetic, propionic and butyric acids are the main intermediates during the degradation of organic matter in the acidogenic stage and are transformed into acetate and then into methane and carbon dioxide through the action of methanogenic *Archaea* [60]. Authors such as Marchaim and Krause [61] and Buyukkamaci et al. [62] suggest that a propionic acid/acetic acid ratio of 1.4 or concentrations of acetic acid higher than 0.8 g/L can trigger a failure of AD, which could be used to warn of disequilibrium and serve as a monitoring variable to establish stability conditions. Other authors such as Giwa et al. [63] suggest that in general, an elevated concentration of propionic acid indicates acidification, leading to a decrease in pH and therefore an increase of CO_2 in the biogas.

Although the natural buffer system for avoiding inhibition by VFAs is based on a balance between the phases or stages of production and the consumption of VFAs (carbonic, bicarbonate and carbonate acids prevent the pH value from drastically decreasing [5]), in the case of easily acidifiable substrates such as FW and BW, it is usually advisable to perform pH conditioning because the substrate and inoculum can lack TA and BA [25, 28].

Pre-treatment of the substrate

Different types of substrate pre-treatment increase methane production; these include physical, thermal, chemical and biological methods [45]. The objective of physical methods is to separate FW from other waste flows, such as plastics [64], and to decrease particle size (crushing) in order to increase surface area to facilitate and accelerate degradation. This pre-treatment is highly related to VFA production and retention times, and ultimately results in lower operating costs [22]. Ultrasound, another type of physical pre-treatment, has been used by researchers such as Rasapoor et al. [47] to increase biogas production by up to 80% compared to a substrate without pre-treatment, both in laboratory and pilot-scale reactors, with a power of 0.2 W/mL.

Thermal pre-treatment is one of the most used methods, and it has even been successfully applied to a full-scale operation [21]. Authors such as Cesaro et al. [65] and Li et al. [21] mention that the effectiveness of this type of treatment is due to accelerated hydrolysis and the consequent decrease in hydraulic retention time given the greater solubility induced in both organic and inorganic compounds, in addition to lower viscosity values [66]. Conversely, studies such as those by Liu et al. [67] show that high temperatures (170 °C) and long exposure times (60 minutes) lead to a decrease in efficiency, which is consistent with the results of Li et al. [21], who stated that this type of process should not be carried out at temperatures above 120 °C or exceed 15 minutes of exposure.

Kondusamy et al. [45] indicated that alkaline chemical pre-treatments are too effective. Studies such as those of Wang et al. [68] and Wang et al. [69] have evaluated the effect of the addition of different low-cost buffer substances (i.e., NaOH, CaO, $Ca (OH)_{2}$, KOH, K_2HPO_4, $Na_2 CO_3$, NaOH) to optimize the process, and each substance has advantages and limitations that must be evaluated in each case. Neves et al. [70] indicated that care must be taken, because high concentrations of sodium or potassium can cause inhibition problems.

The main objective of biological pre-treatment is to simplify molecularly complex wastes, such as lipids or lignocellulosic components, so that the microorganisms involved in AD can assimilate them more easily. This type of pre-treatment includes enzymatic pre-treatment, which aims to reduce the duration of the hydrolysis step with a mixture of enzymes (e.g., hemicellulase, xylanase, araninase) [71], and the addition of fungi such as *Trichoderma viride* to boost methane production by increasing the availability of nutrients and organic acids. Given that certain species of fungi are more associated with particular materials, prior characterization of the substrate is essential [72].

Nutrients

Some elements, such as nitrogen, phosphorus, potassium, calcium, selenium, nickel, zinc, aluminium and iron, are necessary for good process performance because they are required for the growth of a large number of the microorganisms responsible for degrading the various compounds present in FW [73, 74].

One of the most important nutrients in biological processes is nitrogen and its form is directly related to pH. Compounds such as free ammonia (NH_3) or ammonium ions (NH_4^+) are produced mainly through protein degradation, in high concentrations, NH_3 can lead to inhibition, especially at high temperatures and high pH values. Authors such as Fernandes et al. [75] report that at a pH of 7 and a temperature of 35 °C, only 1% of

NH_3 is in its free form, while at a pH of 8 and at the same temperature, the concentration increases to 10%. Contrary to the behaviour of NH_3, NH_4^+ will act as a buffer that contributes to VFA equilibrium [5].

Authors such as Parawira et al. [76] suggest that concentrations of NH_4^+ in the order of 1100 mg/L can create an adequate buffer capacity. However, authors such as Parra-Orobio et al. [77] report that only a third of this concentration (324 mg/L) is necessary to ensure enough BA is available to neutralize the VFA acidity in substrates such as BW and FW. It is therefore important to quantify NH_4^+ levels in the substrate and inoculum, in order to identify whether an external nutrient source should be added.

The traditional way to analyse the presence of adequate nutrient levels in the AD process is through the carbon/nitrogen (C/N) ratio, for which there is no established fixed value. However, authors such as Lee et al. [78] and Zhang et al. [60] claim that the most appropriate value is between 15- 35. When the C/N is low, an increase in NH_3 levels is favoured, making the AD process unfeasible [79]. Ariunbaatar et al. [33] reported a value of 3.8 g/L for the inhibitory concentration of NH_3, while Hansen et al. [80] indicated a value of 1.1 gN/L. However, optimal conditions depend upon the characteristics of the substrate and the inoculum, as well as on pH and temperature conditions, so this behaviour should be investigated, and potentially inhibitory values should be identified for different scenarios [75].

Potassium concentrations lower than 400 mg/L lead to better production in the mesophilic and thermophilic ranges, whereas when concentrations are higher, the inhibitory effect is more pronounced in the thermophilic range; thus, bacterial groups that degrade acetate are more sensitive to the toxicity associated with high potassium concentrations [81].

Essential micronutrients include nickel (Ni), iron (Fe) and zinc (Zn) because for some microorganisms, these cations play an important role in maintaining enzymatic activity. Ni is required due to its relationship with coenzymes such as F_{430}, because it serves as a central element [82], and the role of Fe is related to the transport of electrons and the conversion of carbon dioxide to methane. Zn concentrations have been found in methanogenic *Archaea* on the order of 50 - 630 mg/L [83], and thus, Zn is also expected to play an important role. However, it should be noted that depending on the concentration, pH and redox potential, these compounds can potentially produce inhibitory effects [60].

Some studies, such as those by Parra-Orobio [51], Wei et al. [84] and Zhang et al. [85], discuss the addition of nutrients in the starting conditions of the AD process, leading to the successful development of the process.

Co-digestion

Co-digestion has emerged as an economic alternative to improve the AD process, because FW is an easily degradable substrate which tends to accumulate large amounts of VFAs, leading to a consumption of BA and therefore a decrease in pH, all of which are variables of interest because they affect the methanogenic *Archaea*, thereby compromising methane production [86]. This type of alternative seeks the simultaneous digestion of two or more substrates, generating synergistic effects induced by the introduction of co-substrates to the process, in order to provide the missing nutrient requirements and other aspects such as buffer capacity [87], so that biogas production can be increased. Co-digestion is considered a sustainable and beneficial strategy for the environment, and is a practice which originated in Europe, in countries such as Germany more than 20 years ago [88].

Among the co-substrates most commonly used in the AD of FW and BW, are sewage sludge from wastewater treatment plants (WWTP), and agro-industrial waste, such as green waste and pig slurry. The first is categorized as a good co-substrate based on its total organic carbon concentration, the contribution of alkalinity to the process and the presence of macro and micronutrients [40].

3.2.2. Inoculum

The inoculum or seed is an important factor since it is the component that contributes the microorganisms to the reactor, its quality, origin and acclimatization will largely depend on the development of the process, therefore it is important to choose an appropriate inoculum according to the characteristics of the substrate to be used. According to Holliger et al. [28], the inoculum should not provide more than 20% of the total methane production of the reactor and should ideally have a pH between 7 - 8.5 units. Next, the main variables and strategies that group this factor are described.

Source of Inoculum

The type and source of inoculum are important in the AD process because granular types are more beneficial for the process than flocculent ones, due to the greater settlement, microbial diversity and methanogenic activity of the former [34]. Similarly, depending on their origin, they can supply nutrients and provide the microorganisms that are necessary for the proper development of the process [51, 89]. The inoculum should ideally be physicochemically characterized, even with respect to its nutrient content, because this along with the characteristics of the substrate, will indicate the need for an external nutrient source [22]. The most commonly used inoculum types are *i.* digester sludge from WWTPs, *ii.* sludge from agricultural waste treatment plants, *iii.* sludge from biological waste [90], *iv.* digester sludge from chicken excreta and *v.* sludge from the degradation of pig and cattle manure [5].

Substrate/Inoculum (S/I) Ratio

The S/I ratio is important in the AD process because according to the characteristics of the substrate and the inoculum used, this ratio can vary [91]. Authors such as Raposo et al. [34] recommend employing ratios between 0.5-1.0 $gVS_{substrate}/gVS_{inoculum}$. This relationship is also expressed in terms of $gCOD_{substrate}/gVS_{inoculum}$, and authors such as Liu et al. [67] and Parra-Orobio et al. [77] show that at a higher S/I ratio, there is less methane production, which according to authors such as Hansen et al. [92], may be due to the accumulation of VFAs and the low buffer capacity of the reactors, which facilitates the acidification of the system and thereby inhibits the AD process.

Inoculum Mixtures

To increase and improve the AD processes, strategies have been studied to increase biogas production, including the inoculum mixture. Studies such as Parra-Orobio et al. [51], focus on the Biochemical Methane Potential (BMP) with different inoculum mixtures; using a granular inoculum from an anaerobic digester of a slaughterhouse and a flocculent sludge from a WWTP, they found that the optimal inoculum mixture was 25:75 V/V. Additionally, Cristancho et al. [93] mixed a flocculent inoculum from a UASB reactor and an inoculum with a tendency to form granules from a pig manure treatment process at 50:50 V/V, and compared their results to the yields obtained for each sludge separately; it was found that the inoculum mixture not only induced an increase in methane production but also showed better pH stability.

Pre-treatment of the Inoculum

Researchers have found that alternatives such as the addition of coagulants and nutrients or incubation of the inoculum, improve its characteristics. However, while the addition of coagulants improves the settlement of sludge, it can inhibit microorganisms, so the concentration to be added must be studied [51]. In addition, depending on the type of coagulant, the behaviour will vary, and therefore, each case should be evaluated. Romero et al. [94] claimed that nutrients have a positive impact on the AD process, not only because of the stability that they provide to the process, but also because they facilitate their own reactions. In any case, the ideal is to show the shortcomings of the system and to supplement the missing nutrients with the objective of optimization [24]. Incubation consists in leaving the inoculum at the temperature at which the process is going to develop so that there is a period of temperature acclimation and a shorter latency or adaptive phase, thus avoiding low production once the reactor starts operating, which could potentially prevent the process from being classified as adequate [68, 89].

3.2.3 Experimental Design

The experimental design of the reactor is one of the main factors to be taken into account in AD, because different production values will be obtained for the same substrate and inoculum used [5]. More attention should be paid at certain stages of the process due to the sensitivity, and in some cases, operating costs will be higher [95]. Some variables and strategies considered in this factor are presented below.

Temperature

Temperature is one of the variables that most affects the AD process, because according to the temperature range, there are differences in the latency phase, microbial communities and stability, among others [64, 96]. Biogas production in wastewater has been identified in both the Arctic (<0 °C) and at very high temperatures (100 °C) [12]. In general, three temperature ranges and optimal points are established; for example, the psychrophilic range is less than 20 °C, the mesophilic range is between 20 and 45 °C and the thermophilic range is between 45 and 65 °C, with 15, 35 and 55 °C being the optimal temperatures, respectively [14, 97].

Authors such as Deepanraj et al. [98], have conducted different studies with temperatures ranging between 30 and 60 °C in both the mesophilic and thermophilic ranges [99], and found greater yields at higher temperatures. Although high temperatures (thermophilic range) have a positive effect on biogas production as a consequence of the higher degradation rate of the substrate (approximately 50%) compared to the mesophilic range, and digestate with a lower amount of pathogens is obtained, i.e., a more adequate material in terms of safety for agricultural use [5, 100], more energy is required to maintain these temperatures [59], and in addition, in this condition the AD process is more sensitive to small changes and prone to the accumulation of propionic acid [101, 18]. Due to the stability of the process and the high organic loadings of FW, authors such as Guo et al. [11] indicate that mesophilic AD (i.e., 35-37 °C) is the most suitable for this type of substrate.

There are zones that due to their climatic and topographic characteristics are considered cold, and as a consequence of the energy expenditure required, some alternatives that enable viable AD in the psychrophilic range have been studied. Authors such as Rajagopal et al. [58] have showed that the co-digestion of FW with substrates such as dairy cattle manure favours methane gas production under psychrophilic conditions. Similarly, [102] carried out pilot-scale application studies at 16.8 °C for the AD of fruit and vegetable waste without particle size conditioning; the digester was constructed with a black geomembrane of different thicknesses (1.5 and 2.0 mm), and expanded polyethylene was used as insulation to prevent heat loss, the design did not involve the heating or mixing of substrates to obtain biogas. In European countries, FW-AD has become an emerging and applied technology due to the subsidies granted [31].

Number of stages

The design of reactors by stages has emerged as a solution for the rapid transformation of FW into VFAs, because the processes that occur in the first stage of AD trigger a drastic drop in pH and therefore an inhibition of the process, mainly of methanogenic *Archaea* [103]. When compared to a single-stage digestion, AD that has been developed in two or three stages presents advantages, such as *i*. An improvement of the hydrolysis phase, *ii*. Process stability, *iii*. An increase in methane production, i.*e.*, a decrease in the hydraulic retention time, i*v*. The use of high organic loads and *v*. A greater microbial diversity [104, 105]. A disadvantages of the two stages includes the higher costs attributable to the needs and operation of another reactor [95].

In staged systems, a slightly acidic pH (5.5-6.5) is maintained in the first reactor with a short hydraulic retention time (HRT) (2-3 days), while in the second reactor, a pH close to neutrality (6-8) is maintained with a HRT of 20 to 30 days, to promote a favourable growth environment for the methanogenic *Archaea* [106]. Additionally, the temperature can vary between the stages; for example, the first stage can be maintained in thermophilic conditions, while the second is maintained in mesophilic conditions. In addition, according to authors such as Xu et al. [103], the recirculation of the leachate from the second to the first stage, can improve the process.

In the treatment of FW through AD, authors such as Nathao et al. [107] and Shen et al. [108] have increased methane gas production by 7.0 to 15.8% compared to single-stage reactors. Zhang et al. [106] demonstrated that a three-stage system (hydrolytic, acidogenic and methanogenic) is also favourable for AD degradation with a co-substrate, increasing production up to 22.7% when compared to a conventional system that used the same co-substrate. They also identified significant differences in the microbial population present and favoured the trophic group of interest corresponding to each stage.

Head Space

Another variable that affects the process and is related to pH is head space, which is especially important when biogas measurements are performed using the manometric method, which consists of introducing the needle of a manometer through a rubber plug and converting the measured pressure into the volume of the biogas produced by means of a series of equations [29, 109, 101]. According to Holliger et al. [111] and a study from 32 laboratories using different substrates, this method of quantifying methane yields inferior results when compared with measurements made by automated volumetric methods.

In agreement with authors such as Angelidaki et al. [25], Holliger et al. [28] and Valero et al. [112], head space can inhibit the process due to alterations in pH because with a greater partial pressure of CO_2 in the head space, there is a greater presence of organic carbon dissolved in solution, and consequently, a lower concentration of CO_2 in the biogas, for which low head space conditions favour the increase in partial pressure and the solubilization of $CO_{2.}$ In addition, because organic carbon is a buffer component (i.e., it contributes to pH equilibrium by preventing declines as a consequence of the VFAs produced from decomposing organic matter), it prevents inhibition of the process caused by the death of microorganisms such as methanogenic *Archaea*, which are responsible for methane production and are highly sensitive to changes in pH [24, 113, 114]. This also avoids the need for additional alkalizing agents which represents a cost saving.

Despite the importance of this variable on the efficiency of the AD process, it is observed that there is no consensus regarding the recommended range of values, and in this review values between 20 and 50% of the useful volume of the reactors were found [83, 85, 115]. In addition, no studies on the influence of this parameter with FW were found, only publications suggesting it should be evaluated [112, 114].

Use of traditional kinetic models and dynamics

Kinetic models enable the design, operation and control of biological systems [116]. Within these models, some models, such as the first-order model, consider environmental or operational influencing variables. Taking into account that overall in the AD of solid waste, the limiting stage of the process is hydrolysis, kinetic models can establish the influence of different factors [22, 46, 117]. In this sense, other models have been developed that enable the biomass-by-product ratio to be predicted in order to prioritize methane production. Furthermore, some of these models can be used to evaluate variables such as the latency phase, which is related to two stages of anaerobic degradation (acidogenesis and acetogenesis).

These models also include the logistical function, transfer function and modified Gompertz models: the first model assumes that the methane obtained is proportional to the microorganisms present and the substrate under biodegradation; the transfer model considers the reactor to be a fixed process, and thus, as a system of inputs and outputs; the Gompertz model assumes that production is proportional to microbial activity and decreases with the solid retention time [21, 30]. It should be noted that the latter is one of the most widely used model in AD processes using an FW substrate, even though some authors claim that production can sometimes be overestimated [118]. For this reason, in general, the use of several models is recommended in order to identify the one that best fits production trends. Furthermore, given that some models also provide information that others do not (i.e., the Gompertz model yields the latency phase, while the first-order model order can determine the hydrolysis constant); it is advisable to use them in a complementary manner, not for comparison purposes, but rather to obtain more information about the AD process.

Another model used to predict methane production is the Anaerobic Digestion Model No. 1 (ADM1) model, which was developed by the International Water Association (IWA). The model is based on mass and load balances, represents both biological and physicochemical reactions [119] and is divided into five steps: disintegration, hydrolysis, acidogenesis, acetogenesis and methanogenesis. The first step is a process in which organic and inorganic matter is transformed into carbohydrates, proteins and lipids; the model also considers the effects of inhibition by variables such as pH and hydrogen and consists of 32 dynamic state variables [120].

Organic Load

Organic load is another variable of incidence in the AD process which affects the performance of the methanogenic *Archaeas* and therefore biogas production. Although higher organic load is expected to increase methane production, in practice it is not entirely true, since it can cause inhibition problems [121]. Some authors such as Kumar et al. [49] jointly evaluated the effect of organic load and temperature (mesophilic and thermophilic regime) on FW-AD; in their study organic loads between 1.0 and 6.1 kg COD / m^3 * day were used. Kumar et al found that an increase in organic load increased methane production in both temperature regimes, and under thermophilic conditions, the acceleration of the hydrolysis of the process is favoured.

Solids Content

The total solids (TS) and volatile solids (VS) content of FW is generally between 18.1 - 30.9 and 17.1 - 26.3 respectively [60], so the moisture content is high (70 - 80 %), and given the high VS / TS ratio which indicates the organic predominance of the substrate [5], it is an easily degradable substrate [101]. From the point of view of the TS content, AD can be classified in two types: when TS <16%, it is a wet process and when 16% <TS <40%, it is a dry process [95]; while according to other classifications, there is the conventional modality (≤20% TS), semi-dry (10% <TS <20%) and dry (TS> 20%). The main advantage of wet AD is the greater contact between the substrate and the inoculum, which is why it is attributed greater efficiency, but its greatest limitation is the requirement of greater area due to the volume of liquid necessary to guarantee moisture [95, 50].

Experiences such as that of Yi et al., [122] conclude that as the content of TS increases and moves in the range of 10-20%, higher yields are obtained, likewise there is a decrease in the VS, which is reflected in increased biogas production.

Retention time (RT)

RT is considered as the time it takes organic matter to decompose completely or the average time that organic matter remains in a reactor or digester [14]. Mao et al., [101] pointed out that temperature is an important variable, since it is closely related to bacterial growth and retention time. According to Kothari et al., [12], a time of 10 to 40 days is enough to treat organic waste at temperatures in the mesophilic range, but in the thermophilic range, this period is shorter. For example, a reactor operated in this range has a RT of approximately 14 days.

There are two kinds of RT, the first is the Solid Retention Time (TRS) and refers to the time in which the microorganisms are in the digester, and the second is the Hydraulic Retention Time (more related to liquid substrates), which is defined as the time in which the liquid passes through the digester [98]. Therefore, prolonged TRS and HRT are recommended in order to facilitate biological acclimatization to toxic compounds and greater degradation, therefore enhancing biogas production.

4. Research and Reflection Trends

According to this technological surveillance, factors such as the substrate, inoculum and reactor are observed to be of great importance in the DA process. Variables such as pH, alkalinity and VFAs, the solids content and nutrients, as well as an evaluation of the process under temperature conditions in the mesophilic and thermophilic ranges, have been extensively investigated. Among the strategies for improving the AD process of BW and FW, co-digestion with other substrates and both substrate and inoculum pre-treatments stand out.

Although it is observed that in general there are ranges considered optimal for the majority of variables identified for each factor, with some being more studied than others, it is necessary to evaluate each particular variable depending on local conditions (characteristics of available substrates and inoculums, temperature modification possibilities, nutrient addition, mode of operation, etc.), in order to first evaluate the conditions that sustainably favour the highest methane yield, thereby minimizing resource consumption and always obtaining the greatest possible amount of renewable energy.

In contrast, among the less evaluated aspects, there is the evaluation of the process performance in conditions of psychrophilic temperatures and head space is also known to have an important impact on the process, being another aspect that requires more research. Another interesting aspect of this study is the identification of the increasing importance that digestate or biosolid has taken, which is the solid material that in most cases is stabilized as a result of the degradation of the AD process itself. This material is rich in nutrients such as nitrogen, phosphorus and potassium and is of great interest for its potential use as a soil improver or partial replacement of chemical fertilizers. Variables such as pH, nutrient content, organic matter content and pathogens should be measured in the digestate once the process is finished, with the aim of assessing its potential use [123], since according to these characteristics, it can increase microbial activity and therefore the flow of CO_2, in addition to generating nitrogen losses and may even have a phytotoxic effect [43].

In addition to the economic and environmental benefits associated with a decrease in the levels of biosolids or digestates, their return to the production chain can also contribute to a reduction of costs associated with the use of chemical fertilizers. For example, in 2009 in the United Kingdom, it was decreed that the digestate must comply with the protocol (The Quality Protocol for Anaerobic Digestate) established by the Environmental Agency, which issues a certificate, without which the material cannot be applied to the ground. In the Colombian case, Decree 1284 [124] was approved in 2014, which establishes criteria for the use of the biosolids generated in WWTP.

The above shows the opportunity for future work along these lines, in order to increase the closing of cycles in this kind of process and favour technologies that point both to a decrease in the generation of waste, and to a minimization in the final disposal of materials of high exploitation potential. Techniques such as Anaerobic Digestion, allow us to obtain materials with both energetic and agricultural potential, while reducing problems such as the generation of GHG and leachate, among other aspects. In this way, the AD is related with the circular economy and green economy, promoting the incorporation of waste to other processes and reducing the quantity which finishes up in landfill [5].

Another aspect observed is that, in general, there are still few studies that jointly or simultaneously evaluate several of the related aspects, therefore future studies which jointly evaluate different variables should be developed, so that their effect upon methane production and the interaction between the different variables is documented.

Finally, it is recommended that the results obtained in different experimental conditions should be contrasted with expected theoretical production values from elementary analysis (C, H_2, O_2, N, S) or from the organic load (COD) of the substrate [125], to identify the variables that most contribute to this aspect, so that cost-effective analyses and multi-criteria techniques can be carried out for the application of improvement strategies.

5. Conclusions

- The anaerobic digestion of biowaste and food waste is an increasingly relevant biological technology, because it contributes to extending the useful life of landfills, decreases the generation of leachates and greenhouse gases through both treatment and recovery, thereby helping to close production cycles.

- While temperature (thermophilic and mesophilic) is one of the variables that has been widely studied, a limited number of studies have been conducted under psychrophilic conditions, which is important to study, especially in places where resources to provide the mesophilic temperature are scarce; a condition that has been reported within the optimum range due to its stability. Another variable for which there is not yet sufficient information is head space, although several articles suggest evaluating it due to its relationship with the carbonaceous system that influences the balance of bicarbonate alkalinity and volatile fatty acids.
- The majority of studies present evaluations focused on a single parameter and ignore the interactions that may exist between two or more variables, so it is recommended to evaluate variables together to determine whether or not there is an interaction between them and to identify the optimal values for each variable.

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CAPÍTULO 3. EVALUACIÓN SIMULTÁNEA DEL HEAD SPACE Y LA TEMPERATURA SOBRE EL PBM

3.1. Síntesis del capítulo

De acuerdo con los resultados obtenidos en el capítulo anterior, se seleccionaron como variables a evaluar experimentalmente el *head space* (X_1) y la temperatura (X_2), con las cuales se evaluó la influencia simultánea sobre el proceso de DA de los RA, los cuales fueron conformados con frutas no cítricas (8,2%), frutas cítricas (25,0%) fibras minerales (3,2) y cáscaras de plátano y papa (56,0%), simulando las características de RA de un municipio que realiza separación en la fuente y recolección selectiva de los RSM (Parra-Orobio et al., 2018; Soto-Paz et al., 2019). Los ensayos se realizaron por triplicado, a través de ensayos PBM y mediante la aplicación de la metodología MSR y un Anova, se analizó la interacción de X_1 y X_2 en tres niveles para cada variable que fueron 20, 35 y 50% del volumen útil y 20, 35 y 55°C, los valores fueron tomados de acuerdo a los resultados de la vigilancia tecnológica del capítulo previo (Zhang y Jahng, 2012; Wei et al., 2014; Deepanraj et al., 2015, Li et al., 2016 y Parra-Orobio et al., 2018); estos valores fueron normalizados en X_1, X_2 y X_3. Los ensayos se llevaron a cabo en reactores de 0.25 L, el seguimiento a la producción se llevó a cabo a través del método manométrico empleando un manómetro marca Wika tipo CPG500 y para mantener cada una de las temperaturas se emplearon incubadoras WTW TS 606/G12 (20 ± 1°C), WTW TS 606-G/2-i (35 ± 0.5°C) y un horno WTB binder E-28 (55 ± 1.5°C).

Así mismo se emplearon los resultados experimentales de producción de metano para aplicar los modelos cinéticos de primer orden y de Gompertz Modificado, para evidenciar si había incidencia o no de las variables evaluadas sobre el proceso y sobre las condiciones de hidrólisis del proceso, que es un aspecto de gran importancia en la DA de residuos sólidos como los RA.

Se logró establecer que el punto donde el *head space* y la temperatura hacen que la producción de metano se maximice, son 20% y 48.9°C, lo cual es consistente si se compara con otras investigaciones como las de Stumm y Morgan, (1996), Kim et al., (2006), Himanshu y Col, (2017) y Mirmohamadsadeghi et al., (2019). Así mismo se logró identificar mediante un análisis Anova, que la variable evaluada que tiene mayor incidencia en el proceso es la temperatura con un $p \leq 0.0001$ en comparación con el *head space*, con un $p = 0.0002$. Así mismo, se identificó que un *head space* mayor al 20% favorece que se alcance una mayor concentración de AGVs, lo que inhibe el proceso. De otro lado, se halló que en cada uno de las configuraciones experimentales evaluadas el aporte del inóculo no superó el 20% de producción total, lo cual se encuentra en concordancia con lo recomendado por Holliger et al., (2016).

A través del análisis cinético realizado, se logró reafirmar que los valores de *head space* (20%) y temperatura (49.8°C) hallados como óptimos, son la mejor combinación para el buen desempeño del proceso, ya que bajo estas condiciones se halló una constante de hidrólisis más alta (0.03) si se compara con las demás obtenidas. El segundo modelo hace una mejor descripción del proceso, tanto por el mayor ajuste como por presentar un error más bajo (R^2=0.99 y RMSE<2.12); además, fue posible evidenciar la incidencia de las variables sobre la fase de latencia, sobre la cual se puede afirmar que no siempre una fase corta favorecerá la DA ya que la sobreproducción de AGVs podría imposibilitar el desempeño del reactor (Rajagopal et al., 2017).

Los resultados del trabajo experimental de este estudio (Objetivos específicos 2 y 3), fueron compilados en el artículo *Evaluation of Simultaneous Incidence of Head Space and Temperature on Biochemical Methane Potential in Food*

Waste, el cual fue publicado en la revista *Cogent Engineering* catalogada como Q2 de acuerdo a Scimago (Ver Anexo 2).

Anexo 2. Evaluation of Simultaneous Incidence of Head Space and Temperature on Biochemical Methane Potential in Food Waste

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Evaluation of simultaneous incidence of head space and temperature on biochemical methane potential in food waste

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Abstract: The anaerobic digestion (AD) of food waste (FW) has been recognised as a low-cost and high-efficiency alternative that mitigates the impacts of the final disposal of FW, while facilitating the production of high value-added by-products, such as biogas and digestate. In this study, the simultaneous incidence of two significant variables in the AD of FW were evaluated: the temperature (20, 35 and 55 °C), which affects the kinetics of the process, and the head space (20, 35 and 50%), which can affect the pH and therefore the biochemical methane potential (BMP). A composite central design was used, and response surface methodology (RSM) and kinetic models (the first-order and Gompertz models) were used to analyse the process. At higher temperatures and smaller head spaces, inhibition phenomena were reduced by the consumption of bicarbonate alkalinity and decreasing the pH, resulting in a higher process performance. The optimal conditions were a temperature of 48.9°C and a head space of 20%. The Gompertz kinetic model fit the process dynamics better and with a lower error than the first-order model.

Subjects: Agriculture & Environmental Sciences; Environment & Resources; Environmental Change & Pollution

Keywords: anaerobic digestion; food waste; head space; process kinetics; temperature

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PUBLIC INTEREST STATEMENT

A consequence of growing population, is the production of Municipal Solid Waste and due to their composition predominantly organic (as Biowaste and Food Waste), the anaerobic digestion is a biological alternative that not only allows to increase the useful life of landfills and minimize the generation of leachate and GHG emissions, but also its valorization for the production of energies such as methane. Biochemical Potential of Methane (BMP) assay, is the main tool of prediction, on controlled scale of the potential energetic of the substrate. Both, the head space and the temperature have influence the process and the methane generation, and is necessary to study their join influence, to stablish the optimal condition for the process.

1. Introduction

In developing countries, the predominant component of municipal solid wastes (MSW) is the organic fraction (50-70%), commonly known as OFMSW or MBW (municipal biowaste) (Oviedo, Marmolejo, & Torres, 2012; Thi, Kumar, & Lin, 2015; Kaza et al., 2018). The MBW consists mainly of food waste (FW: 40-60%) (Cabeza, María, Aura, Paola, & Marioc, 2016; Parra-Orobio et al., 2018). Unlike other solid wastes, such as industrial or agricultural waste (Wang & Nie, 2001), FW is considered a heterogeneous flow, mainly because of the difficulty of separating the FW stream from other waste streams, such as paper, plastics and inert waste (De Gioannis, Diaz, Muntoni, & Pisanu, 2008), in addition to the low frequency of source separation and selective collection of MSW (Parra-Orobio et al., 2018; Soto-Paz, Oviedo-Ocaña, Manyoma-Velásquez, Torres-Lozada, & Gea, 2019).

Final disposal (FD) in landfills (LF) is not an appropriate management strategy for MSW (Saer, Lansing, Davitt, & Graves, 2013; Schott, Wenzel, & la Cour Jansen, 2016) because the subsequent environmental impacts, such as the generation of greenhouse gases (GHG) and leachates, however, LF remains the most commonly used management alternative for MBW and FW in most developing countries, such as Colombia, which is covered by Decree 2820 (2010) from the Ministry of Environment, Housing and Territorial Development (MAVDT 2010). Silpa, Yao, Bhada-Tata, and Van Woerden (2018) have reported that the GHG produced worldwide from MSW disposal in LF are on the order of 1.6×10^9 tons of carbon dioxide ($CO_{2equivalent}$), representing 5% of global emissions, which deteriorate the ozone layer and negatively impact human health and the environment (Christensen, Manfredi, & Knox, 2011). This form of management also has other unfavourable effects, such as the depletion of the useful life of the FD sites, which are in increasingly limited supply (Manyoma, Orejuela, Torres, Marmolejo, & Vidal, 2015).

However, being predominantly organic, these residues have a high use potential through strategies such as anaerobic digestion (AD) and composting. AD is a biochemical process that occurs in the absence of molecular oxygen and consists of multiple stages involving different microorganisms; the process occurs via the transformation of complex compounds, such as carbohydrates, proteins and lipids, into simpler products such as 90% biogas (mainly methane -CH_4, which has a high energy potential) and 10% biosolids or digestate (Foresti, Florêncio, Van Haandel, Zaiat, & Cavalcanti, 1999; Satchwell et al., 2018), which has a high potential for use in soil recovery and agricultural activities (Tampio, Salo, & Rintala, 2016). Thus, research on AD technology has increased in recent years (Lin, Xu, Ge, & Li, 2018).

The biochemical methane potential (BMP) assay is a traditional form that is widely used to evaluate the AD viability of a substrate. There is still no consensus on a methodology to determine BMP development, despite the efforts of researchers, such as Angelidaki et al. (2009), Raposo, De La Rubia, Fernández-Cegrí, and Borja (2012), Holliger et al. (2016) and Cárdenas-Cleves, Parra-Orobio, Torres-Lozada, and Vásquez-Franco (2016). The temperature and the head space are two significant AD incidence variables (Komilis et al., 2017; Valero, Montes, Rico, & Rico, 2016; Sasha et al., 2019; Mirmohamadsadeghi, Karimi, Tabatabaei, & Aghbashlo, 2019; Ohemeng-Ntiamoah et al., 2019); the temperature affects the process kinetics (Boulalagui et al., 2004; Kim, Oh, Chun, & Kim, 2006; Labatut, Angenent, & Scott, 2014), being the mesophilic (20–45 °C) and thermophilic (46–60°C) ranges typically considered (with the former being most frequently used), and few studies have been performed over the psychrophilic range (≤ 20 °C) (Connaughton, Collins, & O'Flaherty, 2006; Kothari, Pandey, Kumar, Tyagi, & Tyagi, 2014). On the other hand, the head space can affect the pH of the medium and destabilise microbial consortia and thus the BMP quantification, and the solubilisation of gases, such as CO_2, can have a buffer effect on the process (Jankowska, Chwiałkowska, Stodolny, & Oleskowicz-Popiel, 2015; Valero et al., 2016). Ranges of head space between 20 and 50% of the total volume of the reactor are generally used (Li, Liu, Su, & Yan, 2016; Wei et al., 2014; Zhang & Jahng, 2012).

In this study, the simultaneous incidence of temperature and head space on the BMP of FW was evaluated. The FW simulated the characteristics of MBW from a locality that performs separation at the source and selective MSW collection, which are fundamental premises of the use of this type of substrate (Han & Zhang, 2017; Lakeh et al., 2019; Razavi, Koupaie, Azizi, Hafez, & Elbeshbishy, 2019; Soto-Paz et al., 2019).

2. Materials and methods

2.1. Substrate and inoculum

A sample was reconstituted with the characteristics of the MBW from a locality that performs separation at the source and selective collection of MSW. The substrate was formed from raw FW that was separated at the source of a university restaurant that serves an average 4338 lunches per day (Parra-Orobio et al., 2018) and a market place in the City of Cali, Colombia. The FW composition was as follows: carbohydrates (55.4%), citrus fruits (25.0%), fibres and minerals (8.2%), non-citrus fruits (8.2%), herbs and others (3.2%) (Parra-Orobio et al., 2018; Soto-Paz et al., 2019).

An inoculum was formed using a 75/25 (V/V) mixture of flocculent sludge and granular sludge, respectively. The first one was obtained from an anaerobic digester of a municipal wastewater treatment plant (WWTP) that serves a population of more than two million inhabitants, and the granular sludge was obtained from an anaerobic reactor of a slaughter plant for cattle and pigs (Parra-Orobio et al., 2018).

2.2. Analytical methods

The FW were characterised in terms of pH (units), total alkalinity (TA, mg $CaCO_3$/L) and bicarbonate alkalinity (BA, mg $CaCO_3$/L), volatile fatty acids (VFA, mg $CaCO_3$/L), chemical oxygen demand (COD, mg O_2/L), total organic carbon (TOC, mg/kg), total solids (TS, mg/L) and volatile solids (VS, mg/L), total nitrogen (TN, mg/kg), total phosphorus (TP, mg/kg), lignin (%), cellulose (%), carbon (C,%), hydrogen (H,%), oxygen (O,%), nitrogen (N,%), carbohydrates (%), proteins (%) and lipids (%), according to APHA et al., (2005), ICONTEC (2004) and ICONTEC, (2011).

The inoculum was characterised according to APHA and WEF (2005), Aquino et al., (2007), ICONTEC (2004), ICONTEC, (2011) and Hu, Wang, and Yu (2004) in terms of the pH, TA, BA, VFA, TS, VS, specific methanogenic activity (SMA, $gCOD_{CH4}$/g VS·d), hydrolytic activity (HA, $gCOD_{Glucose}$/gVS.d) and specific acidogenic activity (SAA, $gCOD_{Glucose}$/gVS.d).

3. Calculation of theoretical methane potential

Different calculation schemes were used to compare the theoretical methane production with the experimental results. In the first calculation, the stoichiometry of the substrate degradation reaction was used with Equations 1 and 2 (Buswell & Muller, 1952 and Sosnowski et al., 2003). A second calculation (Equation 3) was performed using the percentage of carbohydrates, proteins and lipids in the sample (Lesteur et al., 2010; Raposo et al., 2012 ; Nielfa, Cano, & Fdz-Polanco, 2015).

$$C_nH_aO_bN_c + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{a}\right)H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} - \frac{b}{4} + \frac{3z}{8}\right)CO_2 + \left(\frac{n}{2} - \frac{a}{8} - \frac{b}{4} + \frac{3z}{8}\right)CH_4 + cNH_3 \quad (1)$$

$$BMP_{theoretical}(mL\ CH_4/gVS) = \frac{22.4 * \left(\frac{n}{2} - \frac{a}{8} - \frac{b}{4} + \frac{3c}{8}\right) * 1000}{12n + a + 16b + 14c} \quad (2)$$

where the number of moles of carbon, hydrogen, oxygen and nitrogen are represented by n, a, b and c, respectively; 22.4 is the volume in litres (L) occupied by an ideal gas at standard conditions; 1000 corresponds to the conversion factor from L to mL; and 12, 1, 16 and 14 are the molecular weights of carbon, hydrogen, oxygen and nitrogen, respectively.

$$BMP_{theoretical}(mL\ CH_4/gSV)\ 415 * \%Carbohydrates + 496 * \%proteins + 1014 * \%lipids \quad (3)$$

where 415, 496 and 1014 are multiplicative constants for the percentages of carbohydrates, proteins and lipids from the FW sample (Lesteur et al., 2010; Raposo et al., 2011; Nielfa et al., 2015).

Table 1. Experimental configurations

Treatment	X_1	X_2	Temperature (° C)	Head space (%)
A	−1	−1	20	20
B	−1	0	20	35
C	−1	+1	20	50
D	0	−1	35	20
E	0	0	35	35
F	0	+1	35	50
G	+1	−1	55	20
H	+1	0	55	35
I	+1	+1	55	50

4. Experimental design

A composite central experimental design was performed in which the simultaneous incidence of the temperature (X_1) and the head space (X_2) were evaluated using response surface methodology (RSM) (Montgomery, Runger, & Hubele, 2009). In this study, each factor was evaluated at three levels corresponding to minimum, intermediate and maximum values (the normalised values were −1, 0, +1, respectively), following studies by Zhang and Jahng (2012), Wei et al. (2014), Deepanraj, Sivasubramanian, and Jayaraj (2015), Li et al. (2016) and Parra-Orobio et al., (2018). Table 1 presents the values used in the design and the different experimental configurations. The BMP was the response variable.

5. Experimental setups and process monitoring

A CB15 industrial food processor was used to grind the substrate to a particle size of less than 30 mm (Izumi et al., 2010; Pearse et al., 2018), with which the PBM assays were developed. A substrate/inoculum (S/I) ratio of 1 $gVS_{substrate}/gVS_{inoculum}$, was maintained, acording Parra-Orobio et al., (2018).

The BMP assays were performed in triplicate in 0.25 L amber glass reactors. To ensure that the manometric measurement of the biogas corresponded mainly to methane, a polypropylene cartridge containing four NaOH beads was placed inside each bottle under the lid to capture CO_2 (Holliger et al., 2016; Souto, Aquino, Silva, & Chernicharo, 2010). A manometer (WIKA, model CPG500) was used to record the pressure of the biogas that accumulated in the headspaces of the reactors. The biogas composition was confirmed by chromatography (Shimadzu GC-2014). Nutrient addition was performed, and a control reactor (target) containing only the inoculum and distilled water was used to subtract endogenous production (Cárdenas-Cleves et al., 2016).

The temperature was controlled by maintaining the lowest temperature (20 ± 1 °C) in a WTW TS 606/G12-i incubator; the intermediate temperature (35 ± 0.5 °C) in a WTW TS 606-G/2-i incubator and the high temperature (55 °C ± 1.5 °C) in a WTB binder E-28 oven. The BMP was determined using equations formulated by Aquino (2007), Giménez, Martí, Ferrer, and Seco (2012) and Cárdenas-Cleves et al. (2016), where the proportion of the dissolved methane was used to determine the volume of methane produced under standard conditions. The assays were manually agitated and monitored four times a day (Parra-Orobio et al., 2018), and biogas samples were withdrawn for chromatographic analysis. The monitoring was terminated upon the observation of asymptotic behaviour, following the recommendations of Holliger et al. (2016).

6. Data processing

The results of the characterisation of the substrate and inoculum were analysed using descriptive statistics tools. The production results obtained from each of the experimental configurations were processed using MATLAB® 2017 software to develop a response surface, showing the optimal points at which the variable of interest (the BMP) was maximised. A two-way ANOVA model was similarly implemented, whereby a second-degree polynomial (Equation 4) was generated to reflect the interaction of the considered variables in the process (Zainudin et al., al., 2005). The data were normalised using the normal standard variation method, as described by Equation 5:

$$Y = b_0 + \sum_{i=1}^{n} b_i x_i + \left(\sum_{i=1}^{n} b_{ii} x_i\right) + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} x_i x_j \quad (4)$$

$$Xinar = \frac{Xi - \bar{x}}{\sigma * Xi} \quad (5)$$

where Y is the response variable; b_0 is a constant coefficient; b_i is the linear coefficient; b_{ij} is the coefficient for the interaction of the variables; b_{ii} is the quadratic coefficient; x_i and y_i are the coded values of the variables; Xi is the intercept of each variable (Figure 2); $\bar{x}$ is the average of the selected values; and σ is the deviation from the selected values (the selected values are presented in Table 1).

7. Process kinetics

The experimental data were fitted to the kinetic models presented in Table 2.

The mean squared error (MSE) was determined for the model fits. The kinetic analysis was performed in the MATLAB® 2017 software using the Levenberg-Marquardt algorithm (Güçlü, Yılmaz, & Ozkan-Yucel, 2011).

8. Results and discussion

8.1. Characterisation of substrate and inoculum

Table 3 presents the results of the physico-chemical characterisation of the substrate and the inoculum.

The pH of the FW is within the reported range for this type of substrate and is consistent with characteristic BA and VFA concentrations of easily acidifiable substrates, showing the need for an external alkalising source (Steinmetz et al., 2016; Parra-Orobio et al., 2018). Although the COD, TOC and VS/TS ratio indicate a high content of organic matter, the $COD_{filtered}/COD_{total}$ ratio confirms the predominance of particulate organic matter, which can be a limiting condition in hydrolysis (Li et al., 2012; Aldin et al., 2011). The TN and TP values show that nutrients are provided for the requirements of the microorganisms involved in the biological process and reflect the presence of legumes in the FW (Elser, 2012). The values for lignin and cellulose (which are slowly degrading organic materials) are similar to those from other studies on AD (Parra-Orobio et al., 2018) and composting (Soto-Paz et al., 2019) of this type of waste, which was separated at the source and obtained by selective collection.

The pH and the BA/TA ratio (0.63) of the inoculum indicate a good buffer capacity, which prevents the accumulation of VFAs produced during AD. These values are similar to previously reported values, such as by de Raposo et al., (2012) and Torres-Lozada, Díaz-Granados, and Parra-Orobio (2015). Although the VS/TS ratio (0.53) confirms that the incorporation of granular sludge into flocculent sludge improves the characteristics of the inoculum, the SMA of 0.15 $gCOD_{CH4}$/gVS is low, while being within the range reported by Angelidaki et al. (2009). The HA and SAA values of 0.26 and 3.77 $gCOD_{Glucose}$/gVS.d, respectively, are close to reported values, such as by Regueiro et al. (2012) and Parra-Orobio et al., (2018).

9. Theoretical potential of methane

The elemental analysis and composition of the FW samples, in terms of carbohydrates, proteins and lipids (Table 3), was used to determine the theoretical production using Equations 1, 2 and 3. Considering the reaction stoichiometry in Equations 1 and 2 results in a theoretical production of 429 $mLCH_4$/gVS; however, these are theoretical methods based on an elemental analysis, in which

Table 2. Kinetic models

Model	Equation	Variable definition	Source
First-order	$BMP = BMP_0\left(1 - e^{-kt}\right)$ **(6)**	**BMP**: Biochemical potential of accumulated CH_4 ($mLCH_4/gVS$)	Nielfa et al. (2015)
Gompertz	$BMP = BMP_0 e\left\{-e\left[\frac{R_{max}e}{PBM_0}(\lambda - t) + 1\right]\right\}$ **(7)**	**BMP_0**: BMP substrate (theoretical) (FW) ($mLCH_4/gVS$) **k**: Hydrolysis constant (d^{-1}) **t**: Assay time (d) **R_{max}**: Production rate of CH_4 ($mL\ CH_4\ gVS^{-1}\ d^{-1}$) **λ** : Lag phase (d) *e* (Exponential value 1) = 2.7183	Lay, Li, and Noike (1997)

Table 3. Physico-chemical characterisation of substrate (FW) and Inoculum

Parameters	FW (n = 3)	Inoculum (n = 3)
pH (units)	4.92 ± 0.01	7.71 ± 0.10
TA (mg $CaCO_3$/L)	3940.23 ± 238.56	1718.26 ± 95.66
BA (mg $CaCO_3$/L)	—	1166.58 ± 90.66
BA/TA	—	0.68
VFAs (mg/L)	34,102.70± 120.36	398.33 ± 83.11
Total COD (mg/L)	109,715.88 ± 198.32	—
Filtered COD (mg/L)	24,149.89 ± 220.36	—
TOC (mg/kg)	47,333.48 ± 23.97	—
TS (mg/L)	101,630.02 ± 2.40	44,850.00 ± 3.54
VS (mg/L)	92,881.68 ± 2.27	25,566.66 ± 2.44
VS/TS	0.91	0.57
TN (mg/kg)	10,860.00 ± 1.09	—
TP (mg/kg)	240.36 ± 0.13	—
Lignin (%)	2.20 ± 0.64	—
Cellulose (%)	1.67 ± 0.57	—
Carbon (%) *	41.16 ± 0.81	—
Hydrogen (%) *	5.04 ± 0.36	—
Oxygen (%) *	40.61 ± 1.99	—
Nitrogen (%) *	1.76 ± 0.03	—
Carbohydrates (%)	9.60 ± 0.6	—
Proteins (%)	6.79 ± 0.8	—
Lipids (%)	5.30 ± 1.2	—
SMA ($gCOD_{CH4}$/g VS·d)	—	0.15 ± 0.15
HA ($gDQO_{Glucose/}$gVS.d)	—	0.26 ± 0.02
SAA ($gDQO_{Glucose/}$gVS.d)	—	3.72 ± 0.25

*Elemental Analysis (n = 8)

100% degradation of the FW is assumed (Fernandez et al., 2008), which corresponds to an ideal theoretical condition. For example, with respect to carbon, the degraded fraction corresponds only to anaerobically biodegradable organic carbon, which is also affected by the presence of materials such as lignin and cellulose (Puyuelo, Ponsá, Gea, & Sánchez, 2011). Some components, while being organic in nature, are difficult to degrade and have limited solubility and as it was shown in the physico-chemical characterisationand of the substrate, they represent an important fraction in these residues. Using Equation 3 resulted in a production of 127 mL_{CH4}/gVS, which is close to the maximum value of 107 $mLCH_4$/gVS obtained in the present study.

By contrast, Equation 3 only accounts for easily degradable monomers in the FW (Mahmoud et al., 2018). Nielfa et al. (2015) have reported an error of only 10% for the experimental production using Equation 3. Thus, different AD incidence variables need to be simultaneously evaluated for FW to better correspond the theoretical conditions, while remaining cognisant that not all of the substrate may be degraded or easily assimilated.

10. Response surface methodology (RSM) for determination of optimal temperature and head space

Figure 1 shows the response surfaces for the interaction between (a) the head space and time; (b) the temperature and time; and (c) the interaction of the two considered variables.

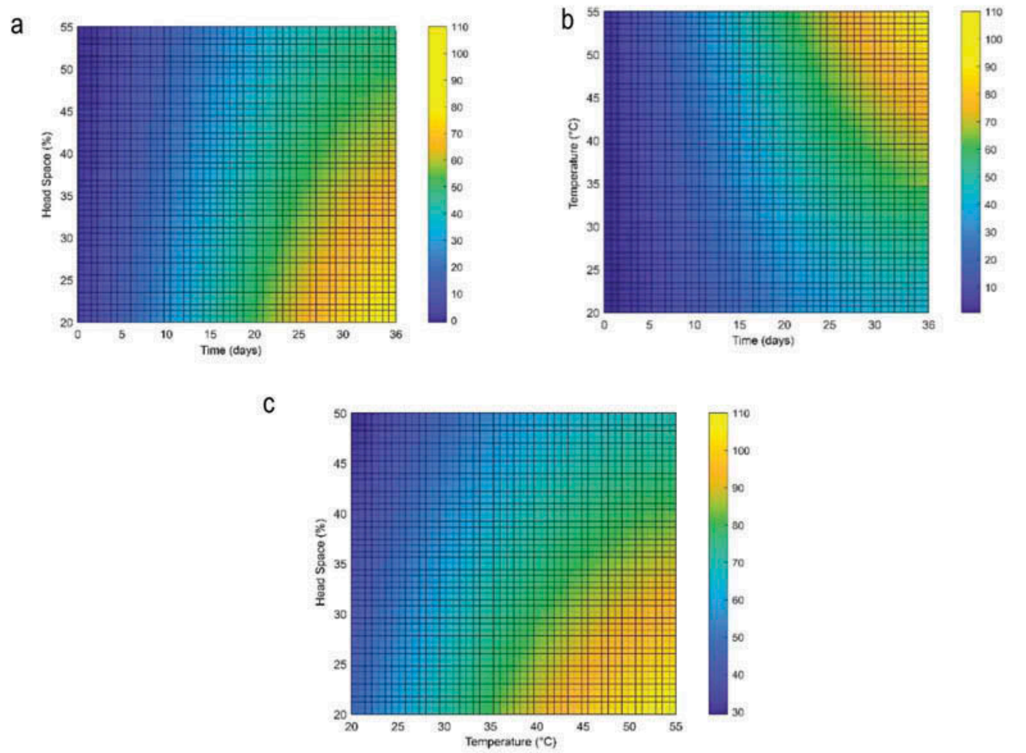

Figure 1. Response surfaces for methane production: (a) head space and time, (b) temperature and time (c) head space and temperature.

Figure 1(a) shows an inverse relationship between the head space and methane production, which is in agreements with the results of Stumm and Morgan (2012) and Himanshu, Voelklein, Murphy, Grant, and O'Kiely (2017), wherein increasing the reactor head space was found to decrease the methane yield, because the pH and the partial pressure of CO_2 are related to the presence of CO_2 in the biogas and the inorganic carbon dissolved in the interior of the reactor.

Rittman and McCarty (2001) and Panigrahi and Dubey (2019) reported that increasing the partial pressure (the lower head space) increases the dissolution of inorganic carbon, which is a natural buffering agent and prevents microbial populations (i.e. methanogenic *Archaea*) from being affected by decreases in the pH. Microbes play a critical role in the degradation process and the transformation of intermediate products to methane. Some of these microbes are highly pH-sensitive, and some bacterial consortia require an almost neutral pH to perform well (Jankowska et al., 2015).

Figure 1(b) shows that thermophilic temperatures result in approximately 50% more methane production than mesophilic temperatures, which is in agreement with reports by Kim et al. (2006) and Mirmohamadsadeghi et al. (2019). High temperatures also favour the solubilisation of this type of compound, facilitating degradation by microorganisms (Frigon & Guiot, 2010; Shi, Guo, Zuo, Wang, & Zhang, 2018), which increases methane production, in contrast to mesophilic or psychrophilic conditions, which lower production.

Figure 1(c) shows the interaction between the temperature and the head space. The area delimited by temperatures between 50 and 55 °C and head spaces between 20 and 25% correspond to the conditions under which the BMP increased in this study. The optimal values for each variable were determined by using Equation 5 to denormalise the values and data from the intercepts in Figure 2. This analysis shows that the optimal conditions are a temperature of 48.9 °C and a head space of 20%, which is consistent with the aforementioned results and reports by Deepanraj et al. (2015) and Shi et al. (2018) of increased methane production under thermophilic temperature conditions (50–55°C).

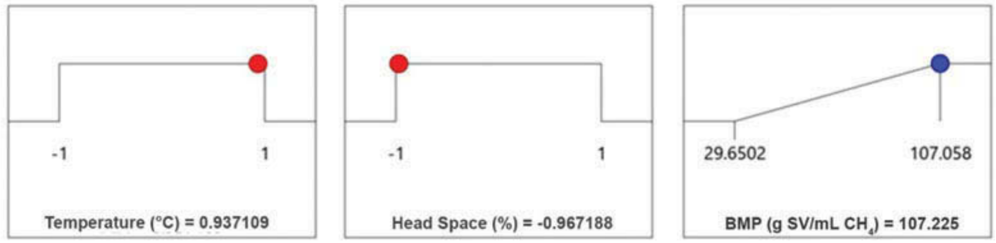

Figure 2. Use of intercepts to determine optimal conditions.

The ANOVA analysis was fitted to a 2FI quadratic interaction model for the interaction of two variables, which has been widely used for authors as Li et al. (2015) and Oliveira, Alves, and Costa (2015). The analysis produced Equation 8 with a R^2 value of 0.98 and $p = 0.02$:

$$y = 65.05 + 25.15 * T - 14.16 * Hs - 5.42 * T * Hs \quad (8)$$

where y is the response variable (BMP), T is the temperature, and Hs is the head space.

Equation 8 shows that temperature variable has the most significant impact on the process, with a coefficient of 25.15 compared to the head space coefficient of −14.16. The coefficient of −5.42 for the interaction between the two variables is less significant. Thus, at higher temperatures, the BMP increases, and the greater the head space is, the lower the BMP. This result is consistent with the significance of each variable: $p < 0.0001$ (temperature), $p = 0.0002$ (head space) and $p = 0.0290$ (interaction of the two variables), where the interaction of the temperature and the head space has the *p*-value closest to 0.05.

Note that although the degradation rate over the thermophilic range is generally faster than over the psychrophilic or mesophilic ranges, there are disadvantages associated with the thermophilic range. *i*. Instabilities are introduced into the process from temperature fluctuations, along with the risk of accumulation of VFAs (i.e. propionic acid) from an enhanced high reaction rate, especially during acidogenesis (Mao, Feng, Wang, & Ren, 2015; Panigrahi & Dubey, 2019; Rajagopal, Bellavance, & Rahaman, 2017). *ii*. More energy must be expended to maintain the temperature above ambient (Shi et al., 2018). Thus, the cost/benefit ratio for production versus the required consumption should be evaluated to determine the feasibility of the proposed strategy. Most AD processes for FW, including industrial scale processes, are carried out under mesophilic conditions to ensure that the process remains stable under the high organic loads supplied (Guo et al., 2014).

Given the above-mentioned limitations of implementing thermophilic conditions and increasing the BMP without incurring cost overruns for reactor heating, it is advisable to explore alternatives, such as *i*. the use of insulating materials for temperature maintenance (Martí-Herrero, Soria-Castellón, Diaz-de-Basurto, Alvarez, & Chemisana, 2019); *ii*. a staged process or phase separation (Wu, Kobayashi, Li, & Xu, 2015; Xiao et al., 2018); *iii*. pre-treatment of the substrate and/or inoculum (Li et al., 2016; Parra-Orobio et al., 2017) and *iv*. innovative studies on the interaction between variables to optimise the process to achieve high biogas production at the lowest possible expense.

11. Kinetic analysis of process

Table 4 shows the kinetic parameters obtained using the first-order and Gompertz models, along with the VFAs obtained for each configuration at the end of the process.

Using the Gompertz model resulted in a maximum production of 133.45 mLCH4/gVS (G), which is far from the maximum production (I) calculated using the first-order model (348.36 mLCH4/gVS), showing that the first-order model overestimates production (Díaz et al., 2015). The theoretical value of 127 mLCH4/gVS obtained using the substrate composition (in terms of the carbohydrates,

Table 4. Kinetic parameters of models

Treatment			Experimental Production	First-order Model				Gompertz Model					VFAs
ID	Tem (° C)	HS (%)		Pmax	k	R^2	RMSE	Pmax	λ (d)	Rmax	R^2	RMSE	
A	20	20	47.96	99.05	0.01	0.99	1.17	58.71	0.40	1.59	0.99	0.98	33,564.80
B	20	35	37.30	56.02	0.01	0.98	1.40	40.51	0.51	1.59	0.99	1.02	34,802.55
C	20	50	29.65	47.40	0.01	0.96	1.75	30.38	2.89	1.57	0.99	0.61	36,241.26
D	35	20	81.47	194.92	0.02	0.99	1.67	95.14	0.78	2.99	0.99	1.53	18,196.71
A	35	35	68.33	159.50	0.01	0.99	1.81	81.37	1.10	2.49	0.99	1.32	20,233.04
F	35	50	54.91	184.04	0.01	0.98	2.09	66.93	1.72	2.06	0.99	1.73	32,711.58
G	55	20	107.06	193.66	0.03	0.99	2.67	133.45	1.24	3.46	0.99	2.12	15,763.24
H	55	35	91.74	211.31	0.02	0.98	2.71	118.86	2.41	2.95	0.99	2.58	17,361.14
I	55	50	67.05	348.36	0.01	0.99	1.97	80.84	4.44	2.54	0.99	1.51	28,203.20

Tem: temperature; **HS**: head space; **units**: experimental production ($mLCH_4/gVS$); Pmax ($mLCH_4/gVS$); λ (d); Rmax ($mLCH_4/gVS$)

proteins and lipids in the FW, Equation 3) is near the experimental value of 107 mLCH4/gVS and the result from the Gompertz model. Thus, both the theoretical method and the Gompertz model can be successfully used to describe the AD of FW because of the small difference between these methods, especially considering that using Equation 3 results in production values with an estimation error below 10% (Nielfa et al., 2015).

Following Donoso-Bravo, Pérez-Elvira, and Fdz-Polanco (2010) and Parra-Orobio et al., (2018), R^2 should be maximised and the error (RMSE) should be minimised. The Gompertz model produced the best fit to the data (R^2 = 0.99 and RMSE < 2.12) and thus describes the process better than the first-order model. A comparison of the kinetic parameters shows that the first-order model, despite its widespread use, did not adequately estimate the methane production for all of the experimental configurations (Díaz, Reyes, Lundin, & Horváth, 2011) and that the Gompertz model better represented the data, in agreement with the results of Cárdenas-Cleves, Marmolejo-Rebellón, and Torres-Lozada (2018). The latency phase increased with the head space (Table 4), indicating the impact of this parameter on methane production via the accumulation of VFAs and the absence of CO_2 dilution because of insufficient pressure (Valero et al., 2016).

The VFA concentrations are consistent with the high presence of acidogenic bacteria (AAE, Table 3), which transform organic matter into VFAs such as valeric, propionic and butyric acids (Zhang et al., 2014). This bacterial presence, in conjunction with the bacterial growth rate (Rmax), explains the high VFA concentrations at the end of the process. In addition, methanogenic *Archaea* are known to be very sensitive to changes in pH, requiring almost neutral conditions (Jankowska et al., 2015). The VFA concentrations at the end of the process showed that a neutral environment did not predominate in most of the assays, which is in agreement with the experimental methane production.

The results from Table 4 for the latency phase of the process (λ) show that a short latency does not always favour AD, because increasing the reaction rate or the degradation of VS also increases the accumulation of VFAs, which can inhibit the process if there is no buffer capacity (Lee et al., 2015). By contrast, a prolonged latency phase may allow the microbial consortia to better adapt to the substrate and increase methane production. These results were reflected in the D and G assays. These assemblies were subjected to different temperatures for the same head space value, and different yields were obtained for different latency phase values.

The first-order model does not take into account the latency phase and overestimates methane production data. However, this model is useful for describing hydrolysis in the process: the data showed that the reaction speed was generally not rapid, except in the G assay (Parra-Orobio et al., 2018). Inhibition phenomena may have occurred in the assays with head spaces above 20%, but at lower rates than at the mesophilic and thermophilic temperatures, especially given the reaction rates at elevated temperatures (Mirmohamadsadeghi et al., 2019). Thus, the incidence of the head space is reflected in Rmax, which increases with the temperature.

In general, it can be stated that the Gompertz model is a non-linear model that can be used to evaluate the entire process easily, whereas the first-order model, as its name indicates, reflects the effect that the variable of interest has on hydrolysis. These models are not comparable but can be complementary: the Gompertz model can be used to provide data on the latency phase, whereas the first-order model can provide information on the critical stage of AD of FW (Rajagopal et al., 2017).

12. Conclusions

- FW contains lignocellulosic compounds that degrade slowly and can thus inhibit high AD yields. Equations have been developed to estimate the potential of methane production from this type of substrate.
- An ANOVA of the considered variables shows that the temperature is the highest incidence variable in the process. The optimal temperature was found to be 48.9 °C, and temperatures

above this value did not result in increased methane production. The optimal head space was found to be 20%, and head spaces greater than this value resulted in the inhibition of the process from the accumulation of VFAs.

- The result of the models implemented in the study showed that the Gompertz model best describes the AD of FW because the latency phase of the process is considered and does not overestimate methane production, unlike the first-order model. The two models are not comparable to each other.

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CAPÍTULO 4. SINTESIS DEL TRABAJO DE INVESTIGACIÓN

La DA ha surgido como una de las alternativas que contribuyen a la economía circular ya que, a través de esta estrategia, es posible valorizar y aprovechar residuos orgánicos como los RA, que son un flujo de residuos importante dadas sus características en cuanto a nutrientes se refiere y a las cantidades generadas en PD y principalmente en PeD. Estos residuos vienen siendo cada vez más estudiados, debido a que son una de las fracciones predominantes en los RSM (Oviedo et al., 2012; Thi et al., 2015; Kaza et al., 2018; Soto-Paz et al., 2019; Parra-Orobio et al., 2019). Fruto del proceso de DA, se obtienen dos subproductos importantes de alto potencial de aprovechamiento como el biogás (fuente de energía renovable como el metano y el hidrógeno (Enitan et al., 2017; Grando et al., 2017; Mirmohamadsadeghi et al., 2019) y el digestato (fuente de aprovechamiento agrícola por su contenido de nutrientes – Owamah et al., 2014; Ortíz et al., 2019), Adicionalmente, el aprovechamiento de estos residuos permite reducir áreas requeridas para disposición final, aumentar la vida útil de los rellenos sanitarios, y reducir la generación de GEI (Manyoma et al., 2015).

Como se ha mencionado previamente el ensayo PBM es un ensayo sencillo y de bajo costo y de gran utilidad para establecer el potencial máximo que tiene un residuo para la producción de biogás pero que aún no se encuentra estandarizado y por tanto existen diferentes configuraciones alrededor del mismo. Por lo anterior, se realizó una vigilancia tecnológica alrededor del tema para establecer las principales variables que inciden en el proceso y su nivel de estudio y desarrollo. La vigilancia tecnológica identificó que variables como *head space*, tiempo de retención, contenido de sólidos entre otros, inciden fuertemente en el proceso y fueron agrupadas en un factor que se denominó Configuración Experimental, el cual ha sido ampliamente evaluado, siendo aspectos como la carga orgánica y el número de etapas, los más estudiados; el sustrato es el segundo factor de mayor estudio y a éste se le asocia el pretratamiento como la variable más desarrollada; al inóculo se le asocian principalmente variables como la mezcla de inóculos, la relación sustrato inóculo (S/I) y el pretratamiento del mismo, siendo esta última variable la de mayor estudio. En cuanto a las variables menos estudiadas, se encontraron el *head* space (2.16%) y el tiempo de retención (3.03%); además, se identificó que, si bien la temperatura es una variable ampliamente estudiada, la mayoría de los estudios hacen referencia principalmente a regímenes mesofílicos y termofílicos en comparación con el estudio en condiciones sicrofilicas, que es más limitado.

En concordancia con los resultados obtenidos en la vigilancia, se decidió evaluar experimentalmente y a escala de laboratorio el *head space* (X_1) y la temperatura (X_2), lo cual se desarrolló por medio de ensayos de PBM. Cada uno de los niveles fueron evaluados, se definió de acuerdo con la vigilancia tecnológica y para desarrollarlo se aplicó un diseño central compuesto para evaluar la incidencia simultánea de las dos variables (X_1 y X_2) en el proceso y hallar lo puntos óptimos. Por medio del análisis se estableció que el punto donde X_1 se maximiza es 20%, mientras que para X_2 es 48.9°C. Además, se identificó que el modelo cinético que mejor describe el proceso es el modelo de Gompertz Modificado, se puede afirmar que no siempre una fase corta favorecerá la DA ya que la sobreproducción de AGVs podría inhibir el proceso (Rajagopal et al., 2017); el modelo de primer orden también fue muy útil, ya que reafirmó que las condiciones halladas como óptimas son las que favorecen una mayor constante de hidrólisis.

A continuación, se presentan las conclusiones y recomendaciones generales producto del presente trabajo de investigación.

4.1. Conclusiones

- ✓ Debido a su composición predominantemente orgánica, los biorresiduos de origen municipal (BOM) y los residuos de alimentos (RA), tienen un alto potencial de aprovechamiento a través de procesos biológicos como la digestión anaerobia (DA); sin embargo, debido a que son un sustrato fácilmente acidificable, requiere ajustes y el monitoreo de variables como el pH, los ácidos grasos volátiles, la alcalinidad bicarbonática y total, además del estudio de aspectos como el tamaño de partículas, entre otras.

- ✓ Desde el punto de vista de aplicabilidad de la Digestión Anaerobia (DA) de BOM y RA se evidencia que, a nivel mundial, el continente europeo es el que lidera las investigaciones en torno a este tema, mientras, que a nivel país, el principal productor de investigaciones en los últimos años es China. A nivel del continente americano, es Estados Unidos y a nivel Latinoamericano, Brasil.

- ✓ En este estudio se determinó que los valores óptimos fueron 48.9°C y 20% para la temperatura y el *head space* respectivamente, así mismo se identificó que la temperatura es la variable que tiene mayor incidencia sobre el proceso. Se estableció además que valores superiores de temperatura no necesariamente incrementan la producción de metano, además que pueden conducir a un alto gasto energético; igualmente, valores superiores de head space (>20%) conllevan a la inhibición del proceso por altas concentraciones de AGVs, que no alcanzan a ser neutralizadas por el CO_2 disuelto.

- ✓ Entre los modelos cinéticos evaluados, el de Gompertz Modificado describe mejor el comportamiento del proceso de DA de RA ya que éste tiene en cuenta la fase de latencia del proceso y no sobreestima la producción de metano, a diferencia del modelo de primer orden; sin embargo, este último también es importante porque brinda información útil como la constante de hidrólisis, la cual se incrementó en condiciones de 20% de head space y 55°C de temperatura, valores cercanos a los hallados como óptimos en este estudio.

4.2. Recomendaciones

- ✓ Si bien se conoce que las temperaturas bajas no son las más adecuadas para desarrollar procesos de DA, deben buscarse mecanismos aislantes que permitan viabilizar este tipo de procesos en zonas donde la temperatura no supere los 20°C, de manera que el proceso de digestión anaerobia se considere factible, sin necesidad de suministrar energía en forma de calor, lo que podría incrementar los costos de la tecnología.

- ✓ Se recomienda continuar realizando este tipo de estudios considerando dos o más variables (i.e número de fases, tiempo de retención hidráulico) de manera simultánea, debido a que aún hay desconocimiento entre las posibles interacciones existentes entre ellas, así como el uso y evaluación del proceso en reactores de tamaño superior (> 5L) y operados en modo continuo o semicontinuo, evaluando además diferentes tamaños de partícula.

- ✓ Se recomienda evaluar diferentes modelos cinéticos como el modelo Modelo de Digestión Anaerobia N° 1 (Anaerobic Digestion Model No.1) o ADM1 por sus siglas en inglés, el cual es un modelo robusto y que contempla 32 variables dinámicas y tiene en cuenta efectos de inhibición por diferentes causas.

- ✓ Se recomienda realizar un protocolo del ensayo PBM que recopile los resultados de varias investigaciones alrededor de la DA con RA y BOM, lo anterior con el fin de estandarizar el ensayo con esta clase de sustratos y a fin de que pueda ser publicado y replicado de tal modo que los resultados se puedan comparar con mayor confianza.

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