OPTIMIZATION OF INFLUENCING PARAMETERS FOR DRY ANAEROBIC CO-DIGESTION OF LIGNOCELLULOSIC BIOMASS

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168049C

Degree of Master of Philosophy

Department of Chemical and Process Engineering

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Thesis submitted in partial fulfillment of the requirements for the degree Master of Philosophy in Chemical and Process Engineering

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Abstract

Anaerobic digestion offers an attractive solution for recovering energy from rice straw (RS) which is a lignocellulosic agricultural residue produced in huge quantities in Asia and Africa. Given the high solids content of this feedstock, high solids anaerobic co-digestion in batch mode is a process that can be applied. In this study, optimal operating conditions for the co-digestion of RS with cow dung (CD) in pure batch reactors and batch reactors with leachate recirculation are assessed. The preliminary experiments carried out in pure batch conditions showed that the initial concentration of RS in the mixture of substrates, i.e., S_0, (g VS rice straw /kg of mixture) is an important parameter. Only the batch reactors with the lowest S_0 values (29g VS_{RS}/kg) produced biogas after a long lag phase of 14 days. The use of digestate from a previous batch as an inoculum was investigated with S_0 values of 29 and 55 g VS_{RS}/kg. Re-use of the digestate as an inoculum source drastically improved both the initial degradation kinetics and the methane yield measured after 60 days for the S_0 of 29 g VS_{RS}/kg, as lag phase time period almost reached to zero and final methane yield of this reactor was 222 ml/g VS. This indicates a 104 % increase of specific methane yield increase compared to the reactor that only has the same S_0 concentration but the substrate mixture comprises only RS and CD. However, for 55 g VS_{RS}/kg, the degradation kinetics were affected: after two months, 32% of the biodegradable organic matter was not eliminated.

Leachate recirculation experiments were conducted in leach-bed reactors (LBRs) with S_0 between 30 and 65 g VS_{RS} /kg, the highest methane yield was recorded at the lowest S_0 value, confirming that in batch mode during high solids anaerobic co-digestion (HS-AcoD) conditions, an initial RS concentration around 30 g VS_{RS}/kg is recommended for industrial applications.

Then mathematical modeling was applied to estimate kinetic parameters related to HS-AcoD process using the modified Gompertz model. Results obtained from Batch experiment no.3 (i.e., the three consecutive batches) were considered for the mathematical modelling. Modified Gompertz model very closely predicted the ultimate methane yield (M_{max}) with R^2 almost 0.99 in each scenario. Degradation kinetics improved drastically with the strategy of re-using digestate, as for the Batch-2 the lag phase period (λ) reduced from 14 days to almost zero. Ultimate methane yield increased by 104% through this approach. Degradation kinetics were negatively affected with the increase of TS% within the substrate mixture even though digestate was reused as an inoculum. In Batch-3 ultimate methane yield was 138 ml/g VS which was a 38% reduction compared to Batch-2, even though digestate was used as the main inoculum source for the both batches. But it was a 27% increase compared to Batch-1 which CD was used as the only inoculum.

Key words: High solids anaerobic co-digestion, lignocellulosic biomass, leachate recirculation, mesophilic, mathematical modelling, kinetic parameters

ACKNOWLEDGEMENT

It is not possible to succeed this work unless the support, guidance and encouragement of people around me. Therefore, I would like to forward my gratitude towards them.

First of all, I would like to express my deepest gratitude to my main supervisor Prof: P.G Rathnasiri for his inspiring and motivating guidance throughout this research. During the research I have received an Internship to conduct laboratory experiments related to this research in a well reputed biotechnology laboratory in France (INRAE, Lbe, Narbonne) due to his international recognition in the field of anaerobic digestion.

I gratefully acknowledge my co-supervisor Prof: M. Narayana for his guidance towards modelling of the experimental results and supervising me to enhance the quality of the research study.

Then I would gratefully remember and forward my gratitude and honor to late Dr. Michel TORRIJOS who was a senior research Engineer in INRAE,Lbe (Laboratory for biotechnology and environment,Narbonne, France), for his kind assistance and guidance for this research project from the very beginning. Life in Lbe and Narbonne was not a difficult one for me because of his kind and patient guidance with all his busy schedules.

Then I would like to forward my gratitude to Research Director at Lbe, Dr. Ranaud ESCUDIE for his guidance throughout all the experiments and publications.

I gratefully acknowledge senior technician Mr. Philippe SOUSBIE for his all assistance throughout all the experiments. His experience and guidance helped me a lot in every time when I was working in the Lbe.

Then I would like to forward my gratitude to all the staff of INRAE,Lbe including Directors, Engineers, Technicians and the research colleagues. I should gratefully remember the financial assistance given by INRAE during my stay and the staff of the Embassy of France, Sri Lanka for their support. I should forward my gratitude to Campus France, Ms. Beatrice BEAUDOUIN for the guidance, thorough the arrival to Narbonne and for the financial assistance during my stay.

I should gratefully remember Prof: J. Manathunga department of Civil Engineering, University of Moratuwa and Prof: A.D.U.S Amarasinghe department of Chemical & Process Engineering for being progress reviewers of this research study and giving guidance to improve the quality of the research study.

I would also like to forward my gratitude for all the academic staff and non-academic staff of the department of Chemical and Process Engineering including Head of the department Prof. S. Walpolage, and former Head of the department Dr. S.H.P Gunawardena for providing necessary facilities for the post graduate studies.

I should gratefully acknowledge Faculty of graduate studies University of Moratuwa and Senate Research Grant University of Moratuwa [SRC/CAP/16/06] providing guidance and financial support for successfully completing research project.

Most importantly, none of these would not have been possible without the love and patience of my family. I am deeply indebted to my parents, sister, brother and loving wife who always supported, encouraged and believed in me, in my entire endeavor.

TABLE OF CONTENT

1 INTRODUCTION 1
1.1 Background 1
1.2 Objectives of the research 2
1.3 Research strategy 3
2 LITERATURE REVIEW 5
2.1 Anaerobic digestion 5
2.1.1 Hydrolysis 5
2.1.2 Acidogenesis 5
2.1.3 Acetogenesis 6
2.1.4 Methanogenesis 6
2.2 Anaerobic co-digestion 7
2.3 Anaerobic digestion of rice straw 8
2.4 Anaerobic co-digestion of rice straw 9
2.5 High solids anaerobic co-digestion of rice straw 10
2.5.1 Significance of cow dung as a co-substrate to digest with rice straw .. 11
3 KINETICS MODELING OF HIGH SOLIDS ANAEROBIC CO-DIGESTION OF LIGNOCELLULOSIC BIOMASS 12
4 MATERIALS AND METHODS 14
4.1 Introduction to materials and methods 14
4.2 Substrates used 15
4.3 Experimental setups and operation 15
4.3.2 Methane based biodegradability 19
4.3.3 Pure batch reactors 19
4.3.4 Leach-bed batch reactors 22
4.4 Analytical methods and experimental protocols 26
4.4.1 Total solids and Volatile solids percentage calculation 26
4.4.2 TS percentage calculation of a substrate mixture for sample preparation 27
4.4.3 Methane volume calculation in 2L batch reactors 27
5 RESULTS AND DISCUSSION 29

5.1 Substrate characterization 29
5.2 Co-digestion of RS and CD in batch conditions 30
5.2.1 Assessment of optimal initial conditions 30
5.2.2 Co-digestion of RS and CD in successive batches 32
5.3 Co-digestion of RS and CD in Leach Bed Reactors 35
5.3.1 Methane production from LBR experiments 36
5.3.2 VFAs concentration, pH and alkalinity 37
5.3.3 Discussion on LBRs 39
5.4 Parameter estimation of modified Gompertz model using experimental data of Co-digestion of RS and CD in successive batches 41
6 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES 45
6.1 Conclusions 45
6.2 Recommendations for future studies 47
7 REFERENCES 48

LIST OF FIGURES

Figure 1. Conceptual diagram of the research study 4
Figure 2. Main steps and intermediates of anaerobic digestion process 5
Figure 3. Lignocellulosic structure (extracted: https://lignofuel.wordpress.com/2010/09/15/lignocellulose/ 9
Figure 4. Experimental methods and parameters 14
Figure 5. Main steps of BMP test 18
Figure 6. Batch reactors with gas collection bags 19
Figure 7. Strategy developed re-using digestate as inoculum 22
Figure 8. Schematic diagram of Leach bed batch reactor setup 23
Figure 9. Leach bed batch reactors Setup 23
Figure 10. BMP test results for rice straw and cow dung 30
Figure 11. Cumulative methane volumes produced (a), TVFA concentration (b) in experiment no.1 and Cumulative methane volumes produced(c), TVFA concentration (d) in experiment no.2 31
Figure 12. Evolution of cumulative CH_4 (a) and specific methane yield (b) of 2L batch reactors with time when digestate re-use strategy was applied 33
Figure 13. Evolution of the total volume of CH_4 produced (a) and of the specific methane yield (b) over the initial RS concentration within the reactor 36
Figure 14.Total VFA and pH variation in first (a & b) and second (c & d) LBR experiments with time 38
Figure 15.Specific methane production rates comparison of 2L batch reactors and 6 L LBRs that have S_0 value 30 and 55 40
Figure 16. Maximum total volatile fatty acids concentration variation in LBRs with initial rice straw VS concentration 41
Figure 17. Evolution of methane production with modified Gompertz fits for three consecutive batch experiments 42

LIST OF TABLES

Table 1.Strengths and weaknesses of HS-AD..... 2
Table 2.Merits and Demerits of AcoD..... 8
Table 3.Results from characterization of rice straw and cow dung..... 15
Table 4.Quantities of substrate added for the 1st batch experiment..... 20
Table 5.Quantities of substrate added for the 2nd batch experiment..... 21
Table 6. Quantities of substrates added for successive batches in experiment No.3 . 21
Table 7. Results from characterization of solid and liquid fractions of digestate..... 24
Table 8. Quantities of substrates, digestate and water added for first and second LBR experiments..... 25
Table 9. Experimental results of methane yields and estimated modified Gompertz model parameters..... 43

LIST OF ABBREVIATION

AcoD- Anaerobic Co-Digestion

AD- Anaerobic Digestion

ADM1- Anaerobic Digestion Model no.1

BMP- Biochemical Methane Potential

CD- Cow Dung

CSTR- Continuous Stirred Tank Reactor

FW- Food waste

GC- Gas Chromatography

HAc- Acetic Acid

HS-AD- High Solids Anaerobic Digestion

LBR- Leach Bed Batch Reactor

LS-AD- Liquid State Anaerobic Digestion

MSW- Municipal Solid Waste

RS- Rice Straw

TS- Total Solids

UASB- Up flow Anaerobic Sludge Blanket

VFAs -Volatile Fatty Acids

VS- Volatile Solids

1 INTRODUCTION

1.1 Background

Biogas generated from anaerobic digestion (AD) process has been regarded as a promising source of renewable energy and it can be used for various applications such as: generation of heat or electricity from burned biogas, liquefied and compressed biogas as a vehicle fuel. Also, during the production of biogas nutrient rich fertilizer generates as a byproduct.

Biogas can be produced from different types of organic materials such as industrial waste water, food waste, sewage sludge, agricultural waste and municipal solid waste (MSW). Lignocellulosic plant residues such as rice straw (RS), wheat straw, sugar cane bagasse and corn stover, represent the most promising renewable feed stock for biogas production as they are widely generated during agricultural activities all over the world.

1.1.1 Wet and dry anaerobic digestion

When total solids (TS) content is below 10-12% in the substrate mixture, the process is described as wet or liquid state anaerobic digestion (LS-AD) [1]. Wet AD requires a large amount of water, which may be either equal or greater than the quantity of substrate. This results in a wasting of large amount of water [2]. In addition, post processing of leachate from wet AD is a capital intensive. Wet digesters are ideally suited to the processing of low dry matter feedstock such as farm slurries and source separated food waste slurries.

Solid state or high solids anaerobic digestion (HS-AD) which is called dry anaerobic systems are characterized by TS content higher than 10-12% [1]. Dry digesters are ideally suited to feedstock with high dry matter content such as lignocellulosic biomass, energy crops, garden wastes and mechanically recovered municipal wastes. However, HS-AD exhibits slower degradation rate at the initial stage as hydrolysis step and become slower due to the low water content. Table 1 presents strengths and weaknesses of HS-AD.

Table 1.Strengths and weaknesses of HS-AD

Strengths	**Weaknesses**
Higher biomass retention	Complex handling of feedstock
Controlled feeding	Material handling and mixing are difficult
Low energy demand	Slower degradation rate at initial stage
Higher methane yield	Acidification and failure of the process

Both LS-AD and HS-AD were applied for AD of lignocellulosic biomass. HS-AD has gained increasing attention in recent years especially for digesting lignocellulosic biomass such as wheat straw or rice straw due to its advantages over LS-AD such as lower need of water addition, no or slowly moving parts, lower energy input for heating and mixing, easier to handle end product ([3],[4]). Also, problems in wet AD such as floating and stratification of fats and fibers are not present in HS-AD. However, methane yields of lignocellulosic biomass during HS-AD are relatively low due to few reasons such as the recalcitrance of the plant cell wall to digestion, retarded mass transfer due to low moisture content, poor nutrient source (nitrogen, phosphorous, trace elements etc.) for degrading microorganisms ([5],[6],[7],[8]).

Therefore, this research is targeted to optimize HS-AD of lignocellulosic biomass by identifying the most critical parameters with the co-digestion of rice straw and cow dung in batch reactors and leach bed reactors (LBRs) under high solids anaerobic co-digestion (HS-AcoD) conditions at mesophilic temperature (35°C).

1.2 Objectives of the research

The main objective of this study is to optimize high solids anaerobic co-digestion process for degradation of lignocellulosic biomass by the development of laboratory and pilot scale experimental strategies combining with mathematical modeling approach. Preliminary experiments were carried out to characterize both rice straw and cow dung to evaluate the biochemical methane potentials (BMPs) of both substrates in order to check the eligibility of anaerobic digestion and co-digestion. Then batch experiments were carried out to identify the effect of most critical

parameters for the HS-AcoD of rice straw with cow dung. Final set of experiments were carried out to evaluate and develop HS-AcoD optimization strategies. Mathematical modeling was applied to estimate parameters of statistical model and to derive model equations under different scenarios.

As summery, the objectives of the study can be listed out as follows:

1. Identify the most critical parameters and investigate the effect of those parameters to HS-AcoD of lignocellulosic biomass.
2. Develop experimental strategies to optimize high solids anaerobic co-digestion (HS-AcoD) of lignocellulosic biomass.
3. Apply mathematical model to simulate experimental data in order to evaluate the parameters of the model and justify with the experimental data.

1.3 Research strategy

A conceptual diagram of the research strategy is shown in the Figure 1. This study was started by focusing the application of anaerobic digestion for the treatment of solid waste. Then under the solid-state anaerobic co-digestion, study is further focused on optimization strategies of HS-AcoD of lignocellulosic biomass. Both experimental strategies and mathematical modeling approach will be considered for the optimization of the HS-AcoD process.

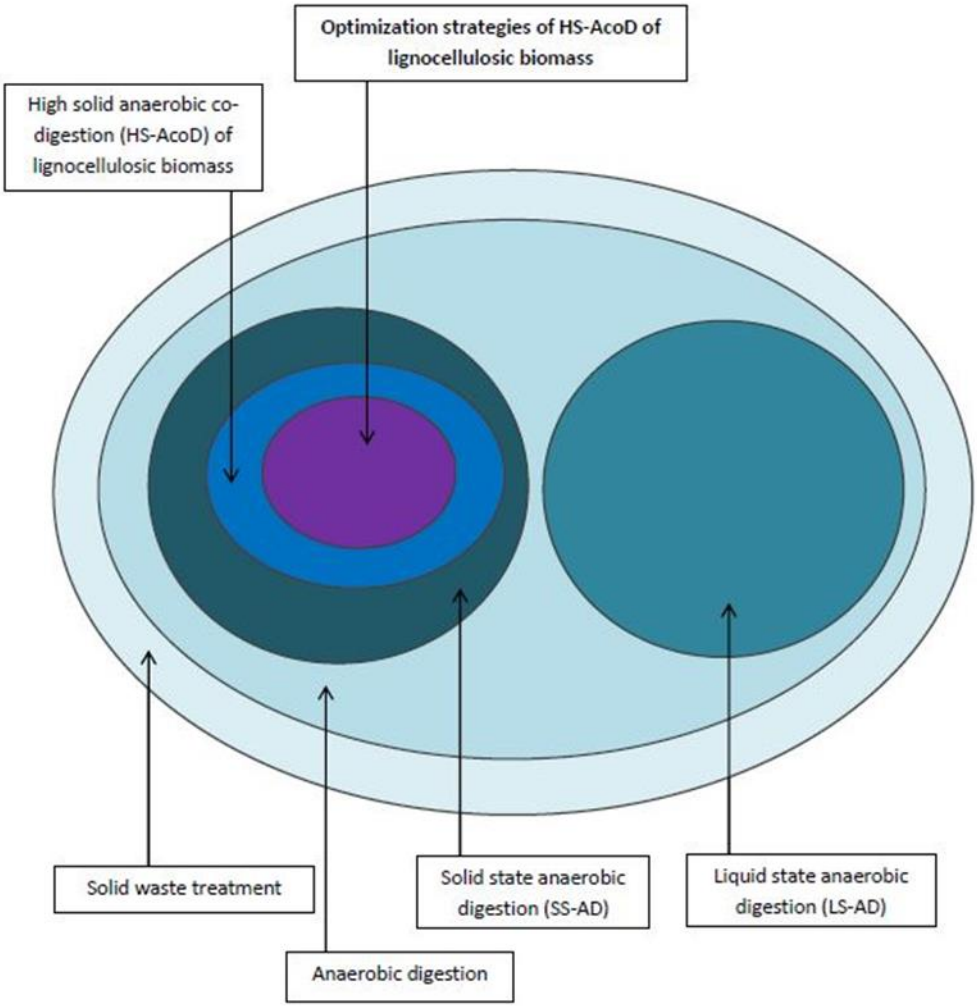

Figure 1. Conceptual diagram of the research study

2 LITERATURE REVIEW

2.1 Anaerobic digestion

Anaerobic digestion (AD) is a process in which microorganisms break down biodegradable material in the absence of oxygen. Anaerobic digestion can be used to treat various organic wastes and recover bio-energy in the form of biogas, which contains mainly CH_4 and CO_2.

Figure 2 presents four main steps hydrolysis, acidogenesis, acetogenesis and methanogenesis in the AD process and the intermediates.

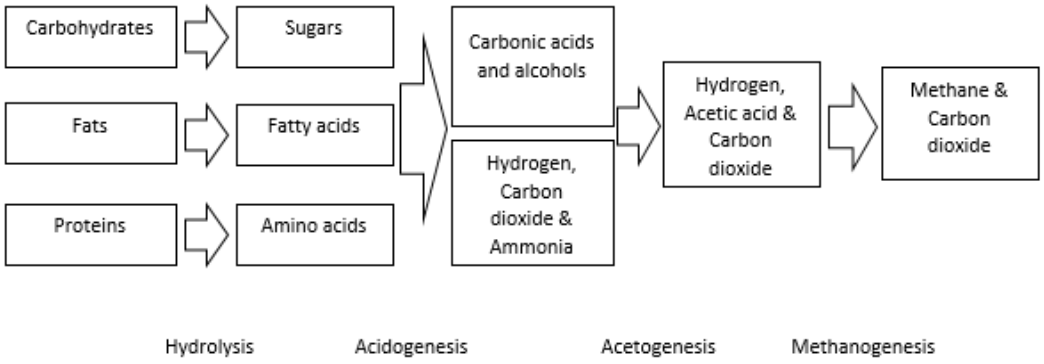

Figure 2.Main steps and intermediates of anaerobic digestion process

2.1.1 Hydrolysis

In the first phase of AD process, complex biopolymers are broken down into smaller soluble molecules (monomers and oligomers) by water and enzymes. The products from this stage are normally monosaccharaides from carbohydrates, amino acids from proteins, long chain fatty acids (LCFAs) and glycerin from lipids. Hydrolysis can be identified as the rate limiting step in AD and is dependent on the type of substrates used, temperature of digester and etc. [9].

2.1.2 Acidogenesis

Acidogenesis (acid formation stage) is the second step of AD. During this step, soluble monomers are converted into small organic compounds such as short chain volatile fatty acids (propionic, formic, lactic), alcohols (ethanol, methanol) and

ketones (acetone). This step is usually the fastest reaction in the overall anaerobic digestion process. The bacteria responsible for this stage called Acidogens. During acidogenesis an acidic environment in the digester is created due to the generation of ammonia, H_2, CO_2, H_2O, volatile fatty acids (VFAs), carbonic acids and alcohols. These VFAs concentration accumulated in the digester have a significant impact on the overall performance of the process as digestion process can be inhibited by the exceeded VFAs concentration.

2.1.3 Acetogenesis

In the acetogenesis phase, the products from acidogenesis are completely converted to acetate, hydrogen and carbon dioxide by group of bacteria known as acetogens. This step is regarded as the most important stage as it produces main substrates for last stage. The performance of this stage is depended on the H_2 partial pressure as acetogenic bacteria survive and function only at low hydrogen partial pressure (lower than 10^{-5} bar). Therefore, acetogenic bacteria live in symbiosis with methanogenic microorganisms as methanogens will assimilate the hydrogen, result in lowering the H_2 partial pressure.

2.1.4 Methanogenesis

This is the final stage of AD process. H_2, CO_2 and acetate are converted to methane by methanogenic bacteria. Methanogenic bacterial group responsible for this step is highly sensitive to changed process parameters such as pH, temperature and substrate concentration. Their growth rate also very slow (generation time, 5-25 days) thus, this phase also a rate limiting step of the overall process.

$$CH_3COOH \leftrightarrow CH_4 + CO_2 \quad \text{(Eq.2.1)}$$

$$4H_2 + CO_2 \leftrightarrow CH_4 + 2H_2O \quad \text{(Eq.2.2)}$$

2.2 Anaerobic co-digestion

Anaerobic co-digestion (AcoD) is the simultaneous digestion of a homogenous mixture of two or more substrates. During the co-digestion, two or more organic materials should be managed properly to increase biogas production as compared to mono-digestion of these substrates. Co-digestion can enhance biogas production from 25% to 400% over the mono-digestion of the same substrates [10].

Digestion process tends to fail, when one readily degradable organic matter is used as sole substrate without external nutrients and buffering agent. Co-digestion could provide more suitable physicochemical property of feedstock and more balanced nutrients for efficient digestion and high biogas production [11]. As an example, it can be used to accomplish better Carbon to Nitrogen (C:N) ratio because it is obvious that this is a main factor to increase the performance of the AcoD process. Lignocellulosic biomass may produce low methane yields during mono-digestion due to their high C:N ratios (usually higher than 50). AD process is stable at the optimum value of the carbon to nitrogen ratio in the range 20-30 [10].HS-AcoD of lignocellulosic biomass with feed stocks that have lower C:N ratios (below 20) has been used to obtain a proper C:N ratio for improving methane yield and stability of digestion process [5]. Buffering capacity of wastes can be balanced and the effect of toxic or inhibitory compounds in the process can be minimized using different substrates as co-digestives while improving the stability and process performance. Therefore, AcoD can be applied for the digestion of poorly biodegradable wastes such as lignocellulosic biomass which cannot be digested alone. Table 2 presents merits and demerits of the AcoD process.

Table 2.Merits and demerits of AcoD

Merits	**Demerits**
Improvement of nutrient balance and digestion	Increase of digester effluent COD
Equalization of particulate, floating, settling, acidifying of wastes, through dilution by manure or sewage sludge	Additional pre-treatment requirements
Increase of biogas (methane)yield	Increase of mixing requirements
Additional fertilizer (soil conditioner) reclamation	High utilization degree required
Improve the process stabilization	Reduction of biogas production rates

2.3 Anaerobic digestion of rice straw

Rice straw (RS) is a lignocellulosic waste material that is widely generated specially in Asia and Africa. For every ton of rice harvested, approximately 1.35 tons of rice straw remains in the field with energy potential[12]. RS is a dry residue with a very high total solids (TS) and volatile solids (VS) content([13],[14]) and its main components are cellulose, hemicellulose and lignin([15],[16]). Cellulose and hemicellulose are the principal biodegradable components, but complex lignocellulosic structure (Figure 3) and relatively high lignin content(i.e., 10-15% dry weight) increase the resistance to anaerobic biodegradation([3],[7]). Methane potentials ranging from 92 to 280 L/kg of VS added, have been reported for RS in different researches ([7],[17]). The considerable variation of methane yield of RS was due to type of pretreatment such as physical pretreatment (hydrothermal, freeze, and crush), chemical pretreatment (sodium carbonate, sodium sulfite, hydrogen peroxide, alkaline, and dilute acid) and biological pretreatment (fungal pretreatment) also combined pretreatment (microwave irradiation and chemical) and digestion conditions [7].

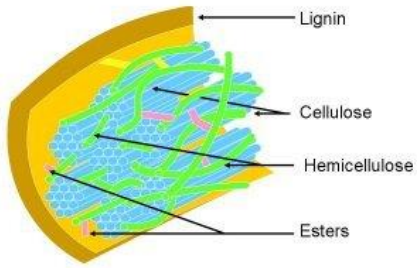

Figure 3. Lignocellulosic structure
(extracted:**https://lignofuel.wordpress.com/2010/09/15/lignocellulose/**)

2.4 Anaerobic co-digestion of rice straw

The high C:N ratio of rice straw (90:1) indicates a risk of having low nitrogen content for bacterial growth in case of mono-digestion of RS. Therefore an external source of nitrogen is essential for its effective digestion taking into account the optimal digestion conditions such as pH (6.5-8.0), temperature (35-40°C), and nutrients (C:N ratio of (25-35):1) which were defined for rice straw [7]. In addition to the limited moisture and nitrogen contents, lignocellulosic biomass such as rice straw also has low micronutrient content. Micronutrients such as Fe and Ni are critical for enzymatic activities and a micronutrient deficiency can lead to failure of AD process. Therefore AcoD of RS is interesting in order to overcome the problems and disadvantages associated with anaerobic mono-digestion of rice straw ([10],[18]). Indeed, several authors have listed the benefits that AcoD can give such as improvement of process stabilization, increasing methane yield, dilution of inhibitory substances, nutrient balance, accomplishment of the required solids content in the digester feed, synergetic effects of microorganisms, and increasing the organic loading rate([19],[20],[21]). Some authors have reported synergistic effects during AcoD [5]. According to a study by Yong et al.(2015) [22], HS-AcoD of food waste (FW) and straw which, composed with maize, sorgos and wheat, at mesophilic temperature has shown 39.5% increase of methane yield of FW and 149.7% increase of methane yield of straw compared to the mono digestion of FW and straw. From a study by Brown et al. (2013) [23], the HS-AcoD of food waste and yard waste has also given 20% increase of volumetric methane production.

2.5 High solids anaerobic co-digestion of rice straw

Both wet anaerobic digestion (i.e., TS content < 10-12%) and high-solids anaerobic digestion (HS-AD) with TS content ≥ 10-12% [1], can be applied for AcoD of rice straw. HS-AD has gained increasing attention in recent years, especially for digesting lignocellulosic biomass such as wheat or rice straw due to its advantages over wet AD such as lower need of water addition, no or slowly moving parts, lower energy input for heating and mixing, easier to handle end product ([3],[4]). Also problems in wet AD such as floating and stratification of fats and fibers are not present in HS-AD [24].

However, long retention time, poor startup performance, incomplete mixing, accumulation of volatile fatty acids (VFAs), relatively low methane yield and potential instability are considered as the main disadvantages of HS-AD of lignocellulosic biomass ([16],[25],[26],[27]). Low methane yield in HS-AD can be caused by both the recalcitrance of lignocellulosic biomass and mass transfer limitations within the digester content resulting in reduction of the hydrolysis rate which is the rate limiting step in HS-AD. Moisture content is a critical factor to facilitate mass transfer. In HS-AD, the high solids content implies high heterogeneity that leads to slow mass transfer between microbes and feedstock, resulting in slow methane production and low methane yield ([16],[28],[29]). High TS concentration of 30-35% may even hinder solid-liquid-gas transfer, leading to accumulation of carbon dioxide (CO_2) and hydrogen (H_2) that inhibit methanogens [30].

Both continuous and batch type reactors can be operated under HS-AD conditions. Continuous processes dominate among HS-AD systems treating municipal solid waste (MSW), but they are not widely applied to the processing of lignocellulosic biomass due to mixing techniques that are not appropriate for HS-AcoD of lignocellulosic biomass ([4],[31]). HS-AcoD of lignocellulosic biomass such as RS in batch mode presents a number of advantages as relative simplicity, minimum maintenance requirement, low energy consumption, minimum capital cost. However, operating conditions of such reactors (temperature, pH, buffering capacity,

inoculation, and management of volatile fatty acid concentration) still need to be optimized in order to maximize the biogas production in a shorter retention time.

Experimental strategies such as re-use of digestate and leachate recirculation were applied by various researches for several types of waste such as food waste or spent animal bedding ([20],[32],[33]). Using batch reactors with leachate recirculation, also called leach bed reactors (LBRs), Riggio et al.(2016)[34] has shown that LBR is an adapted process for the treatment of spent animal bedding since an average of 89% ± 11% of the bio-chemical methane potential (BMP) was reached after 60 days of operation.

2.5.1 Significance of cow dung as a co-substrate to digest with rice straw

Lignocellulosic biomass such as rice straw does not naturally contain methanogens; therefore, a methanogens rich inoculum is required for HS-AD of lignocellulosic biomass. Water content is critical in facilitating mass transfer. In HS-AD, the high solids content leads to slow mass transfer between microbes and substrate mixture. This will lead to slow methane production and low methane yield. Rice straw has high carbon content and a high C:N ratio, cow dung has a low C:N ratio [10]; thus obtaining proper C:N ratio, acquiring required moisture content, nutrient content, buffering capacity and methanogens within the substrate mixture, cow dung (CD) can be used as co-substrate to co-digest with RS.

3 KINETICS MODELING OF HIGH SOLIDS ANAEROBIC CO-DIGESTION OF LIGNOCELLULOSIC BIOMASS

Kinetics are important to understand the anaerobic degradation, reactor design and operation. The results of the kinetic modelling are important in estimation of treatment efficiencies and system characteristics of full-scale reactors operating at similar conditions. Kinetic parameters depend on the nature and the characteristics of feed stock. Due to the structural complexity of fat and lignocellulosic structure, enzymatic degradation of lignocellulosic biomass is much slower than pure carbohydrates and proteins. Based on this; hydrolysis can be the rate limiting step of dry anaerobic digestion of lignocellulosic materials such as rice straw.

Many models have been proposed for wet anaerobic digestion, and anaerobic digestion model no.1 (ADM1) was the mostly used structural model that describes the multiple steps of biochemical and physico-chemical process in AD [35],[36]. But ADM1 does not seem to be adapted to HS-AcoD of lignocellulosic biomass because of these reasons: (1) Original ADM1 was applied for continuous stirred tank reactor (CSTR), but complete mixing of solid substrate or homogenized substrate mixture can't be obtained for the HS-AcoD of lignocellulosic biomass in a batch reactor, (2) mass transfer coefficients are applied to liquid AD, can't be used for HS-AcoD because of soluble compounds are transported by diffusive convection that may cause to create pockets of local environment (pH, VFAs, dissolved H_2 and CO_2) which can inhibit the methanogenesis due to the low moisture content and poor mixing efficiency within dry digester [30],[37].

Modified Gompertz model as shown in Eq.3.1 was considered for the mathematical modeling. Modified Gompertz model can be used to estimate the cumulative biogas volume or cumulative methane volume of a batch AD reactor. This model is based on the hypothesis that the rate of biogas yield follows a sigmoid curve (it may not increase linearly with time due to the lag phase).

The Gompertz model, which was set on an exponential relationship between specific growth rate and population density, was originally developed to fit human mortality data and it has also been used to predict organ growth (Causton, 1977). Gibson et al. (1987) modified the Gompertz model to a function that describes cell density during bacterial growth periods in terms of exponential growth rates and lag phase duration. An assumption of methane production rate in a batch digester corresponding to the specific growth rate of methanogenic bacteria led to equation (Lay et al., 1998). This equation has been identified as a good empirical non-linear regression model and commonly used in the simulation of methane production [38],[39].

$$M(t) = M_{max} * e^{-e\left[\left(\frac{\mu_{max} * e}{M_{max}}\right)(\lambda - t)+1\right]} \quad \text{(Eq.3.1)}$$

Where;

$M(t)$ = Cumulative methane yield at digestion time t (ml CH_4/g VS)

M_{max} = Methane potential of substrate (ml CH_4/g VS)

μ_{max} = Maximum methane production rate (ml CH_4/g VS/day)

λ = Lag phase (day)

t = Batch duration (day)

e = 2.7183

4 MATERIALS AND METHODS

4.1 Introduction to materials and methods

Experimental methods followed in this study can be divided into three main sections: (1) preliminary experiments were conducted to characterize rice straw and cow dung and to evaluate biochemical methane potential (BMP) of both substrates, (2) batch experiments were conducted in 2L reactors to identify the effect of inoculum source and identify the most critical parameters for the HS-AcoD of rice straw and cow dung, (3) batch experiments were conducted in 6L Leach bed reactors with the application of leachate recirculation to evaluate the effect of identified critical parameters and to define optimum values of the critical parameters for HS-AcoD of RS and CD. The experimental methods followed and parameters that were considered during each experiment are shown in Figure 4.

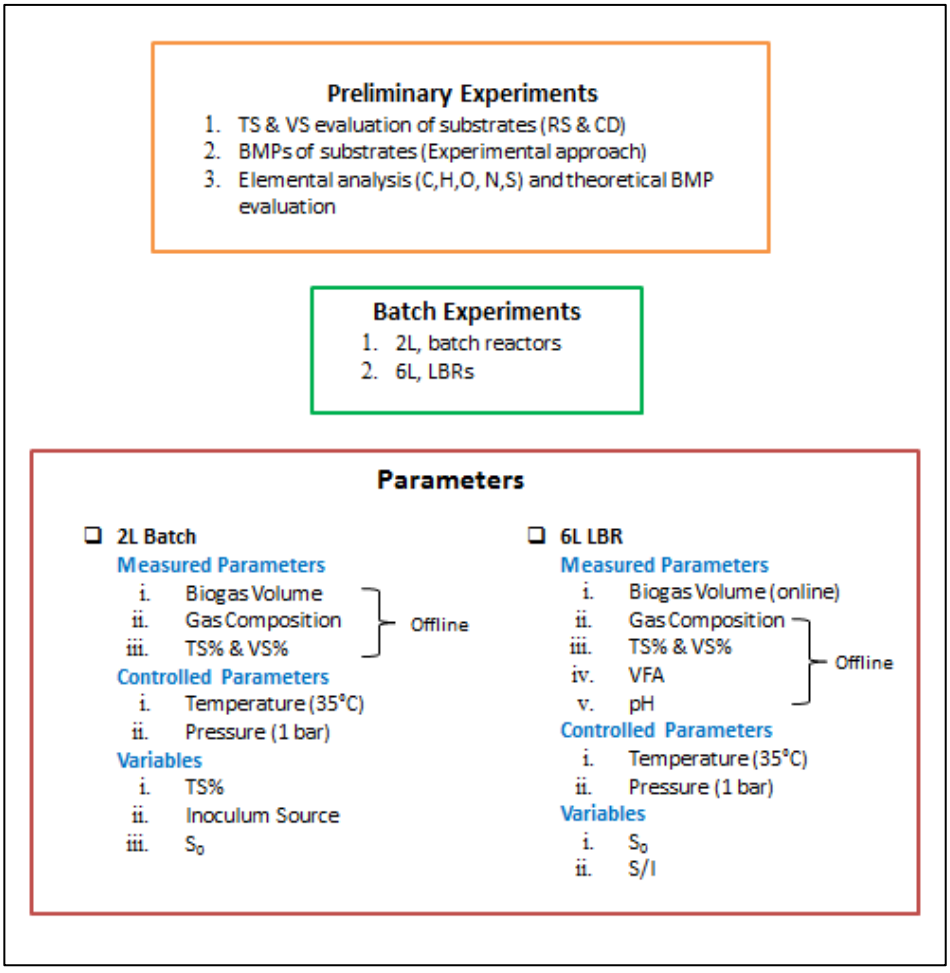

Figure 4. Experimental methods and parameters

4.2 Substrates used

Rice straw (RS) and Cow dung (CD) were used as substrates in this study. They were collected at 2 different farms located in South of France. Rice straw was grinded to 30-40 mm pieces with a 3 kW Blick shredder BB230. The solids composition of the two substrates and the elemental composition (mass %) of RS are presented in Table 3.

Table 3.Results from characterization of rice straw and cow dung

Substrate	TS concentration (%)	VS Concentration (%)	VS/TS %	C%	H%	O%	N%	S%	C: N[a]
RS	92.82 ± 0.29	79.42 ± 0.44	86 ± 0.24	38.5	5.8	41.8	0.4	0.5	90/1
CD	12.28 ± 0.21	10.62 ± 0.16	86 ± 0.23	ND	ND	ND	ND	ND	ND

[a]Calculated using elemental composition

ND – not determined

4.3 Experimental setups and operation

4.3.1.1 Experimental BMP evaluation (Protocol)

Biochemical methane potentials (BMPs) of rice straw and cow dung were evaluated by following a procedure based on the protocol described by Angelidaki et al. (2009) [40] . This method is based on the measurement of methane production in a closed bottle in which, a known amount of substrate and inoculum are put in contact. The culture medium comprises of: (i) 6.9 ml of macro elements solution which contains NH_4Cl, KH_2PO_4, $MgCl_2.6H_2O$, $CaCl_2.2H_2O$ in 26.6g/l, 10g/l, 6g/l, 3g/l concentrations respectively; (ii) 4 ml of micronutrients solution which contains $FeCl_2.4H_2O$, $CoCl_2.6H_2O$, $MnCl_2.4H_2O$, $NiCl_2.6H_2O$, $ZnCl_2$, H_3BO_3, Na_2SeO_3, $CuCl_2.2H_2O$, $Na_2MoO_4.2H_2O$ in 2 g/l, 0.5g/l, 0.1g/l, 0.1g/l, 0.05g/l, 0.05 g/l, 0.05 g/l, 0.04g/l, 0.01g/l concentrations respectively and; (iii) 20.8 ml of 50g/l bicarbonate buffer solution ($NaHCO_3$). Substrate samples were put into the 600 ml glass bottles and culture medium was added to each bottle up to 400 ml while maintaining inoculum concentration within the bottle at 4 g VS/L and initial substrate to

inoculum ratio (S/I) at 1:1 as VS basis. After purging N_2 gas to the head space, each bottle was sealed with rubber stoppers together with aluminum crimps and kept in a shaking machine at 90 r.p.m and at 35 $^{\circ}$C temperature. Eliminate the methane produced by inoculum, bottles only with culture medium (control) also kept in the same shaking machine at the same conditions. At different time intervals, pressure readings of the head space of bottles were taken to indirectly measure the biogas volumes produced. Biogas composition of the head space was analyzed using gas chromatography (GC perkin 580). Then degassing of the reactor bottles was performed to equalize the pressure inside the head space of the bottle to atmospheric pressure. Degassing was performed through water, as to be verified not entering atmospheric air into the bottle. After the degassing, again a pressure reading of the headspace was taken and finally the bottles were kept inside the shaking machine and the same procedure was followed for 60 days. Figure 5 shows the main steps in BMP test.

Number of CH_4 moles produced can be calculated as follows,

$$\Delta N_{(j)} = \left[y_{(j)} P_{(j)} \frac{V_h}{RT}\right] - \left[y_{(j-1)} P_{atm\,(j-1)} \frac{V_h}{RT}\right] \quad \text{(Eq.4.1)}$$

Where,

$\Delta N_{(j)}$ = CH_4 moles produced (moles)

$y_{(j)}$ = CH_4 percentage of current day (%)

$y_{(j-1)}$ = CH_4 percentage of previous day (%)

$P_{(j)}$ = Pressure reading (Manometer reading) of current day (Pa)

$P_{atm(j-1)}$ = Atmospheric pressure reading of previous day (after degassing the bottle) (Pa)

V_h = Head space (gas space) volume of reactor (m^3)

R = Universal gas constant (8.314 J /mol/K)

T = Room temperature (35°C / 308.15 K)

Then the CH_4 moles can be converted to CH_4 volume (ml) in Standard temperature and pressure (STP) conditions using equation 4.2

$$\Delta V_{(j)} = \left[\Delta N_{(j)} \frac{RT_0}{P_0} \right] = \left[\Delta N_{(j)} \frac{8.314 \times 273.15}{10^5} \right] \times 10^6 \quad \text{(Eq.4.2)}$$

Then the correction of actual methane volume production can be done as below,

$$V_a = V_s - V_i \quad \text{(Eq.4.3)}$$

$$V_a = V_s - \frac{V_b}{m_b} \times m_i \quad \text{(Eq.4.4)}$$

Where,

$\Delta V_{(j)}$ = CH_4volume produced (ml)

V_a = actual methane volume (ml)

V_s = raw methane volume (total methane volume produced by substrate and inoculum) (ml)

V_i = methane volume produced by inoculum (ml)

V_b = methane volume in blank sample (reactor only with inoculum) (ml)

m_b = inoculum mass in controlled (or blank) reactor(g)

m_i = inoculum mass in sample (g)

Experimental Bio-methane potential (BMP) can be calculated as below.

$$BMP = \frac{V_{CH4}(\text{ml @ STP})}{g\ VS_{sample}} \quad \text{(Eq.4.5)}$$

Where,

V_{CH4} = methane volume at STP (Nml)

g VS_{sample} = Volatile solids (VS) mass of the sample (g)

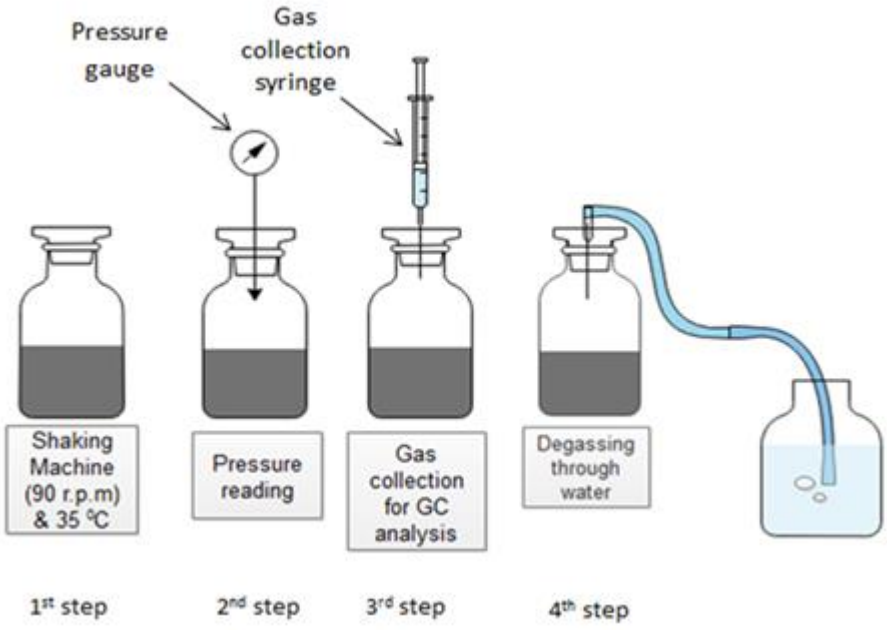

Figure 5. Main steps of BMP test

4.3.1.2 Theoretical BMP calculation of RS

For this calculation, the formula for the substrate was assumed to be $C_aH_bO_cN_dS_c$ and molar ratio, a: b: c: d: e was calculated according to the elemental composition of RS as given in Table 3.

The Buswell equation([17],[41]) mentioned below (Eq.4.6) can be used to estimate theoretical methane volume by assuming 100% degradation.

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right) H_2O \rightarrow \left(\mathbf{\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}}\right) \mathbf{CH_4}$$

$$+ \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right) CO_2 + dNH_3 + eH_2S$$

$$BMP_{theoretical} = \frac{\left(\mathbf{\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}}\right) \times 22.4 \times 10^3}{(a\times12)+b+(c\times16)+(d\times14)+(e\times32)} \quad \text{(Eq.4.6)}$$

Calculated Theoretical BMP = 428 NmL/g VS

4.3.2 Methane based biodegradability

Methane based degradability is defined as follows,

$$\text{Methane based biodegradebility} = \frac{(\text{Experimental BMP})}{(\text{Theoretical BMP})} \times 100\% \qquad \text{(Eq.4.7)}$$

Therefore, for the rice straw;

Methane based biodegradability = (254/428) ×100%

= 60%

4.3.3 Pure batch reactors

All batch experiments were carried out in 2L glass bottles with a working volume between 0.8 and 1.1 L. The bottles were sealed after the substrate mixtures were introduced, and maintained at 35°C without agitation. All batch conditions were duplicated. Each bottle was connected to a gas collection bag (Tedlar bag, Zefon International, Inc.) in order to collect and store the biogas produced (Figure 6). Biogas volume in gas collection bags was measured regularly using water displacement method. For all reactors, parameters monitored were biogas volume and composition and at the end of the digestion period, TS and VS concentrations of the digestate and VFAs concentration were determined. Different experimental conditions, described here after, were used in three sets of experiments.

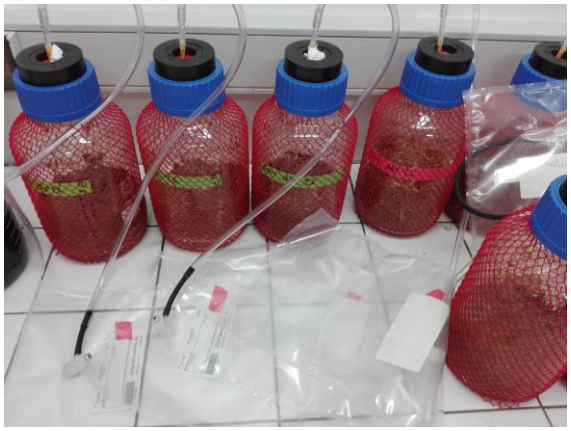

Figure 6. Batch reactors with gas collection bags

4.3.3.1 Batch experiment No.1

In the first batch experiment reactors which have substrate mixtures of four different initial TS contents: 36, 29, 22 and 15% were used. To reach the different TS% within the reactors, two mixing strategies were applied. In the first strategy, only rice straw and cow dung were mixed together to obtain the relevant TS% in the mixture. As a consequence, the quantity of CD was increased to reduce the initial TS content of the mixture. In the second strategy the quantity of RS and CD was identical in all the batch reactors, and water was added to modify the TS content. The respective quantities added are presented in Table 4. It is important to state that CD was the only source of methanogenic inoculum in this experiment.

Table 4.Quantities of substrate added for the 1st batch experiment

	Mixing strategy-1 (E-1)				Mixing strategy-2 (E-2)			
Initial TS concentration of mix (%)	36	29	22	15	36	29	22	15
RS (g)	50	50	50	25	50	50	50	50
CD (g)	120	188	355	650	120	120	120	120
Water (g)	-	-	-	-	-	40	107	236
S_0 (g VS straw/ kg mix)	232	166	98	29	232	188	143	97

4.3.3.2 Batch experiment No.2

In the second experimental test, sludge from Up flow anaerobic sludge blanket (UASB) reactor which had 2.8% TS and 2.6% VS concentrations was used as an external inoculum source. Reactors which have substrate mixtures of four different initial TS contents: 7, 10, 15 and 20% were used. RS, CD, inoculum and water were mixed together to obtain these TS percentages within the bottles (Table 5). This table also reports the initial VS concentration (S_0) of RS, which constitutes the main biodegradable substrate in the batch reactors.

Table 5.Quantities of substrate added for the 2nd batch experiment

TS concentration of mixture (%)	20	15	10	7
RS (g)	50	50	50	50
CD (g)	175	175	175	175
Inoculum (g)	141	141	141	141
Water (g)	-	122	366	686
S_0 (g VS straw/ kg mix) of mixture	108	81	54	38

4.3.3.3 Batch experiment No.3

Under third experimental study, the re-use of digestate generated from a previous batch experiment as an inoculum source, was investigated. In this experiment, three consecutive batches were carried out with the quantities shown in Table 6. Batch-1 corresponds to the batch with 15% TS of batch experiment No.1 (see paragraph 4.3.3.1). Digestate recovered at the end of digestion period from the two 15% TS bottles in first batch, was used as inoculum source for the second batch and digestate recovered at the end of digestion period from the bottles in second batch was used as inoculum for the third batch (Figure 7). In the first and second batches initial TS concentration of the mix were almost same (15% TS for Batch-1 and 16%TS for Batch-2 respectively) and rice straw VS concentration of the mix was same (29 g VS rice straw/kg mixture). In the third batch both TS concentration and rice straw VS concentration of the mix were increased to 20% and 55 g VS rice straw/ kg mixture, respectively.

Table 6.Quantities of substrates added for successive batches in experiment No.3

	Batch-1	**Batch-2**	**Batch-3**
RS (g)	25	25	50
CD (g)	650	350	480
Solid Digestate (g)	-	300	190
TS concentration of mixture (%)	15	16	20
S_0 (g VS straw/kg mixture) of mixture	29	29	55

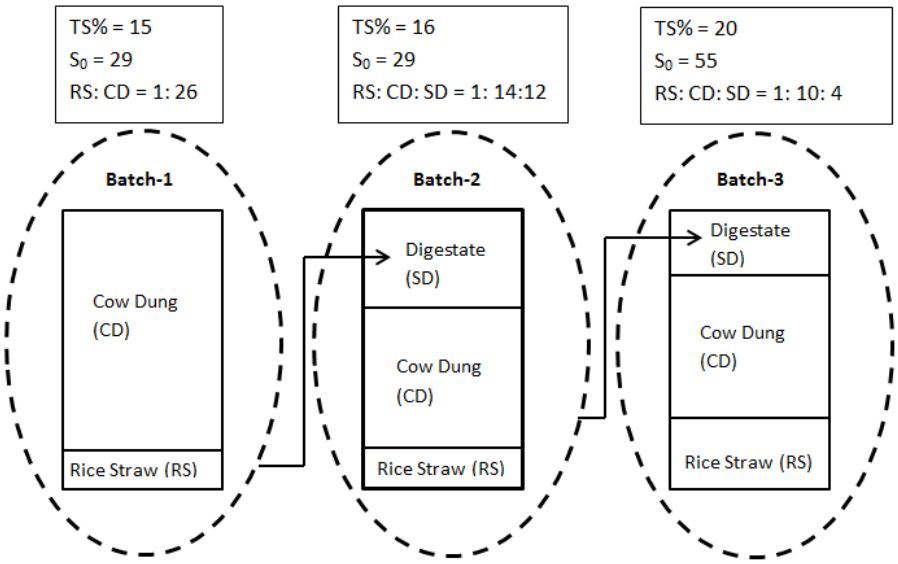

Figure 7. Strategy developed re-using digestate as inoculum

4.3.4 Leach-bed batch reactors

Four leach-bed batch reactors (LBRs) made of glass with a total volume of 6 L, 14.5 cm internal diameter and 43 cm internal height were used (Figure 8 & Figure 9). In each reactor, the solid and liquid fractions were kept apart by a sieve (1 mm holes) placed at 10 cm up from the bottom of the reactor, which enabled the leachate to be retained in the lower volume at the bottom of the reactor. Peristaltic pumps were connected to each reactor to recirculate leachate. A tube connecting the headspace and the volume under the mesh was added in order to equalize the pressure between two compartments (Figure 8). Each reactor was connected to a water bath which was maintained at 35°C and water was being circulated through the jacket. Ritter gas flow meters (MGC-1 V3.1 Milligascounter) were connected to automatically measure the biogas volumes and gas collection port was included in each reactor to collect biogas for composition analysis. Leachate collection port was connected to leachate stock of each reactor in order to collect leachate to measure pH and analyze VFA concentration [34].

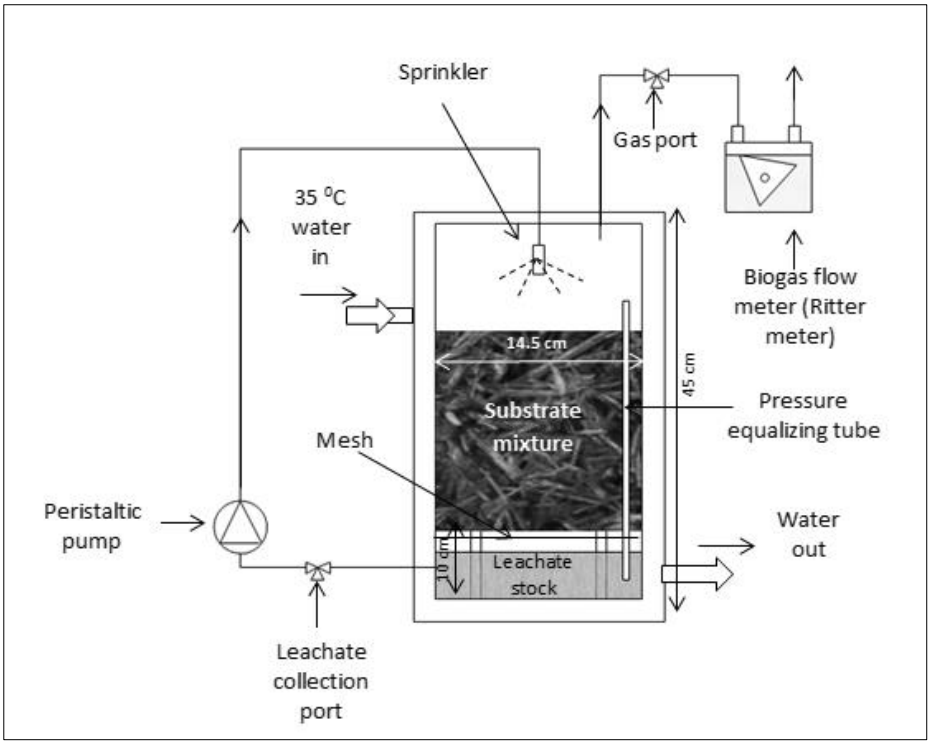

Figure 8. Schematic diagram of Leach bed batch reactor setup

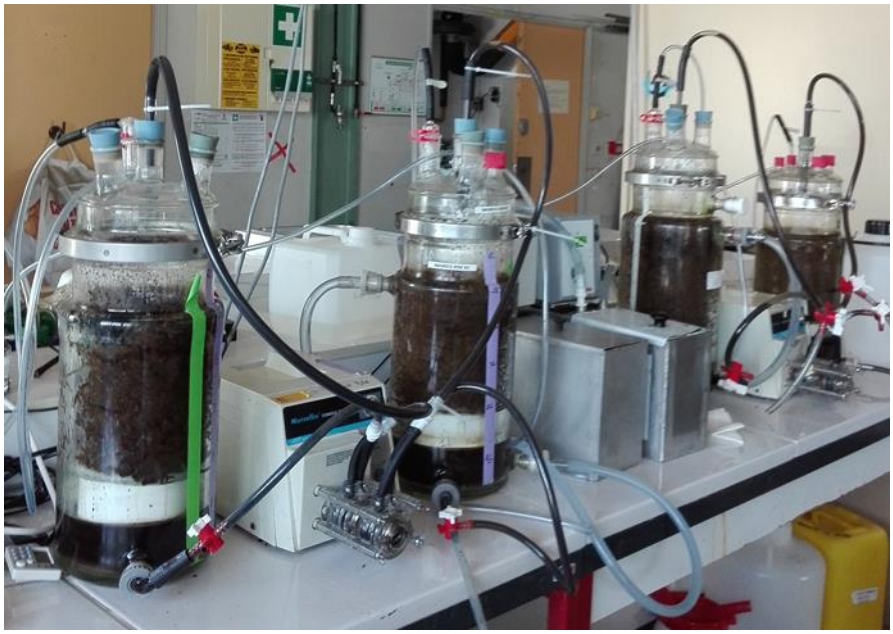

Figure 9. Leach bed batch reactors Setup

4.3.4.1 Inoculation and leachate preparation

Solid and liquid fractions of digestate collected from an actively running batch digester in a farm, which used lignocellulosic substrate and cow manure as a feedstock, were used as inoculum and for liquid recirculation in the first LBR experiment. Characteristics of solid and liquid fractions of digestate are shown in Table 7. Leachate used for recirculation within the LBRs was prepared by mixing liquid fraction of digestate with water at a ratio of 1:1.

Table 7.Results from characterization of solid and liquid fractions of digestate

Parameter	**Initial Value**
TS concentration of solid fraction	17 %
VS concentration of solid fraction	11.8 %
TS concentration of liquid fraction	4.5 %
VS concentration of liquid fraction	2.6 %
Alkalinity of liquid fraction	17.9 g $CaCO_3$/L
VFA concentration of liquid fraction	0.766 g/L
pH of liquid fraction	8.5

4.3.4.2 Operating conditions

Two setups of experiments were carried out using LBRs. In the first setup of experiment, four 6L leach-bed reactors were used. First three reactors were operated with different rice straw quantities in order to increase the initial concentrations of rice straw (S_0). Rice straw, cow dung and solid digestate were mixed together to obtain rice straw concentrations in each reactor of 30, 36 and 47 g VS rice straw/ kg mixture and initial TS concentrations around 12.5%. Substrates to inoculum (S/I) ratios in VS basis (rice straw VS mass / solid digestate VS mass) were 0.7, 1 and 2, respectively (Table 8). The fourth reactor was operated only with digestate and served as control reactor.

Table 8.Quantities of substrates, digestate and water added for first and second LBR experiments

	1st LBR experiment (P-1)				**2nd LBR experiment (P-2)**			
Reactor	**1-(1)**	**1-(2)**	**1-(3)**	**1-(4) (control)**	**2-(1)**	**2-(2)**	**2-(3)**	**2-(4) (control)**
RS (g)	132	156	205	0	205	250	300	0
CD (g)	450	600	778	0	771	900	1000	0
Solid digestate (g)	1262	1044	686	2500	779	950	1139	2865
Liquid digestate (g)	820	830	900	500	850	760	615	500
Water (g)	820	830	900	500	850	760	615	500
S_0 (g VS_{RS}/kg)	30	36	47	0	47	55	65	0
Global TS content (%)	12.3	12.5	12.7	12.8	13	14.7	16.9	10.8
S/I (VS mass rice straw/VS mass solid digestate)	0.7	1	2	-	2	2	2	-

After filling each reactor with a prepared substrate mixture, 7 kg of weight was applied for 10 minutes to the top of substrate mixture in order to reduce its bulk volume. Then the reactors were closed and leachate was recirculated at the top of the reactors using peristaltic pumps. Initially, leachate recirculation was conducted for 5 minutes in order to saturate the bulk with water. No further leachate recirculation was performed for the blank reactor. Leachate recirculation was performed automatically for other three reactors for 2 minutes at a rate of 600 ml/min every 12 hours. After 55-60 days all the reactors were opened and solid digestate and leachate were collected.

Solid digestate from each reactor except the blank reactor were mixed together to prepare inoculum for the second LBR experimental setup. For the first three reactors, RS, CD and Solid digestate recovered from first LBRs experiment were mixed together to obtain initial rice straw VS concentration S_0 values of 47, 55 and 65 g VS rice straw/ kg mixture respectively, and initial TS concentrations in the range 13-17%. S/I ratio (VS basis) was kept at 2 for first three reactors (Table 8). Remaining

digestate was put in fourth reactor and it was operated as the blank reactor. Operation, data monitoring and analyzing were performed similarly to the previous experimental setup.

4.4 Analytical methods and experimental protocols

For all the experiments biogas composition was analyzed using gas chromatography (GC perkin 580). Argon gas was used as the carrier gas for the GC.

For batch experiment No.1 and batch experiment No.2, VFAs of the digestate were measured at the end of digestion period, after preparation of the digestate samples by dilution with water in order to obtain liquid fraction. For batch experiment No.1, 10 g of digestate from failed reactors (bottles which didn't continuously produce biogas) were taken and diluted up to 10% TS concentration by adding water. For batch experiment No.2, 10 g of digestate from failed reactors were taken and diluted up to 7% TS concentration by adding water. Then liquid fractions were filtered out and centrifuged for 2 min at 6000 r.p.m in EBA 200 centrifuge equipment to obtain samples for VFA analysis. Internal standard solution (2-Ethyl-butyric acid) was added to centrifuged sample in 1:1 ratio. VFAs were analyzed using gas chromatography (GC perking 580) using N_2 as the carrier gas. For LBRs pH values of the leachates collected from leachate collection ports were measured using pH probe.

4.4.1 Total solids and Volatile solids percentage calculation

Total solids (TS) and volatile solids (VS) concentrations, alkalinity of substrates and digestate were measured according to the APHA standard methods (APHA, 2005) [42]. Triplicates of each substrate samples were taken to obtain average values of TS and VS percentages. Elemental composition (C, H, O, N, and S) of rice straw was determined using Vario-MICRO analyzer.

$$\text{g\_TS/g\_Sample} = \frac{W_3 - W_1}{W_2 - W_1} \quad \text{(Eq.4.8)}$$

$$\text{g\_VS/g\_Sample} = \frac{W_3 - W_4}{W_2 - W_1} \quad \text{(Eq.4.9)}$$

Where;

W_1 – weight of the dried crucible (g)

W_2 – weight of the raw sample + crucible (g)

W_3 – weight of the dried sample + crucible in 105°C oven (g)

W_4 – weight of the ignited sample +crucible in 550°C oven (g)

4.4.2 TS percentage calculation of a substrate mixture for sample preparation

Required TS percentages were obtained by mixing substrates (RS and CD) and inoculums or substrates, inoculums and water according to the different ratios.

$$\text{TS\% of mixture} = \frac{M_1x_1 + M_2x_2}{M_1 + M_2} \quad \text{(Eq.4.10)}$$

Where;

M_1 = mass of first substrate (g)

M_2 = mass of second substrate (g)

x_1 = TS% of first substrate

x_2 = TS% of second substrate

But some TS% couldn't achieve by only mixing two substrates (Here RS and CD). Therefore, to obtain those TS percentages within the mixture, water should be added to substrates.

In that case:

$$\text{TS\% of mixture} = \frac{M_1x_1 + M_2x_2}{M_1 + M_2 + M_w} \quad \text{(Eq.4.11)}$$

Where;

M_w = mass of water (g)

4.4.3 Methane volume calculation in 2L batch reactors

Produced biogas volumes in 2L batch reactors were collected to gas collection bags and volumes were measured using water displacement method.

Produced methane volume can be calculated as follows.

$$V_{(i)} = V_{bg(i)} \times X_{(i)} + V_h \times \{X_{(i)} - X_{(i-1)}\} \quad \text{(Eq.4.12)}$$

Where,

$V_{(i)}$ = Methane volume of present day (ml)

$V_{bg(i)}$ = Biogas volume of gas bag at present day (ml)

V_h = Head space volume of the reactor (ml)

$X_{(i)}$ = Methane composition of present day

$X_{(i-1)}$ = Methane composition of previous day

5 RESULTS AND DISCUSSION

5.1 Substrate characterization

The results of the characterization of rice straw and cow dung are presented in Table 3. TS and VS percentages were 92.8% and 79.4%, respectively, for rice straw and 12.3% and 10.6% for cow dung. Rice straw had a low N content with a high C: N ratio of 90:1 and cow dung had a lower C:N ratio of (15-28): 1 ([43],[44]). These ratios made it possible to keep optimum C:N ratio of (25-35):1 in the substrate mixture for HS-AcoD of rice straw ([7],[19],[45]). For co-digestion of these two substrates under HS-AcoD conditions, either rice straw with high value of TS% and cow dung with lower value of TS% or RS, CD and water were mixed in order to obtain desired TS% of the mixture.

Experimental BMP value of rice straw was 254 ± 11 NmL/g VS (Figure 10). Theoretical BMP of rice straw was calculated using Buswell formula ([17],[41]), its elemental composition is shown in Table 3 and assumed 100% degradation. Calculated theoretical BMP value was 428 NmL/g VS giving a biodegradability (ratio between experimental and theoretical BMPs) of 60%. That value was in accordance with the study of Contreras et al.(2012) [13].

BMP value obtained for cow dung was 173 ± 1 NmL/g VS,as shown in Figure 10. BMP value of rice straw was almost 50% higher compared to cow dung. Lower BMP value of cow dung compared to rice straw indicates that the biodegradable volatile solids fraction of cow dung is lower than that of rice straw, as CD had already been digested within the digestive system of cow.

After the 9^{th} day, methane production rates of both substrates tended to decrease, indicating that the rapidly biodegradable fraction of both substrates had been eliminated. Within 60 days, methane production rates reached to zero (Figure 10) indicating that the entire biodegradable fractions from added substrates were eliminated.

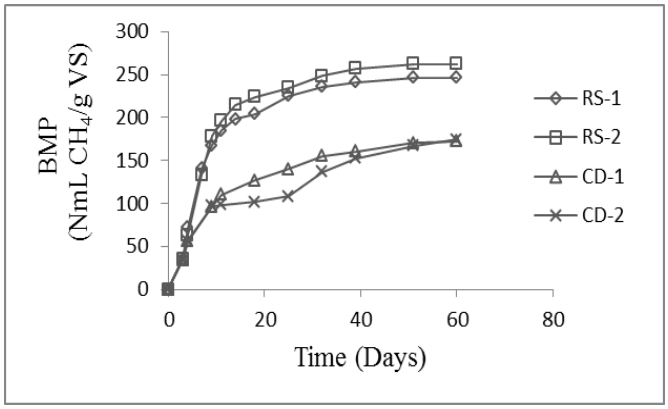

Figure 10. BMP test results for rice straw and cow dung

5.2 Co-digestion of RS and CD in batch conditions

The main objective of the results presented below is to gain better understanding of, first, the initial conditions for the substrate mix (i.e., initial TS% and initial rice straw VS concentration (S_0)) and second, of the inoculation conditions for co-digestion of RS and CD in batch reactors operated with high solids concentrations.

5.2.1 Assessment of optimal initial conditions

5.2.1.1Results from batch experiment No.1

The first set of 2L batch experiments were carried out to investigate the effect of the initial TS concentration of the mixture of substrates on the anaerobic digestion process and of the potential role of cow dung as inoculum. The desired TS concentrations of the mixture of substrates were obtained by mixing only RS and CD or, by mixing RS, CD and water in order to reduce the quantity of CD to be added. The respective quantity of RS, CD and water added are presented in Table 4. As RS contained an important fraction of quite rapidly biodegradable organic matter [13], the initial VS concentration of RS in the mixture (g VS rice straw/ kg mixture) was also calculated and presented in Table 4. Figure 11 (a) shows the evolution of cumulative specific methane volumes with time, for the eight conditions tested. The only actively biogas producing reactor was the 15 % TS reactor with only RS and CD, which contained a mixture of 25 g RS and 650 g CD. Furthermore, the same reactor also has shown a 15-day lag phase. All the other reactors did not produce

significant volumes of biogas in 80 days. In addition, Figure 11 (b) shows that increase of final VFAs concentration in all these reactors with the increase of the initial TS concentration for the two feeding strategies. As a consequence, a strong acidification of the reactors occurred, except for the batch digesters carried out at 15% TS and at the lower initial S_0 of 29 g VS straw/kg mixture.

5.2.1.2.Results from batch experiment No.2

The main objective of the second set of experiment was to evaluate another range of initial TS concentration of mixture of substrates and the effect on anaerobic digestion process of inoculating with another source of inoculum i.e., anaerobic sludge. Taking into account the previous results, the initial TS concentrations of the mixture of substrates was lowered in the range 7 to 20% were used. The quantities of substrates used to make the mixtures are presented in Table 5.

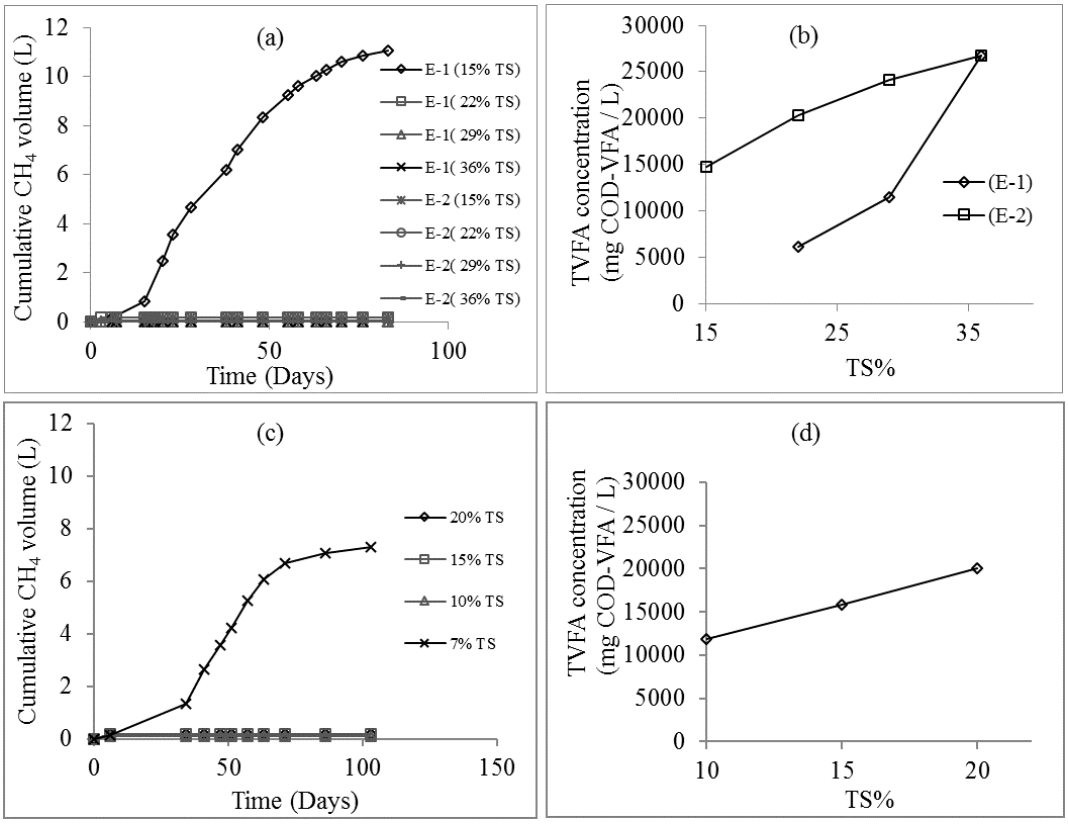

Figure 11. Cumulative methane volumes produced (a), TVFA concentration (b) in experiment no.1 and Cumulative methane volumes produced(c), TVFA concentration (d) in experiment no.2

Figure 11(c) shows that only the reactor operated at 7% TS produced biogas in the second experiment with a long lag phase of 34 days. Figure 11(d) shows the accumulation of volatile fatty acids in the digestate of the reactors of second set of experiments, which did not continuously produce biogas.

The results obtained suggest that an important parameter is the initial VS concentration of RS (S_0) in the mixture of substrates. The mode of preparation of the initial mixture of the different batches led to different initial RS contents, even for a same initial TS content (Tables 4 and 5). As an example, during the batch experiment no.1, for initial TS content of 15%, S_0 was 29 for (E-1)and 97 for (E-2) in terms of g VS rice straw/ kg mixture, for a mixture of RS and CD and for a mixture of RS, CD and water, respectively. According to the previous two experimental setups, only the reactors operated under the lowest RS concentrations (29 and 38 g VS rice straw/ kg mixture) produced biogas. High substrate concentrations, and particularly high RS concentrations, lead to digester failure by acidification. This can be identified in Figure 11 (b) and Figure 11 (d), where the digestate in failed reactors have very high total VFAs concentrations in mg COD-VFA/L.

As a consequence, during HS-AcoD of RS and CD, it is not an appropriate strategy to dilute rice straw with water in order to decrease the TS content, Instead it is preferable to use only RS and CD and eventually add a small quantity of inoculum.

Furthermore, the activity of cow dung as an inoculum source seems to be rather low as the reactors which continuously produced biogas in experiment no.1 (i.e., the reactors which has 15%TS content and initial rice straw concentration of 29) had a 15 day lag phase and quite low degradation rates.

5.2.2 Co-digestion of RS and CD in successive batches

The source of inoculum plays an important role during AcoD of rice straw and cow dung [46]. Therefore, the effect of inoculum on HS-AcoD of RS and CD was investigated by digestate re-use from one batch to the next one. Three successive batches namely Batch-1, Batch-2 and Batch-3 were carried out with the operating conditions presented in Figure 7 and Table 6. As previously, S_0 represents the initial

VS concentration of rice straw in the initial mix. Digestate of the 15% TS reactor from the Batch-1 was re-used for the Batch-2 as inoculum with very close operating conditions: TS concentration varied in the range of 15-16%, respectively, under the same S_0 concentration of 29 g VS rice straw/kg mixture. For the Batch-3, higher TS concentration of 20% and higher S_0 concentration of 55 g VS rice straw /kg mixture were tested and digestate from Batch-2 was used as the inoculum.

Figure 12(a) presents the evolution of the methane production and Figure 12(b) presents the evolution of specific methane yield with time. Methane yields were calculated on the basis of the quantity of volatile solids added to each reactor to normalize this parameter in each reactor. Methane production in each reactor was corrected by deducting the contribution of methane production by the digestate. Therefore, VS added in the calculation consist only of RS and CD.

In the Batch-2, almost 50% of cow dung of the initial mixture was replaced by digestate from Batch-1. The re-use of digestate as an inoculum source positively affected the anaerobic digestion performance. Indeed, the lag phase period was drastically reduced from 15 days to almost 0 days, and a 103% increase in the methane yield was achieved.

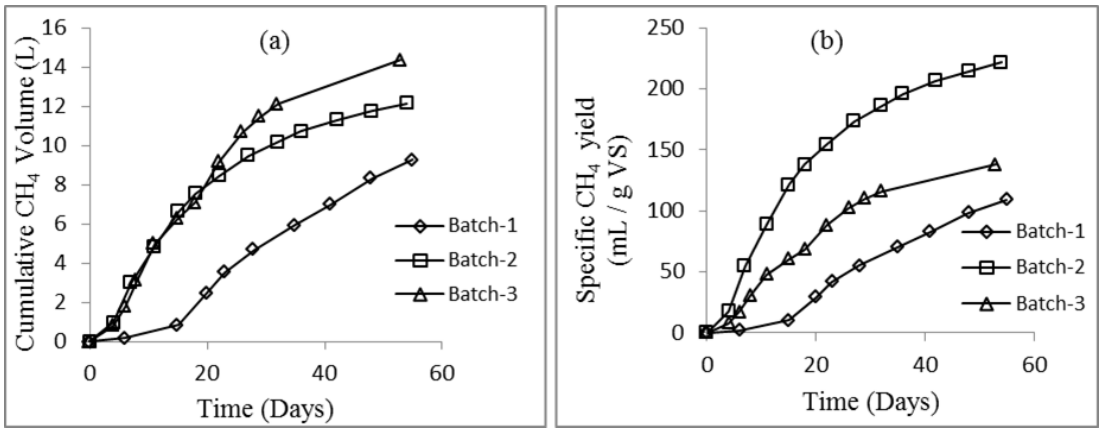

Figure 12. Evolution of cumulative CH4 (a) and specific methane yield (b) of 2L batch reactors with time when digestate re-use strategy was applied

From the individual BMP values of RS and CD, the expected cumulative methane volume and methane yield of the Batch-2 were calculated (11.1 L and 202.7 mL CH_4/ g VS, respectively) and compared to the experimental values (12.1 L and

221.8 mL CH_4/ g VS, respectively). The difference between the expected and actual values was less than 10% for both parameters suggesting that all the added biodegradable VS had been eliminated in the experimental conditions of the Batch-2.

In the Batch-3, both TS% and S_0 were increased compared to Batch-1 and Batch-2 reactors. However, only an 18% increase in the total methane production was observed compared to the Batch-2. As a consequence, the methane yield was much lower in Batch-3 compared to Batch-1. Calculated value of expected methane yield for the Batch-3 using individual BMPs was 203.6 mL CH_4/g VS and experimental value was 137.7 mL CH_4/g VS which indicates that 68% of the biodegradable organic matter has actually been removed. This lower methane production in the Batch-3 can be explained by two factors: the higher TS concentration of the initial mixture and the higher initial RS concentration (S_0). It was shown in the first part that the initial concentration in rice straw is a critical parameter and in the third batch it seems that this concentration was too high (55 g VS rice straw /kg mixture). Furthermore, data from the literature suggests that increasing the TS concentration of the digestate could lead also to a negative impact on the performances of anaerobic digestion. Abbassi-Guendouz et al.(2012) [1] investigated the effects of TS on AD by using cardboard as a substrate, and found that the VS based methane production rate continued to decrease when the reactor TS increased from 10% to 35%. Similarly, Motte et al. (2013)[47] used wheat straw as a substrate and also showed a declining VS based methane production rate as TS increased from 15% to 25%. Xu et al (2014) [48] also investigated the effect of TS content on anaerobic digestion of rice straw and found that there was a TS content threshold between 15% and 20% for AD of lignocellulosic biomass, below which the methane production rate tends to increase with TS, and decreases when exceeding it. TS content is a key parameter that affects the mass transfer between gas-liquid-solid phases in HS-AcoD, which affects the rate of substrate degradation and accumulation of inhibitors such as VFAs ([1],[48],[49]). TS content has been assumed to affect the hydrolysis rate constant, maximum microbial growth rate and diffusion constant as well [48].

5.3 Co-digestion of RS and CD in Leach Bed Reactors

As shown in the previous experiments, one of the main problems associated with co-digestion in batch at high solids content is the production of VFAs in high concentrations which causes pH drop, inhibition of the microbial population, decrease of methane production and finally leading to digester failure. Furthermore, high TS content implies high heterogeneity which makes it difficult to distribute microorganisms and nutrients in the substrate mixture.

Leachate re-circulation is a strategy that was used in batch high solids anaerobic digestion, in reactors called Leach Bed Reactors (LBRs), in order to control VFAs production at the beginning of a batch. From a physico-chemical point of view, leachate recirculation is used to increase the moisture content of the bulk of substrate, thus improving mass transfer and access to organic matter ([24],[25]), to leach out the produced VFAs, to dilute inhibitor compounds and to increase the buffer capacity of the medium. From a biological point of view, increasing moisture content improves the growth of microorganisms [35]. Therefore, that will result in higher biodegradation of lignocellulosic substrates. Furthermore, re-use of digestate permits to improve the start-up of a reactor as it makes it possible to recycle biomass already acclimated to the substrate.

In this work, two sets of experiments were carried out to further investigate both the effect of leachate re-circulation and the initial RS concentration on high-solids co-digestion of RS and CD in LBR.

Table 8 shows the substrates quantities added in each set of experiment. In the first set of LBR experiments, three conditions were tested with initial rice straw VS concentrations (S_0) of 30, 36 and 47 g VS rice straw/ kg mix. In the second set of experiments initial rice straw VS concentrations of 47, 55 and 65 g VS rice straw/ kg mix were tested. The maximum reaction time was fixed at 60 days for all conditions. In these experiments, the quantities of RS and CD were increased but the proportion of RS and CD in terms of VS remained very close with rice straw VS representing on average 67.4 ±1.3 % for the 6 mixtures used. As a result, the cumulated volume of

methane produced from RS represented 75.2 ± 1.1 % of the expected total volume of methane produced and the average expected methane yield of the mixes was 228 ± 1 mL CH_4/g VS. The only difference between the two sets of experiments is the digestate used for inoculation. In the first experiments, digestate was sampled from an industrial scale LBR whereas for the second experiment, digestate collected at the end of the first experiment was used.

5.3.1 Methane production from LBR experiments

Figure 13(a) and 13(b) present, respectively, the total volume of methane produced by the LBRs and the methane yield as a function of the initial VS concentration of rice straw (S_0). Initial RS concentration was chosen to plot these curves for three reasons: (i) 75% of methane is produced from RS, (ii) organic matter of RS has the highest degradation kinetics and (iii) it was shown previously in batch mode that S_0 is a critical parameter for reactor acidification and failure.

Figure13 (a) shows that total methane production increased when increasing S_0 values. However, this increase was not proportional to the additional quantity of VS added. For example; if the two extreme values are compared, only 50% additional CH_4 was produced when the initial RS quantity was doubled. As a consequence, the methane yield decreased when S_0 was increased as presented in Figure 13 (b). For the lowest quantities of substrate added, the methane yield observed in LBRs was close to the BMP of the mixtures calculated from the BMP of RS and CD (228 ± 1 mL CH_4/g VS) but for the highest substrate load, the methane yield was only 70% of the expected value, indicating that in two months, all the added organic matter was not degraded.

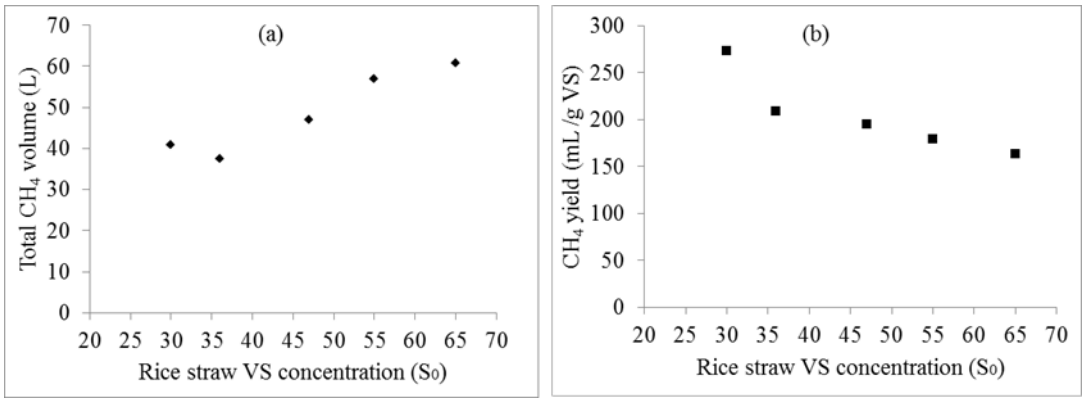

Figure 13. Evolution of the total volume of CH_4 produced (a) and of the specific methane yield (b) over the initial RS concentration within the reactor

5.3.2 VFAs concentration, pH and alkalinity

Total volatile fatty acids concentration (TVFA) and pH were measured regularly in the liquid fraction (leachate) of each reactor and Figure 14 presents the evolution of total VFA concentration and pH throughout the digestion time period. VFAs production was high at the beginning of the batches with VFA accumulation in the leachate indicating that methanogenesis was slower than acidogenesis. The maximum total VFAs concentration in each reactor reached after 5-6 days of operation and ranged between 6000 mg-COD VFA/L and 12000 mg-COD VFA/L (Figure 14 (a) and14 (c)). At the same time, pH decreased as shown in Figures 14 (b) and 14 (d) but remained always above 6.6. This is due to the high alkalinity of the mix of digestate and substrates (above 8.3 g $CaCO_3$/L).

In the following days, VFA concentrations of the leachate reduced rapidly to reach 0 before day 20. Several authors have reported similar results., Mussoline et al.(2012) [50] have observed an initial peak of VFA in terms of acetic acid (3375 mg HAc/L) which occurred at the beginning of the experiment on dry AD of rice straw and piggery waste water. In this study, on day 57, VFA concentration fell below 200 mg HAc/L. Similar observations could be found in a study on hybrid solid anaerobic digestion of organic fraction of municipal solid waste conducted by Massaccesi et al. (2013) [51]. From these results, stable operation of the reactors and optimization to the leachate recirculation time period can be identified as those are important facts when these types of reactors are operated in large scale.

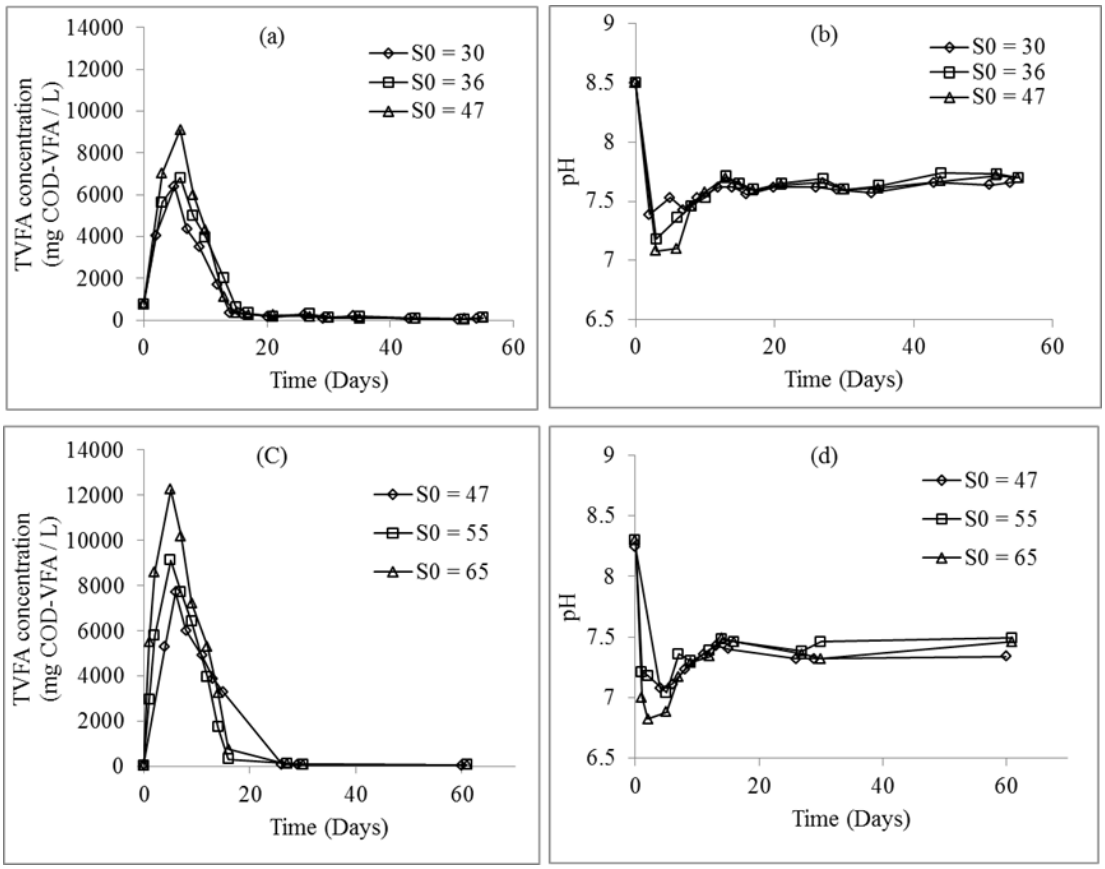

Figure 14.Total VFA and pH variation in first (a & b) and second (c & d) LBR experiments with time

5.3.3 Discussion on LBRs

5.3.3.1 Effect of leachate re-circulation

In order to evaluate the influence of leachate recirculation, results obtained without recirculation in pure batch (2L bottles) and with recirculation in 6L LBRs were compared at two initial RS concentrations of 30 and 55 g VS rice straw/ kg mix (Figure 15). In terms of methane yield, the performances observed in LBRs were slightly better than those in pure batch reactors. As an example specific methane yield observed in LBR which initial RS concentration (S_0) of 30 was 273 mL CH_4 / g VS, and for the pure batch rector which had same initial RS concentration, specific methane yield was 222 mL CH_4/ gVS. Therefore it was a 23% increase of specific methane yield in LBR compared to pure batch reactor. For the initial RS concentration of 55, specific methane yield of 179 mL CH_4/g VS in LBR was a 30% increase compared to specific methane yield of 138 mL CH_4/ g VS in pure batch reactor. Specific methane production rates were slightly higher in LBR compared to batch at the beginning of the operation for a S_0 of 30 g VS rice straw/ kg mixture. However, after 1 month of operation, the slowly biodegradable organic matter had the same rates of degradation. At S_0 of 55 g VS rice straw/ kg mixture, rates were quite close except in the period day10-day20 where they were lower for the batch without recirculation, probably linked to some partial inhibition in that period due to VFA production and pH drop. After day 20, rates were again very close. As already shown, both specific methane production rates and methane yields reduced with the increase of rice straw concentration.

In conclusion, leachate recirculation slightly improves the performances and the management of the reactors but operation in pure batch still remains possible at RS concentrations up to about 50 g VS rice straw/ kg mixture.

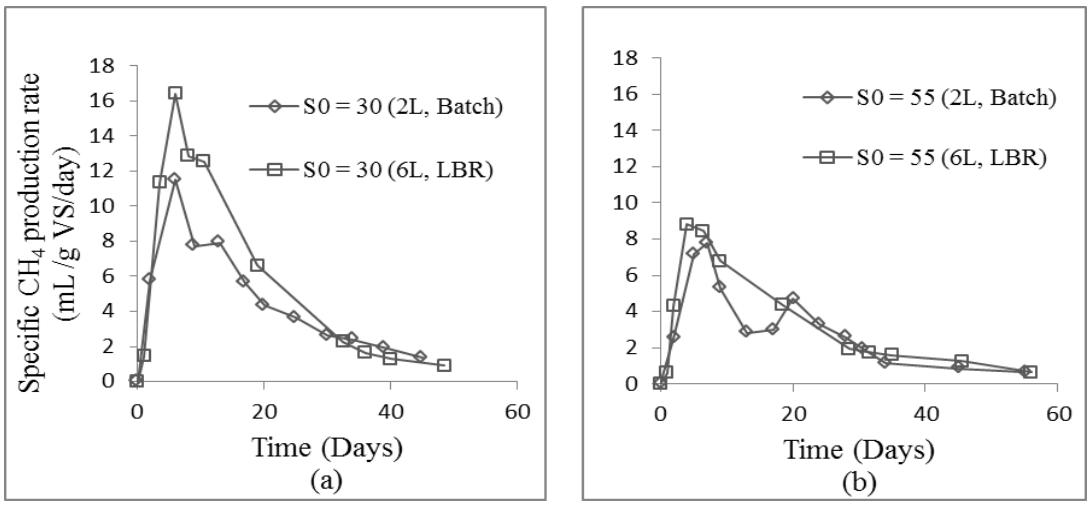

Figure 15.Specific methane production rates comparison of 2L batch reactors and 6 L LBRs that have S_0 value 30 and 55

5.3.3.2 Optimal operating conditions of LBRs

From the results obtained in the two sets of experiments with LBRs, it is possible to assess the optimal operating conditions. Figure 16, clearly demonstrates that maximum TVFA concentration increased with the increase of initial rice straw concentration (S_0) but reactors were operated safely even at the higher S_0 values used in this work due to the buffering capacity provided by the leachate and washout of VFAs. However, use of higher initial RS concentration may lead to potential inhibition at the beginning of the batch as the maximum TVFAs concentration measured at the beginning of the batch with a S_0 of 65 g VS rice straw/ kg mix was 12,200 mg-COD VFA/L and minimum pH was 6.82, that is to state, close to the critical value of 6.6 for methanogenesis inhibition ([7],[35]). Furthermore, VS degradation decreased for increasing substrate loads (Figure 13 (b)) and VS removal was only 70% of the calculated value after two months of operation for the highest substrate load. As a result, for optimal methane production in LBRs and safe operation, initial RS concentration in the mix S_0 should be around 30 g VS rice straw/ kg mixture.

The results obtained in this work suggest also that leachate re-circulation can be stopped after 15 days of operation as total VFA production has dramatically

decreased and the accumulated VFAs concentration is below 740 mg COD-VFA/L even for the highest substrate load. André et al. (2015) [52] also found that during dry AD of cattle manure, after 19 days of degradation the leachate recirculation had no effect on methane production rates.

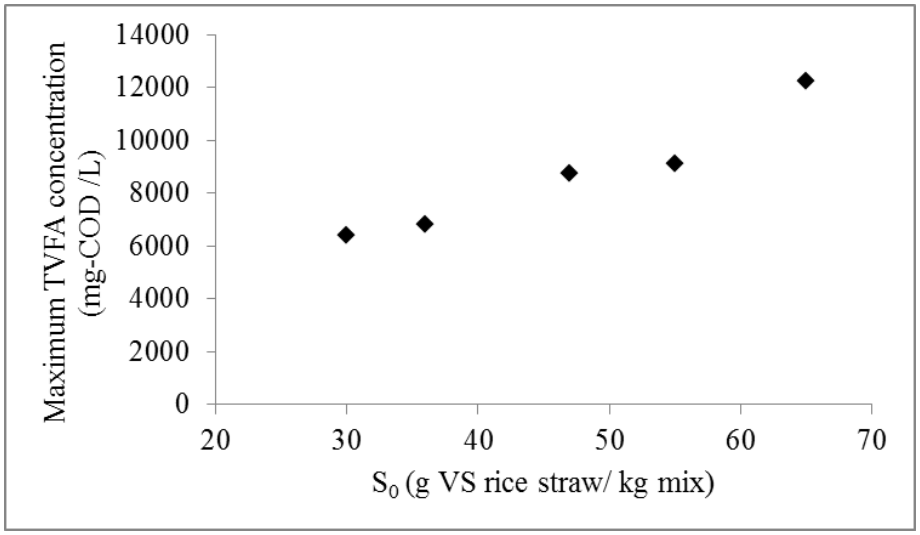

Figure 16. Maximum total volatile fatty acids concentration variation in LBRs with initial rice straw VS concentration

5.4 Parameter estimation of modified Gompertz model using experimental data of Co-digestion of RS and CD in successive batches

MATLAB curve fitting tool (Matlab R2015a) was used to estimate the parameters. Figure 17 shows the comparisons of methane productions and the modified Gompertz model fits for 55 days of digestion time period in the three batches of batch experiment no.3. It can be clearly identified that model very closely follow the experimental results (R^2 = 0.99). Therefore, the ultimate methane yield was very closely predicted by modified Gompertz model for the three batches. Table 9 shows the estimated kinetic parameters of modified Gompertz model together with experimental methane yields of each experiment. M_{max} corresponds to experimental methane yield. In Batch-1 the long lag phase (λ) of 11 days predicted from the Gompertz model indicates that the lack of inoculation efficiency of cow dung as described in the section 5.21.

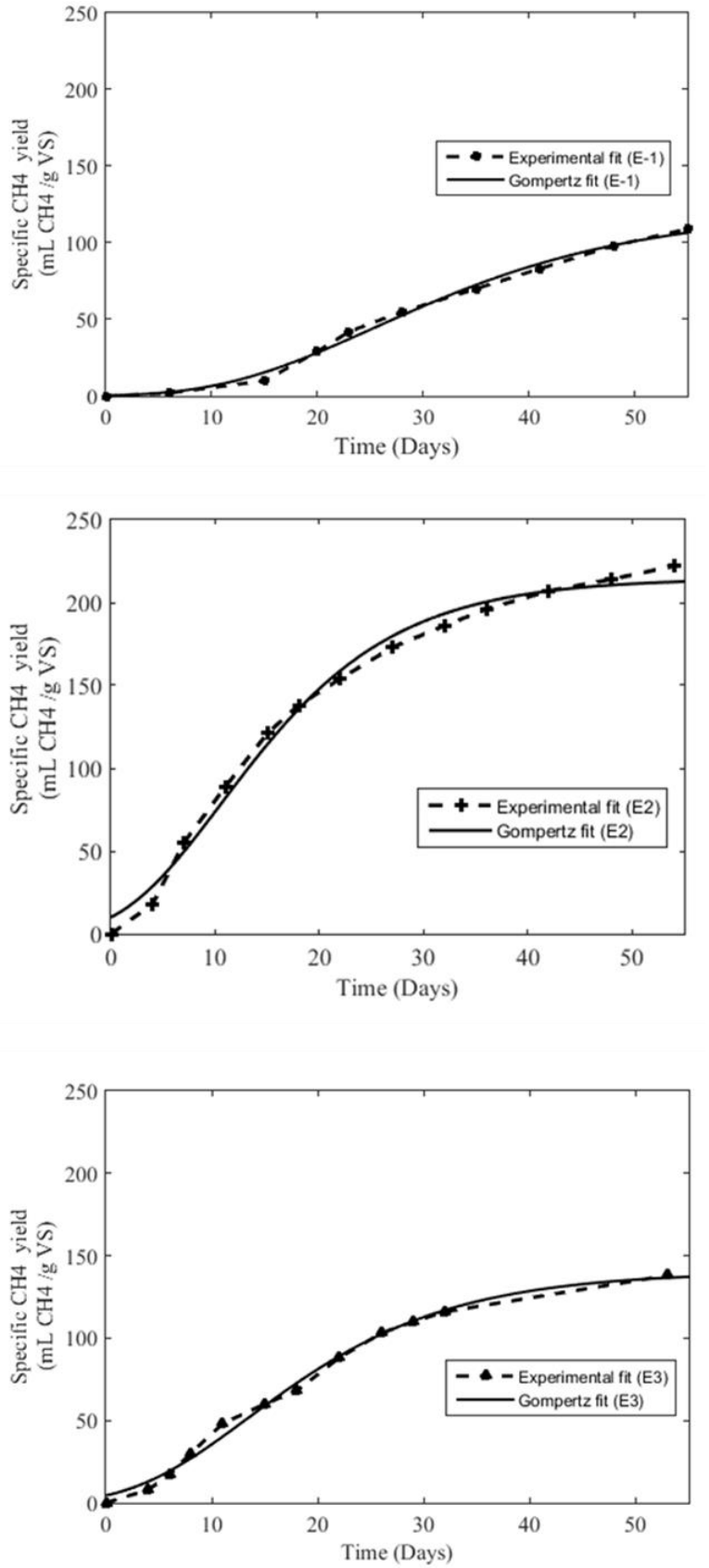

Figure 17. Evolution of methane production with modified Gompertz fits for three consecutive batch experiments

Compared to the Batch-1, in Batch-2 lag phase considerably decreased (by almost 90% reduction), and maximum methane production rate (μ_{max}) also 2.7 times greater than in Batch-2, compared to Batch-1. Although initial RS concentration S_0 and total substrate quantity of Batch-1 and Batch-2 were same, in Batch-2, introduction of solid digestate as an inoculum to the reactor has positively affected on co-digestion process kinetics [53],[46],[54].

In Batch-1 and Batch-2, TS% of the substrate mixtures were almost the same (15% TS and 16% TS respectively), but in the third experiment TS% is 33% higher than the first experiment (20% TS). In terms of initial RS concentrations, Batch-1 and Batch-2 had S_0 value of 29 and for Batch-3, S_0 value was 55. Therefore, it was a 90% increase of initial RS concentration within the reactor. Lag phase (λ) was almost 2 times longer than in Batch-3 compared to Batch-2. But it's not a significant increase with compared to first experiment as, λ decreased by 77% in Batch-3 compared to Batch-1. As well as μ_{max} was reduced by almost 50% in Batch-3 compared Batch-2

Table 9. Experimental results of methane yields and estimated modified Gompertz model parameters

Experiment	**Experimental methane yield (ml/g VS)**	**Experimental maximum methane production rate (ml/g VS/d)**	**Modified Gompertz parameters (modeled parameters)**			**R^2**
			M_{max} (ml/g VS)	**λ (d)**	**μ_{max} (ml/g VS/d)**	
Batch-1	109	4.18	121	10.98	3.09	0.9941
Batch-2	222	12.3	214.7	1.13	8.33	0.9913
Batch-3	138	6.46	140.2	2.56	4.74	0.9936

From these results it can be concluded that kinetics were not only affected by the inoculum but also the TS content within the Batch reactor. In the third Batch (Batch-3), although the lag phase decreased considerably, ultimate methane yield and methane production rate decreased compared to second Batch (Batch-2). When TS content increases methanogenic activity can be inhibited. Abbassi-Guendouz et al. (2012) [55] also investigated the effects of TS on AD by using cardboard as a substrate and found that TS content is a key parameter that affects the mass transfer

between gas-liquid-solid phases in dry AD which affects the rate of substrate degradation and the accumulation of inhibitors such as VFAs.

Another significant result was that the experimental methane yield of the second Batch (Batch-2) was somewhat higher than the predicted methane yield. This may due to synergistic effect of the anaerobic co-digestion. This will indicate that proper inoculation and proper TS content lead to a maximum degradation of the substrates during HS-AcoD of lignocellulosic biomass.

6 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES

6.1 Conclusions

- HS-AcoD of rice straw and cow dung provides more balanced nutrient requirement for the AD process (i.e. optimal C: N ratio, macro and micro nutrients) and buffering capacity.
- However, lower efficiency and effectiveness of cow dung as an inoculum source in HS-AcoD process was identified from the Batch experiments carried out. In order to successfully operate a Batch reactor, initially rice straw and cow dung should be mixed in the ratio of 1: 26 respectively in % W/W.
- Through the re-use of digestate strategy and leachate recirculation strategy, significant reduction of lag phase (from 15 days to almost 0 days) and increase of specific methane production rates were achieved.
- For 55 days of digestion time period 103% increase of cumulative specific methane volume was achieved in 16% TS reactor (S_0 = 29) which digestate re-use strategy applied, compared to 15% TS reactor (S_0 =29) which only operated with a mixture of rice straw and cow dung.
- Operation of Leach Bed Reactors (LBRs) was a more sophisticated technique. Operation is somewhat complex in LBRs compared to simple Batch reactors.
- But with the application of leachate re-circulation, reactors with higher rice straw concentrations (S_0) can be safely operated. VFA accumulation within the substrate mixture can be avoided by flushing out, through the leachate recirculation. Also, stable pH, reduction of lag phase, increase of specific methane production rate and cumulative specific methane volume can be achieved.
- Leachate recirculation can be stopped after 15 days as reactors were reached stable conditions after this period.
- For the same rice straw VS concentrations (S_0) LBRs showed higher specific methane yields compared to simple Batch reactors which digestate re-use was applied. For example specific methane yield observed in LBR which initial RS

concentration (S_0) of 30 was 273 mL CH_4 / g VS, and for the pure batch rector which had same initial RS concentration, specific methane yield was 222 mL CH_4/ g VS. So it can be concluded that the degradation kinetics were improved in LBRs compared to pure batch reactors as maximum specific methane production rate of LBR which initial RS concentration (S_0) of 30 was 16 ml/g VS/day compared to pure batch reactor which has the same initial RS concentration having maximum specific methane production rate of 12 ml/g VS/day.

- Higher biodegradability of rice straw was achieved with the application of digestate re-use and leachate re-circulation strategies. And also, from these strategies it was able to achieve successful and stable reactor operation with much higher TS% (above 15% TS) and higher VS concentration of rice straw (i.e., S_0values >29).
- However specific methane yields and specific methane production rates were reduced with the increase of lignocellulosic concentration, particularly initial RS concentration within the reactors. Therefore, further investigations should be done to find the threshold values (i.e. for TS% and S_0) of HS-AcoD of lignocellulosic biomass and to increase the performance of HS-AcoD of lignocellulosic biomass.
- Modified Gompertz model is a statistical model, but it can be used to closely predict the ultimate methane yield and lag phase period of the dry anaerobic co-digestion process of lignocellulosic biomass.
- For the three experiments modified Gompertz model has very closely complied with the experimental data with Root mean square error (R^2) value of 0.99. From the estimated parameters of λ and μ_{max}, positive effects on kinetic parameters by the introduction of solid digestate as inoculum to the dry AcoD system can be identified and also negative effects on kinetics with the increase of TS content (particularly RS proportion) can be identified.

6.2 Recommendations for future studies

- It is suggested to use several types of lignocellulosic substrates such as wheat straw, sugar cane bagasse etc. rather than rice straw to compare the results.
- Studies can be done by applying different pretreatment options for rice straw during pure batch digestion to optimize further the digestion process.
- Semi-continuous feeding options for the HS-AcoD process of the lignocellulosic biomass can be studied in order to get continuous methane yield with the benefit of digestate re-use strategy applied.

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