

TRABAJO ESPECIAL DE GRADO

OPTIMIZACIÓN DE LA PRODUCCIÓN DE BIOGÁS MEDIANTE FERMENTACIÓN ANAERÓBICA EN DOS ETAPAS DE DESECHOS ORGÁNICOS PRETRATADOS ALCALINAMENTE

(OPTIMIZATION OF BIOGAS PRODUCTION IN TWO- STAGE ANAEROBIC FERMENTATION OF ORGANIC WASTE MARKET USING ALKALINE PRETREATMENT)

Presentado ante la Ilustre
Universidad Central de Venezuela
Por el Br. Mejías R., Rubén E.
Para optar al Título
de Ingeniero Químico

Caracas, 2014

TRABAJO ESPECIAL DE GRADO

OPTIMIZACIÓN DE LA PRODUCCIÓN DE BIOGAS MEDIANTE FERMENTACIÓN ANAERÓBICA EN DOS ETAPAS DE DESECHOS ORGÁNICOS PRETRATADOS ALCALINAMENTE

(OPTIMIZATION OF BIOGAS PRODUCTION IN TWO- STAGE ANAEROBIC FERMENTATION OF ORGANIC WASTE MARKET USING ALKALINE PRETREATMENT)

TUTORES ACADÉMICOS: Prof. Bernardo Ruggeri (Politecnico di Torino)
Profa. Alejandra Meza (U.C.V)

Presentado ante la Ilustre
Universidad Central de Venezuela
Por el Br. Mejías R., Rubén E.
Para optar al Título
de Ingeniero Químico

Presentado ante el Ilustre
Politecnico di Torino
Por el Br. Mejías R., Rubén E.
Para optar al Título
de Ingeniero Químico y de los Procesos Sostenibles

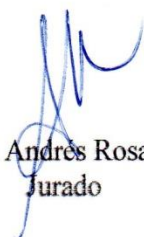
Caracas, 2014

Caracas, 7 de marzo de 2014

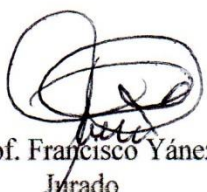
Los abajo firmantes, miembros del Jurado designado por el Consejo de Escuela de Ingeniería Química, para evaluar el Trabajo Especial de Grado presentado por el Bachiller Rubén Eduardo Mejías Rojas, titulado:

**"OPTIMIZACIÓN DE LA PRODUCCIÓN DE BIOGAS
MEDIANTE FERMENTACIÓN ANAERÓBICA EN DOS
ETAPAS DE DESECHOS ORGÁNICOS PRETRATADOS
ALCALINAMENTE"**

Consideran que el mismo cumple con los requisitos exigidos por el plan de estudios conducente al Título de Ingeniero Químico, y sin que ello signifique que se hacen solidarios con las ideas expuestas por el autor, lo declaran **APROBADO**.



Prof. Andrés Rosales
Jurado



Prof. Francisco Yáñez
Jurado



Profa. Alejandra Meza
Tutor Académico



**POLITECNICO
DI TORINO**

Area Gestione Didattica

n. 131210113337

Student Id.

n. 182918

Page 1 / 1

IT IS HEREBY CERTIFIED THAT

Mr Ruben Eduardo MEJIAS ROJAS
Place of birth CARACAS, VENEZUELA
Date of birth 08/09/1990

graduated on 05/12/2013 in

CHEMICAL AND SUSTAINABLE PROCESSES ENGINEERING

Laurea Magistrale (Master of science-level of the Bologna process)

CHEMICAL ENGINEERING DEGREE CLASS

with the following grade: 103/110.

Final project title:

Optimization of biogas production in two-stage anaerobic fermentation of organic waste market using alkaline pretreatment

Torino, 10/12/2013

The Officer in charge

Luigi Rinaldi



APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. PAESE **ITALIA**
Il presente atto pubblico
 2. è stato firmato dal Sig. **RINALDI LUIGI**
 3. nella sua qualità di **RESPONSABILE PRESIDIO
TASSE E DIRITTO ALLO STUDIO**
 4. e porta il sigillo-timbro **POLITECNICO DI TORINO**
- ATTESTATO**
5. a Torino
 6. il **12 dic 2013**
 7. dalla Prefettura di Torino
 8. sotto il numero d'ordine **0033209/2013**

9. Timbro

10. Firma
p. IL PREFETTO
coadiutore amministrativo contabile
CAMPANA Roberta

AGRADECIMIENTOS

Un especial agradecimiento a la ilustre y distinguida Universidad Central de Venezuela por ofrecerme la oportunidad de formarme como profesional y como persona durante los últimos años. Me siento realmente orgulloso de pertenecer a esta prestigiosa casa de estudio que vence las sombras.

También me gustaría agradecer al Politecnico di Torino por la oportunidad que me ofreció esta reconocida institución de estudiar en Italia. Al Prof. Bernardo Ruggeri, quien con su sabiduría y paciencia tuvo siempre la disposición para guiarme durante la última etapa de mis estudios. Aprovecho para expresar mi gran admiración y respeto por el Prof. Ruggeri, por ser una persona que sabe ser exigente en su labor, creando a la vez un ambiente armónico y confortable de trabajo.

A mis profesores en la Escuela de Ingeniería Química de la U.C.V., a quienes agradezco la excelente formación y preparación que me proporcionaron y a quienes debo gran parte de mis conocimientos profesionales. A la Ing. Andrea Luongo por su paciencia, liderazgo y soporte durante todas las labores que desempeñé en el laboratorio para el desarrollo de mi trabajo de investigación.

También quisiera aprovechar estas líneas para expresar mi más profunda gratitud a Dios y a mi familia, por la oportunidad que me han dado de estudiar y convertirme en un profesional, luego de haber transitado un camino lleno de experiencias inolvidables. En los últimos seis años he tenido la oportunidad de aprender, no solo desde el punto de vista académico, sino también desde una perspectiva humana. Especialmente durante mi preparación en el extranjero los últimos dos años, viví numerosas experiencias que me hicieron crecer en el ámbito personal y que sobretodo, disfruté con total plenitud, aún cuando me aterraba la idea de abandonar mi hogar hace dos años atrás. Hoy en día, me encuentro nuevamente de regreso en mi hogar, de vuelta al calor familiar, agradecido con todas aquellas personas que durante tanto tiempo me apoyaron y ayudaron a superar mis temores.

Quisiera agradecer enormemente a mis padres por el apoyo irrestricto que me han dado durante todos estos años de formación profesional, y por el apoyo que sé que siempre me brindarán para alcanzar cualquier meta que me proponga en un futuro. Nunca me habría sido posible culminar mis estudios sin la guía y consejos de la maravillosa familia que tengo suerte de tener.

Les doy gracia a mis tíos, tías, abuelas, primos y hermano, quienes estuvieron siempre apoyándome y alentándome con sus bendiciones y mejores deseos. Ellos siempre estuvieron ahí para recordarme el lugar del que provengo y lo importante que es la humildad.

Finalmente quisiera mostrar mi gratitud a todos mis amigos. A mis amigos en Venezuela con los que durante tanto tiempo crecí y compartí. A todos aquellos amigos que dejé en Torino, quienes me ayudaron a hacer más grata mi estadía en Italia y a quienes sin duda, siempre recordaré con nostalgia por todos los buenos momentos compartidos. Me complace también dar las gracias a mis amigos en España, Francia, Colombia e Italia, en donde sé que siempre seré bien recibido en un hogar.

Mejías R., Rubén E.

**OPTIMIZACIÓN DE LA PRODUCCIÓN DE BIOGAS
MEDIANTE FERMENTACIÓN ANAERÓBICA EN DOS
ETAPAS DE DESECHOS ORGÁNICOS PRETRATADOS
ALCALINAMENTE**

**Tutores Académicos: Profa. Alejandra Meza (U.C.V), Prof. Bernardo Ruggeri
(Politecnico di Torino). Tesis. Caracas, U.C.V. Facultad de Ingeniería Escuela
de Ingeniería Química. Torino, Politecnico di Torino. Escuela de Ingeniería
Química y Materiales, Año 2014, 200 p.**

Palabras Claves: Fermentación anaeróbica, Desechos orgánicos, Biogás.

Resumen. El presente trabajo tiene como objetivo optimizar la producción de biogás que se obtiene mediante la digestión anaeróbica en dos etapas de desechos orgánicos. Este tipo de procesos requiere realizar un pretratamiento a la materia prima antes efectuar la fermentación. Por esta razón se desea evaluar el rendimiento que se obtiene al realizar un pretratamiento alcalino que, en comparación a otros tipos de pretratamientos como el alcalino/térmico, tiene un bajo requerimiento energético. Para ello, fueron calculados dos parámetros energéticos: la “eficiencia” y la “eficacia”, los cuales permiten realizar comparaciones con investigaciones realizadas anteriormente. Adicionalmente se calculó un índice de sostenibilidad para determinar si el proceso es sostenible desde un punto de vista energético. Estableciendo como punto de comparación el pretratamiento alcalino/térmico, los resultados demuestran que aunque el pretratamiento alcalino no favorece la producción de hidrógeno, sí mejora la producción de metano, el cual posee un mayor valor energético y por lo tanto, conduce a obtener una mayor recuperación energética. La “eficiencia” del proceso mejora en un 21% y el proceso es sostenible desde el punto de vista energético con un índice de sostenibilidad de 10.5. La producción de hidrógeno fue de 1.17 L/L sustrato y la de metano 15.33 L/L sustrato.

RESUMEN

OPTIMIZACIÓN DE LA PRODUCCIÓN DE BIOGAS MEDIANTE FERMENTACIÓN ANAERÓBICA EN DOS ETAPAS DE DESECHOS ORGÁNICOS PRETRATADOS ALCALINAMENTE

Rubén Eduardo Mejías Rojas¹

¹ *rbmejias@hotmail.com*

INTRODUCCIÓN

Hoy en día la contaminación ambiental y el agotamiento progresivo de los combustibles fósiles se han convertido en problemas de gran interés global, fomentado de esta manera la búsqueda de fuentes alternativas de energía y técnicas para la reducción de la contaminación. Uno de los métodos más interesantes para lograr este objetivo consiste en recuperar la energía presente en desechos a través de procesos de bioconversión. Entre los distintos métodos disponibles para el tratamiento de desechos orgánicos, la digestión anaeróbica, estudiada en este trabajo, es una de las alternativas más destacadas debido a la alta concentración de metano e hidrógeno que puede ser obtenida en el biogás producido, además de la obtención de un residuo estable al final del proceso.

La digestión anaeróbica (DA) es un proceso biológico efectuado por microorganismos que conduce a la producción de biogás, y por lo tanto de energía, mediante la degradación de sustancias orgánicas en condiciones anaeróbicas, es decir, en ausencia de oxígeno molecular. El proceso involucra varios grupos de bacterias: hidrolíticas, acidificantes y metanógenas, cuyo metabolismo conlleva a la producción de CO₂, hidrógeno y metano. El hecho de que los dos últimos compuestos sean de notable valor energético, hace que el biogás obtenido represente una fuente importante de energía alternativa.

La digestión o fermentación anaeróbica es un proceso complejo que se divide en cuatro fases sucesivas de degradación: hidrólisis, acidogénesis, acetogénesis y metanogénesis. En cada fase actúan diferentes grupos de microorganismos que requieren de condiciones de reacción diferentes para su crecimiento. Tanto la primera y la segunda fase, como la tercera y la cuarta están estrechamente ligadas entre ellas, por lo cual el proceso puede ser efectuado eficientemente en dos etapas (Deublein y Steinhauser, 2008).

Durante la primera fase de fermentación (la hidrólisis) las sustancias de compleja composición son degradadas y transformadas en oligómeros y monómeros, quedando así disponibles para ser transportadas a las paredes celulares de los microorganismos acidogénicos. Estos últimos llevan a cabo la segunda fase de fermentación, la acidogénesis, en la cual los monómeros son transformados en ácidos orgánicos de cadena corta, alcoholes, hidrógeno y CO₂. Seguidamente en la fase acetogénica, a partir de los ácidos grasos formados en la fase anterior, se obtiene la producción de ácido acético, ácido fórmico, CO₂ e hidrógeno molecular. Finalmente, en la cuarta y última fase de fermentación, ocurre la producción de metano, el cual es el producto con mayor valor energético producido durante el proceso. El metano, de hecho, es el único compuesto no reactivo presente durante la digestión anaeróbica y debido a esto puede ser considerado el producto final del proceso (Deublein y Steinhauser, 2008).

El pH es un parámetro que puede ser empleado como indicador de la estabilidad del sistema, dado que su variación está asociada a la capacidad amortiguante del medio de reacción y a

cambios en el equilibrio existente entre las especies involucradas en la cadena trófica durante el proceso (Cecchi et al., 2005). Las bacterias presentes en cada fase de fermentación tienen diversas exigencias de pH para llevar a cabo su actividad metabólica: las bacterias acidogénicas y acetogénicas muestran un máximo de actividad con valores de pH entre 5 y 5.5, mientras que las bacterias metanígenas tienen un máximo de actividad con condiciones de pH superior a 6.5 (Camarota, 2004). Por lo tanto, el pH resulta ser un parámetro que es utilizado para separar la etapa acidogénica de la metanígena, en reactores que operan en dos etapas, tal como se explica más adelante en este trabajo.

La temperatura dentro de un digestor anaeróbico determina la velocidad de degradación de los compuestos. La fermentación se efectúa comúnmente en dos intervalos óptimos de temperatura: condiciones mesófilas, siendo la temperatura óptima de 35 °C; y condiciones termófilas, con una temperatura óptima de 55 °C. Sin embargo, las bacterias mesófilas pueden tolerar mayores variaciones de los parámetros ambientales como la temperatura, facilitando así el funcionamiento de los reactores. Por este motivo, la estabilidad del proceso mesófilo lo convierte en el más popular en las plantas de digestión anaeróbica (Zaher *et al.*, 2007).

La agitación favorece el proceso de digestión anaeróbica ya que distribuye uniformemente las bacterias, el sustrato y las sustancias nutritivas en todo el digestor. La actividad metabólica de las bacterias acidogénicas, al igual que aquella de las bacterias metanígenas, requiere que estas se encuentren en estrecho y lento contacto espacial entre ellas. Por otro lado, la presión en un digestor puede ser atmosférica, aunque con frecuencia se prefiere operar con una ligera sobrepresión de 10 a 20 mbar para garantizar que el reactor se encuentre en condiciones anaeróbicas.

La digestión puede ser realizada en una sola etapa, o bien, en dos etapas tal y como ha sido realizado en este trabajo. Mientras que la fermentación efectuada en una sola etapa implica la presencia simultánea de las bacterias acidogénicas y metanígenas en un sistema que, a causa de la menor velocidad de reproducción de los metanígenos es notablemente sensible a las variaciones de las condiciones operativas, la fermentación efectuada en dos etapas comprende la separación de la hidrólisis y la acidogénesis de la acetogénesis y metanogénesis. Este sistema de dos etapas es considerado un proceso prometedor debido a que las condiciones de operación como el pH pueden ser reguladas para satisfacer los requerimientos de cada grupo de microorganismos.

Por otra parte, la degradabilidad y potencial de producción de biogás a partir de desechos sólidos en un digestor anaeróbico, depende en gran medida de la cantidad en la que se encuentren los componentes principales. Entre ellos, los lípidos son las sustancias que se digieren con mayor facilidad durante el proceso, mientras que la lignocelulosa es aquella que se digiere con mayor lentitud (Nayoto, 2010). Durante la primera fase de fermentación (hidrólisis), se produce la solubilización de las partículas y la descomposición biológica de los polímeros orgánicos en monómeros o dímeros que favorecen la producción de sustancias necesarias para los microorganismos acidogénicos. Sin embargo, este mecanismo representa la fase limitante del proceso de digestión anaeróbica (Ghyoot y Verstrate, 1997) y por lo tanto pretratamientos térmicos, químicos, biológicos y mecánicos, así como combinaciones de estos son empleados para hacer la materia más accesible a los microorganismos.

Entre la amplia gama de pretratamiento que han sido estudiados, el alcalino y el mecánico son particularmente importantes para este trabajo. El pretratamiento alcalino es uno de los más eficientes, debido a las distintas ventajas que presenta con respecto a otros métodos. En primer lugar, el álcali tiene la capacidad de solubilizar la lignina (Hendriks y Zeeman, 2008). En segundo lugar, se neutralizan varios ácidos producidos durante el proceso y en tercer lugar, el álcali impide una posterior reducción del pH durante la fase de acidogénesis

(Pavlostathis y Gossett, 1985); en la mayor parte de los casos se utiliza NaOH. Por otro lado el pretratamiento mecánico tiene como objetivo reducir el tamaño de las partículas y por lo tanto, aumentar la superficie específica disponible para el proceso biológico.

El objetivo de este trabajo es evaluar el rendimiento de la digestión anaeróbica en dos etapas de desechos orgánicos (frutas y verduras recolectadas en un mercado local) pretratados alcalinamente con NaOH; la selección del pretratamiento alcalino para este proceso se realizó en base al bajo requerimiento energético para efectuar el mismo.

El rendimiento de la digestión ha sido evaluado calculando la “eficiencia” y la “eficacia” del proceso y comparando estos resultados con los obtenidos en una investigación previa en la cual los desechos orgánicos fueron tratados empleando un pretratamiento alcalino/térmico; el objetivo es el de determinar si el pretratamiento térmico que requiere de una gran cantidad de energía, es realmente eficaz para mejorar la producción de metano y la eficiencia de la fermentación. Por otra parte, los resultados han sido comparados con estudios previos en los cuales se efectuó la digestión anaeróbica de la concha de las semillas de café (sustrato con elevada presencia de compuestos lignocelulósicos) y la digestión de la glucosa (monómero de elevada biodegradabilidad).

METODOLOGÍA

La experimentación consiste en la preparación del inóculo, preparación del sustrato y la fermentación en el reactor. Estas actividades fueron llevadas a cabo en base a la metodología empleada en investigaciones precedentes, con la finalidad de maximizar la producción de gas y establecer referencias con respecto a las cuales realizar las comparaciones.

Los cultivos de bacterias necesarios para efectuar la fermentación fueron proporcionados con la adición de deyecciones (estiércol) bovinas, de modo tal de introducir tanto las bacterias acidogénicas en la primera etapa, como las bacterias metanógenas en la segunda etapa de fermentación.

Para seleccionar la microflora acidogénica para la producción de biohidrógeno, el estiércol bovino fue pretratado con HCl 2N para llevar el pH a un valor de 3 y posteriormente dejarlo en reposo en una cámara termostática a 30 °C durante 24 h. Posteriormente el inóculo fue congelado a -24 °C (Tommasi, 2010). El pretratamiento efectuado se realizó con la finalidad de inhibir el crecimiento de las bacterias metanógenas las cuales poseen una escasa resistencia a las condiciones de excesiva acidez; en cambio, las bacterias productoras de hidrógeno forman esporas resistentes a las condiciones extremas (Brock y otros, 1994). La microflora metanógena para la segunda etapa estuvo proporcionada por el inóculo de estiércol bovino sin ningún tipo de pretratamiento.

La preparación del sustrato se realizó a partir de frutas y verduras recolectadas en un mercado local. El material orgánico fue triturado con una licuadora de cocina con la finalidad de efectuar un pretratamiento mecánico para reducir las dimensiones de las partículas. El caldo obtenido se diluyó en proporción 1:1 con agua corriente para alcanzar una concentración de sólidos totales (ST) de 51 g/L (Rametta, 2013). La dilución permite además disminuir la viscosidad del sustrato, evitando así problemas de agitación y problemas de transferencia de calor y materia.

La caracterización del caldo se realizó en base a la masa agregada de cada fruta, vegetal y la composición porcentual de estas presente en la base de datos INRAN (INRAN, 2013). El poder calorífico del caldo se determinó experimentalmente con una bomba calorimétrica. También se efectuó un pretratamiento alcalino, agregando NaOH 2N hasta obtener un pH de

12, para luego dejar en reposo el sustrato en una cámara termostática a 30 °C durante 24 h (Ruggeri et al., 2013).

La fermentación fue efectuada en dos etapas en un reactor batch durante un período de 109 días. En la primera etapa se obtuvo biohidrógeno como producto, mientras que en la segunda biometano. La separación entre la primera y segunda etapa de digestión fue hecha a través de un control de pH en el reactor, al igual que mediante la adición del inóculo. Durante la primera etapa el pH en el reactor fue mantenido en torno a un valor de 5.5, ya que estas condiciones favorecen el metabolismo de las bacterias productoras de hidrógeno y no de aquellas productoras de metano. En cambio, durante la segunda etapa de fermentación el pH fue llevado a un valor cercano a 7.2, dado que estas condiciones favorecen el crecimiento de los metanógenos, pero no el de las bacterias acidogénicas.

En la primera etapa del proceso, el almacenamiento del biogás (fundamentalmente hidrógeno) se realizó con la técnica de desplazamiento de líquido, para lo cual se utilizaron gasómetros: estos consistían en recipientes de vidrio inicialmente llenos con agua acidulada con pH=2. Midiendo la masa de líquido desplazada por el gas durante el proceso se determinaba el volumen de gas producido.

En la segunda etapa, a causa de la mayor velocidad de producción de biogás, en su mayor parte metano, el volumen de gas fue medido a través de un medidor de flujo a trampa de agua “Miligascounter” y el gas fue almacenado en bolsas de plástico diseñadas para esta finalidad.

El volumen de reacción fue de 1.52 L de los cuales 10% era inóculo. El reactor se encontraba dotado de instrumentos adecuados para el monitoreo y control de los parámetros más influyentes en el proceso: pH, agitación, presión, temperatura y potencial redox. El pH se controló a través de una lógica de control On/Off que accionaba una bomba para suministrar NaOH 2N cuando fuese necesario. La agitación estuvo garantizada por dos agitadores tipo Rushton posicionados en el fondo y en la interfase gas/líquido para evitar la formación de espuma, y tres baffles. En la primera etapa se experimentó con una agitación de 250 rpm, mientras que en la segunda se trabajó con un valor de 100 rpm dada la mayor sensibilidad al estrés mecánico de los metanógenos.

La temperatura estuvo controlada con una lógica de control On/Off a través de una camisa de calentamiento eléctrica y una termocupla, operando con un set point de 35 °C para efectuar la fermentación en condiciones mesófilas. La presión en el reactor era de 20 mbar por encima de la presión atmosférica.

Las mediciones efectuadas fueron: la medición del volumen de gas producido, su composición, la concentración de sólidos totales (ST) y sólidos volátiles (SV) en el interior del reactor y el pH. El volumen de gas producido fue medido con el método anteriormente explicado, mientras que el análisis de ST y SV fue efectuado con el procedimiento estándar (APHA, 1995). La medición del pH se llevó a cabo con un pH-metro Mettler en el interior del reactor.

Por lo que respecta al tratamiento de los datos experimentales, la producción de biogás fue modelada matemáticamente con el modelo de Gompertz modificado a uno y dos parámetros (Lo y otros, 2011). Se emplearon estos modelos debido a que son ampliamente usados en procesos de digestión anaeróbica. El modelo de Gompertz y Gompertz modificado a dos parámetros se muestran respectivamente a continuación:

$$G = G_{max} e^{-e^{\left(\frac{R_{max} e}{G_{max}}(\lambda - t) + 1\right)}} \quad (1)$$

Donde:

G: producción acumulada de gas (L/L);

G_{max} : producción máxima de biogás (L/L);

R_{max} : productividad máxima de biogás (L/Lh);

λ : duración del tiempo de retreaso (h);

t: tiempo de avance del experimento (t).

$$G = G_{1,max} e^{-e^{\left(\frac{R_{1,max}}{G_{1,max}}(\lambda-t)+1\right)}} + G_{2,max} e^{-e^{\left(\frac{R_{2,max}}{G_{2,max}}(\lambda-t)+1\right)}} \quad (2)$$

Los parámetros de procesos fueron obtenidos a través de una regresión matemática realizada con el instrumento “solver” del software “Microsoft Excel”.

Los cálculos del rendimiento energético fueron realizados empleando parámetros descritos por Ruggeri and Tommasi (2012), los cuales son: la eficiencia(ξ) y la eficacia (η). Estos son calculados de la siguiente manera:

$$\xi_{OWM} = \frac{E_{p,OWM}}{E_{0,OWM}} \quad (3)$$

Donde:

$E_{p,OWM}$: energía producida utilizando desechos orgánicos como sustrato;

$E_{0,OWM}$: energía presente en el sustrato.

$$\eta = \frac{\xi_{OWM}}{\xi_{Glu}} \quad (4)$$

Donde:

ξ_{OWM} : eficiencia de la digestión anaeróbica de desechos orgánicos;

ξ_{Glu} : eficiencia de la digestión anaeróbica de la glucosa.

También se definió un parámetro denominado índice de sostenibilidad (SI) con la finalidad de establecer una relación entre la energía producida en forma de biogás y la energía consumida durante el pretratamiento. Este índice se expresa como:

$$SI = \frac{E_p}{E_s} \quad (5)$$

Donde:

E_p : energía producida en forma de biogás (kJ).

E_s : energía gastada durante el pretratamiento (kJ).

RESULTADOS Y DISCUSIÓN

La digestión se realizó en condiciones controladas de pH, debido a que las bacterias acidogénicas y metanogénicas tienen requerimientos diferentes para su crecimiento. Por lo tanto, las dos etapas de producción se llevaron a cabo de manera tal de optimizar las condiciones adecuadas para cada grupo de microorganismos. En la Figura N°1 se muestra el comportamiento del pH en el reactor, el volumen acumulado de biogás por volumen de sustrato alimentado y la composición del gas en el tiempo.

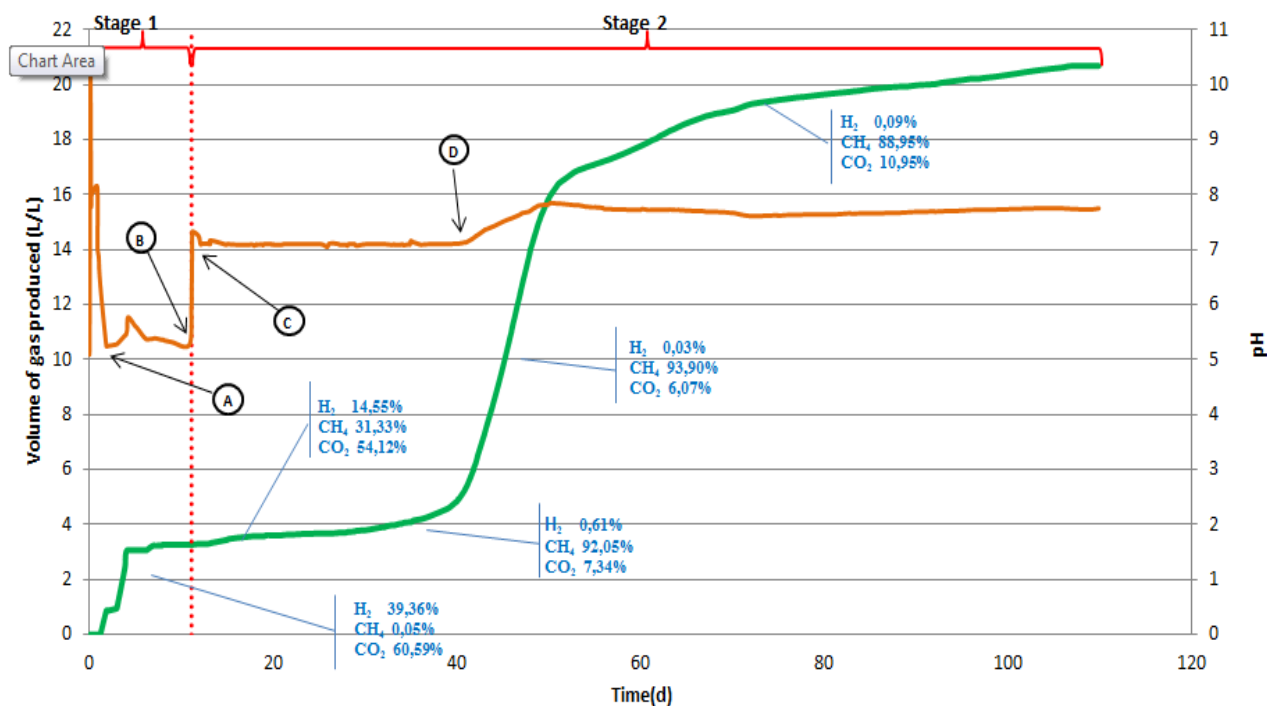


Figura N°1. Comportamiento del volumen de biogás (curva verde), composición y pH (curva naranja) durante la fermentación anaeróbica.

El experimento inició con un valor de pH de 5.09 como resultado de mezclar 1500 mL de sustrato pretratado (pH=12) con 150 mL de inóculo pretratado (pH=3). Seguidamente el pH fue llevado manualmente hasta un valor de 8.5, debido a que la alta viscosidad del sustrato hacía difícil el control automático. Durante los días posteriores el pH disminuyó progresivamente hasta un valor de 5.3 como consecuencia de la producción de ácido por parte de los microorganismos acetogénicos y de la disminución de la viscosidad como consecuencia de la fermentación. Desde el punto A (día 2) al punto B (día 11) de la Figura N°1, este valor fue mantenido constante mediante el control de pH para evitar la acumulación excesiva de ácidos que podrían superar la capacidad de metabolización celular y por lo tanto, producir inhibiciones (Cecchi et al., 2005). El día 5 la producción de biogás se había reducido notablemente, siendo el volumen acumulado hasta el momento de 4.10 L/L con una composición de 39.36% de hidrógeno y 60.59% de CO₂; la presencia de metano era casi despreciable (0.05%). Después de 7 días sin producción de gas, se consideró finalizada la primera etapa (punto B de la Figura N°1), por lo cual en el punto C, se dio inicio a la segunda etapa de fermentación con la adición de inóculo y el cambio del set point a pH=7.1 (Rametta, 2013). El período comprendido entre los puntos C y D, corresponde al tiempo de retardo en el cual los metanógenos se adaptan a las condiciones ambientales y se encuentran en competencia con las bacterias acetogénicas (principalmente *Clostridium*) por la sobrevivencia. Un parámetro que evidencia esta situación es la composición del gas

acumulado durante este período. Luego de la inoculación, el porcentaje de metano que anteriormente era prácticamente despreciable, aumentó hasta 31.33% mientras que el porcentaje de hidrógeno cayó a 14.55%. Un poco antes del inicio de la metanogénesis (punto D), la composición de H_2 había ya disminuido hasta un valor de 0.6%, mientras la de CH_4 se había elevado hasta 92.05%. Por lo tanto, se puede destacar como el cambio de grupo bacteriano predominante en el interior del reactor es evidenciado en la composición del gas.

La Tabla N°1 compara los resultados obtenidos en este trabajo con los resultados obtenidos por Rametta (2013), quien trabajó con una composición de alimentación muy similar. Como se muestra en la tabla, la concentración de sólidos volátiles al inicio de la reacción (VS_0) de Rametta (2013) es muy parecida, así como lo es la cantidad de energía presente en el sustrato por kilogramo de sólidos volátiles (E_{OWM}/VS_0). Sin embargo, la energía recuperada (E_{Prod}/VS_0) durante este trabajo fue superior a la recuperada en la investigación previa y por lo tanto, el rendimiento del proceso en términos de eficiencia resulta ser un 21% mayor.

Tabla N° 1. Comparación del rendimiento energético

Alimentación	VS_0 (g/L)	E_{OWM}/VS_0 (kJ/g VS_0)	E_{Prod}/VS_0 (kJ/g VS_0)	Eficiencia (%)	Referencia
Desechos orgánicos	31.07	24.20	16.20	66.90	Este trabajo
Desechos orgánicos	33.46	24.13	11.15	46.21	Rametta, 2013

El aumento del rendimiento respecto al trabajo precedente obedece a una mayor cantidad de CH_4 producido durante la fermentación. Como se puede apreciar en la Tabla N°2, la producción de CH_4 por litro de sustrato fue 40% superior, mientras que la producción de H_2 resultó ser 41% inferior. A pesar de la reducción en la obtención de H_2 considerando el hecho de que la cantidad de CH_4 producida fue alrededor de 13 veces superior a la de H_2 y destacando el hecho de que el poder calorífico del metano es alrededor de 3 veces superior al del hidrógeno, un incremento en la producción de CH_4 representa un aumento global de la cantidad de energía recuperada, y por lo tanto la eficiencia resulta ser mayor.

Tabla N° 2: Comparación del volumen de H_2 y CH_4 producido

Alimentación	Pretratamiento	H_2 producido (L/L)	CH_4 producido (L/L)	Referencia
Desechos orgánicos	Alcalino	1.17	15.53	Este trabajo
Desechos orgánicos	Alcalino/Térmico	1.97	11.19	Rametta, 2013

La diferencia fundamental entre la prueba conducida por Rametta (2013) y la efectuada en el presente trabajo es el tipo de pretratamiento aplicado al sustrato. Mientras que en la investigación precedente se empleó un pretratamiento alcalino/térmico para aumentar la biodegradabilidad del sustrato, en este trabajo se realizó sólo un pretratamiento alcalino. De acuerdo a Ruggeri et al. (2013), la combinación de un pretratamiento alcalino con uno

térmico, es el modo más adecuado de mejorar la biodegradabilidad de los desechos orgánicos para la producción de hidrógeno. Este argumento es confirmando a través de la comparación mostrada en la Tabla N°2 entre los volúmenes de hidrógeno producidos en ambos casos. Sin embargo, también se evidencia que el pretratamiento térmico no favorece el aumento en la producción de metano que, por el contrario disminuye con este.

Una posible explicación de los resultados obtenidos podría residir en la formación de sustancias inhibitorias a altas temperaturas que comprometan la producción de metano (Haug et al., 1978). Colberg et al. (1980) experimentaron con pretratamientos térmicos en lodos activados a temperaturas superiores a 175 °C, observando la formación de compuestos tóxicos, cuya toxicidad aumentaba al elevar la temperatura, agregar NaOH o HCl, y al aumentar la concentración del lodo. Determinaron así que el pretratamiento podría haber liberado las unidades más simples que constituían los compuestos nitrogenados, las cuales tienden a ser más tóxicas que el compuesto original. Mientras más complejo es un compuesto orgánico, menor es la probabilidad de este de ser tóxico. Esta hipótesis se basa en el hecho de que mientras más grande sea una molécula, es más difícil que la misma pueda penetrar las paredes celulares e inhibir la actividad microbica. Por esta razón, complejos orgánicos nitrogenados como el colágeno, la albúmina y el ARN no resultan tóxicos, mientras que los aminoácidos sí. De acuerdo a Colberg et al. (1980), en el pretratamiento básico/térmico, puede presentarse toxicidad a causa de la solubilización de la fracción nitrogenada del lodo a baja temperatura, que luego es convertida a temperaturas elevadas en sustancias tóxicas.

La Tabla N°3 muestra los parámetros de eficiencia y eficacia obtenidos en el presente trabajo, estableciendo una comparación con los resultados de la digestión anaeróbica en dos etapas de la concha de la semilla de café y la glucosa. Se puede observar como la digestibilidad de los desechos orgánicos es intermedia entre estas dos, ya que la glucosa es un compuesto con una gran facilidad de degradación, mientras que la concha de las semillas de café resultan ser de más difícil degradación por el alto contenido de compuestos lignocelulósicos.

Tabla N°3. Comparación de la recuperación energética empleando diferentes sustratos

Alimentación	Composición promedio del gas		Energía recuperada (kJ)		Energía recuperada Total (kJ)	Energía presente en los desechos (kJ)	Eficiencia ξ (%)	Eficacia η (%)
	H ₂	CH ₄	Etapas 1	Etapas 2				
Glucosa	26.2	81.0	55	2665	2320	3191	72.70	100
Desechos orgánicos (pretratamiento alcalino)	5.7	75.1	19	746	764	1143	66.95	92.09
Desechos orgánicos (pretratamiento alcalino/térmico)	11.2	63.5	30	567	597	1292	46.21	63.6
Concha de semillas de café	0.8	77.5	0.5	2174	2175	8443	25.76	35.43

La Tabla N°4 compara el índice de sostenibilidad de este trabajo con el de Rametta (2013). Ciertamente el gasto energético por litro de sustrato es considerablemente menor aplicando un pretratamiento alcalino al sustrato. Por esta razón se observa que en el presente trabajo, la energía recuperada es aproximadamente 10.5 veces, la energía gastada durante el

pretratamiento (SI=10.5), es decir, el proceso es sostenible desde un punto de vista energético. En Rametta (2013) la energía consumida supera a la energía producida a través de la fermentación (SI=0.37), y por lo tanto el proceso no es energéticamente sostenible.

Tabla N°4. Comparación de la energía gastada durante el pretratamiento y los índices de sostenibilidad

Pretratamiento	Gasto de energía en pretratamiento (kJ/L)	Índice de sostenibilidad (SI)	Referencia
Alcalino	48	10.49	Este trabajo
Alcalino/Térmico	1004.42	0.37	Rametta, 2013

El gasto energético de los pretratamientos alcalinos en ambas investigaciones fue calculado multiplicando la masa de NaOH añadida durante el pretratamiento, por el Gross Energy Requirement (GER) del NaOH. El gasto adicional de energía del pretratamiento térmico de Rametta (2013) se calculó sumando el calor sensible necesario para elevar la temperatura de los desechos desde la temperatura ambiente hasta los 120 °C, con el calor necesario para mantener esta temperatura durante los 30 min que duró el pretratamiento.

CONCLUSIONES

Debido a que la digestión anaeróbica conducida en dos etapas implica la producción por separado de hidrógeno (primera etapa) y metano (segunda etapa), la influencia del pretratamiento alcalino es diversa en cada una de las fases si se le compara con un pretratamiento alcalino/térmico. Someter los desechos orgánicos a altas temperaturas en combinación con la adición de NaOH, representa un pretratamiento eficaz para incrementar la producción de biohidrógeno en la primera etapa de fermentación. A partir de la digestión efectuada en este trabajo aplicando sólo pretratamiento alcalino, se obtuvo una producción específica de hidrógeno de 1.17 L/L sustrato, que es inferior a la reportada en un estudio precedente en el cual se empleó un pretratamiento básico/térmico.

Sin embargo, la producción de metano mejora cuando el pretratamiento térmico no es empleado. La producción específica de metano obtenida utilizando sólo pretratamiento alcalino fue de 15.33 L/L sustrato, siendo este un incremento del 40% respecto a la producción obtenida aplicando pretratamiento alcalino/térmico. La utilización de un pretratamiento químico/térmico para aumentar la biodegradabilidad de los desechos orgánicos, podría producir inhibiciones como consecuencia de la solubilización a bajas temperaturas de compuestos nitrogenados presentes en el sustrato, seguida de una conversión a temperaturas más altas de tales productos solubles en sustancias tóxicas. Al aumentar la temperatura del pretratamiento, los compuestos nitrogenados solubles son hidrolizados a aminoácidos, y finalmente degradados térmicamente a NH_3 , que inhibe la producción de biogás cuando se encuentra en elevadas concentraciones.

La cantidad de metano producido supera alrededor de 13 veces la cantidad de hidrógeno. Dado que el poder calorífico del metano es aproximadamente 3 veces superior al del hidrógeno, el incremento de la producción de CH_4 representa globalmente un aumento de la recuperación energética. La eficiencia y la eficacia del proceso fueron de 66.95% y 92%

respectivamente, que representa un notable aumento respecto a la digestión anaeróbica realizada con pretratamiento básico/térmico.

La baja energía requerida para efectuar el pretratamiento alcalino con NaOH permite realizar un proceso de digestión de manera sostenible, ya que la energía recuperada de la fermentación en forma de biogás es superior a la energía gastada durante el pretratamiento. Este argumento es respaldado con el índice de sostenibilidad obtenido que posee un valor de 10.5.

Por último, comparando con estudios previos sobre la digestión de la concha de las semillas de café y la glucosa, se confirma que los desechos orgánicos tienen una digestibilidad intermedia, ya que las frutas y verduras no son tan ricas en compuestos lignocelulósicos (difícilmente fermentables) como es el caso de las conchas de las semillas de café, pero su fermentabilidad tampoco es tan alta como la de la glucosa pura.

INDEX

Introduction	1
Chapter I. BASIS OF THE RESEARCH	3
Chapter II. THEORETICAL FRAMEWORK.....	6
Biogas	6
Anaerobic digestion	8
Biogas production models	24
Types of anaerobic reactors	26
Substrates	29
Pretreatment	36
CHAPTER III. MATERIALS AND METHODS	41
Experimentation.....	41
Analytical methods	49
Experimental data treatment	52
CHAPTER IV. RESULTS AND DISCUSION.....	55
Fermentation analysis	55
Energy recovery and comparison with other works	58
Experimental data modelling	63
Chapter V. Conclusions	68
REFERENCES.....	70

INDEX OF FIGURES

Figure 1. General scheme of the anaerobic digestion process	9
Figure 2. Quantitative scheme of the different metabolic pathways involved in anaerobic digestion process.....	12
Figure 3. Ammonia-ammonium buffer system.	15
Figure 4. Temperature range for anaerobic digestion.	17
Figure 5. Biogas production in a function of retention time	19
Figure 6. Two stage anaerobic digestion scheme.....	29
Figure 7. Influence of the grinding on the time of fermentation for different substrates	34
Figure 8. Broth obtained after milling.....	42
Figure 9. Broth percentage composition (wet basis).....	44
Figure 10. Broth percentage composition (dry basis)	44
Figure 11. Milligascounter used to measure the volume of gas produced.....	47
Figure 12. "MINIFORS INFORS" reactor.....	48
Figure 13. Experimental data of two-stage fermentation.....	55
Figure 14. Experimental data and Modified Gompertz model fitting.....	63
Figure 15. Experimental data and Gompertz model fitting for first stage of fermentation	64
Figure 16. Experimental data and Gompertz model fitting for second stage of fermentation	65
Figure 17. Experimental data and sum of Gompertz model fitting applied separately to each stage	66

INDEX OF TABLES

Table 1. Average biogas composition of anaerobic digestion	7
Table 2. Amount of fuel necessary to obtain the same energy produced by combustion of 1 m ³ of biogas	7
Table 3. Retention time in a function of the temperature in the digester.....	19
Table 4. Advantages of Mixing Digester Content	22
Table 5. Favorable concentrations of trace elements.	32
Table 6. Time of regeneration of different anaerobic).	35
Table 7. Organic elements added to prepare the substrate and percentage composition	43
Table 8. Experimental data of the two-stage digestion.....	57
Table 9. Energetic performance comparison	58
Table 10. Comparison of volume of H ₂ and CH ₄ produced.....	59
Table 11. CH ₄ and H ₂ yield obtained in researches about anaerobic digestion of organic waste.....	60
Table 12. Energetic recovery in two-stage fermentation	61
Table 13. Pretreatment energy expenditure and sustainability index comparison	62
Table 14. Modified Gompertz model parameters	64
Table 15. Gompertz model parameters for first stage of fermentation	65
Table 16. Gompertz model parameters for second stage of fermentation	66
Table 17. Parameterization of Gompertz model	67

INTRODUCTION

INTRODUCTION

Nowadays, environmental pollution and fossil fuel depletion have become issues of considerable global concern that encourage efforts for finding alternative energy sources and pollution control. One of the most attractive ways to achieve both goals is to recover energy and resources from waste streams through bioconversion processes.

In a society characterized by the population growth and a large consumptions, removal of solid organic waste that is produced daily by households, offices, industry, and the agricultural sectors as well as by food productions has become an ecological problem (Khalid et al., 2011; Ruggeri and Tommasi, 2013). Conveniently, in recent years the organic fraction of solid waste has been recognized as a rewarding resource that can be converted into an energy source via microbial activity.

Among the various methods available for treatment of organic waste, anaerobic digestion, which is conducted in this work, is one of the most promising alternatives because of the high concentration of fuel gas obtained and the stabilized residue remaining at the end of the process which can be used as organic fertilizer. This process involves four phases through which fermentable compounds are transformed into biogas: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Appels et al., 2008). The main fermentation products, hydrogen and methane, are considered comparatively cleaner than fossil fuel so increase the opportunity to decrease environmental pollution (Khalid et al., 2011).

While in fermentation conducted on one-stage systems, all phases and reactions take place simultaneously in a single reactor, in two-stage systems methanogenesis and acetogenesis take place sequentially after hydrolysis and acidogenesis, allowing in this way the optimization of acidification and methanogenesis separately. About 90% of the full scale plants, currently in use in Europe for anaerobic digestion of municipal solid waste operate on one-stage systems because of their simpler designs and lower investment costs (Bouallagui

INTRODUCTION

et al., 2005). Nonetheless, considerable amount of researches, including this, look for optimization of two-stage digestions due to the optimum results they involve. Enhancing of two-stage processes also deals with the fact that biological hydrolysis is identified as the rate-limiting step (Ghyoot and Verstrate, 1997); hence organic waste must receive an appropriated pretreatment to optimize the process performance.

This work is focused on the performance of the anaerobic digestion of fruits and vegetables collected in a local market, pretreated with NaOH and conducted on two-stage system; among others pretreatment alkaline one was implemented because of their low energy requirement. The results are compared with previous works.

CHAPTER I BASIS OF THE RESEARCH

CHAPTER I

BASIS OF THE RESEARCH

APPROACH TO THE PROBLEM

As the environmental pollution and fuel depletion have become global concerns, the anaerobic digestion might represent an alternative energy source that can provide solutions to both problems. Every day large amount of organic material is discarded without taking advantage of the energy present in it, but nowadays is well known that all this amount of waste that normally accumulates in large landfills, can be decomposed in controlled environments and the biogas generated instead of being released into the atmosphere, can be harnessed as an alternative energy source.

In the last decades several researches have improved the yield and performance of the anaerobic fermentation for biogas production. Parameters like temperature have been studied and the optimal range of operation has been determined. pH is another parameter which control has been noted as import and fundamental to ensure the growth of microorganisms involved in the bioconversion process, as each group of bacteria has different requirements. Two-stage anaerobic digestion was also proposed some decades ago, allowing to enhance hydrogen production in the first stage and drawing on the remaining volatile acids which are useful to produce methane, thereby increasing the energy recovery. In this case the most appropriate techniques to inoculate the microflora in both stages has been determine, as well as the ranges of mixing as the acidifying and methanogenic bacteria differ in their sensibility to mechanical stress.

The substrate and type of pretreatment however are elements that must still be studied to improve the energy recovery during fermentation. In the first phase

CHAPTER I BASIS OF THE RESEARCH

of the digestion (hydrolysis) complex compounds are transformed into monomers and oligomers at such a rate that this step becomes the limiting phase. It is therefore important to perform a pretreatment in order to increase the biodegradability of the substrate and render this more accessible to the microorganisms, thus facilitating and reducing the fermentation time.

There are numerous research studies that have investigated the use of organic waste for producing biogas. Many of these, point out to using combination of chemical and thermal pretreatment for increasing hydrogen production in the first stage. Nonetheless, there are not many researches about the optimal pretreatment for enhancing methane production through fermentation of organic waste conducted in two stages.

Previous researches found out that alkaline-thermal pretreatments are suitable methods to improve the yield of hydrogen production in the anaerobic digestion of organic market waste (fruits and vegetables). Moreover it was found in a previous inquiry that this kind of pretreatment offer significantly high yields in both hydrogen and methane production performed in two-stage fermentation processes; alkaline solutions solubilize the lignocellulosic compounds present in the fibres and prevent drops of pH during the acidogenesis while thermal effect helps brake down large molecule into simpler units. Notwithstanding the high yields of biogas production, the energy expenditure during thermal pretreatment is greater than the energy recovered through digestion, so the process is unsustainable from an energy point of view. For this reason this study is focused in determine whether thermal pretreatment is really essential to conduct the anaerobic digestion of organic market waste in two stages, or conversely, if good results can be obtained, especially during methane production, without the need to use large amounts of energy for the pretreatment.

CHAPTER I BASIS OF THE RESEARCH

OBJETIVE OF THE WORK

The goal of this work is to evaluate the performance of the anaerobic digestion of the organic waste market (fruits and vegetables collected in a local market) pretreated with NaOH and conducted on two-stage system; among others pretreatment alkaline one was implemented because of their low energy requirement.

The digestion performance will be based on calculating the efficiency and efficacy of the process and compare the results with a previous work in which organic waste market was treated with alkaline-thermal pretreatment; the purpose is to determine whether thermal pretreatment which requires a large expenditure of energy, is really effective for enhancing methane production and fermentation efficiency. Moreover, a sustainability index was defined to evaluate the sustainability of the process from the energy perspective. The results also were compared with previous researches wherein were fermented coffee seed skin because of their hard fermentability (high presence of lignocellulosic compounds), and glucose because its easier digestion.

CHAPTER II THEORETICAL FRAMEWORK

CHAPTER II

THEORETICAL FRAMEWORK

BIOGAS

Biogas produced by anaerobic digestion is a gas mixture that is primarily made up of methane (CH_4) and carbon dioxide (CO_2), along with water vapour and various trace gases (Table 1).

The most important of these is the methane content, since this is the combustible component of biogas and thus directly influences its calorific value. There is only limited opportunity for influencing the composition of biogas by means of selective process control. First and foremost the composition of the biogas is dependent on the composition of the input material. In addition, the methane content is affected by process parameters such as the digestion temperature, reactor loading and hydraulic retention time, as well as by any disruptions to the process and the method of biological desulphurization used.

The achievable methane yield is essentially determined by the composition of the substrate, in other words by the proportions of fats, proteins and carbohydrates (FNR, 2010).

CHAPTER II THEORETICAL FRAMEWORK

Table 1. Average biogas composition of anaerobic digestion (FNR, 2010)

Constituent	Concentration
Methane (CH ₄)	50-75 vol. %
Carbon dioxide (CO ₂)	25-45 vol. %
Water (H ₂ O)	2-7 vol. % (20-40 °C)
Hydrogen sulphide (H ₂ S)	20-20,000 ppm
Nitrogen (N ₂)	< 2 vol. %
Oxygen (O ₂)	< 2 vol. %
Hydrogen (H ₂)	< 1 vol. %

The calorific value of biogas produced by anaerobic fermentation is lower than that of methane and other fuels. Table 2 shows the amount of other fuels that must be burned to obtain the same energy contained in 1 m³ of biogas (Rametta, 2013).

Table 2. Amount of fuel necessary to obtain the same energy produced by combustion of 1 m³ of biogas (Cecchi et al., 2007)

Fuel	Amount
Gasoline	0.8 L
Ethyl alcohol	1.3 L
Carbide	2 kg
Diesel and heavy diesel	0.7 L
Coal gas	1.5 m ³
Charcoal	1.4 kg
Wood	2.7 kg
Methane	0.7 m ³
Propane	0.3 m ³
Butane	0.2 m ³
Biogas	1 m ³

CHAPTER II THEORETICAL FRAMEWORK

ANAEROBIC DIGESTION

Anaerobic digestion (AD) is a bacterial fermentation process that operates without free oxygen and results in a biogas containing mostly methane and carbon dioxide (Rapport et al., 2008). It involves different microbial groups interacting with each other: the hydrolytic bacteria, the bacteria acidifying (acetogenic and homoacetogenic) and, finally, the methanogenic bacteria, which produce methane and CO₂, which are the gases with more energetic interest and represents approximately 2/3 of the biogas produced. The methanogenic bacteria occupy only the final position of the anaerobic food chain. The methane, slightly soluble in water, is transferred to the gaseous phase, while the CO₂ is distributed in both gaseous and liquid phases (Cecchi et al., 2005).

AD is a complex process which requires strict anaerobic conditions (oxidation reduction potential (ORP) < -200 mV) to proceed, and depends on the coordinated activity of a complex microbial association to transform organic material into mostly CO₂ and methane (CH₄). Despite the successive steps, hydrolysis is generally considered as rate limiting (Appels et al., 2008).

Stages of anaerobic fermentation

Methane fermentation is a complex process, which can be divided up into four phases of degradation, named hydrolysis, acidogenesis, acetogenesis, and methanogenesis, according to the main process of decomposition in this phase (Figure 1). The individual phases are carried out by different groups of microorganisms, which partly stand in syntrophic interrelation and place different requirements on the environment. The first and second as well as the third and fourth phase are linked closely with each other. Therefore, one can accomplish the process well in two stages (Deublein and Steinhauser, 2008).

CHAPTER II THEORETICAL FRAMEWORK

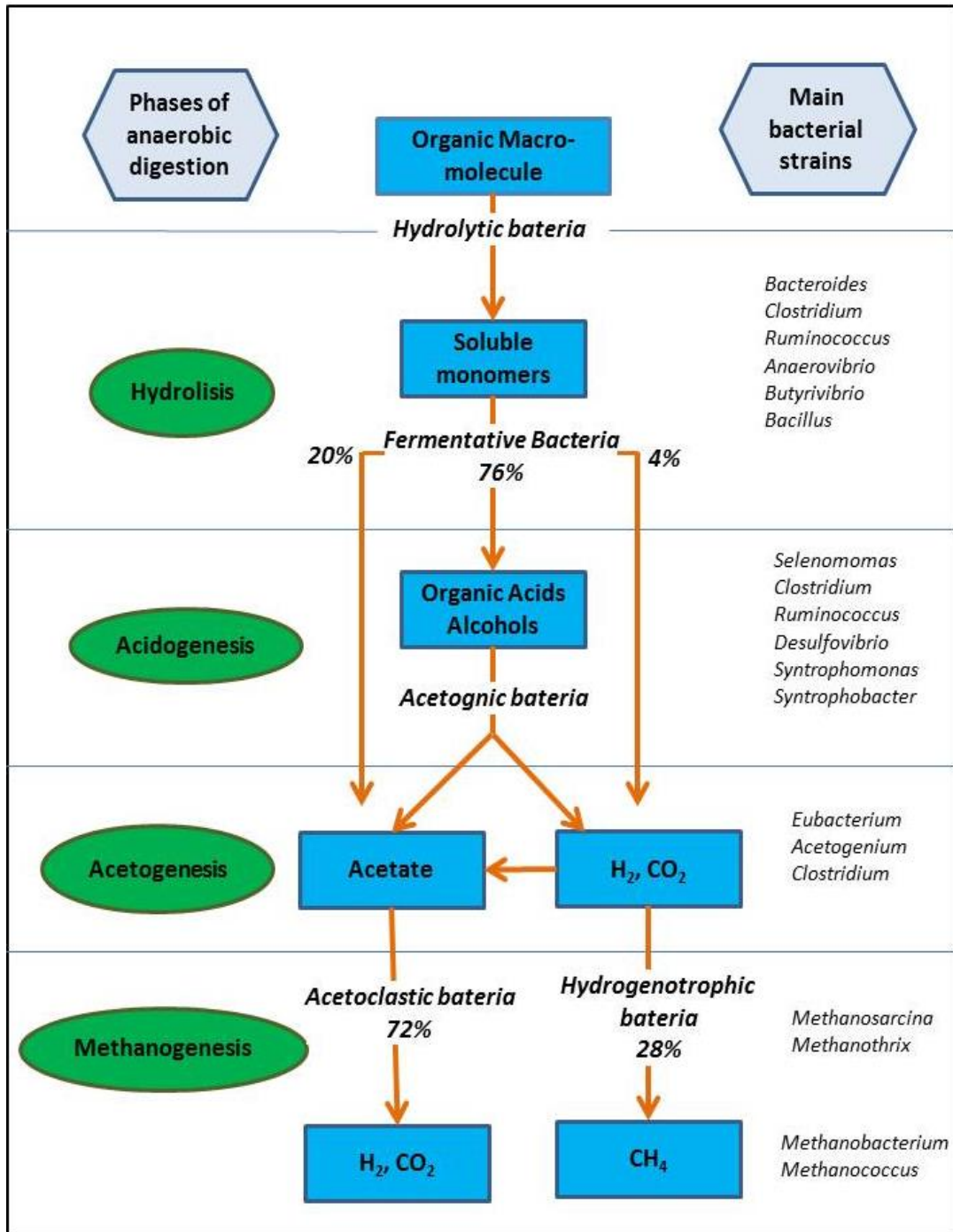


Figure 1. General scheme of the anaerobic digestion process (Cecchi et al., 2005)

Hydrolysis

In the first phase (the hydrolysis) compounds, like cellulose, proteins, and fats are cracked into monomers (water-soluble fragments) by exoenzymes (hydrolase) of facultative and obligatory anaerobic bacteria (Deublein e Steihauser, 2008).

CHAPTER II THEORETICAL FRAMEWORK

Particularly, the hydrolysing microorganisms can colonize the particulate material and degrade or produce extra cellular enzymes capable of breaking down complex organic molecules in oligomers and monomers that become available for being transported into the cells of acidogenic microorganisms. These microorganisms generally produce the oxidation of simple organic substrates to pyruvate which is then converted into volatile fatty acids, alcohols and ketones that are the starting substrates for the subsequent acetogenic phase (Cecchi et al., 2005).

The hydrolysis of carbohydrates takes place within a few hours, the hydrolysis of proteins and lipids within few days. Lignocellulose and lignin are degraded only slowly and incompletely. The facultative anaerobic microorganisms take the oxygen dissolved in the water and thus cause the low redox potential necessary for obligatorily anaerobic microorganisms (Deublein e Steinhauser, 2008).

Acidogenic phase

The monomers formed in the hydrolytic phase are taken up by different facultative and obligatorily anaerobic bacteria and are degraded in the second, the acidogenic phase, to short - chain organic acids, C1 – C5 molecules (e.g., butyric acid, propionic acid, acetate, and acetic acid), alcohols, hydrogen, and carbon dioxide. The concentration of the intermediately formed hydrogen ions affects the kind of the products of fermentation. The higher the partial pressure of the hydrogen, the fewer reduced compounds, like acetate, are formed (Deublein e Steinhauser, 2008).

Acetogenic phase

The products from the acidogenic phase serve as substrate for other bacteria, those of the acetogenic phase.

Acetogenic bacteria are obligatory H₂ producers. The acetate formation by oxidation of long chain fatty acids (e.g., propionic or butyric acid) runs on its own and is thermodynamically possible only with very low hydrogen partial pressure. Acetogenic bacteria can get the energy necessary for their survival and growth, therefore, only at very low H₂ concentration.

CHAPTER II THEORETICAL FRAMEWORK

Acetogenic and methane producing microorganisms must therefore live in symbiosis. Methanogenic organisms can survive only with higher hydrogen partial pressure. They constantly remove the products of metabolism of the acetogenic bacteria from the substrate and so keep the hydrogen partial pressure at a low level suitable for the acetogenic bacteria.

When the hydrogen partial pressure is low, H_2 , CO_2 , and acetate are predominantly formed by the acetogenic bacteria. When the hydrogen partial pressure is higher, predominantly butyric, capronic, propionic, and valeric acids and ethanol are formed. From these products, the methanogenic microorganisms can process only acetate, H_2 , and CO_2 .

About 30% of the entire CH_4 production in the anaerobic sludge can be attributed to the reduction of CO_2 by H_2 , but only 5-6% of the entire methane formation can be attributed to the dissolved hydrogen. This is to be explained by the “interspecies hydrogen transfer”, by which the hydrogen moves directly from the acetogenic microorganisms to the methanogenic one, without being dissolved in the substrate.

The anaerobic conversion of fatty acids and alcohols goes energetically at the expense of the methanogenic, where these, however, in return, receive the substrates (H_2 , CO_2 , acetic acid) needed for growth from the acetogenic bacteria.

The acetogenic phase limits the rate of degradation in the final stage. From the quantity and the composition of the biogas, a conclusion can be drawn about the activity of the acetogenic bacteria (Deublein e Steinhauser, 2008).

Methanogenic phase

In the fourth stage, the methane formation takes place under strictly anaerobic conditions. This reaction is categorically exergonic (Deublein e Steinhauser, 2008). The CH_4 production is the end of the anaerobic trophic chain. The methane is in fact the only nonreactive compound in the whole anaerobic digestion process; therefore it can be considered the final product of the entire process.

The production of methane can take place essentially through two different pathways of reactions: a way is the methanogenesis by bacteria hydrogenotrophic,

CHAPTER II THEORETICAL FRAMEWORK

which operate the anaerobic oxidation of hydrogen, while the second way, the so called via acetoclastic, involves the dismutation of anaerobic acetic acid with the formation of methane and carbon dioxide. Most of the methane production takes place via this second mechanism. Figure 2 quantifies the percentage distribution of the different metabolic pathways involved in the process of digestion.

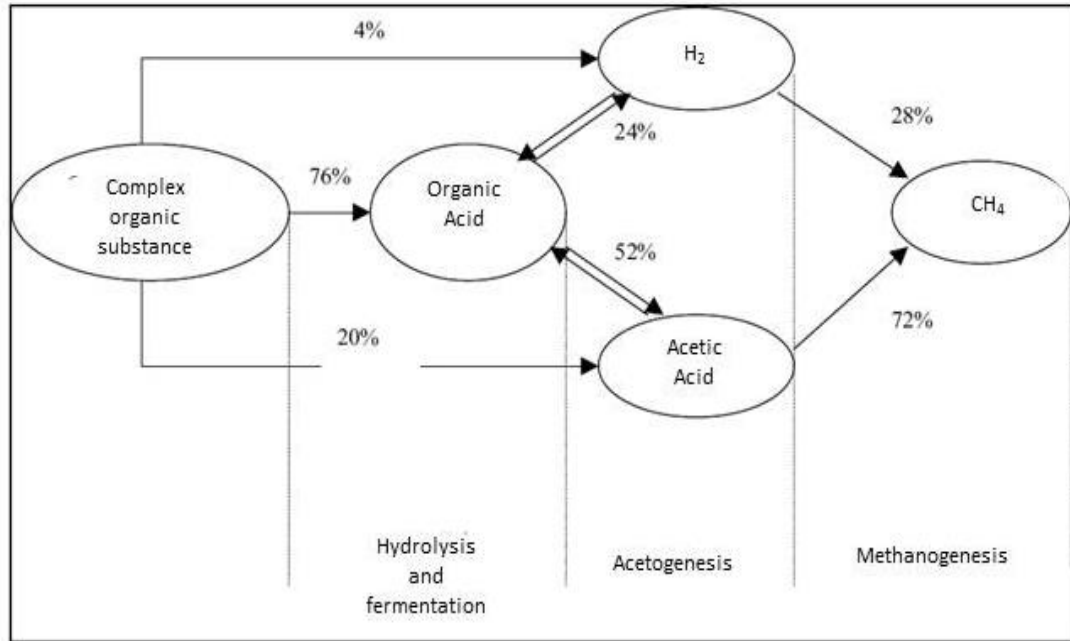


Figure 2. Quantitative scheme of the different metabolic pathways involved in anaerobic digestion process (Cecchi et al., 2005)

The two strains of methanogenic bacteria perform two important functions in the anaerobic trophic chain: on the one hand degrade acetic acid and formic acid to CH₄, removing the acids from the medium and thus preventing the inhibition of degradation of organic substrates for excess of acidity, and on the other hand, maintain low levels of H₂ concentration so as to allow the conversion of long chain fatty acids and alcohols to acetate and H₂.

The most important factor that influence the possibility of use of acetate by methanogenic bacteria is the chemical form as such substrate is present in the medium. In particular, if it is present in the undissociated form (CH₃COOH), the acetic acid can cross the bacterial membrane and therefore be usable (this phenomenon is favoured in a narrow range of pH, generally between 6 and 8). A higher value of pH leads acetic acid to be present mostly in the dissociated form (CH₃COO⁻), so the concentration of undissociated form in the medium is not sufficient to ensure a gradient of concentration such as to enable the trans-

CHAPTER II THEORETICAL FRAMEWORK

membrane transport of the metabolite. In the case of environments characterized by relatively low pH (less than 5) it is present a high concentration of undissociated acid that crosses the cell membrane, so the concentration of such acid may be beyond the capacity of cellular metabolism resulting in inhibition by excess substrate (Cecchi et al., 2005).

Influencing parameters in anaerobic fermentation

There are several factors which can affect both positively and negatively the process of anaerobic digestion and, globally or in one of its phases (Caramiello, 2010). Particularly important are parameters such as pH, temperature, agitation, pressure, concentration of volatile fatty acids (VFA), alkalinity, the ratio of volatile fatty acids and alkalinity, the production and percentage composition of biogas.

As occurs in all biological processes, in this case the constancy of the living conditions is of importance. A temperature change or changes in the substrates or the substrate concentration can lead to shut down the gas production. It can last up to three or even more weeks, until the ecological system has adapted to the new conditions and starts biogas production again without any intervention from outside. But in the case of human interference it can take a further three weeks.

So that, for an optimum fermenting process, numerous parameters must be taken into consideration and be controlled. Furthermore, the environmental requirements of the fermentative bacteria, by which the hydrolysis and acidification of the substrates occur, differ from the requirements of the methane forming microorganisms (Deublein e Steinhauser, 2008).

pH

The pH provides an indication of the stability of the reaction medium, since its variation is associated to the buffering capacity of the system and changes in the balance between the species involved in the trophic chain of the process (Cecchi et al., 2005).

Many aspects of the complex microbial metabolism are greatly influenced by pH variations in the digester. Although acceptable enzymatic activity of acid

CHAPTER II THEORETICAL FRAMEWORK

forming bacteria can occur at pH 5.0, methanogenesis proceeds only at a high rate when the pH is maintained in the neutral range. Most anaerobic bacteria including methane forming bacteria function in a pH range of 6.5 to 7.5 (Nayoto, 2010).

The bacteria involved in the anaerobic fermentation, show different sensitivity to pH, so maximum activity at different values of pH, even if considerably close (Camarota, 2004).

a) Acidogenic and Acetogenic phases: 5-5.5;

b) Methanogenic phase: above 6.5.

The pH value in the digester is determined mainly by the presence of CO₂ in the liquid medium, and therefore by its partial pressure in the biogas, and by the values of concentration of volatile fatty acids and ammonia (Olmetto, 2008).

If pH value sinks below 6.5, then the production of organic acids lead to a further decrease in the pH value by the hydrolytic bacteria and possibly to cessation of the fermentation. In the reality, the pH value is held within the neutral range by natural procedures in the fermenter. Two buffering systems ensure this.

A too strong acidification is avoided by the carbon dioxide/hydrogen carbonate/ carbonate buffer system. During the fermentation, CO₂ is continuously evolved and escapes into air. With falling pH value, more CO₂ is dissolved in the substrate as uncharged molecules. With rising pH value, the dissolved CO₂ forms carbonic acid, which ionizes (Equation 1). Thus, hydrogen ions are liberated.



At pH=4 all CO₂ is as free molecules; at pH=13 all CO₂ is dissolved in the form of carbonate in the substrate. The centre around which the pH value swings with this system is at pH=6.5.

A too weak acidification is avoided by the ammonia-ammonium buffer system (Figure 3). With falling pH value, ammonium ions are formed with release of hydroxyl ions. With rising pH value, more free ammonia molecules are formed (Deublein e Steinhauser, 2008).

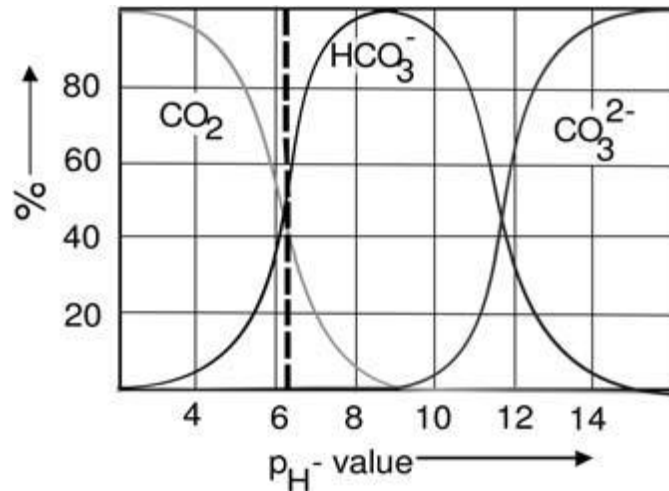


Figure 3. Ammonia-ammonium buffer system (Deublein e Steinhauser, 2008).

Hydrogen partial pressure

A narrow spatial symbiosis is necessary for an undisturbed process between the hydrogen producing acetogenic bacteria and the hydrogen consuming methanogenic. The hydrogen concentration should be well balanced: on the one hand methanogenic need enough hydrogen for the methane production. On the other hand the hydrogen partial pressure must be so low that acetogenic bacteria are not surrounded by too much hydrogen and consequently stop the hydrogen production. The maximum acceptable hydrogen partial pressure depends on the species of bacteria and also on the substrates (Deublein e Steinhauser, 2008).

Redox potential

Since the strictly anaerobic requirements of bacteria involved in the digestion, the system must be oxygen free, so that they can carry out their metabolic functions; the redox potential is an indicator of the reducing level in the system and it is used as an indicator of the oxygen concentration.

In order to encourage the anaerobic digestion, the redox potential must be negative, with values lower than -200 mV in the acid and acetogenic phases, while lower than -400 mV in the methanogenic phase. The methanogenic bacteria are more affected by changes in the redox potential than other bacteria (Camarota, 2004).

CHAPTER II THEORETICAL FRAMEWORK

Temperature

It has been observed that in anaerobic conditions bacterial activity is developed in a range of temperature between +5 °C and +70 °C. Varying the reaction temperature it is possible to change the type of microbial communities active. In fact, the bacteria can be classified on the basis of optimum temperature range for growth and metabolism, and therefore be distinguished in (Caramiello, 2010):

- a) psychrophilic: optimal growing temperature below 20 °C
- b) mesophilic: optimal growing temperature between 20 °C and 45 °C
- c) thermophilic: optimal growing temperature above 45 °C

Most of the methanogenic microorganisms belong to the mesophilics. Only a few are thermophilic. They are also more sensitive to rapid changes of temperature. Thermophilic methanogens are more temperature sensitive than mesophilics. Even small variations in temperature cause a substantial decrease in activity. Therefore, the temperature should be kept exactly within a range of ± 2 °C. Otherwise, gas losses of up to 30% have to be taken into consideration (Deublein e Steinhauser, 2008).

Temperature is one of the major important parameters in anaerobic digestion. It determines the rate of anaerobic degradation processes, particularly the rates of hydrolysis and methanogenesis. Moreover, it not only influences the metabolic activities of the microbial population but also has a significant effect on some other factors such as gas transfer rates and settling characteristics of bio solids (Nayoto, 2010). Anaerobic digestion commonly applies two optimal temperature ranges: mesophilic, with optimum temperature around 35 °C, and thermophilic, with optimum temperature around 55 °C (Mata-Alvarez, 2002, see also Figure 4).

CHAPTER II THEORETICAL FRAMEWORK

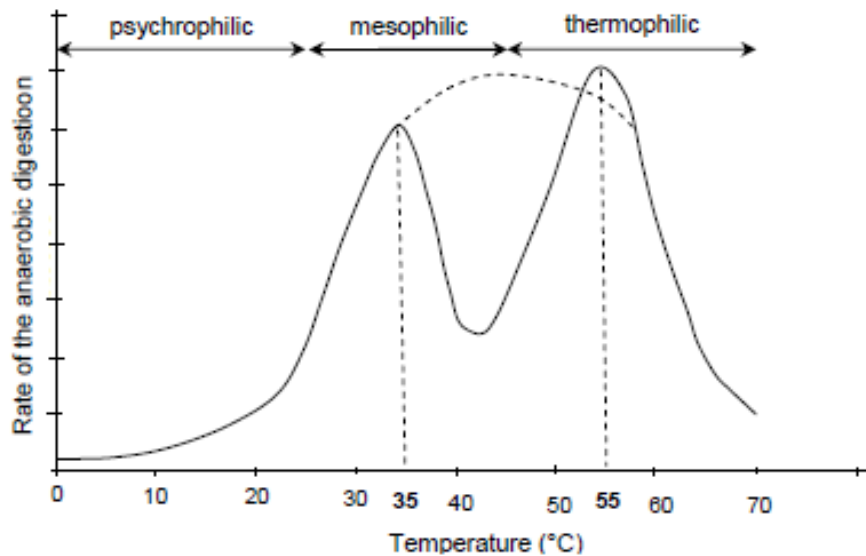


Figure 4. Temperature range for anaerobic digestion. Optima are for mesophilic around 30-35 °C and for thermophilic around 55-60 °C (Mata-Alvarez, 2002)

Mesophilic bacteria are supposed to be more robust and can tolerate greater changes in the environmental parameters, including temperature. Smaller digesters, poorly insulated digesters, or digesters in cold climates are susceptible for extreme temperature fluctuations thus these would be beneficial if the digester is being run in the mesophilic range to minimize system crashing. Although it requires longer retention time, the stability of the mesophilic process makes it more popular in current anaerobic digestion facilities (Zaher *et al.*, 2007).

Thermophilic process offers faster kinetics, higher methane production rates and pathogen removal. Several advantages of increasing temperature can be mentioned (Boe, 2006), e.g.:

- a) Increase solubility of organic compounds which makes them more accessible to the microorganisms.
- b) Increase chemical and biological reaction rates, thus, accelerates the conversion process so the reactor can be smaller and can run with shorter hydraulic retention time (HRT).
- c) Improve several physical-chemical properties such as improve diffusivity of soluble substrate, increase liquid-to-gas transfer rate due to lower gas solubility, decrease liquid viscosity which makes less energy required for mixing and also improve liquid-solid biomass separation.

CHAPTER II THEORETICAL FRAMEWORK

- d) Increase death rate of pathogenic bacteria especially under thermophilic condition, which decreases retention time required for pathogen reduction.

Moreover, the organic acid oxidations reactions become more energetic at higher temperature which is advantage for degradation of long chain fatty acids (LCFA), volatile fatty acids (VFA) and other intermediates (Van Lier, 1995).

This method, however, is more sensitive to toxic substances and changes of operation parameters (Nayoto, 2010). Increasing temperature decreases pKa of ammonia, thus, increases the fraction of free ammonia (NH_3) which is inhibitory to microorganisms. In addition, increasing temperature increases also pKa of VFA, which raise its undissociated fraction, especially at low pH (4-5) such as in the acidogenic reactor (Van Lier, 1995). This makes the thermophilic process more sensitive to inhibition. Moreover, thermophilic process is sometimes considered as less attractive from the energy point of view since it requires more energy for heating (Zaher *et al.*, 2007).

Retention time

The “retention time” or residence time is an important parameter for the success of the biogas production and it is defined as the residence time of the organic mass in the digester.

The production of biogas increases with the retention time and has a bell-shaped trend: initially there is not production, after some days it reaches a maximum and then decreases more slowly with a bell-shaped trend (Figure 5).

CHAPTER II THEORETICAL FRAMEWORK

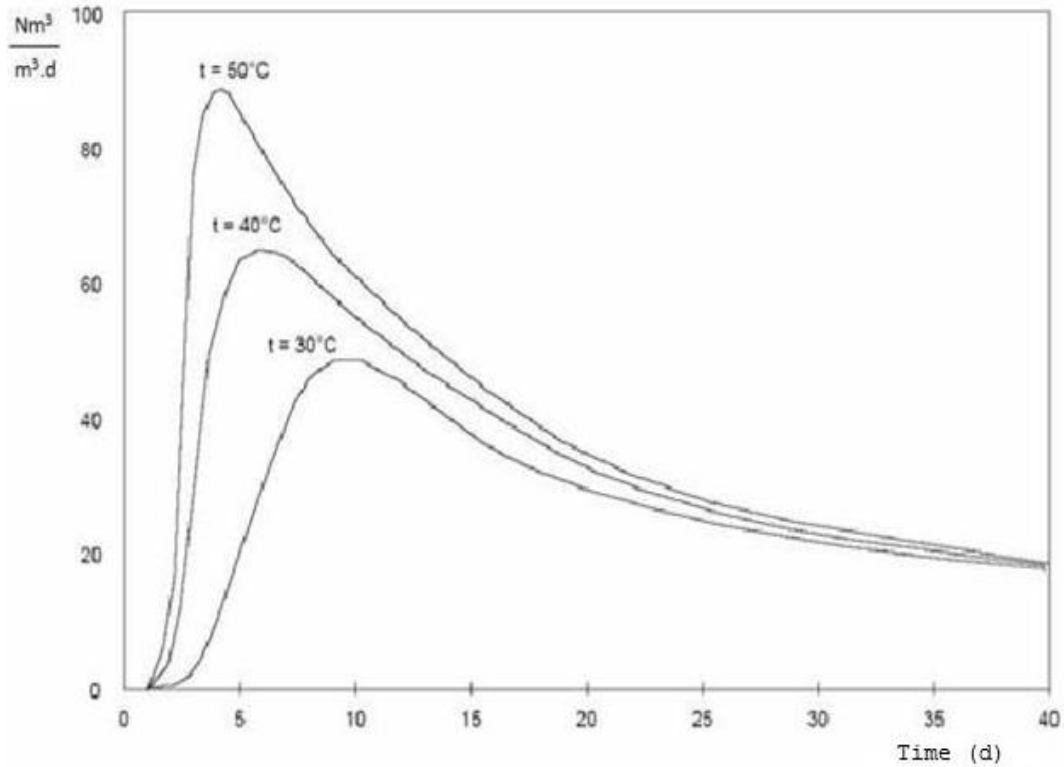


Figure 5. Biogas production in a function of retention time (Reale et al., 2009)

The optimum retention time is a function of the temperature inside the digester and it decreases with that (Table3).

Table 3. Retention time in a function of the temperature in the digester (Reale et al., 2009)

Temperature (°C)	Time (days)	
	Minimun	Maximun
20	11	28
25	8	20
30	6	14
35	4	10
40	4	10

It is important to establish differences between the Hydraulic Retention Time (HRT) and Solid Retention Time (SRT). The first is the ratio between the occupied volume of the reactor and the hydraulic feed flow. It represents the residence time of each fluid element within the reactor. The SRT is given by the ratio between the mass of volatile solids present in the reactor and the flow of volatile solids extracted from the reactor, so it represents the time that the microorganisms remain in the mass indigestion (Reale et al., 2009).

CHAPTER II THEORETICAL FRAMEWORK

Volatile fatty acids (VFA's)

The concentration of volatile fatty acids and alkalinity are the two parameters that show a more rapid variation when the system tends to move away from stability conditions (Reale et al., 2009).

This type of acids, are produced by hydrolytic and acidifying bacteria during digestion, and are chemically represented by the formula $R-COOH$, where R is an alkyl group of the type $CH_3(CH_2)_n$, containing up to 3 carbon atoms. The level of concentration is usually expressed in terms of acetic acid or COD depending on the type of the substrate treated, and ranges from about 200 up to 4000 mgAc per liter (Olmetto, 2008).

Generally it is not the absolute concentration of VFA's the parameter which indicates the stability of the process, but its variation: rapid increases in volatile fatty acids indicate that the process is going towards processes acidogenic rather than methanogenic. The data about the concentration of these organic acids should always be evaluated together with information related to parameter such as production and biogas composition, pH or alkalinity. Specially, it can be useful to assess the ratio "concentration of fatty acids", expressed in terms of acetic acid and "alkalinity", expressed as calcium carbonate content. These are, in fact, the two values that, in the case of imbalance, tend to change more rapidly: the fatty acids tend to increase, while simultaneously the alkalinity is reduced. Normally if values are about 0.3, then the system is considered stable, while higher values indicate possible problems in the system (Caramiello, 2010).

Alkalinity

This parameter represents the capability of a system to neutralize protons, and is usually expressed in terms of the concentration of calcium carbonate in the liquid phase inside the reactor. Typical values for digesters operating in optimal and stable conditions are between 3000 and 5000 mg of $CaCO_3$ per liter (Olmetto, 2008).

Alkalinity is fundamental important in the anaerobic processes. Considering that the growth rates of methanogenic biomass is extremely low, it may occur that an increase in the organic loading, leads to rise the hydrolytic and

CHAPTER II THEORETICAL FRAMEWORK

acidifying capacity of the system, so as to cause an imbalance of the bacterial population in favour of the acidogenic component rather the methanogenic component. Therefore, it will be observed a transitional phase in which it would take place an increase of concentration of volatile fatty acids. In these cases it is essential the buffering capacity of the system, which must be available to counteract the dropping of pH value determined by the accumulation of organic acids. The alkalinity of an anaerobic digester is determined essentially by the presence of a buffer system due to the coexistence of ammonia, originated from the degradation of proteins, and bicarbonate, resulting from the dissolution of carbon dioxide in the medium (Cecchi et al., 2005).

Ammonia concentration

Ammonium-nitrogen ($\text{NH}_4^+\text{-N}$) or ammonium ions (NH_4^+), a reduced form of nitrogen may be transferred to the anaerobic digester or it can be produced during the degradation of the organic nitrogen compounds like proteins and amino acids. The ammonium ions play a positive and negative role during the anaerobic digestion process. Ammonium ions are used in the anaerobic digestion process by the bacteria as a nutrient source.

If the concentration of the ammonia in the digester is around 50–200 mg/L then it is beneficial for the digestion process. If the concentration is around 200–2000 mg/L then it will not affect the process very much and on the other hand, if the concentration is about 2000–4000 mg/L it inhibits the methane production. High concentration of the ammonia in the sludge leads to toxicity. When toxicity occurs in the digester, it leads to serious issues, like accumulation of volatile acids, drop in pH, loss of alkalinity and low methane production (Ravikumar and Gopalam, 2011).

Mixing

Anaerobic digester content should be mixed. Mixing enhances the digestion process by distributing bacteria, substrate, and nutrients throughout the digester as well as equalizing temperature. The metabolic activities of acetate forming bacteria and methane forming bacteria require that they be in close spatial contact. Slow, gentle mixing ensures that contact. Also, mixing provides for

CHAPTER II THEORETICAL FRAMEWORK

efficient hydrolysis of wastes and production of organic acids and alcohols by acid-forming bacteria.

Mixing minimizes the settling of grit and reduces the build-up of scum. Over lengthy periods of operation, solids accumulation can reduce digester performance as the reactor hydraulics become restricted by localized dead volumes and short-circuiting of sludge flow. The advantages of mixing digester content are listed in Table 4.

Table 4. Advantages of Mixing Digester Content (Gerardi, 2003)

Eliminating or reducing scum buildup
Eliminating thermal stratification or localized pockets of depressed temperature
Maintaining digester sludge chemical and physical uniformity throughout the tank
Rapid dispersion of metabolic wastes (products) produced during substrate digestion
Rapid dispersion of any toxic materials entering the tank (minimizing toxicity)
Prevent deposition of grit

Mixing can be accomplished through mechanical methods or gas recirculation. Mechanical mixers are more effective than gas recirculation, but they often become clogged or fouled with digester solids (Gerardi, 2003).

It is important to be aware about the aspects affected by the presence of agitation. For example, it must not be excessively disturbed the symbiosis between methanogenic and acetogenic bacteria, moreover, it must be noted that the acidogenic are less sensitive to fluidynamics stress than methanogenic. Finally, it must always be considered the aspect of energy, trying to minimize the expenditure (Calzata et al., 1984).

Pressure

The operating pressure in the anaerobic digester may be atmospheric, but usually it is preferred to work in slight overpressure (10-20 mbar) to prevent ingress of air that would be in opposition to the needs required by the anaerobic bacteria.

Elevated pressures may slow the course of the reactions due to reduction of gas diffusion at the gas/liquid interface, so as to increase the concentration of biogas in the liquid phase, which causes a drop in the biogas production (Camarota, 2004).

CHAPTER II THEORETICAL FRAMEWORK

Biogas production

Monitoring the biogas composition and quantity (at least in terms of methane and carbon dioxide) is fundamentally important for controlling the stability of anaerobic digestion processes.

If the reactor is operating in stable conditions, the production and composition of biogas are constants. A decrease in the biogas overall production and an increase in the CO₂ percentage may indicate inhibition problems and detriment of the methanogenic bacteria due, for example, to the excessive presence of volatile fatty acids.

The analysis of biogas production and percentage composition should always be associated with the control of parameters such as volatile fatty acids concentration and alkalinity of the system.

It has been observed that in the presence of excessive substrate, the percentage of CO₂ tends to grow at the expense of the methane production, which is closely related to the variations of the volatile fatty acid concentration. In particular, we can observe three different situations:

- a) Low concentration of VFA and high biogas production, in which the CO₂ concentration is low (between 25% and 33%) indicating that the process is stable and has a good transferability from acidifying bacteria to methanogenic bacteria.
- b) Increasing concentrations of VFA and a progressive growth in the concentration of CO₂ (values higher than 2/3 of the produced biogas), which indicate that the acidifying bacteria populations are taking advantage over the methanogenic and therefore there is a progressive accumulation of VFA in the reactor.
- c) Both increasing concentrations of VFA and progressively decrease of biogas production may indicate inhibition problems or toxicity (Reale et al., 2009).

BIOGAS PRODUCTION MODELS

Process kinetic studies of anaerobic degradation of organic substrates can contribute to the basic process analysis, optimal design and operation, and maximum substrate utilization rate in anaerobic digestion. Modelling of biological processes in anaerobic digestion is a good tool to describe and convert complex biological problems and extensive experimental data into a simple formula.

Kinetic models for anaerobic digestion

The microbial growth rate in anaerobic digestion is described by the following autocatalytic equation based on the nature of microbial binary fission (Chen, 2010):

$$\frac{dX}{dt} = \mu X \quad (2)$$

Where:

dX/dt : Microbial growth rate ($\text{mg VSS/L} \cdot \text{h}^{-1}$)

X: Biomass concentration (mg/L)

T: Time (h)

μ : Specific growth rate, (h^{-1}).

Stoichiometric reactions between substrate utilization and microbial growth suggest that the relationship between microbial growth rate and substrate growth rate is coupled as follows:

$$\frac{dS}{dt} = -\frac{1}{Y} \frac{dX}{dt} \quad (3)$$

Where:

dS/dt : Substrate utilization rate ($\text{mg substrate/L} \cdot \text{h}^{-1}$)

S: Substrate concentration (mg/L)

Y: Microbial growth yield coefficient ($\text{mg substrate/mg VSS} \cdot \text{h}^{-1}$)

The changes of substrate and biomass concentration in batch reactors must be solved simultaneously with equations (2) and (3). To do so, Monod equation is needed to link substrate concentration with bacterial growth. Over the years, the

CHAPTER II THEORETICAL FRAMEWORK

empirical based Monod equation was verified experimentally. The Monod equation is described as follows:

$$\mu = \hat{\mu} \frac{S}{K_s + S} \quad (4)$$

Where:

$\hat{\mu}$: Maximum specific growth rate (h^{-1})

K_s = Substrate concentration at one half the maximum rate (mg/L)

The Monod equation is similar to the equation from Michaelis-Menten Model. Combined equation 2.3 and 2.4 results in:

$$\frac{dS}{dt} = -\frac{\hat{\mu} X S}{Y K_s + S} \quad (5)$$

This equation describes that the rate of substrate utilization rate depends on multiple factors including substrate concentration. However, some organic substrates show significant inhibition on anaerobic reactions at high substrate concentrations. Consequently, a modified Monod equation-Andrews equation including an inhibition coefficient is introduced as follows.

$$\mu = \hat{\mu} \frac{S}{K_s + S + \frac{S^2}{K_I}} \quad (6)$$

Where

K_I = Inhibition coefficient (mg/L)

Biogas physical-chemistry

The biogas produced during the anaerobic digestion consists primarily of methane, carbon dioxide, and molecular hydrogen. The methane is practically insoluble in the liquid phase and therefore passes to the gaseous phase, while the carbon dioxide reaches a dynamic balance between the two phases. Even though the hydrogen produced in relatively small amounts is insoluble, is unable to leave the liquid phase, because it is rapidly converted to methane by hydrogenotrophic microbes.

CHAPTER II THEORETICAL FRAMEWORK

In general the rate of mass transfer from the gas phase to the liquid is expressed through the relation:

$$\frac{dS}{dt} = K_s a \left(S - \frac{P_p}{H} \right) \quad (7)$$

Where:

dS/dt : Gas transfer rate from liquid phase to gas phase ($\text{mol/m}^3\text{s}$)

S : Concentration of gas dissolved in the liquid phase (mol/m^3)

K_L : Overall mass transfer coefficient ($\text{m}^3/(\text{m}^2 \text{ s})$)

a : Specific surface area of the gas bubble (m^2/m^3)

C_p : Concentration of component in gas phase, (mol/m^3)

H : Henry constant (dimensionless)

According to the equation shown, the gas rate transfer from the liquid phase to the gas phase depends, through a characteristic coefficient K_L , from the surface in exchange of the bubble and the driving force, expressed by the term in parenthesis. Various models for the calculation of the K_L transfer coefficient in the case of oxygen are reported in literature.

The bubbles of methane, after desorption from the substrate, reach the gas phase with a speed that depends on their size and the density difference with respect to the liquid phase (Olmetto, 2008).

TYPES OF ANAEROBIC REACTORS

Typically anaerobic reactors or processes of solid waste can be distinguished into several types, mostly according to the feeding mode (continuous mode: single stage, two stages and batch mode) and the moisture content of the substrate (wet or dry digestion). Furthermore with those basic types, the anaerobic reactors can be arranged according to the digestion process temperature (mesophilic or thermophilic) and the shape of the reactors (vertical or horizontal) (Nayoto, 2010).

CHAPTER II THEORETICAL FRAMEWORK

Wet and dry anaerobic digestion:

Anaerobic digestion processes can be termed as “wet” and “dry” digestions depending on the total solids concentration of the feed substrate. Anaerobic digestion is defined as a wet process if the total solids concentration of the substrate is less than 15% and as a dry process if the concentration reaches 20–40% (Lissens *et al.*, 2001).

In wet digestion processes, the solid waste has to be conditioned to the appropriate solids concentration by adding process water either by recirculation of the liquid effluent fraction, or by co-digestion with a more liquid waste. The application of a wet digestion process offers several advantages such as dilution of inhibitory substances by process water and requirement of less sophisticated mechanical equipment. However, disadvantages, such as complicated pretreatment, high consumption of water and energy for heating and the reduction of working volume due to sedimentation of inert materials have to be taken into account.

The reactors used in dry anaerobic digestion processes generally do not apply mechanical mixers and may use biogas injection to perform mixing of the digester content. However, using this technique, complete mixing of the digestate is almost impossible; thus, the ideal contact of microorganisms and substrate cannot be guaranteed (Nayoto, 2010).

Batch and continuous feeding systems

Two feeding modes are generally used in anaerobic digestion of solid waste: the batch system and the continuous system. In the batch system, digesters are filled once with fresh feedstock, with or without addition of inoculum, and sealed for the complete retention time, after which it is opened and the effluent removed. In the continuous system, fresh feedstock continuously enters the digester and an equal amount of digested material is removed (Nayoto, 2010).

Batch reactors are the simplest, filled with the feedstock and left for a period that can be considered to be the hydraulic reaction time, after which they are emptied. Anaerobic batch reactors are useful because they can perform quick digestion with simple and inexpensive equipment, and also are helpful in

CHAPTER II THEORETICAL FRAMEWORK

assessing the rate of digestion easily. On the other hand, batch reactors have some limitations such as high fluctuations in gas production as well as gas quality, biogas losses during emptying the bioreactors and restricted bioreactor heights (Khalid et al., 2011).

In a continuous AD process, fresh organic substrate is added and an equal amount of effluent is removed in an on-going process. With consistent feedstock input, all reactions occur at a fairly steady state rate resulting in approximately constant biogas production. Because there is constant movement, however, the substrate inside the tank digester is mixed and does not become stratified. This allows for more optimal use of the tank volume. The disadvantage of the continuous process is that the removed effluent is a combination of completely digested and partially digested material (Zaher et al., 2007).

One-stage and two-stage anaerobic digestion

A typical single-stage digester consists of only one tank or reactor. Digester operations consist of sludge addition and withdraw, mixing, heating and gas collecting. These digesters are more easily upset than two-stage digesters. This is because of the presence of the simultaneous activities of two groups of bacteria, the acid forming bacteria and the methane forming bacteria. Because acid forming bacteria grow more quickly than methane forming bacteria and are more tolerant of fluctuations in operational conditions, an imbalance between acid production rate and methane production rate often occurs. This imbalance may cause a decrease in alkalinity and pH those results in digester failure (Gerardi, 2003).

Two-stage digester systems consist of at least two separate tanks or reactors in which biochemical processes of hydrolysis-acidification and acetogenesis-methanogenesis take place separately (Figure 6). Two-stage system is considered a promising process to treat organic wastes with high efficiency in term of degradation yield and biogas production. According to Demirer and Chen (2005), a two stage system allows the selection and enrichment of different bacteria in each phase. The complex organic materials are degraded by acidogenic bacteria to volatile fatty acids and alcohols, which are then easily metabolized into

CHAPTER II THEORETICAL FRAMEWORK

methane and carbon dioxide by methanogens or archaea. Further, this type of system increases the stability of the process by controlling the acidification phase through optimization of the hydraulic retention time to prevent overloading and the build-up of toxic material. The biomass concentration and other conditions can also be optimized independently for each stage (Khalid et al., 2011). In a single stage anaerobic digestion process, a variety of higher organic acids, such as propionic, butyric, and lactic, as well as alcohols and ketones, are also formed during the breakdown of the organic substrates by acidogens. However, in a well operated process, these products are mostly converted to acetic acid and hydrogen, which, in turn, are converted to methane gas.

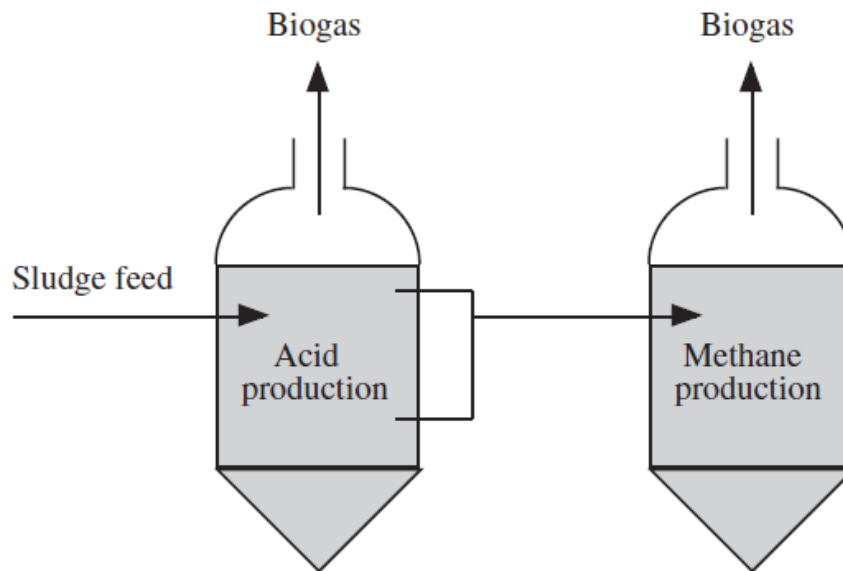


Figure 6. Two stage anaerobic digestion scheme (Gerardi, 2003)

SUBSTRATES

In general, all types of biomass can be used as substrates as long as they contain carbohydrates, proteins, fats, cellulose, and hemicelluloses as main components. It is important that the following points are taken into consideration when selecting the biomass:

- a) The content of organic substance should be appropriate for the selected fermentation process.

CHAPTER II THEORETICAL FRAMEWORK

- b) The nutritional value of the organic substance, hence the potential for gas formation, should be as high as possible.
- c) The substrate should be free of pathogens and other organisms which would need to be made innocuous prior to the fermentation process.
- d) The content of harmful substances and trash should be low to allow the fermentation process to take place smoothly.
- e) The composition of the fermentation residue should be such that it can be used, e.g., as fertilizer.

Influence of lipids and lignocellulosic components

The type of substrate determines the rate of the anaerobic degradation and must be taken into consideration in the process technology and process operation. If a substrate component of vital importance runs out, the microorganisms stop their metabolism.

Therefore, it is often necessary to feed possibly lacking substances (carbohydrates, fat, proteins, mineral substances, and trace elements) as well as the substrate (Deublein e Steinhauser, 2008). The degradability and biogas production potential from solid waste in an anaerobic digester are dependent on the amount of the main components. Among them, lipids are the most significant substances in the anaerobic digestion, since the methane yield from lipids is higher than from most other organic materials. The theoretical gas yield of glyceride trioleate is, for example, 1.4 m³ per kilogram of oil with a methane content of 70%. Although, the organic waste with a high content of lipids is an attractive substrate for biogas production. Neves *et al.*, (2008) reported that the lowest hydrolysis rate constants were obtained in the assays fed with kitchen waste that contained an excess of lipids. This was presumably due to a synergetic effect on the degradation of the other components since lipids adsorb onto solid surfaces and may delay the hydrolysis process by reducing the accessibility of enzyme attack. Lignocellulosic (cellulose and hemicelluloses which are tightly bound to the lignin) waste can be found in abundant amount in the form of garden waste, paper residue or agricultural waste. Due to the presence of lignin, lignocellulosic waste is considered to be quite resistant to anaerobic digestion and

CHAPTER II THEORETICAL FRAMEWORK

hydrolysis is the rate limiting step in the overall process. In order to improve the rate of enzyme hydrolysis and increase yields of fermentable sugars from cellulose or hemicellulose in lignocellulosic waste, several pretreatment methods were proposed by several authors (Nayoto, 2010).

According to the composition of the substrates, intermediate products of the decomposition can also limit or inhibit the degradation. Thus, for example, the degradation of fats can give rise to fatty acids, which limit the further degradation. With the decomposition of proteins, methane fermentation can be restrained by the formation of ammonia and hydrogen sulphide. Lignin, the main constituent of wood, and most synthetic organic polymers (plastics) simply decompose slowly (Deublein e Steinhauser, 2008).

C/N ratio

The composition of waste also determines the relative amounts of organic carbon and nitrogen present in the waste substrate (C/N ratio). A solid waste substrate with high C/N ratio is not suitable for bacterial growth due to deficiency of nitrogen. As a result the gas production rate and solids degradability will be low. On the other hand, if the C/N ratio is very low, the degradation process leads to ammonia accumulation which is toxic to the bacteria. Kayhanian and Hardy (1994) found that a C/N ratio (based on biodegradable organic carbon and nitrogen) within the range of 25–30 is considered to be optimum for an anaerobic digester. To maintain the C/N level of the digester material at optimum levels, substrates with high C/N ratio can be co-digested with nutrient rich organic wastes (low C/N ratio) like animal manure or food waste (Nayoto, 2010).

Micro and macronutrients

The microorganisms involved in anaerobic degradation have species-specific needs in terms of macronutrients, micronutrients and vitamins. The concentration and availability of these components affect the rate of growth and the activity of the various populations. There are species-specific minimum and maximum concentrations, which are difficult to define because of the variety of different cultures and their sometimes considerable adaptability (FNR, 2010).

CHAPTER II THEORETICAL FRAMEWORK

Macronutrients, for example, nitrogen and phosphorus, are nutrients that are required in relatively large quantities by all bacteria. They are made available to anaerobic bacteria, including methane-forming bacteria, as ammonia-nitrogen ($\text{NH}_4^+\text{-N}$) and orthophosphate-phosphorus ($\text{HPO}_4^-\text{-P}$). These nutrients, like all nutrients, are available to bacteria only in a soluble form.

Micronutrients are nutrients that are required in relatively small quantities by most bacteria. Methane-forming bacteria possess several unique enzyme systems, so they have micronutrient requirements that are different from those of other bacteria. Especially the presence of cobalt, iron, nickel, and sulphide, is critical because they are required by methane-forming bacteria to convert acetate to methane (Table 5). Additional trace elements on which enzymes of methane-forming bacteria are dependent include selenium and tungsten. The incorporation of micronutrients in enzyme systems is essential to ensure not only proper degradation of substrate but also efficient operation of the digester (Gerardi, 2003).

Table 5. Favorable concentrations of trace elements (FNR, 2010).

Trace element	Concentration range [mg/l]			
	as in [2-18]	as in [2-19]	as in [2-16] ^a	as in [2-17] ^b
Co	0.003-0.06	0.003-10	0.06	0.12
Ni	0.005-0.5	0.005-15	0.006	0.015
Se	0.08	0.08-0.2	0.008	0.018
Mo	0.005-0.05	0.005-0.2	0.05	0.15
Mn	n.s.	0.005-50	0.005-50	n.s.
Fe	1-10	0.1-10	1-10	n.s.

a. Absolute minimum concentration in biogas plants

b. Recommended optimum concentration

Specific surface of material

To support a biochemical reaction a material surface as large as possible is necessary. The material surface often varies proportionally to the square of the

CHAPTER II THEORETICAL FRAMEWORK

particle size. In order to increase the material surface, comminution of the biomass is in many cases recommended before fermentation.

Comminution does not have a big influence on the biogas yield when easily degradable materials (95% or 88% grade of decomposition) are used, which have only a very low content of structural materials (cellulose, lignin, etc.). Such material is easy accessible for the microorganisms. With substrates like hay and foliage, which are rich in structured materials and enable only a grade of decomposition of 50% without comminution, the biogas yield can be increased by up to 20% depending on the grade of comminution (Figure 7). The increase in the particle surface area and the loosening of the fibre structure are the reasons.

The technical residence time is the time, at which 80% of the maximum biogas yield is reached. A tendency can be clearly seen, in the case of all substrates, for the technical residence time to be reduced as a result of comminution. The best reduction is achieved with substrates which have the longest technical residence time without comminution. The technical residence time of different substrates can be equalized by comminution.

It can be assumed that the increase in the specific surface increases especially the microbiological degradation processes and not the physical solution processes, which would start after a short time (Deublein e Steinhauser, 2008).

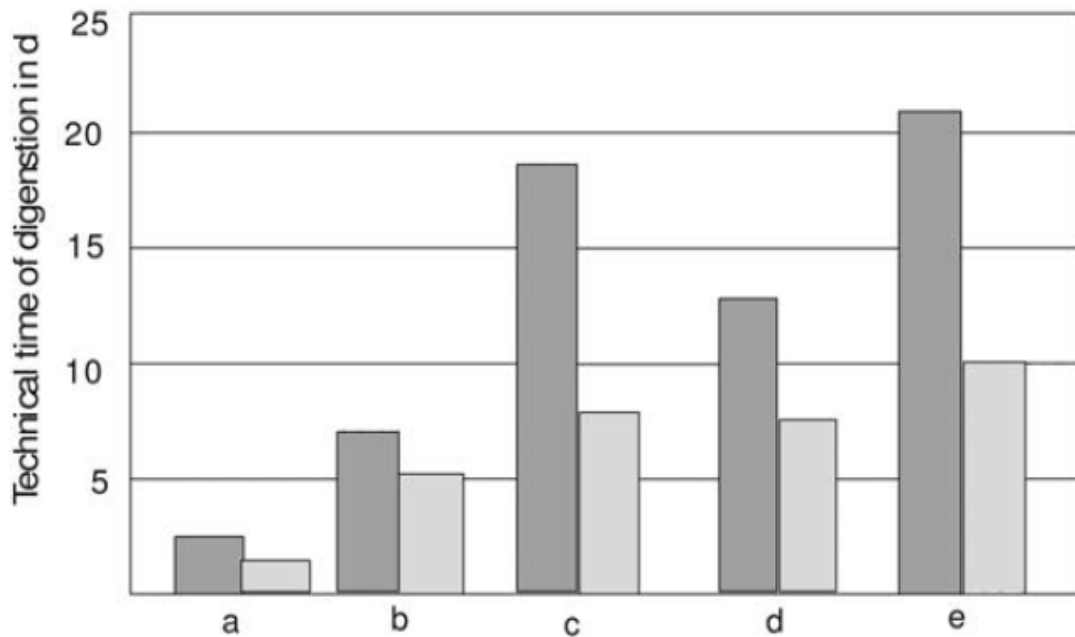


Figure 7. Influence of the grinding on the time of fermentation for different substrates (a: mix of apples, potatoes, and carrots; b: meat; c: sunflower kernels; d: hay; e: leaves) left columns: without grinding, right columns: with grinding (Deublein e Steinhauser, 2008)

Microorganism

The anaerobic digestion process can be catalyzed by a variety of microorganisms that convert complex macromolecules into low molecular weight compounds. An inoculum source is crucial for the optimization of the waste/inoculum ratio. Sludge is commonly used as inoculum for the treatment of waste although naturally selected strains or artificially mixed strains of microorganisms are also employed. In addition, cell aggregates in the form of flocks, biofilms, granules, and mats, with dimensions that typically range from 0.1 to 100 mm may also be used in the treatment system (Jeong et al., 2010).

A wide variety of microbial communities have been reported to be involved in the anaerobic decomposition process. Fricke et al. (2007) reported that organic material is most likely decomposed by heterotrophic microorganisms. Lee et al. (2009) reported that *Clostridium* species are most common among the degraders under anaerobic condition.

However, it is very unusual for a biological treatment to rely solely on a single microbial strain and generally a microbial consortium is responsible for the anaerobic digestion process. According to Ike et al. (2010), a group of

CHAPTER II THEORETICAL FRAMEWORK

microorganisms such as *actinomyces*, *Thermomonospora*, *Ralstonia* and *Shewanella* are involved in the degradation of food waste into volatile fatty acids, but *Methanosarcina* and *Methanobrevibacter/Methanobacterium* mainly contribute in methane production. High concentration of organic acid like acetic acid (>5000 mg/L) and butyric acid (>3000 mg/L) in the digester has been found to inhibit the growth of microorganisms and consequently the production of energy rich compounds (Khalid et al., 2011).

Methanogenic microorganisms have a long regeneration time in general (Table 6). To avoid washing out from the reactor, hydraulic residence times must be at least 10-15 days with reactor systems which do not have facilities for retaining and returning biomass.

In comparison with this, the regeneration times of hydrolytic and acid-forming bacteria are significantly shorter, so that with them there is hardly any risk of washout (Deublein e Steinhauser, 2008).

Table 6. Time of regeneration of different anaerobic (Deublein e Steinhauser, 2008).

<i>Anaerobic microorganism</i>	<i>Time of regeneration</i>
Acidogenic bacteria	
Bacterioids	<24 h
<i>Clostridia</i>	24–36 h
Acetogenic bacteria	80–90 h
Methanogenic bacteria	
<i>Methanosarcina barkeri</i>	5–16 d
<i>Methanococcus</i>	ca. 10 d
Aerobic microorganism	
<i>Escherichia coli</i>	20 min
Active sludge bacteria	2 h
Bacteria living on earth	1–5 h

Inhibition

Inhibition in anaerobic digestion process by the presence of toxic substances can occur to varying degrees, causing upset of biogas production and organic removal or even digester failure (Stronach *et al.*, 1986). These kinds of substances can be found as components of the feeding substrate (organic solid

CHAPTER II THEORETICAL FRAMEWORK

waste) or as by-products of the metabolic activities of bacteria consortium in the digester. The inhibition depends on the concentration of the inhibitors, the composition of the substrate, and the adaptation of the bacteria to the inhibitor. Anaerobic bacteria need a low concentration of the inhibitors as trace elements. Previous publications on anaerobic digestion show a wide variation in the inhibition/toxicity levels for most substances. The main reason for these variations is the significant influence by microbiological mechanisms such as acclimation, antagonism, and synergism (Chen *et al.*, 2008). Acclimation is the ability of microorganism to rearrange their metabolic resources to overcome the metabolic block produced by the inhibitory or toxic substances when the concentrations of these substances are slowly increased within the environment. Antagonism is defined as a reduction of the toxic effect of one substance by the presence of another, whereas synergism is an increase in the toxic effect of one substance by the presence of another. Several substances are inhibitory/toxic potential to anaerobic digestion, such as ammonia, sulphide, light metal ions, heavy metals and organic substances (Nayoto, 2010).

PRETREATMENT

A way to increase biogas production is to increase the biodegradability by pretreatment of the substrate such as domestic sludge, solid waste, and most of the agricultural with high content of cellulose and hemi-cellulose (BOE, 2006).

In anaerobic digestion, the biological hydrolysis is identified as the rate-limiting step (Ghyoot and Verstrate, 1997). Most soluble organic materials which can be converted into biogas are produced during hydrolysis process. Consequently, the biogas production depends for the most part on the biodegradability and hydrolysis rate (E1 and H.E., 2003). In this step, both solubilisation of particulate matter and biological decomposition of organic polymers to monomers or dimers take place. That is, cell walls are ruptured and extracellular polymeric substances are degraded resulting in the release of readily available organic material for the acidogenic micro-organisms. Thermal, chemical, biological and mechanical processes, as well as combinations of these,

CHAPTER II THEORETICAL FRAMEWORK

have been studied as possible pre-treatments cause the lysis of or disintegration of sludge cells permitting the release of intracellular matter that becomes more accessible to anaerobic microorganisms. This fact improves the overall digestion process velocity and the degree of sludge degradation, thus reducing anaerobic digester retention time and increasing methane production rates (Hanjie, 2010).

Mechanical pre-treatment

Mechanical pretreatment is almost always applied before any other kind of pretreatment, and actually refers to milling, chipping or grinding, through which reduction of particle size and crystallinity of biomass is achieved. Size reduction of particles and the resulting increase in the specific surface available to the medium improves the biological process. Two effects have been reported: first, if the substrate has high fibre content and low degradability, their comminution leads to improved gas production; and second, size reduction can lead to more rapid digestion (Mata-Alvarez et al., 2000). The final size of biomass particles is usually 10-30 mm after chipping, while milling or grinding leads to smaller sizes of 0.2-2 mm. Mechanical pretreatment is necessary, because it facilitates feedstock handling and treatment (Mudhoo, 2012).

Chemical pre-treatment

Chemical pretreatment is an efficient and cost-effective method to hydrolyze the cell wall and membrane and thus increase solubility of the organic matter contained within the cells. According to different principles, chemical methods can be divided to acid and alkaline (thermal) hydrolysis and oxidation.

Oxidation

An oxidative pretreatment consists in the addition of an oxidizing compound, like hydrogen peroxide or peracetic acid, to the biomass, which is suspended in water. The objective is to remove the hemicellulose and lignin to increase the accessibility of the cellulose.

During oxidative pretreatment several reactions can take place, like electrophilic substitution, displacement of side chains, cleavage of alkyl aryl ether linkages or the oxidative cleavage of aromatic nuclei (Hon and Shiraishi, 2001).

CHAPTER II THEORETICAL FRAMEWORK

In many cases the used oxidant is not selective and therefore losses of hemicellulose and cellulose can occur. A high risk on the formation of inhibitors exists, as lignin is oxidized and soluble aromatic compounds are formed. Teixeira et al. (1999) have investigated the use of peracetic acid at ambient temperatures as a pretreatment method for hybrid poplar and sugar cane bagasse. Peracetic acid is very lignin selective and no significant carbohydrate losses occurred. The enzymatic hydrolysis of the cellulose increased from 6.8% (untreated) to a maximum of about 98% (pretreated) at a 21% peracetic acid pretreatment. Teixeira et al. (1999) showed similar results for digestibility at using a mixture of NaOH and peracetic acid.

Gould (1984) demonstrated the use of H_2O_2 for delignification with a maximum at pH 11.5. According to Gould (1984) the hydrogen peroxide concentration should be at least 1% and the weight ratio between H_2O_2 and biomass should be 0.25 for a good delignification (Hendriks and Zeeman, 2008).

Acid pretreatment

Pretreatment of lignocellulose with acids at ambient temperature are done to enhance the anaerobic digestibility. The objective is to solubilize the hemicellulose, and by this, making the cellulose better accessible.

The pretreatment can be done with dilute or strong acids. The main reaction that occurs during acid pretreatment is the hydrolysis of hemicellulose, especially xylan as glucomannan is relatively acid stable. Solubilized hemicelluloses (oligomers) can be subjected to hydrolytic reactions producing monomers, furfural, HMF and other (volatile) products in acidic environments. During acid pretreatment solubilised lignin will quickly condensate and precipitate in acidic environments (Liu and Wyman, 2003; Shevchenko et al., 1999). The solubilisation of hemicellulose and precipitation of solubilised lignin are more pronounced during strong acid pretreatment compared to dilute acid pretreatment.

Alkaline pretreatment

Alkaline pretreatment is one of the leading pretreatments, due to several advantages over the other methods, including acid, steam and biological

CHAPTER II THEORETICAL FRAMEWORK

pretreatments. First, alkaline solubilises lignin (Hendriks and Zeeman, 2008). Second, alkaline neutralizes various acidic productions, and third, alkaline prevents a drop of pH during subsequent acidogenesis process (Pavlostathis and Gossett, 1985). It has been reported that a 72.9% increase in total biogas production was achieved with 2.0% NaOH-treated corn stover for 3 days at 88% of moisture content (Yao et al., 2013). Alkaline pretreatment has several advantages, i.e. simple devices, easy to operate and high efficiency. Most of the investigations exhibited an increase in methane production and decrease in volatile suspended solids (VSS), especially during low-dose alkaline treatment.

In several studies about alkaline pretreatment of waste activated sludge (WAS), the preferred reagent in, in most cases, was sodium hydroxide (NaOH), which was reported to yield greater solubilisation efficiency than calcium hydroxide ($\text{Ca}(\text{OH})_2$) (López and Espinosa, 2008). The dose of NaOH was commonly controlled at a low level. If a high dose of NaOH was used in alkaline sludge pretreatment, the residual NaOH in the pretreated sludge could destroy the bicarbonate buffer system in anaerobic digesters, and the excessive increase in pH could inhibit or even inactivate the anaerobic microorganisms (Li et al., 2012).

Thermal pre-treatment

While the carbohydrates and the lipids of the substrates are easily degradable, the proteins are protected from the enzymatic hydrolysis by the cell wall. Heat applied during thermal treatment destroyed the chemical bonds of the cell wall and membrane, thus makes the proteins accessible for biological degradation.

Thermal pretreatment has been studied using a wide range of temperatures ranging from 60 to 270 °C. In practice, the optimum temperature is in range of 160-180 °C and treatment times from 30 to 60 min. Pressure associated to these temperatures may vary from 600 to 2500 kPa. Various experiments and research of thermal pretreatment have been done to proclaim this conclusion.

The temperature and duration of the optimum pretreatment depend on the nature of the sludge: the greater the proportion of difficult hydrolyzing biological sludge substrates, higher the intensity of pretreatments needed.

CHAPTER II THEORETICAL FRAMEWORK

In general, thermal pretreatment in waste activated sludge can considerably increase methane production for mesophilic anaerobic digestion and to a lesser extent for thermophilic anaerobic digestion, for that thermophilic digestion is already more efficient at volatile suspended solids reduction and methane production as compared with mesophilic digestion, hence reduces benefits of pretreatment.

Evidently, the thermal pretreatment requires the input of a considerably amount of heat, since the biomass feedstock needs to be preheated to the operating temperature at the expense of using some of the biogas produced. Moreover, the biogas production is not in proportion to the temperature. Most works have shown that excessively high temperatures (higher than 170-190 °C) lead to decreased biomass biodegradability in spite of achieving high solubilisation efficiencies. Indeed, in some cases, there is formation of toxic, refractory compounds during pretreatment which is a major drawback (Hanjie, 2010).

Biological pretreatment

The aim of biological pretreatment is to prepare the substrates for the enzymatic degradation and the best method and conditions of pretreatment depend greatly on the type of substrate. The microorganisms can be used to degrade organic substrates, several fungi, e.g. brown-, white and soft-rot fungi, and bacteria have been used for this purpose. Bacteria can be used for biological pretreatment of substrates in anaerobic conditions. Low energy requirement, no chemical requirement, and mild environmental conditions are the main advantages of biological pretreatment. However, the treatment efficiency is very low in most biological pretreatment processes (Mudhoo, 2012).

CHAPTER III

MATERIALS AND METHODS

EXPERIMENTATION

The experimental method consists of three steps: preparation of inoculum, preparation of substrate and fermentation in the reactor. These tasks, which are described below, were carried out on the basis of previous works in such a way to enhance gas production and have a reference to compare results.

Inoculum preparation

In order to develop consecutively two different stages of fermentation, the first for biohydrogen production and the second for methane production, two inoculums with different preparation were used to ensure the selective growth of appropriate microorganisms in each stage.

The anaerobic microflora used for producing biohydrogen was provided by cattle manure pretreated with 2N HCl to adjust the pH to 3 and then laid in a thermostatic chamber at 30 °C during 24 h. After pretreatment the inoculum was frozen at -24 °C (Tommasi, 2010). The pretreatment has the purpose of inhibiting the methanogenic population, since it is not resistant to conditions of extreme acidity. In addition, the hydrogen-producing bacteria form spores resistant to these conditions (Brock et al., 1994). When the environment is favourable, germination and metabolic processes begin again, therefore hydrogen and other metabolic products are produced.

While the anaerobic microflora used for producing biohydrogen was provided by cattle manure, the microflora used for producing biomethane was provided by cattle manure without any treatment (Rametta, 2013).

CHAPTER III MATERIALS AND METHODS

Substrate preparation

The organic material used to carry out the fermentation consisted in a mix of fruit and vegetable waste collected in a fruit market. A kitchen blender was used to mill the material so that to obtain a viscous broth (Figure 8) which was sampled to measure total solids (TS) of the mixture and know its concentration.



Figure 8. Broth obtained after milling

On the basis of previous works (Rametta, 2013) it was decided to have an initial concentration (TS) of 51 g/L and therefore tap water was added in a ratio 1:1 in order to dilute the substrate to the desired value. Moreover, dilution reduces the viscosity avoiding mixing problems and difficulties in heat and mass transferring during the operation of the reactor. Before milling, every organic element was identified and its weight was recorded for determining the percentage composition of the mix by using the INRAN's database (INRAN, 2013). Table 7 shows the mass of each element added to the mix and its percentage composition according to the database.

CHAPTER III MATERIALS AND METHODS

Table 7. Organic elements added to prepare the substrate and percentage composition

Waste	Weight (g)	Carbohydrates	Proteins	Lipids	Fibres	Water	Others
Strawberry	172.7	5.30	0.90	0.40	1.60	90.50	1.30
Pear	202	8.40	0.30	0.10	3.80	87.40	0.00
Apple	114.4	10.70	0.40	0.10	1.70	86.90	0.20
Tomato	287.7	3.53	0.88	0.22	1.99	93.16	0.22
Zucchini	79.3	1.40	1.30	0.10	1.20	93.60	2.40
Pepper	113.3	4.20	0.90	0.30	1.90	92.30	0.40
Onion	141.4	5.70	1.00	0.10	1.00	92.10	0.10
Sedano	25.5	2.40	2.30	0.20	1.60	88.30	5.20
Potato	52.6	16.80	2.10	1.00	1.60	78.50	0.00
Orange	22.2	7.80	0.70	0.20	1.60	87.20	2.50
Parsley	17.7	1.00	3.70	0.60	5.00	87.20	2.50
Lettuce	23.4	2.20	1.80	0.40	1.30	94.30	0.00
Total	1252.2	69.43	16.28	3.72	24.29	1071.46	14.82

The figures show below, contains the percentage composition in wet (Figure 9) and dry basis (Figure 10) of the broth prepared.

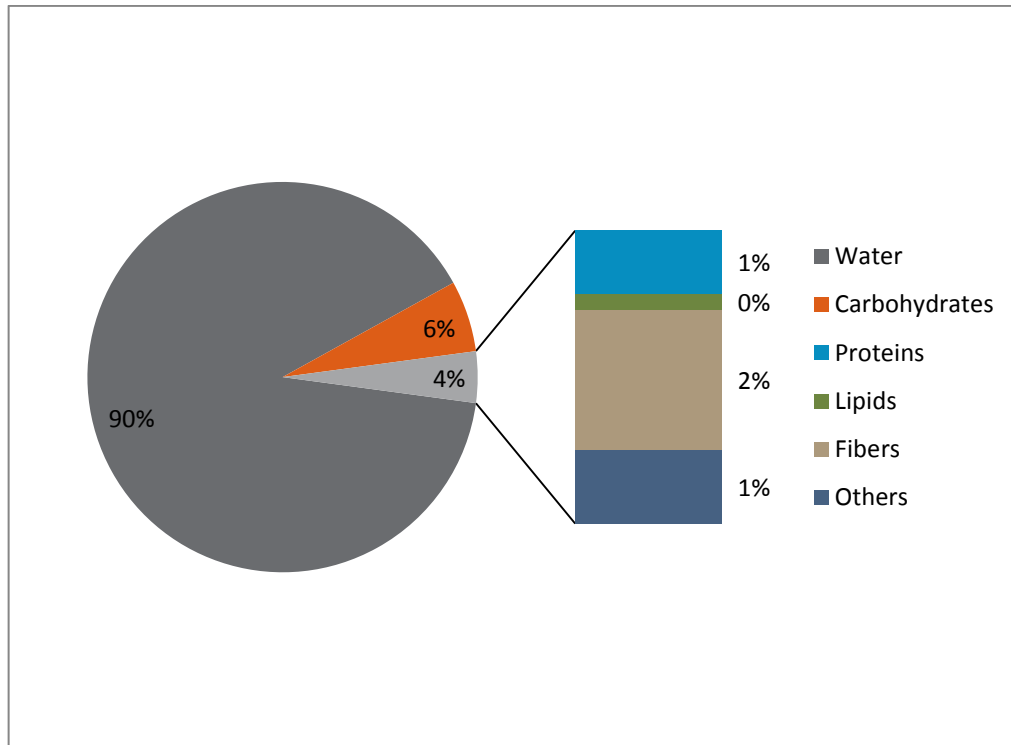


Figure 9. Broth percentage composition (wet basis)

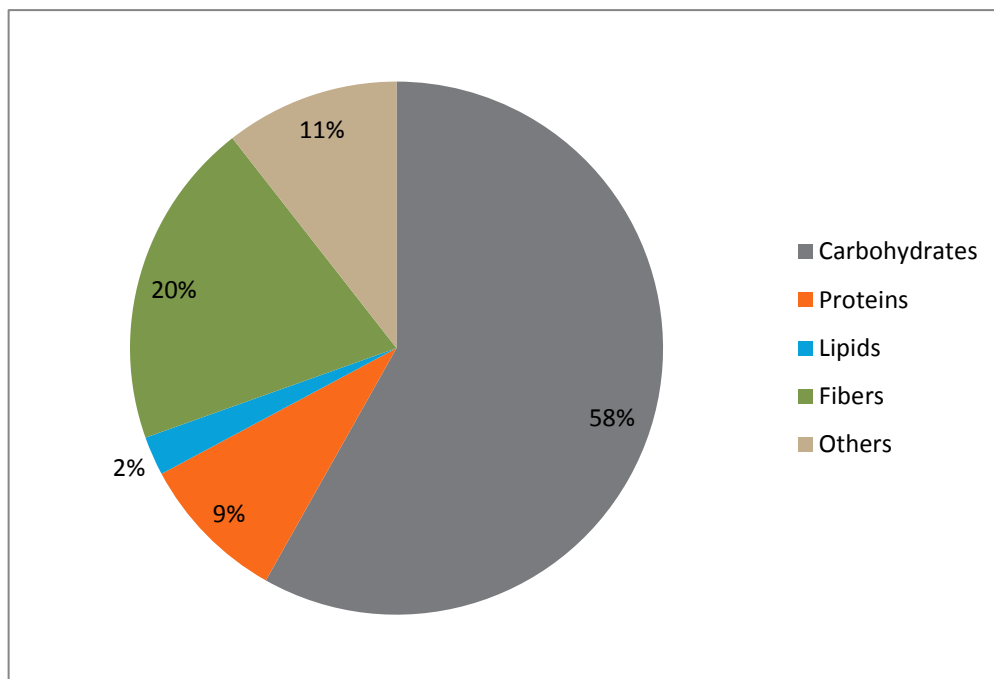


Figure 10. Broth percentage composition (dry basis)

As pretreatments can enhance the bio-digestibility of waste constituents and increase the accessibility of the enzymes to the materials, so as to improving biogas yield, alkaline pretreatment was used because of its low energy

CHAPTER III MATERIALS AND METHODS

requirement and the capability of the alkaline agent to neutralize various acidic productions, hence preventing excessive drops of pH (Pavlostathis and Gossett, 1985). This pretreatment was carried out in a thermostatic chamber at 30 °C and at pH=12 for 24 h using 2N NaOH (Ruggeri et al., 2013). Previous researches (Rametta, 2012; Ruggeri et al., 2013) show that combination of thermal and basic pretreatment greatly increases the biodegradability of the organic waste material, however only basic pretreatment was used in this work in order to reduce the energy requirement of the process.

The Lower Heating Value (LHV) of the broth was determined using the PARR 1261 bomb calorimeter produced by Parr-Instrument. The value was used for calculation of the energy recovery.

Fermentation in reactor

The fermentation process was realized batch wise and in two stages. Only one reactor was used to develop the digestion, hence the separation between the first stage (biohydrogen production) and the second (methane production) was determined by changing the pH conditions and adding inoculum. The instruments, reagents and equipment employed during the experimentation were:

Instruments:

- a) Silicon and neoprene tubes
- b) Plastic Bottle
- c) Conical lids 29/321816 Kartell: two tube inputs
- d) Glass Flask (2,5 L)
- e) Plastic bags for gas storage.
- f) Drechsel bottle

Equipment

- g) pH-meter
- h) Reactor “MINIFORS INFORS”
- i) Analytic Balance
- j) Gasometer “Miligascounter”

Reagents

- k) Distillate water

CHAPTER III MATERIALS AND METHODS

- l) 2N HCl
- m) 2N NaOH
- n) Cattle manure
- o) Substrate (organic waste market)

The procedure performed to carry out the fermentation is described below:

To collect the biogas produced during the anaerobic digestion, two different arrangements were implemented to each stage. The production of biohydrogen is a phase wherein the amount of biogas released from the chemical reactions is significantly less than the accumulated during the phase of biomethane production and therefore, gas meter flasks were used to pick up the gas in this first stage.

The gas meters were made up by a glass container filled with liquid and sealed with a plastic lid previously greased with silicone for preventing entry of air. The two openings of the lid were used as gas inlet and water outlet; the biogas flowing from the reactor was led to the inlet opening using a neoprene tube which ensures gas transport without losses by permeation. As the gas went cumulatively into the gas meter, it displaces the liquid that was forced to leave the container through a silicon tube connected to the water outlet opening. The liquid was collected in plastic bottles that were weighted from time to time to determine the biogas production. The liquid used to fill the gas meter was distillate water acidified to pH=2 with HCl to prevent absorption of the CO₂ contained in the biogas.

Given that during the second stage the production rate is higher, a gas counter “Miligascounter” (Figure 11) was connected to the reactor outlet through a neoprene tube for measuring the production instead of using a gas meter; the biogas was stored in plastic bags for analysis.

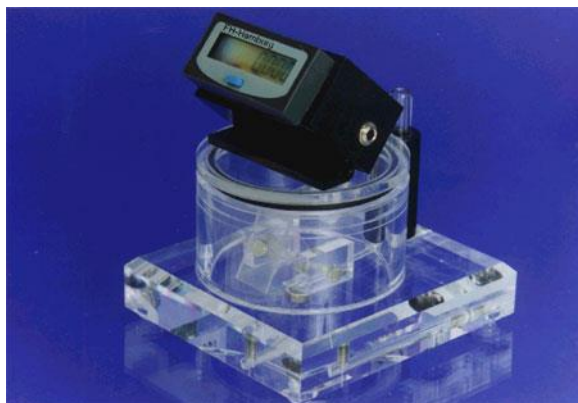


Figure 11. Milligascounter used to measure the volume of gas produced

The fermentation was performed in the reactor MINIFORS INFORS (Figure 12) whose whole volume was 2.5 L. However, the reaction volume was 1.52 L of feedstock containing 10% v/v of inoculum. The reactor was equipped with suitable instruments to monitor and control the more influential parameters in the digestion: pH, mixing, temperature and redox potential.



Figure 12. "MINIFORS INFORS" reactor

pH was controlled by control logic ON/OFF using a pH-meter Mettler and two peristaltic pumps: one pump supplied 2N NaOH with the purpose of maintaining the pH that varies because of the acidifying reactions, within a range determined by the stage of production. In the first stage the pH was above 5.4 while between 7.1 and 7.8 in the second (Bettoli, 2010). The second pump was used just in the start up for stabilizing the initial pH value by using 2N HCl. The pH is a significant influence parameter because it permitted to select the microbial flora to be developed: acetogenic or methanogenic.

The agitation was made with Rushton impellers located in the bottom and in the gas-liquid interface (for avoiding crust formation) with three baffles. It was maintained constant during the fermentation, but at different values in each step. It was decided to work at 250 rpm for the first stage and at 100 rpm for the second

CHAPTER III MATERIALS AND METHODS

stage, taking as reference previous researches (Bettoli, 2010, and Calzata et al., 1984), in which it is shown that it is necessary to have high stirring speed in the first stage to increase the production of hydrogen and low speeds in the second stage, since the micro-organisms that take place in this phase (methanogenic) are sensitive to mechanical stress. The agitation was occasionally raised to 500 rpm during 2 or 3 min when the crust was formed on the liquid surface, in such a way to remove it for preventing gas diffusion problems.

The temperature was controlled by control logic ON/OFF using an electric heating jacket and a thermocouple. The temperature set point was 35 °C during the fermentation so as to provide mesophilic conditions in the reactor.

The inoculum for biohydrogen production (pretreated cattle manure) was defrosted at room temperature and then mixed with the feedstock to proceed with the fermentation. Once completed the first stage (not biogas production during 5 days), the cattle manure without pretreatment was added to the reactor as the inoculum for methanogenic stage. Both after the start up and after the second inoculum, nitrogen was blown into the reactor for 10 min to create anaerobic conditions. This condition was maintained by keeping an overpressure of 20 mbar in the reactor for preventing air flow in.

The gas produced leaved the reactor passing through a condenser and then a Drechsel bottle filled with the same liquid used in gas meters. It had a water head of 20 cm to ensure 20 mbar of overpressure inside the reactor.

ANALYTICAL METHODS

The methods used to measure and assess the fermentation performance are described below.

Measurement of gas produced.

During the first stage of fermentation the volume of gas produced was measured by determining the volume of liquid displaced in the gas meter flasks through the procedure below.

A plastic bottle was used to collect the liquid displaced by the gas. It was weighted and the measured value recorded (A).

CHAPTER III MATERIALS AND METHODS

The bottle was located in position for collecting liquid. The measure was carried out by removing the bottle and recording its weight (B1), after which it was emptied and fitted again for collecting liquid. This procedure was repeated as necessary, recording the weights (B2), (B3)... (B_{n-1}), (B_n).

The cumulative volume of gas produced (mL) per reactor volume (L) at a time “t” is calculated by using the equation bellow.

$$V_{c(n)} = \frac{(B_n - A + B_{c(n-1)}) \cdot \rho}{\frac{R}{f}} \quad (8)$$

Where:

B_n: Bottle and liquid weight recorded at the measure n, at the time t_n, (g).

A: Empty bottle weight, (g).

B_{c(n-1)}: Bottle and liquid weight at the time t_{n-1} and measure n-1, (g).

ρ: Water density at 35 °C, (0.994 g/L).

f: Conversion factor from (mL) to (L), (1000).

R: Reactor volume.

V_{c(n)}: Gas cumulative volume at time t_n, (mL biogas/L reactor).

During the second stage of fermentation it was used the gas counter “Miligascounter” for determining the volume of biogas produced, cause a higher production rate. In this case the biogas was stored in plastic bags instead of gas meter flasks for its subsequent analysis.

Measurement of gas composition

The gas composition was determined by chromatography, using the equipment “Varian CP 4900” which incorporates a thermal conductivity detector with two capillary columns of 10 m that use Argon as gas carrier. The procedure to make the analysis consists of joining one of the two output tubes in the gas meter flask to the equipment input. The remaining tube is used to inject water slowly through a syringe in order to force the gas to flow at a constant rate toward the gas chromatograph. The connection between the tube/chromatograph and tube/syringe was performed taking care not to let in air. Before and after the

CHAPTER III MATERIALS AND METHODS

measurements, the unit was calibrated by using a gas with known composition. The gas composition reported to make analysis is free of O₂ and N₂.

Total solid (TS)

The total solids were measured by the method (APHA, 1995) weighing in gravimetric balance the residue remaining after water removal of the sample in an oven at 105°C.

Materials:

- a) Ceramic crucibles.
- b) Stove
- c) Gravimetric balance, precision 0.1(mg)

Procedure:

The ceramic crucible was weight and the value recorded (D), (g). Then a sample of 6 mL was added and it was put on stove at 105 °C for at least 24 h. After the stipulated time, the sample was cooled in a desiccator and then weighs, recording the value (E), (g). The total solids shall be calculated with the following equation:

$$TS = \frac{E - D}{V} 1000 \quad (9)$$

Where:

TS: Total solid, (g/L).

V: Sample volume, 6 (mL).

Volatile solids (VS)

The volatile solids (VS) were calculated after calcination of the sample at 500-600 °C (APHA, 1995).

Materials:

- d) Ceramic Crucibles
- e) Stove
- f) Oven 500-600 °C
- g) Gravimetric balance, precision 0.1 (mg)

Procedure:

CHAPTER III MATERIALS AND METHODS

Repeated the procedure for the determination of total solids (TS), the same sample then was moved into the oven at 500-600 °C during 24 h. The sample was cooled in a desiccator and weighed in the gravimetric balance (C). The volatile solids are calculated by the equation.

$$VS = \frac{C - D}{V} 1000 \quad (10)$$

Where:

VS: Volatile solids, (g/L).

pH

The pH measurement was made whenever necessary to the fresh reactor samples, inoculum, and feed by using a pH-meter. It was always calibrated before measurements with buffer solutions at pH 4 and 7 at room temperature.

In the reactor the pH was measured and controlled continuously by the pH-metro Mettler and the logic controller ON/OFF of the MINIFORS INFORS reactor.

EXPERIMENTAL DATA TREATMENT

Biogas production analysis

The biogas production has been mathematically modelled through the Gompertz (Lo et al., 2011) equation which is widely used to describe the evolution of biogas production in batch operations and to obtain parameters biologically important.

$$G = G_{max} e^{-e^{\left(\frac{R_{max}}{G_{max}}(\lambda - t) + 1\right)}} \quad (11)$$

Where:

G: Cumulative biogas production (L/L);

G_{max} : Maximum biogas production (L/L);

R_{max} : Maximum biogas productivity (L/Lh);

λ : Lag phase duration (h);

t: Test time progress (t).

The modified Gompertz equation (4.5) has also been used to describe the behaviour of the biogas production because has better results in terms of best fit for the experimental curves. In this equation first terms represent the H₂ production step, while the second term the methane production ones.

$$G = G_{1,max} e^{-e^{\left(\frac{R_{1,max}}{G_{1,max}}(\lambda-t)+1\right)}} + G_{2,max} e^{-e^{\left(\frac{R_{2,max}}{G_{2,max}}(\lambda-t)+1\right)}} \quad (12)$$

In both models the parameter that make fit the experimental cumulative curve with the Gompertz curve are calculated by a best fitting algorithm that relies on finding the parameter values that minimize the sum of least squares, i.e. minimizing the following function:

$$SS(P) = \sum_{i=1}^n w_i (y_i - \hat{y}_i)^2 \quad (13)$$

Where:

SS: Least square sum;

P: Equation parameters;

n: Number of experimental data;

y_i: i-th experimental value.

$\hat{y}_i$: i-th estimated value with the equation to adjust.

w_i: Weight i-th to give more freedom to the algorithm.

To obtain the parameters that allow obtaining the minimum in the expression above it was used “Solvtool” available in the software “MicrosoftExcel”. From the mathematical regression it could be obtained the parameters of lag phase (λ), maximum productivity of biogas ($R_{1, \max}$ and $R_{2, \max}$) and maximum production of biogas ($G_{1\max}$ and $G_{2\max}$). Furthermore, these parameters obtained are all reported in function of the culture volume to make independent the values from the volume of the reactor.

Energy assessment

To assess the energy efficiency of the tests were implemented two energy parameters (Ruggeri and Tomas, 2012): Energy Efficiency (ξ) and Efficacy (η).

CHAPTER III MATERIALS AND METHODS

The energy efficiency (ξ) evaluates the amount of energy extracted in the form of methane and hydrogen due to pretreatment and bioreaction, comparing it with the energy contained in the substrate. The efficacy (η) is used to compare the efficiency of the bioprocess (using organic waste), with the efficiency obtained using glucose as substrate, under the same reaction conditions. Glucose was used as a reference parameter because is considered the most easily degradable substrate due to the absence of lignocellulosic substances. Mathematically the two parameters can be defined as:

$$\xi = \frac{E_{obtained}}{E_{contained}} \quad (14)$$

In the experiment conducted:

$$\xi_{OWM} = \frac{E_{p,OWM}}{E_{0,OWM}} \quad (15)$$

Where:

$E_{p,OWM}$: Energy produced using organic waste as substrate;

$E_{0,OWM}$: Energy contained in the substrate.

The efficacy is given by the expression:

$$\eta = \frac{\xi_{OWM}}{\xi_{Glu}} \quad (16)$$

Dove:

ξ_{OWM} : Efficiency of waste organic market anaerobic digestion;

ξ_{Glu} : Efficacy of glucose anaerobic digestion.

CHAPTER IV RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

FERMENTATION ANALYSIS

The digestion was performed under controlled conditions of pH, since acidogenic and methanogenic bacteria have different requirement for their growth and thus, two stages of production were developed in order to optimize suitable conditions for each group of microorganism. In Figure 13 it is shown the pH in the reactor, the volume and composition of the gas accumulated over time. The biogas production is shown as the volume of gas produced per litre of reactor for establishing a benchmark to compare to previous or future experiments.

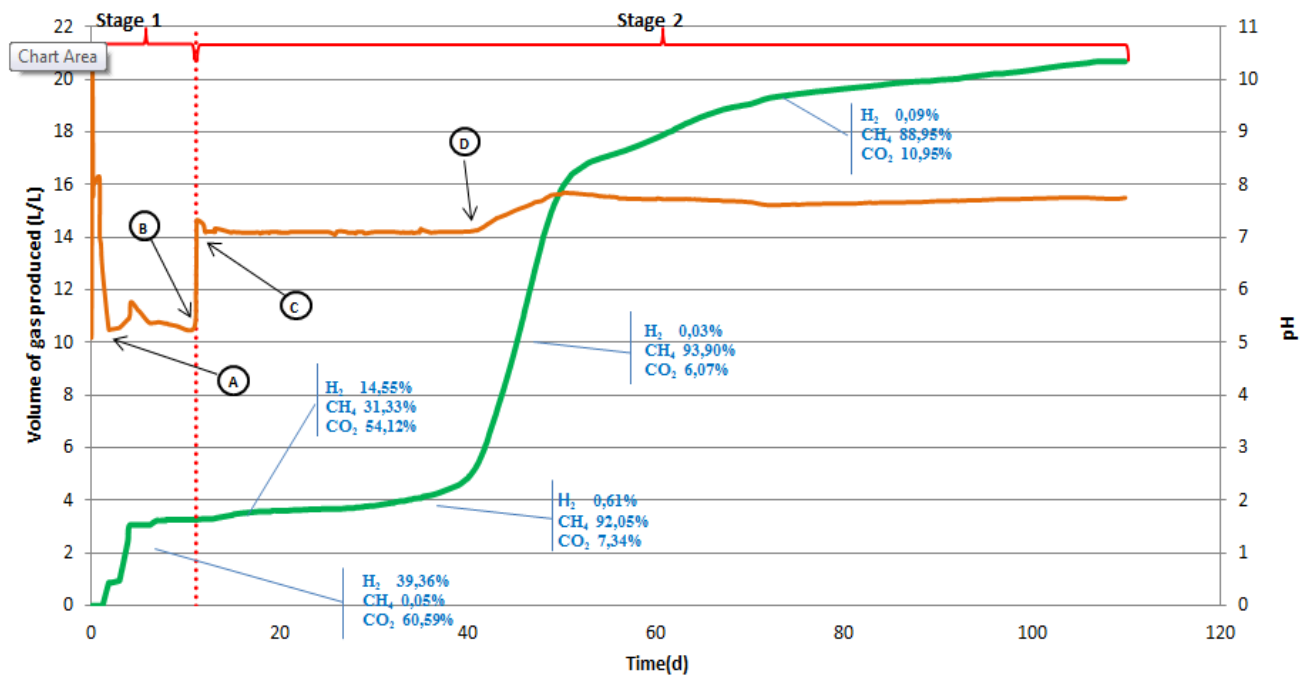


Figure 13. Experimental data of two-stage fermentation. Green line (biogas volume); Orange line (pH)

The experiment began with a pH value of 5.09 as a result of mixing 1500 mL of pretreated substrate (pH=12) with 150 mL of inoculum pretreated at pH 3.

CHAPTER IV RESULTS AND DISCUSSION

According to Li e Chen (2007) the optimal initial pH to obtain the higher yield of hydrogen production is the range of 7.0–7.5. Li e Cheng (2007) demonstrated that pH too high or too low lead to a longer lag phase primarily due to the adjustability of microorganism to unfavourable initial pH. Therefore, the initial pH value was raised manually using 2N NaOH solution. However, despite the continuous stirring in the reactor, the high initial viscosity of the substrate resulted in transfer problems that conducted to raise the initial pH value to 10.12 and in consequence, it must be stabilized manually by adding HCl for achieving the desired range (8.5).

The pH decreased progressively till 5.3 as a consequent of the acid production by acetogenic microorganism. From the point A (day 2) to the point B (day 11) of Figure 13, this value was kept constant by the pH control because pH lower than 5 lead to an excessive concentration of acids that could be superior to the cellular metabolizing capacity, causing inhibition (Cecchi et al., 2005). A small increase of pH was observed during this period as results of delays in the pH control response. On the day 5 the production rate dropped significantly and the volume of gas accumulated at this moment was 4.10 L/L with a composition of 39.36% of H₂ and 60.59% of CO₂; the presence of CH₄ was almost neglected (0.05%). After 7 days of not gas production the first stage had been finished (point B), so at the point C the second stage started by adding the inoculum and changing the pH set point to 7.1 (Rametta, 2013).

From the point C to the point D, pH was constant given the intervention of the automatic pH control which added NaOH solution when necessary. This period corresponds to the lag phase in which methanogenic microorganism were adapting to the environmental conditions and competing with acetogenic bacteria (mainly *Clostridium*) for survival. Prove of this bacteria competition are the composition of the accumulated gas during this period. After inoculation, the percentage of CH₄ that previously was neglected became 31.33% and the percentage of H₂ dropped to 14.55%. Just before starting of methanogenesis at the point D, the H₂ composition dropped even more to 0.61% and CH₄ was 92.05%. The presence of both groups of microorganism causes the changes observed in the percentage composition of the biogas. Evidently, pH condition at this point

CHAPTER IV RESULTS AND DISCUSSION

favoured the development of methanogenic micro-organism even though their growing rate is five times lower than that of *Clostridium*.

At the point D the biogas produced started to raise exponentially with a methane composition of 93.93%; just between the days 41 and 51 the accumulated volume of gas went up from 5.3 L/L to 16.3 L/L which represent 53% of the total gas produced during fermentation. In this period the pH rose naturally to a maximum of 7.84 and then decreased progressively to 7.73 as the curve of accumulated gas tended to decrease its slope. At the day 81 the concentration of CH₄ had decreased slightly to 88.95% and the rest was CO₂. Despite the high concentration of methane, the gas production became slowed till the day 109 when the second stage was considered finished.

Table 8 shows experimental data of the anaerobic digestion process at the beginning and end of each stage. It could be noted the differences in pH between the first and second stage of fermentation. It also shows the volumes of methane and hydrogen produced without any mathematical manipulation as well as concentration of volatile solids and volatile solids.

Table 8. Experimental data of the two-stage digestion

Parameter	Start of 1° Stage(*)	End of 1° Stage	Start of 2° Stage(*)	End of 2° Stage	Specific production
pH	5.09	5.95	7.25	7.75	-
TS (g/L)	56.02	48.68	62.01	48.81	-
VS (g/L)	43.84	31.5	43.77	23.12	-
ΔTS	-	7.4	-	13.20	-
ΔVS	-	11.99	-	20.65	-
CH ₄ produced (L)	-	0.10	-	23.61	-
H ₂ produced (L)	-	1.68	-	1.78	-
H ₂ /ΔVS (L/g)	-	-	-	-	0.04
H ₂ /VS ₀ (L/g)	-	-	-	-	0.03
CH ₄ /ΔVS (L/g)	-	-	-	-	0.48
CH ₄ /VS ₀ (L/g)	-	-	-	-	0.35

(*) Concentration of total solids (TS) and volatile solids (VS) were measured after introducing the inoculum in the reactor; all calculation takes into account the presence of inoculum.

CHAPTER IV RESULTS AND DISCUSSION

ENERGY RECOVERY AND COMPARISON WITH OTHER WORKS

Table 9 shows a comparison of the results obtained in this work with those obtained in a previous research in which composition of substrate and operation conditions were similar. As the table shows, solids concentration of the substrate (VS_0) used by Rametta (2013) is close to this work. However, despite the amount of energy contained on the substrates per gram of VS were also similar, the amount of energy produced per gram of VS_0 was considerable higher in this work, hence when it is evaluated the performance of the digestion in terms of rate energy produced to substrate energy content, in this work were obtained better results. This parameter defined in the Chapter II as Efficiency, was 21% higher in this work, meaning a better recovering of the available energy.

Table 9. Energetic performance comparison

Feed	VS_0 (*) (g/L)	E_{OWM}/VS_0 (kJ/g VS_0)	E_{Prod}/VS_0 (kJ/g VS_0)	Efficiency(%)	Reference
Organic Market Waste	31.07	24.20	16.20	66.90	This work
Organic Market Waste	33.46	24.13	11.15	46.21	Rametta, 2013

(*) Concentration of total and volatile solids was measured before introducing the inoculum in the reactor; all calculations are based in the concentration of the substrate before inoculate in the first stage.

The increase in the efficiency with respect to the previous work corresponds to a higher amount of CH_4 obtained during fermentation. As seen in Table 10, in contrast with Rametta (2013) the production of CH_4 per litre of the substrate was 40% higher, while H_2 production was about 41% lower. Despite the reduction in H_2 production, given that the amount of CH_4 produced exceeds over 13-fold that of H_2 and noticed that the calorific value of methane is over 3-fold that of H_2 , the increment in the CH_4 production represent a large increase in the energetic recovery i.e. more efficiency.

CHAPTER IV RESULTS AND DISCUSSION

Table 10. Comparison of volume of H₂ and CH₄ produced

Feed	Pretreatment	H ₂ produced (L/L)	CH ₄ produced (L/L)	Reference
Organic Market Waste	Alkaline	1.17	15.53	This work
Organic Market Waste	Alkaline-Thermal	1.97	11.19	Rametta, 2013

The fundamental difference between fermentation of Rametta (2013) and this work is the type of pretreatment done to the substrate; while in the first alkaline-thermal pretreatment was used to increase the biodegradability of the substrate, in this work only basic pretreatment was implemented. According to Ruggeri et al. (2013), combination of basic and thermal pretreatment is the most appropriate way to treat organic waste market (OWM) and obtaining high efficiency in H₂ production, which is confirmed by comparing the results shows in Table 10. Nevertheless, it can be noticed that alkaline-thermal pretreatment did not enhance methane production more than just alkaline pretreatment. A possible explanation for this result could be the formation of inhibitory materials at high temperature that adversely affected the digester performance for methane production (Haug et al., 1978). Colberg et al. (1980) showed that thermal pretreatment above 175 °C of waste activated sludge (WAS) resulted in the formation of toxic compounds, and this toxicity increased with temperature, NaOH and HCl addition, and concentration of WAS. They concluded that this may be due to pretreatment releasing the building block constituents of nitrogen compounds, which are more toxic than large molecules, or by their subsequent thermal degradation into even more toxic components. The more complex an organic compound is in gross terms, the less likely it is to be toxic. This hypothesis rests on the fact that the larger a molecule, the less likely it is able to penetrate the cell wall and inhibit growth. Hence, more complex nitrogenous organics like collagen, albumin and RNA are not toxic, while the mixture of amino acids is. According to Colberg et al (1980), in the chemical-thermal pretreatment, toxicity appears due to solubilisation of the nitrogen fraction of

CHAPTER IV RESULTS AND DISCUSSION

WAS at low temperatures followed by the conversion of these soluble products at higher temperature in toxicants; as the pretreatment temperature increases, the soluble organic nitrogen was hydrolyzed to amino acids, and then finally thermally degraded to NH_3 , which can inhibit the biogas production at high concentration. Haug et al (1978) also experimented with alkaline-thermal pretreatment applied to WAS for methane production and observed that biogas production was initially enhanced by the pretreatment, but after a few days the production drop suddenly until its complete inhibition.

A comparison between the results obtained in this research and literature data is shown in the Table 11. It can be observed that the yield of CH_4 production per gram of volatile solid was similar in comparison with Rametta (2013) who also conducted fermentation in batch system. The vantages of providing separately appropriate conditions for acetogenic and methanogenic microorganism through a two-stage digestion are evident by observing the lower yield of CH_4 production obtained by Rajeshwari et al., 1998 and Bouallagui et al., 2001, who performed digestion in single stage.

Table 11. CH_4 and H_2 yield obtained in researches about anaerobic disgestion of organic waste

Feed	Type of process	H_2 Yield (L/g VS ₀)	CH_4 Yield (L/g VS ₀)	Reference
Organic Market Waste	Batch Two-stage	0.037	0.47	This work
Organic Market Waste	Batch Two-stage	0.059	0.334	Rametta, 2013
Fruit and Vegetable Waste	Batch Single stage	-	0.16	Rajeshwari et al., 1998
Fruit and Vegetable Waste	Batch Single stage	-	0.26	Bouallagui et al., 2001
Fruit and Vegetable Waste	Continuous Single stage	-	0.47	Mata-Alvarez et al., 1992
Fruit and Vegetable Waste	Continuous Single stage	-	0.37	Verrier et al., 1987
Food waste	Continuous Two-stage	0.205	0.46	Chung et al., 2008

On the basis of the parameters defined in Chapter II (Efficiency and Efficacy), the fermentation performance has been evaluated by comparing two-stage anaerobic digestion of OWM with fermentation of glucose and coffee seed skin as shown in Table 12. It can be observed that OWM has an intermediate

CHAPTER IV RESULTS AND DISCUSSION

digestibility, since the composition of vegetables and fruits are not as rich in lignocellulose (hard fermentable compound) as coffee seed skin is, but their fermentability is not as easy as that of pure glucose. Given that fermentability of the monosaccharide is the highest cause of its easy decomposition by microorganism, the efficacy of glucose is 100 and it is used as comparison reference. It is also important to notice in the Table 5.5 that the efficacy of alkaline pretreated OWM carried out in this work is higher than that of thermal-alkaline pretreated OWM due to the better efficiency obtained and previously commented.

Table 12. Energetic recovery in two-stage fermentation

Feed	Gas average composition		Recovered energy (kJ)		Total energy recovered (kJ)	Available energy in refuse(*) (kJ)	Efficiency ξ (%)	Efficacy (η)
	H ₂	CH ₄	Stage 1	Stage 2				
Glucose	26.2	81.0	55	2665	2320	3191	72.70	100
Organic waste market (alkaline pretreatment)	5.7	75.1	19	746	764	1143	66.90	92.0
Organic waste market (thermal-alkaline pretreatment)	11.2	63.5	30	567	597	1292	46.21	63.6
Coffee Seed Skin	0.8	77.5	0.5	2174	2175	8443	25.76	35.43

(*)The values have been evaluated taking into account the concentrations of the feeds and the volumes used for the fermentation tests.

From table 12 also can be noticed that in all cases the energy contribution of the first stage is low compared to the total energy recovered. During the fermentation of coffee seed skin the energetic recovery in the first stage represents only 0.02% of the total energy recovered, while thermal-alkaline pretreated OWM has the best recovery in this phase (5% of total energy recovered), which is similarly low. Nonetheless, despite its low energetic contribution this phase serves as a pretreatment of the organic compounds to make them more accessible to the next stage and increase in this way the amount of methane produced. This could

CHAPTER IV RESULTS AND DISCUSSION

be explained by the fact that the acidogenic stage converts sugars (glucose, xylose, mannose) inorganic acids, alcohols and organic compounds that have a higher lineal structure, which are more biodegradable than cyclic one (Ruggeri and Tommasi., 2012).

It is important not only to evaluate the performance of the process in terms of yield of hydrogen and methane production, but also the energy spent during pretreatment must be considered to evaluate the net energy gained through fermentation of OWM. Thus, a sustainability index (SI) has been defined in this work as the ratio of energy produced (E_p) in the form of H_2 and CH_4 , to the energy spent (E_s) for pretreatment ($SI = E_p/E_s$); if this parameter is less than 1 then the energy used for pretreatment is higher than the energy produced in the form of biogas, therefore the process is not sustainable. On the other hand, if the sustainability index is higher than 1, the energy recovered is higher than the spent in pretreatment, so the process is sustainable from an energy point of view.

Table 13 compares the sustainability index in the present work with that of Rametta (2013). Certainly the expenditure of energy per litre of substrate is considerable lower when just alkaline pretreatment is used instead the of alkaline-thermal, so much that in the first case the energy produced exceed 10.5-fold the energy expended, while in the second the energy requirement of alkaline-thermal pretreatment is so high that exceed the energy produced, i.e. SI value is lower than 1 (0.37) which means that process is not sustainable.

Table 13. Pretreatment energy expenditure and sustainability index comparison

Pretreatment	Pretreatment energy expenditure (kJ/L)	Sustainability Index (SI)	Reference
Alkaline	48	10.49	This work
Alkaline-Thermal	1004.42	0.37	Rametta, 2013

The energy expenditure of alkaline pretreatments was calculated in both researches by multiplying the mass of NaOH added during pretreatment by the Gross Energy Requirement (GER) of NaOH. GER represents the amount of commercial energy that is required directly and indirectly by the process of making a good or service (Franzese et al., 2009), in this case NaOH; it was

CHAPTER IV RESULTS AND DISCUSSION

obtained from Ecoinvent (2007). Additional energy expenditure for thermal pretreatment of Rametta (2013) was calculated as the sensible heat needed to raise the temperature of OWM to 120 °C plus the heat required to maintain that temperature during 30 min of pretreatment.

EXPERIMENTAL DATA MODELLING

The Modified Gompertz model has been employed to obtain a trend line that fits the experimental data as described in Chapter III. This model is widely used because of its good fitting in biogas production and in this particular case it does well for the second stage of fermentation (Figure 14). Nonetheless, the curve provided by this model is not the most appropriated to describe the first stage of fermentation since it does not fit well neither the lag phase nor the increment in the volume of biogas during this phase.

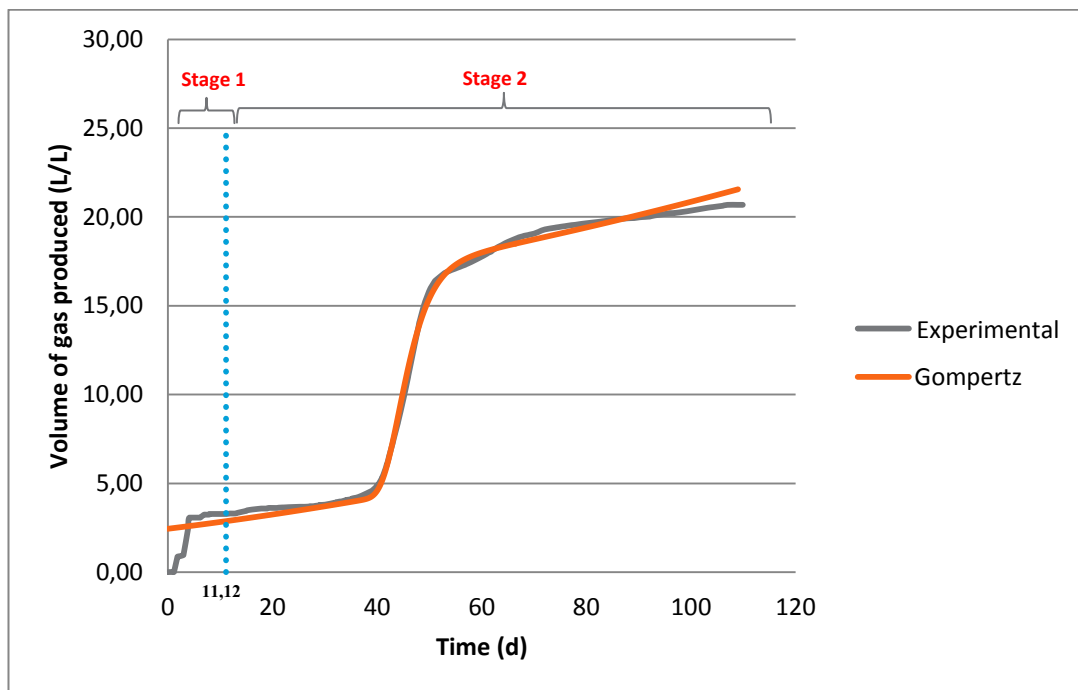


Figure 14. Experimental data and Modified Gompertz model fitting

Another drawback with using this model is that the maximum biogas production given by the sum of parameters $G_{1,max}$ and $G_{2,max}$ (Table 14) is over 3-folds the real amount of gas produced during the experiment (20.68 L/L).

CHAPTER IV RESULTS AND DISCUSSION

Therefore, unmodified Gompertz was implemented at each stage separately in order to get a better fit.

Table 14. Modified Gompertz model parameters

Parameter	Value
$G_{1,max}(L/L)$	12.78
$G_{2,max}(L/L)$	64.52
$G_{max} = G_{1,max} + G_{2,max} (L/L)$	77.3
$R_1 (L/L d)$	1.40
$R_2 (L/L d)$	0.11
$\lambda(d)$	40.89
R^2	0.9923

As seen below in the Figure 15, the unmodified equation is more suitable to fitting a curve to experimental data of the first stage.

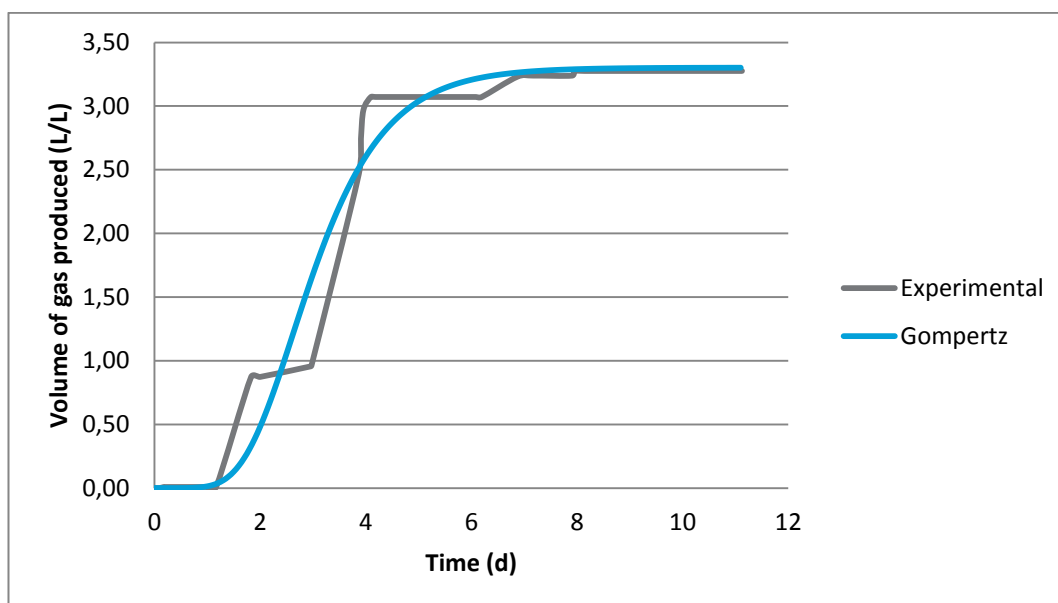


Figure 15. Experimental data and Gompertz model fitting for first stage of fermentation

The lag phase parameter (λ) showed in the Table 15 match the real value of 1.6 d. Moreover, G_{max} parameter is also close to the real value of 3.28 L/L.

CHAPTER IV RESULTS AND DISCUSSION

Table 15. Gompertz model parameters for first stage of fermentation

Parameter	Value
$G_{\max}(\text{L/L})$	3.30
$R(\text{L/L d})$	1.27
$\lambda(\text{d})$	1.67
R^2	0.9741

Similar results were obtained using the Gompertz equation for experimental data in the second stage of fermentation (Figure 16).

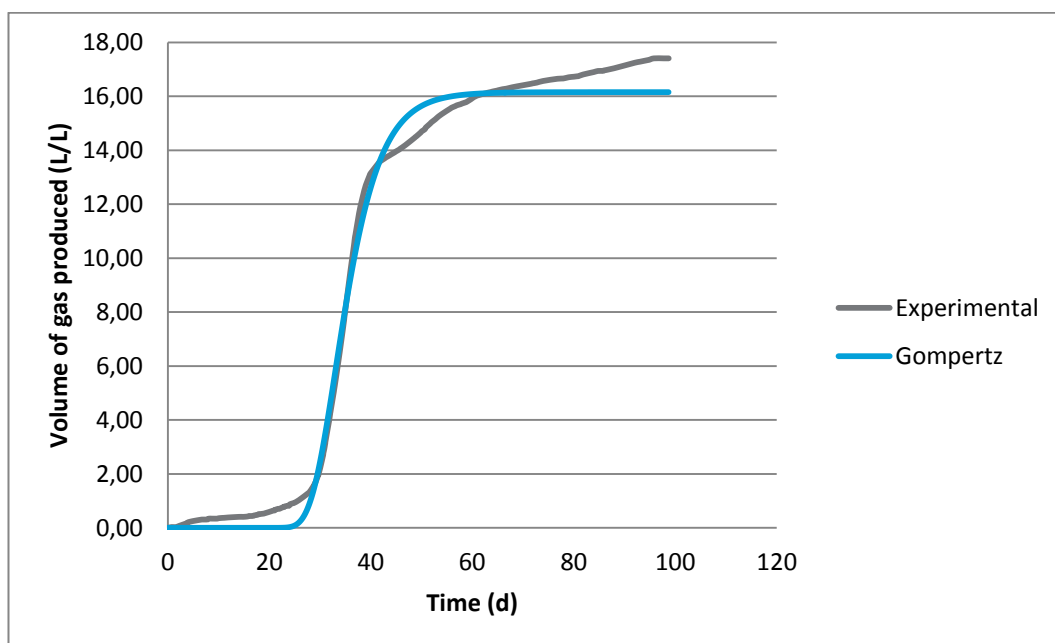


Figure 16. Experimental data and Gompertz model fitting for second stage of fermentation

Lag phase parameter (see Table 16) which represents the adaptability period of methanogenic bacteria, matches the real value of 28 d. Moreover, the maximum amount of biogas produced in this stage (17.4 L/L) is similar to the parameter $G_{\max}$. In this case R^2 value is close to 1, indicating a good fitting.

CHAPTER IV RESULTS AND DISCUSSION

Table 16. Gompertz model parameters for second stage of fermentation

Parameter	Value
$G_{\max}(\text{L/L})$	16.15
$R(\text{L/L d})$	1.21
$\lambda(\text{d})$	28.20
R^2	0.9935

Finally, by modelling the fermentation separately it is possible to describe completely the fermentation, with a suitable matching of the sum of $G_{\max}$ parameter of each stage with the real maximum production of biogas (20.68 L/L). The resultant curve is shown in the Figure 17.

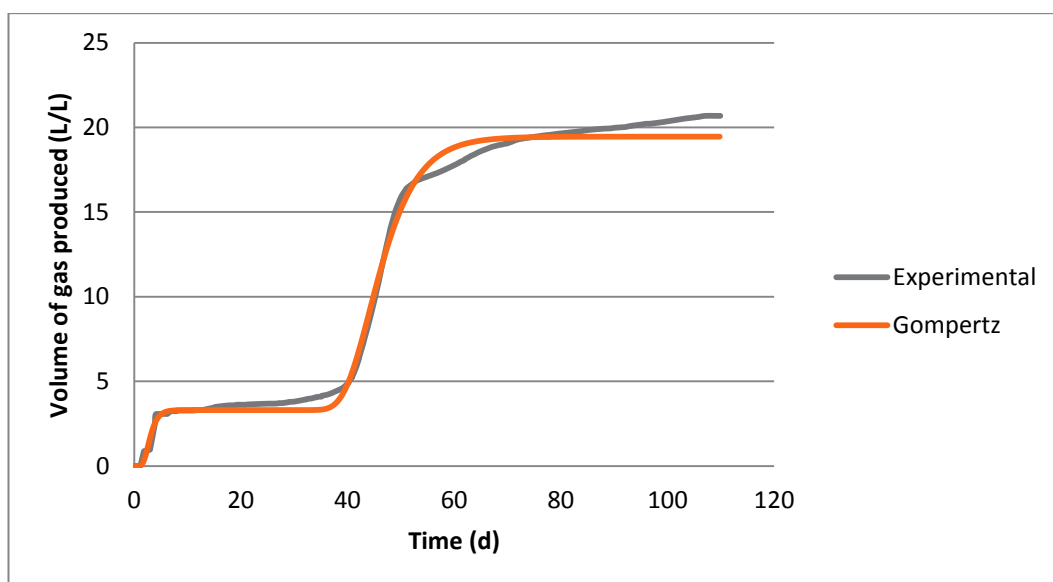


Figure 17. Experimental data and sum of Gompertz model fitting applied separately to each stage

Table 17 shows the parameterization used to unify both fitting and obtaining a continuous curve for the whole process.

CHAPTER IV RESULTS AND DISCUSSION

Table 17. Parameterization of Gompertz model

Stage 1	0 < t (d) < 11	$G = G_{max} e^{-e^{\left(\frac{R_{max} * e}{G_{max}}(\lambda - t) + 1\right)}}$	$G_{max} \text{ (L/L)} = 3.30$
			$R_{max} \text{ (L/L d)} = 1.27$
			$\lambda \text{ (d)} = 1.67$
Stage 2	11 < t (d) < 109	$G = G_{max} e^{-e^{\left(\frac{R_{max} * e}{G_{max}}(\lambda - (t - 11)) + 1\right)}} + 3.30$	$G_{max} \text{ (L/L)} = 15.15$
			$R_{max} \text{ (L/L d)} = 1.21$
			$\lambda \text{ (d)} = 28.20$

CHAPTER V CONCLUSIONS

CHAPTER V

CONCLUSIONS

The current study aimed to evaluate the performance of anaerobic digestion of organic waste market pretreated with NaOH, through comparison of results with a previous work in which alkaline-thermal pretreatment was implemented. The parameters established for comparison were Efficiency, Efficacy and Sustainability Index.

Since anaerobic digestion conducted in two-stage involves the production of hydrogen (first stage) and methane (second stage) separately, the influence of alkaline pretreatment is different in each stage when compared with alkaline-thermal pretreatment. The combination of high temperature with the addition of NaOH is a better pretreatment for enhancing biohydrogen production in the first stage of fermentation. Implementation of just alkaline pretreatment leaves to a hydrogen production of 1.17 L/L substrate, which is not as high as in the previous study.

Nonetheless, methane production is greater when no thermal pretreatment is used. The volume of methane obtained by using alkaline treatment was 15.53 L/L substrate; representing 40% more methane that obtained in the preceding study in which alkaline-thermal pretreatment was used. Seemingly, in the chemical-thermal pretreatment, toxicity could appear due to solubilization of the nitrogen fraction of the substrate at low temperatures followed by conversion of these soluble products at higher temperature to toxicants; as the pretreatment temperature increases, the soluble organic nitrogen compounds are hydrolyzed to amino acids, and then finally thermally degraded to NH_3 , which can inhibit the biogas production at high concentration.

The amount of CH_4 produced exceeds over 13-fold that of H_2 and noticed that the calorific value of methane is over 3-fold that of H_2 , the enhancement of CH_4 production represent an increase of the energy recovery. The efficiency and

CHAPTER V CONCLUSIONS

efficacy of the process were 66.9% and 92.0 respectively, which represent an improvement with respect to anaerobic digestion conducted with alkaline-thermal pretreatment.

The low energy expenditure required by alkaline pretreatment with NaOH makes the anaerobic digestion a sustainable process, since the recovered energy from fermentation in the form of biogas is higher than the energy spent in pretreatment. This is evidenced by the sustainability index which is $SI=10.5$.

Finally, in comparison with the coffee seed skin and glucose, it is confirmed that the organic waste market has an intermediate digestibility, since the composition of vegetables and fruits is not as rich in lignocellulose (hard fermentable compound) as coffee seed skin is, but their fermentability is not as easy as that of pure glucose.

REFERENCES

American Public Health Association, American Water Works Association, Water Environment Federation, 1995. *Standard Methods for the Examination of Water and Wastewater*, 19th ed. AWWA, Hanover, MD.

Appels L., Baeyens J., Degève J., Dewil R., 2008. *Principles and potential of the anaerobic digestion of waste-activated sludge*, Progress in Energy and Combustion Science, Vol. 34: 755-781.

Bettoli S., 2010; *Indagine sperimentale con reattore batch su Rifiuti Mercatali per la produzione di bioidrogeno*, Tesi di Laurea Magistrale, Politecnico di Torino.

Boe K., 2006; *Online monitoring and control of the biogas process*, Institute of Environment & Resources Technical University of Denmark, ISBN 87-91855-10-1.

Bouallagui H., Ben C.R., Marouani L., Hamdi M., 2001; *Fermentation méthanique des déchets solides en batch*. Premières journées scientifiques de l'Association Tunisiennede Biotechnologie du 9–11.

Bouallagui H., Touhami Y., Cheikh R.B., Hamdi M., 2005; *Bioreactor performance in anaerobic digestion of fruit and vegetable wastes*, Process Biochemistry, 40, 989-995.

Brock T.D., Madigan M.T., Martino J.M., Parker J., 1994; *Biology of Microorganisms*, Pentice-Hall, USA.

Calzata J.F., Porres E., Yurrita A., Arriola M.C., Micheo F., Rolz C., Menchù J.F., 1984; *Production from coffee pulp juice: one and two-phase systems*, Agricultural Wastes,9, 217-230.

Camarota C., 2004; *Monitoraggio di un impianto pilota di codigestione anaerobica di residui di concerie*, Tesi di laurea, Politecnico di Torino.

Cecchi F., Battistoni P., Pavan P., Bolzonella D., 2007; *Digestione anaerobica. In linee guida per la progettazione, realizzazione e gestione degli impianti a tecnologia complessa per lo smaltimento dei rifiuti urbani*, Hyper Edizioni, Padova, Capitolo F, 89-118.

Cecchi F., Battistoni P., Pavan P., Bolzonella D., Innocenti L., 2005; *Digestione anaerobica della frazione organica dei rifiuti solidi. Aspetti fondamentali, progettuali, gestionali, di impatto ambientale ed integrazione con la depurazione delle acque reflue*, APAT, Roma.

Chen Q., 2010; *Kinetics of Anaerobic Digestion of Selected C1 to C4 Organic Acids*. Master of Science Thesis, University of Missouri-Columbia.

Chen Y., Cheng J.J., Creamer K.S., 2008; *Inhibition of anaerobic digestion process: A review*, Bioresource technology, Vol. 99: 4044 - 4064.

Chun C.F., Li Y.Y., Xu K.Q., Ebie Y., Inamori Y., Kong H.N., 2008; *A pH- and temperature-phased two-stage process for hydrogen and methane production from food waste*, International Journal of Hydrogen Energy, 33, 4739-4746.

Colberg P.J., Baugh K., Everhart T., Bachmann A., Harrison D., Young L.Y., McCarty, 1980; *Heat Treatment of Organic for Increasing Anaerobic Biodegradability*, Environmental Engineering and Science Department of Civil Engineering of Stanford University: Annual Progress Report.

Demirer G.N., Chen S., 2005; *Two-phase anaerobic digestion of unscreened dairy manure*, Process Biochemistry. 40, 3542–3549.

Deublein D., Steinhauser A., 2008; *Biogas from waste and renewable resources*, WILEY-VCH, Germany, 93-128.

Ecoinvent, 2007; *Ecoinvent data v2.0., Final reports Ecoinvent 2000 N.o 1–25*. Swiss Centre for Life Cycle Inventories, Dübendorf.

El-Mashad, H.E.-M.H., 2003; *Solar Thermophilic Anaerobic Reactor (STAR) for Renewable Energy Production* Agrotechnology and Food Sciences. Wageningen University, p. 238.

Fachagentur Nachwachsende Rohstoffe e.V. (FNR), 2010; *Guide to Biogas From production to use*, Fachagentur Nachwachsende Rohstoffe e.V. (FNR).

Franzese P.P., Rydberg T., Russo G.F., Ulgiati S., 2009; *Sustainable biomass production: A comparison between Gross Energy Requirement and Emergy Synthesis methods*, Ecological Indicators, 9, 959-970.

Fricke K., Santen H., Wallmann R., Huttner A., Dichtl N., 2007; *Operating problems in anaerobic digestion plants resulting from nitrogen in MSW*, Waste Management., 27, 30–43.

Gerardi M.H., 2003; *The Microbiology of Anaerobic Digesters*. John Wiley & Sons, Inc. 81-117.

Ghyoot W., Verstraete W., 1997; *Anaerobic digestion of primary sludge from chemical precipitation*. Water Sci Technol; 6-7:357-65.

Gould J.M., 1984; *Alkaline peroxide delignification of agricultural residues to enhance enzymatic saccharification*, Biotechnology and Bioengineering, 26, 46–52.

Hanjie Z., 2010; *Sludge Treatment To Increase Biogas Production*. Royal Institute of Technology (KTH), Trita-LWR Degree Project 10-20.

Haug R.T., Stuckey D.C., Gossett J.M., McCarty P.L., 1978; *Effect of Thermal Pretreatment on Digestibility and Dewaterability of Organic Sludges*, Water Pollution Control Federation, Vol. 50, No.1, pp. 73-85.

Hendriks A.T.W.M, Zeeman G., 2008; *Pretreatments to enhance the digestibility of lignocellulosic biomass*, Bioresource Technology, 100, 10–18.

Hon D.N.S., Shiraishi N., 2001; *Wood and Cellulosic Chemistry*, Second ed. Dekker, New York.

Ike M., Inoue D., Miyano T., Liu T.T., Sei K., Soda S., Kadoshin S., 2010; *Microbial population dynamics during startup of a full-scale anaerobic digester treating industrial food waste in Kyoto eco-energy project*, Bioresource Technology, 101, 3952–3957.

INRAN, Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione.
<http://www.inran.it/646/tabelle_di_composizione_degli_alimenti.html>
(accessed 28.09.13).

Jeong E., Kim H., Nam J., Shin H., 2010; *Enhancement of bioenergy production and effluent quality by integrating optimized acidification with submerged anaerobic membrane bioreactor*, Bioresource Technology, 101, 1873–2976.

Kayhanian M., Hardy S., 1994; *The impact of four design parameters on the performance of a high-solids anaerobic digestion of municipal solid waste for fuel gas production*, Environmental technology, Vol. 15: 557- 567.

Khalid A., Arshad M., Anjum M., Anjum M., Mahmood T., Dawson L., 2011; *The anaerobic digestion of solid organic waste*, Waste Management 31, 1737-1744.

Lee J., Song J., Hwang S., 2009; *Effects of acid pre-treatment on bio hydrogen production and microbial communities during dark fermentation*, Bioresource Technology, 100, 1491–1493.

Lee M., Hidaka T., Hagiwara W., Tsuno H., 2009; *Comparative performance and microbial diversity of hyperthermophilic and thermophilic co-digestion of kitchen garbage and excess sludge*, Bioresource Technology, 100, 578–585.

Li D., Chen H., 2007; *Biological hydrogen production from steam-exploded straw by simultaneous saccharification and fermentation*, Int. J. Hydrogen Energy, 32, 1742-1748.

Li H., Li C., Liu W., Zou S., 2012; *Optimized alkaline pretreatment of sludge before anaerobic digestion*, Bioresource Technology 123, 159-184.

Liu C., Wyman C.E., 2003; *The effect of flow rate of compressed hot water on xylan, lignin and total mass removal from corn stover*. Industrial & Engineering Chemistry Research, 42, 5409– 5416.

Lissens G., Vandevivere P., De Baere L., Biey E.M., Verstraete W., 2001; *Solid waste digestors: process performance and practice for municipal solid waste digestion*, Water science and technology, Vol. 44 (8): 91-102.

- López M., Espinosa M.C., 2008; *Effect of alkaline pretreatment on anaerobic digestion of solid wastes*. Waste Manage, 28, 2229–2234.
- Mata-Alvarez J., Macé S., Llabrés P., 2000; *Anaerobic digestion of organic solid wastes: an overview of research achievements and perspectives*, Bioresource technology, Vol. 74: 3 – 16.
- Mata-Alvarez J., 2002; *Biomethanization of the Organic Fraction of Municipal Solid Wastes*. IWA Publishing, London, 10-16, ISBN: 1 900222 14 0.
- Mata-Alvarez J., Cecchi F., Llabrés P., Pavan P., 1992; *Anaerobic digestion of the Barcelona central food market organic wastes: experimental study*, BioresourceTechnology, 39, 39–48.
- Mudhoo A., 2012; *Biogas Production. Pretreatment methods in Anaerobic Digestion*. Wiley, ISBN 978-1-118-06285-2.
- Nayoto S. E., 2010; *Anaerobic digestion of organic solid waste for energy production*, KIT Scientific Publishing, Karlsruhe.
- Neves L., Gonçalo E., Oliveira R., Alves M.M., 2008; *Influence of composition on the biomethanation potential of restaurant waste at mesophilic temperatures*. Waste management, Vol. 28: 965-972.
- Olmetto D., 2008; *Co-digestione anaerobica di fanghi di depurazione e frazione organica dei R.U.. Sperimentazioni full-scale del processo BT* Apresso la Fisia Italimpianti di Villacidro, Tesi di laurea, Università di Bologna.
- Pavlostathis S.G., Gossett J.M., 1985; *Alkaline treatment of wheat straw for increasing anaerobic biodegradability*. Biotechnology and Bioengineerin, 27, 334–344.
- Rajeshwari K.V., Panth D.C., Lata K., Kishore V.V.N., 1998; *Studies on biomethanation of vegetable market waste*, Biogas Forum, 3, 4–11.
- Rametta G., 2013; *Verifica sperimentale della valorizzazione energetica di rifiuti mercatali mediante fermentazione anaerobica bistadio*, Tesi di Laurea Magistrale, Politecnico di Torino.

Rapport J., Zhang R., Jenkins B.M., Williams R.B., 2008, *Current Anaerobic Digestion Technologies Used for Treatment of Municipal Organic Solid Waste*, California Integrated Waste Management Board.

Ravikumar G.M.M., Gopalam K. K., 2011; *Investigations on the nitrogen inhibition during an anaerobic co-digestion process*. Master of Science thesis, University College of Borås.

Ruggeri B., Luongo Malave' A.C., Bernardi M., Fino D., 2013; *Energy efficacy to score organic refuses pretreatment processes for hydrogen anaerobic production*, Waste Management.

Ruggeri B., Tommasi T., 2012; *Efficiency and efficacy of pre-treatment and bioreaction for bio-H₂ energy production from organic waste*, International Journal of Hydrogen Energy, 37, 6491-6502.

Shevchenko S.M., Beatson R.P., Saddler J.N., 1999; *The nature of lignin from steam explosion/enzymatic hydrolysis of softwood*. Applied Biochemistry and Biotechnology, 867– 876.

Stronach S.M., Rudd T., Lester J.N., 1986; *Anaerobic digestion of wastewater treatment*. Berlin: Springer.

Teixeira L.C., Linden J.C., Schroeder H.A., 1999; *Alkaline and peracetic acid pretreatments of biomass for ethanol production*. Applied Biochemistry and Biotechnology, 19–34.

Tommasi T., 2010; *Experimental evaluation of design bioreactor parameters for dark Bio H₂ production using organic wastes*, Tesi di dottorato, Politecnico di Torino.

Van Lier J.B., 1995; *Thermophilic Anaerobic Wastewater Treatment; Temperature Aspects and Process Stability*, Wageningen University, ISBN 90-5485-436-7.

Verrier D., Ray F., Albagnac G., 1987; *Two-phase methanization of solid vegetable wastes*, Biological Wastes, 22, 163-77.

Yao Y., He M., Ren Y., Ma L., Luo Y., Sheng H., Zhang H., Li Q., An L., Xiang Y., 2013. *Anaerobic digestion of poplar processing residues for methane production after alkaline treatment*, *Bioresources Technology*, 134, 347-352.

Zaher U., Cheong D., Wu B., Chen S., 2007; *Producing Energy and Fertilizer From Organic Municipal Solid Waste*, Project Deliverable #1, *Washington State University*.

Zhu B., Gikas P., Zhang R., Lord J., Jenkins B., Li X., 2009; *Characteristics and biogas production potential of municipal solid wastes pretreated with a rotary drum reactor*, *Bioresource Technology*, 100, 1122–1129.