

DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

BIOHYDROGEN PRODUCTION
FROM RICE HUSK



by
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August, 2019
İZMİR

BIOHYDROGEN PRODUCTION FROM RICE HUSK

**A Thesis Submitted to the
Graduate School of Natural and Applied Sciences of Dokuz Eylül University
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**by
Gülsün Gizem TAYLAN**

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M.Sc THESIS EXAMINATION RESULT FORM

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BIOHYDROGEN PRODUCTION FROM RICE HUSK

ABSTRACT

Biohydrogen production from agro-industrial wastes such as rice husk, as a promising approach is a renewable and a clean transportable fuel producing no greenhouse gases. In this study, *Clostridium termitidis* and *Clostridium intestinale* were evaluated under mesophilic conditions to improve biohydrogen production in co-culture and consolidated fermentation. First the cultures were tested alone for their biohydrogen production performances. Then, the experiments were carried out to determine the best co-culture ratio (1:1-20:1), rice husk particle size (<2000 μ m, <300 μ m, <74 μ m) and substrate concentration (1-20 g L⁻¹) enhancing biohydrogen production yield and rate.

The highest cumulative hydrogen formation by *C. intestinale* was obtained from 10 g L⁻¹ thermal treated rice husk (14 mL), and by *C. termitidis* from 5 g L⁻¹ ground rice husk (14.6 mL) where thermal treatment was not applied. Significant amounts of hydrogen production yields and rates were obtained with pure cultures. In raw rice husk the highest CHF was obtained with the co-culture ratio of 5:1 (29.15 mL). The highest hydrogen production yield with raw rice husk was obtained with the co-culture ratio of 5: 1 (38.86 mL H₂ g⁻¹ substrate) and the highest HPR was obtained with a co-culture ratio of 1:1. The highest substrate concentration gave the best cumulative volume of hydrogen (41.1 mL). The highest hydrogen production yield (90 mL H₂ g⁻¹ substrate) and hydrogen production rate (0.29 mL H₂ g⁻¹ substrate h⁻¹) were obtained with 1 g L⁻¹ rice husk.

Keywords: Biohydrogen, rice husk, consolidated fermentation, co-culture, *Clostridium termitidis*, *Clostridium intestinale*

ÇELTİK KAVUZUNDAN BİYOHİDROJEN ÜRETİMİ

ÖZ

Çeltik kavuzu gibi tarımsal sanayi atıklarından elde edilen biyohidrojen üretimi, yenilenebilir ve sera gazı üretmeyen temiz bir taşınabilir yakıt olması ile umut verici yaklaşımdır. Bu çalışmada, *Clostridium termitidis* ve *Clostridium intestinale*, ko-kültür ve konsolide fermentasyon ile biyohidrojen üretimini geliştirmek için mezofilik koşullar altında değerlendirildi. Kültürler biyohidrojen üretim performansları için tek başlarına test edilmiştir. Daha sonra biyohidrojen üretim verimini ve hacimsel biyohidrojen üretim hızını arttırmak amacı ile en iyi ko-kültür oranını (1:1-20:1), çeltik kavuzu partikül boyutunu ($<2000\ \mu\text{m}$, $<300\ \mu\text{m}$, $<74\ \mu\text{m}$) ve substrat konsantrasyonunu ($1\text{-}20\ \text{g L}^{-1}$) belirlemek için deneyler yapılmıştır.

En yüksek kümülatif hidrojen oluşumu *C. intestinale*'de, $10\ \text{g L}^{-1}$ ısıtılmış işlem görmüş çeltik kavuzu (14 mL) ve *C. termitidis*'te $5\ \text{g L}^{-1}$ öğütülmüş ve ısıtılmış uygulanmayan çeltik kavuzu (14,6 mL) ile elde edilmiştir. Saf kültürlerle önemli miktarda biyohidrojen üretim verimi ve hacimsel biyohidrojen üretim hızları elde edilmiştir. Ham çeltik kavuzunda, en yüksek kümülatif hidrojen oluşumu (29,15 mL) 5: 1 ko-kültür oranı ile elde edilmiştir. Ham çeltik kavuzu ile en yüksek hidrojen üretim verimi, 5: 1 ($38,86\ \text{mL H}_2\ \text{g}^{-1}\text{substrat}$) ko-kültür oranı ile elde edilmiş ve en yüksek biyohidrojen üretim hızı, 1: 1 ko-kültür oranıyla elde bulunmuştur. En yüksek substrat konsantrasyonu, en iyi kümülatif hidrojen hacmini (41,1 mL) vermiştir. En yüksek hidrojen üretim verimi ($90\ \text{mL H}_2\ \text{g}^{-1}\text{substrat}$) ve hacimsel hidrojen üretim hızı ($0,29\ \text{mL H}_2\ \text{g}^{-1}\text{substrat h}^{-1}$), $1\ \text{g L}^{-1}$ çeltik kavuzu ile elde edilmiştir.

Anahtar kelimeler: Biyohidrojen, çeltik kavuzu, konsolide fermentasyon, ko-kültür, *Clostridium termitidis*, *Clostridium intestinale*

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CHAPTER ONE

INTRODUCTION

1.1 Preface/ The Problem Statement

One of the biggest environmental problems is associated with energy utilization which is known as the greenhouse effect or global warming. Economic and other activities of mankind contribute for increasing various greenhouse gas amounts. Fossil fuel combustion, increased human activities, deforestation are several reasons to the release of gases (Dincer, 2000). Combustion of fossil fuels has negative effects on the environment. Fossil fuel combustion can cause significant amounts of air pollution and the emission of greenhouse gases such as CO₂, CH₄ NO_x, etc. Depletion and negative effects of the utilization of fossil fuels are the reasons to the search of new, sustainable energy sources (Das & Veziroğlu, 2001). In addition, energy demand has ascended extremely in the recent decades, because of increasing population, extensive urbanization, and industrialization. Fossil fuels are used to provide this demand in due to huge amounts in nature. It is a fact that, fossil fuel sources will eventually run out.

Energy resources can be categorized as fossil fuels, renewable sources, and nuclear power. Renewable energy sources are solar, wind, biomass, hydropower, geothermal sources which play a profound role in the world. Renewable energy sources are also called alternative energy sources which are sustainable (solar energy, wind energy, biomass energy, geothermal energy) and provide energy with non or very little emissions of both air pollutants and greenhouse gases (Panwar et al., 2011). Renewable energy sources obtained from biomass received a huge interest in the last decades. Especially, biomass from wastes such as kitchen, agricultural wastes, food industry wastes etc. are being used for bio-gas, bio-hydrogen, bio-ethanol, bio-butanol etc. production (Ozmihci & Kargi, 2016). In the production of biofuels such as ethanol and hydrogen, energy crops (sugar beet, corn, wheat, kasava, etc.) as renewable biomass with high carbon content lead to a global discussion on the efficiency and availability of usable agricultural land against food needs (Charles et al., 2007). Therefore, the use of lignocellulosic biomass in biofuel production (2nd and 3rd generation biofuels) with advanced technologies is extremely important (Antizar-Ladislao & Turrion-Gomez,

2008). Researches show that the use of lignocellulosic raw materials can potentially produce 130-410 EJ / year by 2050 and that the desired goal can be achieved by using less fertile soils instead of agricultural land (Hoogwijk et al., 2005).

Hydrogen gas has a high energy content (122 kJ g^{-1}). Hydrogen gas is seen as an alternative fuel by developed countries and it has an accelerating economic impacts. Hydrogen is the most plentiful element in the universe (Azwar et al., 2014). Using bio-hydrogen gas instead of using fossil fuels improves air quality and has less greenhouse emissions. Nowadays hydrogen can be produced with cost-intensive chemical and biological processes. Fermentative hydrogen production from renewable raw materials has advantages. Fermentative processes are preferred due to cheap feedstock, utilization of special organisms and operation conditions when compared with the chemical methods used for hydrogen production (Kapdan & Kargi, 2006; Das & Veziroğlu, 2001). Biohydrogen as a clean renewable fuel has been found an important place to itself as an energy carrier in the clean energy plan in Europe. European Union has a target for 2020 to use 10% of renewable energy sources instead of petroleum or diesel fuel in transportation. The countries in Europe obliged to use 20% of the total energy from renewable energy sources (EU directives 2009/28/EU). In the Turkish National Development Plans of 2011-2023, it is aimed to reduce fossil fuel consumption while increasing clean renewable energy utilization (ETKB, 2014).

Hydrogen gas is not in an available form in the universe like fossil fuels. This is the major problem of using hydrogen gas as a fuel. Production of hydrogen needs energy, high temperature, and pressure and several different pretreatments. However, to produce a sustainable, environmentally friendly energy source, green technologies need to be developed (Argun, 2010). Bio-hydrogen gas production can be categorized as bio-photolysis, photo fermentation, and dark fermentation. Carbohydrate-rich renewable substrates make fermentation more economic and sustainable. Fermentative biomasses contain sugary substances (sugar beet, sugar cane), starchy biomass (potato, cereal), and lignocellulosic feedstock (grass, tree, straw).

Feedstock energy as a renewable energy source is promising, however, it must be obtained from crops that are not used as food but like agricultural waste (called “second generation”) because of competition with food sources. Lignocellulosic materials are mainly used as the second generation for the production of biomass energy (Cheng et al., 2011). The use of lignocellulosic biomass for the production of biofuels has recently been investigated. Lignocellulosic biomass consists of cellulose, hemicelluloses, and lignin.

Bio-hydrogen production with different processes is a long term alternative obtained from renewable sources and has gained attention (Levin et al., 2004). Biophotolysis, dark-fermentation, photo fermentation, and two-stage dark/photo-fermentation are the main processes used for bio-hydrogen production (Kapdan & Kargı, 2006).

Renewable energy sources from lignocellulosic substrates have increasing attention in the literature. Especially, agricultural wastes such as wheat straw, rice straw, corn stalk, rice husk, barley straw, wood fibers etc. may be used for bio-hydrogen gas production. The utilization of lignocellulosic substrates for bio-hydrogen gas production needs pretreatment to obtain sugar syrup due to its strong polymeric structure. The pretreatment steps need chemicals, heat or enzymes which increase the cost of the process. The conversion of lignocellulosic substrates is a difficult process and may need special organisms which are capable of reducing complex sugars into simple ones. Consolidated processing including enzyme hydrolysis and hydrogen fermentation of the hydrolysed cellulosic substrate may be a solution for conversion of lignocellulosic substrates. However using only one type of lignocellulosic degrading organism needs long fermentation periods. Consolidated bioprocessing was evaluated with a co-culture on rice husk to understand the hydrogen production potential in these conditions. Two different types of hydrogen producing organisms were combined to enhance biohydrogen production under consolidated fermentation conditions. One of the main idea to degrade the need of pretreatment was evaluated with the chosen cultures.

CHAPTER TWO

LITERATURE SURVEY

2.1 Hydrogen as a Chemical and Energy Source

Hydrogen is an atom consisting of only one proton and one electron. It is also a widespread element in the world. Hydrogen gas is always combined with other elements like water. Many organic compounds consisting of hydrogen, especially hydrocarbons such as gasoline, methanol, and propane. Hydrogen can be separated from hydrocarbons by applying heat with a process called steam reforming. Nowadays, hydrogen gas is being produced via steam reforming of natural gas. An electrical current can also be used to separate water into oxygen and hydrogen. Some algae and bacteria, using sunlight as their energy source, even give off hydrogen under certain conditions which is known as photolysis. After combusting hydrogen gas the only end product of the reaction is water. Hydrogen is a colorless, odorless gas and has a high calorific value (Momirlan & Veziroglu, 2005).

In the industry, hydrogen is used for ammonia manufacturing, petroleum refinement, and methanol synthesis. Fuel cells convert the hydrogen into electricity. NASA's space program uses hydrogen as a fuel. They used hydrogen in fuel cells to provide heat, electricity and drinking water for astronauts. Generation of electricity and heat can be provided by a fuel cell. Fuel cell technology has been developed in Japan and America lately. There is a wide utilization area for the fuel cell technology. Chemical energy of input fuel can be converted to electricity and heat. Yield of fuel cell is quite high. Granovckii and Dincer (2016) compared the conventional, hybrid, electric and hydrogen fuel cell vehicles. Their study showed that the hybrid and electric cars have advantages. The environmental impacts associates with the use of renewable energy sources.

Hydrogen made from renewable energy resources to help reduce our reliance on the outdoor energy. Hydrogen gas produced from renewable energy sources provides energy for businesses, factories, electric utilities, homes, vehicles, ships, and airplanes. The transportation sector is the first widespread use of hydrogen as a fuel where it will

help to reduce pollution. Internal combustion engines can use pure hydrogen or mixed different fuels. Vehicles can be powered with hydrogen fuel cells three times more efficient than gasoline. Fuel cells can also supply heat and electricity for domestic needs like homes, buildings.

The combustion of hydrogen does not produce pollutants such as CO_2 , CO , SO_2 , however, it can emit water vapor and NO_x . NO_x is related to combustion temperature and duration. Engine design can be developed with considering the wide flammability range of hydrogen so that the NO_x emission can be reduced (Momirlan & Veziroglu, 2005)

2.2 Hydrogen Production Methods

Steam reforming and thermal cracking of natural gas, coal gasification and non-catalytic partial oxidation of fossil fuels, electrolysis and photolysis of water and thermochemical cycles etc. are methods of production of hydrogen (Kapdan & Kargı, 2006; Das & Veziroğlu, 2001).

Hydrogen production method as mentioned above are mainly related to the utilization of fossil fuels which result end up with emissions (Sagnak, 2011). Therefore, production methods of hydrogen need to be clean, economic and sustainable. Electrolysis of water may be the cleanest technology for hydrogen gas production in which electricity requirement is around 80% of the total operating cost. Depending on the fact, electrolysis should be used in areas where electricity is inexpensive. Steam reforming of natural gas and water electrolysis are the most commonly used processes for the production of hydrogen (Kotay & Das, 2008). Due to the low energy efficiency of those processes, other hydrogen production methods are searched (Das & Veziroğlu, 2001). Hydrogen gas production from renewable energy sources and carbohydrate-rich waste materials by bioprocesses offers more energy efficiency and other advantages (Kapdan & Kargı, 2006; Manish & Banerjee, 2008). Some problems of biohydrogen production are; low hydrogen production yields and rates (Kapdan & Kargı, 2006; Levin & Chahine, 2010).

2.3 Bio-hydrogen Production Processes

Biological hydrogen production is a sustainable alternative to the above-mentioned methods for hydrogen gas production. Bio-photolysis, photo fermentation and dark-fermentative hydrogen production during the acidogenic phase of anaerobic digestion of organic matter are main processes for bio-hydrogen gas production (Kapdan & Kargı, 2006).

Biological hydrogen production requires less energy and mild environmental conditions. Bio-photolysis includes direct bio-photolysis and indirect bio-photolysis. Bio-photolysis of water under sunlight is considered as the cleanest approach for bio-hydrogen production. However, low H₂ gas production yield, the requirement of light and oxygen inhibition are the main problems in bio-photolysis of water (Kapdan & Kargı, 2006; Yu & Takahashi, 2007). Photo-fermentation and dark-fermentation can be used together as it is used separately (Hawkes et al., 2002). Hydrogen gas production with fermentation of carbohydrates is a faster method than bio-photolysis (Dasgupta et al., 2010; Manish & Banerjee, 2008; Levin et al., 2004; Afsar et al., 2011; Perera et al., 2010).

Bio-hydrogen production with fermentation can be generated under mesophilic (35°C) and thermophilic (55°C) conditions depending on microorganism species. Mesophilic fermentation can be achieved by using different bacteria such as *Thermoanaerobacterium*, *Thermococcus* sp., and *Clostridia* Sp. Different thermophilic bacteria such as *Thermoanaero bacterium*, *Desulfotomaculum* sp., *Thermococcus* sp., *Klebsiella oxytoca* were used for bio-hydrogen production at 55 °C owing attractive advantages like high hydrogen yield and pathogenic bacteria destruction. (Kargı et al., 2012). Species such as *C. bejerinkii*, *C. pasterianum*, *C. butyricum* and *Clostridium* species with high hydrogen production potential, which can generally grow on hexose and pentoses, have been used in mild conditions for hydrogen production (Wang et al., 2008; Özmıhçı & Kargı, 2011).

Biological hydrogen production methods can use waste materials as carbon sources which makes it a sustainable process. Fermentative biomass can be divided into three

by chemical content. These are sugar containing biomass such as sugar beet, sugar cane, starchy biomass like potato, cereal and lignocellulosic biomass as grass, tree, straw. Bio-hydrogen gas production from renewable sources has gained paramount attention in recent years (Levin et al., 2004).

The substrate used should be pre-processed first to produce available sugar. The substrate used is subjected to enzymatic / acid hydrolysis to break the lignocellulosic chain and transfer carbohydrates (sugar) to the solution. Enzymatic hydrolysis is expensive and requires long retention times. Hydrolysis by physicochemical or chemical methods is a process in which simple sugars are detected in 1-2 hours. However, the main disadvantage of physicochemical methods is that they form furfural, 5-HMF inhibiting fermentation, which needs to be purified (Kapdan & Kargı, 2006; Velioglu et al., 2019).

There are many studies focusing on bio-hydrogen production with different species, a reactor configuration, biological methods, two or three stage systems, light-dark combined fermentation and also with processes where the substrate was pretreated with different methods (Kapdan & Kargı 2006; Argun & Kargı 2011). Table 2.1 gives examples using different types of processes for dark bio-hydrogen production in batch, continuous mode and light fermentation using simple sugars. The table depicts hydrogen production yields ranging between 0.9-4.8 mol mol⁻¹ glucose with 1-20 g COD L⁻¹. Conventional biofuel production is based on the release of existing sugars from substrates with as little pretreatment as possible and conversion by organisms.

Table 2.1 Hydrogen Production with different substrates and organisms with different operational modes

Culture	Operation Mode	Substrate Type	Substrate Concentration (g L ⁻¹)	Hydrogen Yield (mol H ₂ /mol substrate)	References
<i>Klebsiella oxytoca</i> HP1	Batch Dark Fermentation	Glucose (50Mm)		1mol mol ⁻¹ glucose	Minnan et al., 2005
<i>E. cloacae</i> IIT-BT 08	Batch Dark Fermentation	Glucose (1%)		2.2mol mol ⁻¹ glucose	Kumar & Das, 2000
<i>E. coli</i>	Batch Dark Fermentation	Glucose	20 g L ⁻¹	4.73×10 ⁻⁸ mol mol ⁻¹ glucose	Podesta et al., 1997
<i>H. alvei</i>	Batch Dark Fermentation	Glucose	10 g L ⁻¹	5.87×10 ⁻⁸ mol mol ⁻¹ glucose	Podesta et al., 1997
<i>Sludge compost</i>	Batch Dark Fermentation	Glucose	10 g L ⁻¹	2.1mol mol ⁻¹ glucose	Morimoto et al., 2004
<i>Mixed culture</i>	Batch Dark Fermentation	Glucose	1g COD L ⁻¹	0.9mol mol ⁻¹ glucose	Logan et al., 2002

Table 2.1 continues

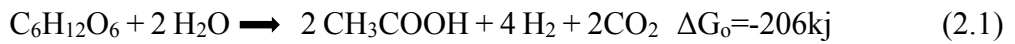
<i>Mixed culture</i>	Batch Dark Fermentation	Sucrose	6 g L ⁻¹	300mL g ⁻¹ COD	Khanal et al., 2004
<i>Klebsiella oxytoca</i> HP1	Batch Dark Fermentation	Sucrose	50 mM	1.5mol mol ⁻¹ sucrose	Minnan et al., 2005
<i>C.pasteurium</i> (dominant)	Batch Dark Fermentation	Sucrose	20 g COD L ⁻¹	4.8mol mol ⁻¹ sucrose	Lin et al., 2004
<i>C. acetobutyricum</i>	Continuous Dark Fermentation	Glucose		2mol mol ⁻¹ glucose	Chin et al., 2003
<i>Mixed culture</i>	Continuous Dark Fermentation	Glucose	20g COD L ⁻¹	1.1mol mol ⁻¹ glucose	Chen et al., 2000
<i>Mixed culture</i>	Continuous Dark Fermentation	Glucose	13.7g L ⁻¹	1.2mol mol ⁻¹ glucose	Oh et al., 2004

Table 2.1 continues

<i>Clostridia sp.</i>	Continuous Dark Fermentation	Glucose	20g COD L ⁻¹	1.7mol mol ⁻¹ glucose	Lin et al., 2004
<i>Mixed culture</i>	Continuous Dark Fermentation	Glucose	7g L ⁻¹	2.1mol mol ⁻¹ glucose	Fang et al., 2002
<i>Mixed culture</i>	Continuous Dark Fermentation	Sucrose	20 g COD L ⁻¹	3.47mol mol ⁻¹ sucrose	Chen et al., 2001
<i>R. sphaeroides OU 001</i>	Photo Fermentation	Sugar refinery effluent+malic acid		13.44 L mol ⁻¹ C	Yetis et al., 2000
<i>R. sphaeroides OU 001</i>	Photo Fermentation	Sugar refinery effluent+malic acid		11.67 L mol ⁻¹ C	Yetis et al., 2000
<i>R. sphaeroides</i>	Photo Fermentation	Tofu WW		0.24mL mg ⁻¹ carbohydrate	Zhu et al., 1999

2.3.1 Dark Fermentation

Some bacteria can ferment carbohydrates to produce hydrogen, volatile fatty acids and carbon dioxide. Monosaccharides which are main feedstock can be obtained with enzymatic or acid hydrolysis from complex substrates. Theoretically, when acetic acid is the only volatile fatty acid produced from sugars converted by organisms, 4 moles of hydrogen can be produced per mole of glucose.



When butyric acid is the final fatty acid, 2 moles of hydrogen can be produced per mole glucose, and propionic acid formation consumes 1 mol of hydrogen per mole of propionic acid. When both acetic and butyric acids are produced in dark fermentation from glucose, theoretically 2.5 mol of H_2 is formed per mole glucose (Argun & Kargi, 2011).



2.3.2 Photo Fermentation

PNS (photosynthetic non-sulfur) bacteria can produce hydrogen and carbon dioxide from volatile fatty acids. *PNS bacteria* can also produce hydrogen using monosaccharides such as glucose, sucrose. *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, *Rhodopseudomonas palustris* are commonly used bacteria species. Nitrogenase is responsible for hydrogen production. Light can be provided as artificial or solar source. Theoretically, when acetic acid is the only volatile fatty acid, 4 mol of hydrogen can be obtained from 1 mol of acetic acid (Argun & Kargi, 2011). There are studies about bio-hydrogen production from food industry wastewaters and organic acids by photo fermentation. Kapdan and Kargi compiled these studies in 2006. Also there are some examples summarized in Table 2.2 and Table 2.3. producing hydrogen from PNS bacteria. Tables reveal studies using VFA and waste substrates, having a hydrogen yield between 0.24 mL mg^{-1} carbohydrate- 13.44 L mol^{-1} C.

Table 2.2 Hydrogen Production from Organic Acids by Photo-Fermentation

Substrate	Inoculum Culture	Concentration	Hydrogen Production Yield	Specific Hydrogen Production Rate	Operation Mode	References
Acetate	<i>R. palustris</i>		2.8 mol H ₂ mol ⁻¹ acetate	9.8 mL g ⁻¹ cell h ⁻¹	Batch	Oh et al., 2004
Acetate	<i>R. capsulata</i>	4 g L ⁻¹	2.5 mol H ₂ mol ⁻¹ acetate	22 mL g ⁻¹ VSS h ⁻¹	Batch	Fang et al., 2005
Acetate	<i>R. capsulate</i>	1.8 g L ⁻¹		19.07 mL g ⁻¹ DW h ⁻¹	Batch	Shi & Yu, 2005
Butyrate	<i>R. capsulate</i>	1 g L ⁻¹	2.8 mol H ₂ mol ⁻¹ butyrate	32 mL g ⁻¹ VSS h ⁻¹	Batch	Fang et al., 2005

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Table 2.3 Hydrogen Production from Wastes by Photo-Fermentation

Wastewater	Inoculum Culture	H ₂ Production Yield	Hydrogen Production Rate	Operation Mode	References
Sugar refinery	<i>R. sphaeroides</i> OU 001	13.44 L mol ⁻¹ C	5mL L ⁻¹ culture	Batch	Yetis et al., 2000
Sugar refinery	<i>R. sphaeroides</i> OU 001	11.67 mol ⁻¹ C	3mL L ⁻¹ culture	Continuous	Yetis et al., 2000
Olive mill WW	<i>R. sphaeroides</i> OU 001		4mL L ⁻¹ culture	Batch	Eroğlu et al., 2004
Tofu WW	<i>R. sphaeroides</i>	0.24 mL mg ⁻¹ carbohydrate	2.1 L (hm ²) ⁻¹ gel	Immobilized	Zhu et al., 1999
Tofu WW	<i>R. sphaeroides</i>		0.393mL mg ⁻¹ DW h ⁻¹	Immobilized	Zhu et al., 2002

2.3.3 Combined Fermentation

Combination of dark and light fermentation is a pretty new method for bio-hydrogen production. There are some studies revealing results about combined fermentation. Volatile Fatty Acids from dark fermentation simultaneously increases hydrogen yield. Theoretically, 12 moles of hydrogen per mole of glucose can be obtained when combined fermentation is used. For instance, in a study the highest yield of 7.1 mol hydrogen mol⁻¹ glucose was obtained with *Lactobacillus delbrueckii* NBRC 13953 and *R. sphaeroides*-RV (Asada et al., 2006). Efficiency of hydrogen production is related with proper temperature, pH, ORP, light source, biomass and microorganisms. Culture selection is one of the most important issue in combined fermentation. PNS bacteria should be adapted to organic acids before inoculation. Different operational modes for combined fermentation are reported in the literature (Argun, 2010). Volatile fatty acids are produced during dark fermentation and bio-hydrogen and carbon dioxide is obtained from photo fermentation in simultaneous dark and photofermentation. Also, enzymatic hydrolysis of biomass and dark fermentation can be actualized in the same medium. Bio-hydrogen production yields are higher than single stage fermentation. However, bio-hydrogen production rates are lower than single stage fermentation (Argun & Kargı, 2011).

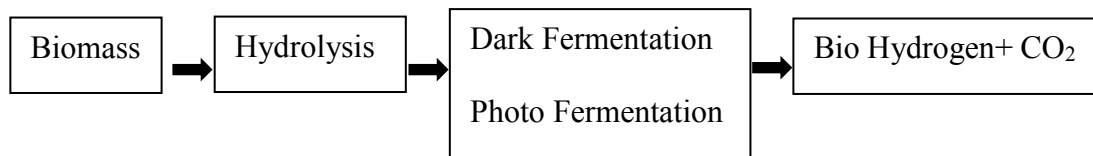


Figure 2.1 Bio-hydrogen production from biomass by simultaneously dark fermentation and photo fermentation (Argun & Kargı, 2011)

2.4 Bio-Hydrogen Producing Microorganisms from Lignocellulosic Substrates

2.4.1 Co-culture Processes

Many species can exist by affecting, each other, improving their abilities with groups. Bio-hydrogen production with co-cultures offers advantages over pure cultures used alone. In co-culture systems cellulose can be hydrolyzed enzymatically. Moreover, the co-culture combination can alter all reducing parameters during the bio-hydrogen fermentation period. The yields and rates are associated with efficiency of biohydrogen production. The co-culture provides high bio-hydrogen production efficiency, less fermentation time by economic and technical advantages. In co-culture systems, microorganisms fulfill complicated tasks which are hydrolyzation of a substrate, alteration of metabolites, simultaneous consumption of substrate (Pachapur et al., 2015). In this regard, there are studies using anaerobic culture and *Rhodobacter sphaeroides*, *C. beijerinckii*, and two types of *Rhodobacter sphaeroides* (RS-NRRL and RS-RV), *C. butyricum* and *C. pasteurianum* under different conditions in suspended cultures to increase hydrogen production efficiency and speed (Ozmihci & Kargı, 2010; Ozmihci & Kargı 2011). *Trichoderma reesei* and *Aspergillus spp.* was used together to increase 50% cellulase glucosidase production and then *Aspergillus ellipticus* and *Aspergillus fumigatus* were used together to increase glucosidase activity in solid state fermentation aiming biohydrogen production (Bhargav et al., 2008).

The most important and expensive step in conversion of lignocellulosic substances is hydrolysis. The conversion of lignocellulosic structure into cellulose, hemicellulose and lignin and then to hexoses and pentoses need enzymes, hydrolysis and combined bioprocesses. Combined processes are preferred to break down the structure and ferment cellulose and hemicellulose together.

Pure *Clostridium intestinale* ATCC 4921 was tested for its hydrogen production potential. The results showed that it's a hydrogen producer which is able to grow on cellobiose, hexose, and alcohol sugar. Also the results revealed that *Clostridium intestinale* is not capable of hydrolyzing cellulose but has short doubling time of 1.5 hours and uses mostly the butyrate producing pathway for hydrogen production

(Ramachandran et al., 2011). There is no literature using *Clostridium intestinale* alone for biohydrogen production, however there are many studies about fermentative biohydrogen production with mixed culture involving *Clostridium intestinale*. For example, Jung et al., (2013) tested anaerobic sludge for glucose and hydrogen production and found that *C. intestinale* was also present in the microbial composition.

The hydrogen production yield and specific hydrogen production rate can be increased by using different cultures. In this study, *Clostridium intestinale* and *Clostridium termitidis* cultures were combined. *Clostridium termitidis*, can grow on cellulosic material. This culture has the capacity to produce hydrogen as well as to break down the substance. Processes that can lead to product formation by enzymatic hydrolysis on lignocellulosic substances are defined as consolidated fermentation. This process is explained in part 2.4.2. The doubling time of *C.termitidis* is high. It is a mesophilic, anaerobic bacterium which can grow on cellulose, cellobiose, glucose, fructose and xylose (Hethener et al., 1992; Ramachandran et al., 2008). There are just a few studies focusing on bio-hydrogen production from *C. termitidis* (Levin et al., 2009; Hethener et al., 1992; Ramachandran et al., 2008). Ramachandran et al. (2009) examined the final products of *C. termitidis* CT1112 species growing on 2 g L⁻¹ cellobiose and α -cellulose and found that the doubling time of the organism were 6.5 hours and 18.9 hours, respectively. The average hydrogen gas production obtained in the case where the substrate is cellobiose and α -cellulose was 4.6 mmol H₂ L⁻¹ culture and 7.7 mmol L⁻¹ culture and specific hydrogen gas production rate was 4.28 mmol H₂ h⁻¹g⁻¹ dry cell mass and 2.79. mmol H₂ is h⁻¹g⁻¹ dry cell mass, respectively.

2.4.2 Consolidated Processes

Miscellaneous researches have used a single-step integrated bioprocess, which combines cellulase production, hydrolysis, and fermentation of both cellulose and hemicellulose to solve these costly processes. For this purpose, microorganisms containing cellulosic and hemicellulosic enzymes are used. (Demain et al., 2005; Lynd, 1996; Lynd et al., 2002; Lynd et al., 2005; Gupta et al., 2012). Simultaneous saccharification and fermentation systems in these processes are more economical, have higher conversion efficiency and less energy requirements of the system.

(Demain et al., 2005; Lynd et al., 2002; Lynd et al., 2005). Examples of anaerobic cellulosic organisms are *Clostridium thermocellum*, *C. phytofermentans*, *C. termitidis*, *C. thermopalmarium*, *C. cellulolyticum*, *C. cellulovorans*. (Carere et al., 2008; Levin et al., 2009; Geng et al., 2010; Gupta et al., 2012).

Factors to be considered in those processes are the capacity and hydrolysis of microorganisms to adhere to cellulose fibers and the necessity of pretreatments such as acid treatment and / or grinding (Levin et al., 2006; Islam et al., 2009). Hydrogen production yields obtained from various studies with cellulosic raw materials have a wide range such as 0.17- 3.2 H₂ mmol g⁻¹ cellulose (Lay, 2001; Ren et al., 2007; Saratale et al., 2008; Wang et al., 2009).

Consolidated bioprocess studies have increased in recent years. Production of biofuels from lignocellulosic biomass contains production of saccharolytic enzymes such as cellulase, hemicellulase, conversion of carbohydrate compounds in biomass structure to fermentable sugars such as glucose, galactose, mannose, xylose, arabinose, etc. (Lynd et al., 2005). Consolidated fermentation is reported to be the most ideal process to reduce the cost of the equipment, reduce the risk of contamination, and eliminate the individual saccharolytic enzyme formation step (Lynd et al., 2002; Olson et al., 2011; Agbor et al., 2014).

Agbor et al. (2014) produced biohydrogen using *Clostridium thermocellum* on hemp, cellulose, hemp fiber and hemp coarse grains and compared the results with alpha cellulose production. It was found that beside hydrogen 8.47 mM ethanol can be produced from alpha cellulose. From waste substrates the highest ethyl alcohol (6.56 mM) was obtained from hemp cellulose. Hemp fibers and grains gave a concentration of 5.48 mM and 3.52 mM ethanol, respectively. In terms of hydrogen production performance alpha cellulose, hemp, hemp fiber and hemp coarse gave 12.70 mM, 11.01 mM, 10.91 mM, 4.72 mM of hydrogen gas, respectively (Agbor et al., 2014).

Consolidated fermentation with *Clostridium thermocellum* and *Thermoanaerobacterium aotearoense* co-culture was tested in cassava silage. The

amount of sugar obtained from lignocellulosic structure in this study is $13.65 \pm 0.45 \text{ g L}^{-1}$ and *C. thermocellum* and *T. aotearoense* are 1.75 and 1.17 times higher than when used alone. In addition, *Saccharomyces cerevisiae* was added to this medium at a ratio of 1: 1 in 84 hours under thermophilic conditions. They produced $8.83 \pm 0.31 \text{ g L}^{-1}$ of ethanol (% Yield = $64.95 \pm 2.71\%$). Hydrogen production was 4.06 mmol H_2 and *C. thermocellum* and *T. aotearoense* cultures were reported to be 1.54 and 2.09 times higher when used alone, respectively (Li & Zhu, 2011).

Paper slurry under thermophilic conditions was subjected to hydrogen production by An et al. (2018). The highest hydrogen production (64.32 mM) was obtained from 7.4% paper slurry. The dynamic bacterial consortium based on 16S rDNA gene screening showed that *Clostridiales* and *Thermoanaerobacterales* were dominant in the medium. In addition, *C. thermocellum* was added to this mixture to increase hydrogen production performance. It was reported that hydrogen production efficiency increased by 96.8% and holocellulose reduction rate increased by 32.98% (An et al., 2018).

2.5 Raw Materials for Bio-hydrogen Fermentation

There are different types of biomass, which can be used as raw materials for bio-hydrogen production. These are sucrose containing, starchy biomass and lignocellulosic materials. Biomass types require some pre-treatments for obtaining water-soluble sugars. The pre-treatment methods for lignocellulosic and starch waste materials are more complex than the pre-treatment methods for simple components. Bio-hydrogen production from those materials, require hydrolysis for converting polysaccharides to simple sugar compositions. Removal of lignin is the main problem. Lignin can not be converted to simple sugars and can have inhibit for microorganisms (Urbaniec & Grabarczyk, 2009).

There are four types of feedstocks and methods, classified as first, second, third and fourth generation biofuels that are used in biofuel production. First generation biofuels are termed for edible biomasses (starch and sugar) which are cultivated as energy crops. The second generation of biofuels is produced from switchgrass, sawdust, low

priced woods, crop wastes and municipal wastes etc. which are alternatives of first generation substrates requiring more steps to produce biofuels with a viable cost. These steps may include thermal, biological, enzymatic or chemical pretreatment techniques to convert lignocellulosic substrates into cellulose, hemicelluloses and lignin. Algae as the aquatic feedstock is classified as the third generation biofuel. Photosynthetic growth of algae reduces CO₂ while producing O₂ which is the additional benefit of using the harvested algae that is converted into biodiesel. Though, it is not easy to produce biofuels from algae because of low growth rate, less stability than other resources, high cost, and unsaturated oil production (more volatile in high temperatures and more likely to degrade). Engineered microorganisms such as engineered algae or crops used as resources are entitled like the fourth generation biofuels. For instance, algae may be genetically modified to consume more CO₂ (Alalvan et al., 2019; Argun & Kargi, 2011; Kapdan & Kargi, 2006; Dinçer, 2000).

There are some concerns about feedstocks. Increasing of energy production is the reason of debating about feedstocks. Industrial bio-energy being produced instead of food. Giant agricultural areas have been used for feedstock for bio-energy production. First generation biofuels involving highly crops. The biggest disadvantage of biofuel is the food versus fuel debate. Rising food prices are also related with production of bio-fuel. Lignocellulosic feedstock can create a potential to afford novel biofuels which are called as 'second generation'. Second generation biofuels are biofuels which can be produced from plant biomass, largely wastes as lignocellulosic substrates. Plant biomass is the most abundant biological resource (Naik et al., 2010; Ntaikou et al., 2010).

2.5.1 Lignocellulosic Materials

Lignocellulosic substances can be classified into six main classes. These are cereal residues (corn straw, corn cob, rice husk, barley husk, rye stalk, oat stalk, rice stalk, corn stalk, cotton stalk, soy stalk, sugar cane pulp), hard timber (eucalyptus, acacia, poplar, carob), softwood (willow, spruce, pine), cellulose waste (newspaper, stationery paper, recycled pulp), herbaceous biomass (legume stalk, grass) and urban organic solid waste (Buriana et al., 2013). Rice Husk is included in cereal waste class.

Lignocellulosic materials contain hemicellulose, cellulose and lignin. Cellulose has a linear structure consisting of multiple regular chain organizations in which the D-glucose molecules bound by β -1,4-glucosidic bonds are equilibrated with a certain number of hydrogen.

The release of cellulose requires the pretreatment of the raw material with acid, base and/ or heat. The purpose of the pre-treatment of lignocellulosic biomass is to increase the surface area of the substrate, to provide enzymatic hydrolysis of cellulose fibers, to reduce the crystallinity of cellulose, to eliminate hemicellulose and to break the lignin network. The increase of the surface area after pretreatment affects the microbial activity depending on its microbial pathway, and creates the final products more efficiently and fastly (Lim et al., 2012; Petersson, 2007).

Lignin content, the degree of polymerization and crystallinity of cellulose are the most important factors limiting enzymatic hydrolysis (Jamradloedluk & Wiriyumpaiwong, 2007; Sluiter et al., 2012). Pre-treatment; when trying to increase carbohydrate formation by hydrolysis, it is necessary to avoid the formation of by-products (5-hydroxymethylfurfur (HMF) and furfural) which will prevent the loss and reduction of carbohydrates and affect the hydrolysis and fermentation processes (Buriana et al., 2013).

Generally, pre-treatment may include both mechanical and thermochemical treatment steps and are costlier than other pre-treatment processes. These may include steam explosion, thermal treatment, CO₂ explosion, AFEX (ammonium fiber / freeze explosion), acid / alkali hydrolysis, wet oxidation, pre-treatment with ozonation, organosolv (usually ethanol, acetone, methanol, ethylene glycol) methods (Buriana et al., 2013; Agbor et al., 2011; Chandra et al., 2007; Sediawan et al., 2011; Soltani et al., 2015). In addition, acidification (delignification) requires the separation of cellulose fibers from lignin and hemicellulose, which is time-consuming. The most expensive step in this process is based on the amount of enzyme or acid consumed during the transformation of cellulose into simple sugars (Moxley et al., 2008).

There are studies focusing on preparation of lignocellulosic materials via enzymatic hydrolysis for biofuel production. Especially, ethanol production from lignocellulosic substrate studies have a massive amount in the literature. For example, delignified corn meal was treated with cellulase and *Penicillium chrysogenum* as an enzyme cocktail obtained from P33; and increased the sugar formation from 21.4% to 106.8% and this value was 78.6% cellulase greater than the one where cellulase was the only hydrolysate inoculated the compost of wheat, rice, maize and soybean stalks with thermophilic *actinomyces*, resulting in the reduction of lignocellulosic materials (Yang et al., 2018; Wei et al., 2019). They have demonstrated that *actinomyces* adjusted well to media and changed the composition of the culture, besides the substrate degradation rate increased and the activities of CMCaz, Xylazine, manganese peroxidase, lignin peroxidase and laccase increased used a grain waste as substrate consisted of rice husk, soybean peel, sugar beet pulp, Yerba mate toothpaste under solid phase fermentation with *Trichoderma reesei* NRRL 3652 to examine its enzyme production performance (Wei et al., 2019; Astolfi et al., 2019). Conditions that maximize cellulase and xylanase activities and where the enzyme activity is highly stable are determined to be pH 4.5-5.5 and temperature 40°C. Michealis-Menten kinetic parameters in the study which had the highest enzyme activity in soybean cellulase (K_m : 19.73 mg mL⁻¹, V_{max} : 0.82 mol min⁻¹), endogluconase (K_m : 0.65 g mL⁻¹, V_{max} : 0.62 mol min⁻¹) and xylase (K_m : 22.64 mg mL⁻¹, V_{max} : 104.17 mol min⁻¹). In this study, the amount of enzyme produced from rice husk was found to be very low. FPase 0.5 U g⁻¹, CMCase 3 U g⁻¹ and xylanase were observed at 30U g⁻¹ (Astolfiv, 2019; Nikzad et al., 2017). obtained 27.11-36.84 g L⁻¹ glucose and 4.23-7.94 g L⁻¹ xylose from the 12% w v⁻¹ rice husk (Nikzad et al., 2017).

2.5.2 Rice Husk as Substrate for Hydrogen Production

Rice is one of the most important cereal crops in the world after wheat and corn, it is a very old cultivated plant and belongs to the genus *Oryzae* of the family *Gramineae* (San, 2005). Asian countries take the first place of rice cultivation in the world. The main rice harvesting areas in Turkey (approx. 7:14 tons / ha) are Marmara (58%), and Karadeniz (37%) and especially Edirne, Çorum, Samsun, Sinop, Izmir, Manisa, Balikesir and Kastamonu are the rice producing cities. In the 2017/18 season rice

production in Turkey was 900 thousand tons/year (Ministry of Agriculture and Forestry, 2019). There are over 150 rice milling factories across Turkey which the installed capacity is over the need of the country (UHK, 2011). They import rice to process and sell back as processed rice. The rice processed in the factories leaves wastes such as unused and unutilized rice husk. When 100 kg of rice is processed, approximately 15-20 kg of husk is formed (approximately 20% of the harvest). Rice as a nutrition covers 20% nutrient energy of the total nutritional energy in the world. It is consisted of 4 layers of epidermis covered with thick upper layer of the cell surrounded by thick lignified and silica walls outside the inner shell of the brass (Dagnino et al.,2012).

As in the other members of the grass family, there is a gnarled stem (trunk), thin long and flat leaves emerging from the nodes and the cluster of flowers formed by the combination of spikelets. Each bunch has an average of 100-150 spikelets, each spikelets have a single flower. Flowers are fertilized to form seeds. Average 5-15 mm long and 2-4 mm wide grains are covered with two globules overlapping the edges. Globular grains are commonly referred to as rough rice, paddy (sometimes referred to as plants). The structure is detailed in Figure 2.2 (Bay 2009).

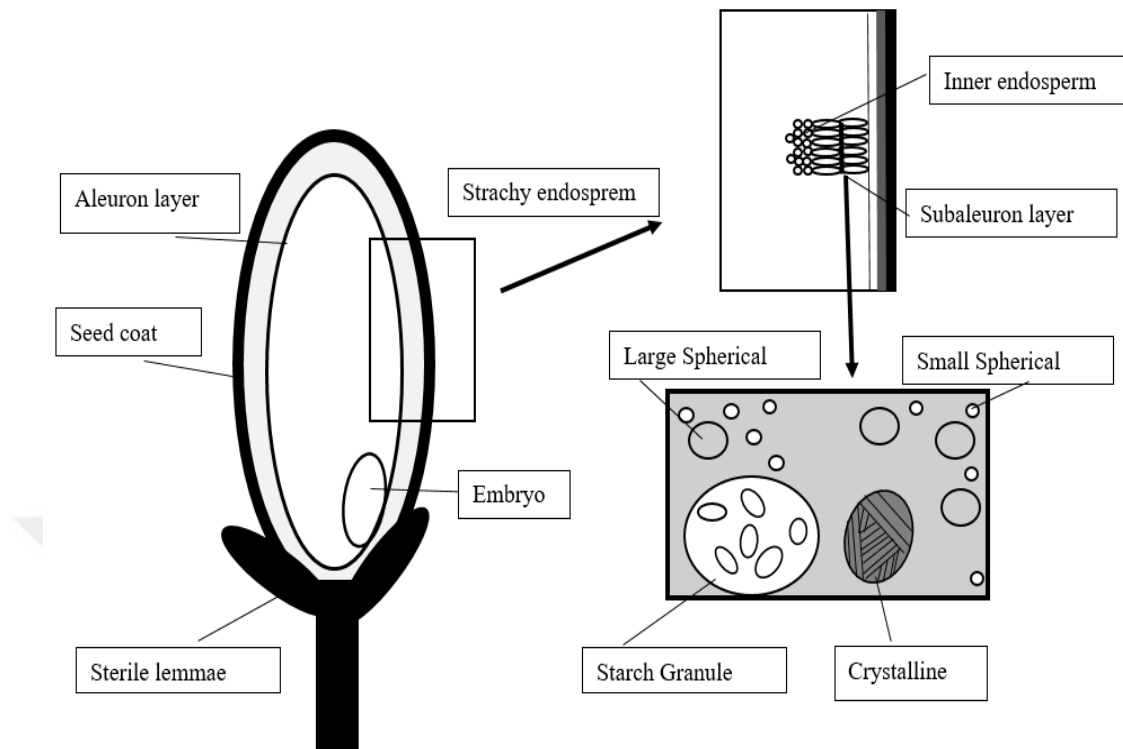


Figure 2.2 The anatomic structure of raw rice (Bay, 2009)

The average components depending on the harvest of rice are 32-47% of cellulose, 19-27% of hemicellulose and 5-24% of lignin. However, rice husk contains 36-40% cellulose and 12-19% hemicellulose (Dagnino et al., 2012). It contains 80% organic volatile material and 20% silica.

Rice husk can also be used as artificial fertilizer. Disposal of rice husk is an important problem. Therefore, it would be preferable to dispose of it either by incineration or burying in open areas. When burned in uncontrolled situations, silica (crystalline) forms and becomes less reactive and also releases CO into the atmosphere (Olawale & Oyawale, 2012; Rafiee et al., 2012). The chemical content of the biomass can affect the combustion efficiency. The high alkali (Na and K) content and the presence of phosphorus can reduce the melting temperature of the powder, which can lead to corrosion and deposit formation on the heat transfer surfaces (Lim et al., 2012). That's why incineration is not a good solution for this waste.

Rice husk consists of approximately %72.8 volatile materials, %9.3 bounded carbon, and %17.9 ash. Except for ash, the structure of rice husk includes %48.9 carbon, %6.2 hydrogen, %0.8 nitrogen and %44.1 oxygen. Chemical components are hemicelluloses (%28.6), cellulose (%28.6), lignin (%24.4) and extractive materials (%18.4) (Wannapeera, 2008; Lim et al., 2012). In this study, components of rice husk which are hemicellulose, cellulose and lignin were investigated with several methods and the most appropriate method was used for characterization which are explained in part 3.4.1.

The efficiency of conversion of the long polymeric chains in the raw material into dissolved sugars (cellobiose, glucose) and the growth rate of the organism on water-insoluble raw materials are the most important factors in cellulose fermentation. Pretreatment of lignocellulosic materials is steam explosion, organosolv extraction, treatment with alkali or acid, treatment with ionic liquids, heat treatment of finely ground particles, etc. Since the aim of this study is to provide hydrolysis without chemical pretreatment, the ground / non-ground particles are used in experiments by thermal treatment without physicochemical pretreatment.

Renewable structure of rice husk is a useful feature, it has low density and no abrasive, along with its reasonable strength and durability. Different polymers such as poly (lactic acid), polybutylene succinate are friendly compounds which are made from rice husk. These compounds have been used in the automobile industry, in insulation applications, in the agricultural area as fertilizer, in the pool edge trim and construction products in building materials. Due to its high silica content (87-97% SiO₂), high porosity, light weight and large outer surface area, rice husk is a valuable material for industrial applications compared to other agricultural wastes. Rice husk can be used as adsorbent (color removal, heavy metal removal, fluoride removal), silica welding, insulation material, cement additive, pigment production material, mushroom production, poultry bed material, organic fertilizer or gasification as fuel or biofuel (Soltani et al., 2015). The main component of rice husk is cellulose. It is thermodynamically stable, crystallized by various hydrogen chains. By providing chemical modification of the cellulose, an oxidized cellulose structure can be formed,

which is also referred to as oxelylcellulose. Oxicellulose is widely used in medical applications and is known as a biocompatible and bioabsorbable polymer. Cellulosic materials can be easily sterilized, have excellent biocompatibility and antioxidant activity. Therefore, it is used in wound control and organ transplantation (Shukla et al., 2013).

There are bio-hydrogen production studies with the by-products from the rice. These studies are about hydrogen and methane production which used dark, light fermentation or combined fermentation with pure cultures or mixed cultures. Hydrogen was produced by dark or light fermentation in different studies and the hydrogen production yield using rice, rice stem etc. ranged between 7.4-155 mL g⁻¹ VS. (Cheng et al., 2008; Chen et al., 2011; He et al., 2014; Kim et al., 2012; Han et al., 2015; Jung et al., 2013). There are limited studies of bio-hydrogen production from rice husk. Most studies in the literature are focusing on the bio-ethanol production (Saha et al., 2005; Singh, 2014; Kaparaju et al., 2009).

2.6 Pre-treatment Methods

2.6.1 Inoculum Pre-treatment

Bio-hydrogen can be produced by anaerobic microorganisms when the final product is acetic acid and butyric acid, hydrogen yields are 4 and 2 mols glucose from 1 mol of glucose respectively (Thauer et al., 1977). Propionic and lactic acid production pathways are not wanted with bio-hydrogen production pathways. As the main products acetate and butyrate, a mixture of volatile fatty acids may be produced during dark fermentation (Alibardi & Cossu, 2016; Pan et al., 2008). Pre-treatment methods are crucial for high bio-hydrogen production yield and rate in dark fermentation. Pre-treatment methods can be classified as inoculum pre-treatments and substrate pre-treatment. Purpose of inoculum pre-treatments is choosing hydrogen producing microorganism, therefore, bio-hydrogen production yields can be increased. Substrate pre-treatment methods can provide better hydrolysis of the complex substrate and biodegradable nutrients for microorganism growth. Also eliminating the hydrogen consuming microorganisms is the other important step for bio-hydrogen

production process with fermentation (R. Rafieenia et al., 2018).

Hydrogen consuming microorganisms can be present because of different factors, different culture conditions. Methanogens are the most important hydrogen consuming microorganisms. Hydrogen consuming microorganisms can reduce bio-hydrogen production yields significantly. Methanogens can consume hydrogen to produce methane. Because of this, inoculum pre-treatment is enriching bio-hydrogen producing microorganisms and eliminating hydrogen-consuming microorganisms, specially methanogens (Rafieenia et al., 2018).

Thermal pre-treatment or heat shock is the most used pre-treatment method for inhibition of hydrogen consuming microorganisms and enrichment of hydrogen-producing bacteria. Heat shock has been used when complex substrates were used as the substrate (Kumar et al., 2015). *Clostridium spp.* and *Bacillus spp.* are spore-forming microorganisms and the most important hydrogen producing microorganisms.

Aeration is another inoculum pre-treatment method in order to inhibit hydrogen consuming microorganisms and enrich hydrogen-producing microorganisms. Methanogens are strictly anaerobic microorganisms and are sensitive to the presence of oxygen. Aeration can inhibit all anaerobic hydrogen consuming microorganism while hydrogen producing and spore-forming microorganisms can survive.

Acid and alkaline pre-treatments are used for eliminating hydrogen consumers with pH changes. Other inoculum pre-treatments, acid and alkaline pre-treatment methods intend to perform extreme conditions for hydrogen consuming microorganisms which are non-spore forming. *Clostridium spp.* and *Bacillus spp.* are spore-forming hydrogen producers and can survive (Wong et al., 2014). Acid and alkaline pre-treatments were used for inoculum pre-treatment in dark fermentation and pre-treated activated sludge and glucose as substrate were used in a study. Hydrogen yield almost increased 3 fold with acid pre-treatment in comparison with untreated activated sludge control (Chang et al., 2011).

2.6.2 Substrate Pre-treatment

Easily degradable substrates are crucial for microorganisms and hydrogen production. Lignocellulosic materials are plentiful and may be regarded as an appropriate substrate for hydrogen production. Lignocellulosic materials are not biodegradable because of their structure which has a crystalline structure also lignocellulosic materials contain cellulose, hemicellulose, and lignin. Cellulose and hemicellulose must be hydrolyzed to biodegradable compounds for microorganisms and increase the hydrogen production yield.

Substrate pre-treatment methods provide to break the complex structure and increase the hydrogen production yield. Hydrogen production yields can be affected by substrate and pre-treatment methods. The most important issue of pre-treatment methods are related with the substrate, cost, and sustainability.

Thermal pre-treatment can be used for breakdown the structure and increase the solubility of the substrate. Also, thermal pre-treatment provide the removal of pathogens. Temperatures above 160 °C may be used for solubilization of lignin and hemicellulose. About 400 °C degree may be used for formation of inhibitory compounds such as phenolic compounds, furfural (Chen et al., 2013; Hendriks & Zeeman, 2009).

Acid or alkaline pre-treatment also has been used for substrate pre-treatment which can increase the solubility of compounds. Acid pre-treatment may improve the solubilization but can not affect the delignification (Hendriks & Zeeman, 2009). Alkaline pre-treatment breakdown the crystalline structure and improve the hydrolysis. HCl and H₂SO₄ are the main acids for acid pre-treatment and NaOH is the main alkaline for alkaline pre-treatment used (R. Rafieenia et al., 2018; Assawamongkholsiri et al., 2013).

Microwave irradiation provides high temperatures and disruption of the cell wall. Thus solubility of the substrate may be increased. When the solubility of the substrate is increased, degradability of the substrate will increase (Banik et al., 2003). There is

not plentiful study about microwave irradiation pre-treatment methods for hydrogen production because of the high cost of this method (R. Rafieenia et al., 2018).

Biological pre-treatment methods are used for breakdown the structure of lignocellulosic materials and improve the solubility. Biological treatments contain, fungal treatment, enzymatic hydrolysis, and aeration. Specific groups of fungi produce extracellular enzymes to degrade lignin, hemicellulose, and cellulose, thus increase the hydrolysis yield and hydrogen production yield (Sun & Cheng, 2002). Biological pre-treatment also contains direct utilization of enzymes for hydrolysis. Different studies were performed with enzymatic hydrolysis as a substrate pre-treatment method for increasing the hydrogen production yield and rate used pretreated cassava wastewater with 0.2% α -amylase and hydrogen yield was 50% higher than control (Cui et al., 2010; Leano & Babel, 2012; Liu et al., 2011) used *Aspergillus fumigatus* for production of bioethanol from yeast in the second stage and first tried to produce enzyme (CMCase, FPase) from lignocellulose with an initial culture density of 50 °C, 80% water content, pH 4 and 7%, Guo et al., (2010) produced cellulase from corn stalk using *Trichoderma viride* in the solid state fermentation process and used it in the second stage for biohydrogen production (122 mL H₂ g⁻¹TVS).

2.7 Biohydrogen Production Processes from Rice Husk

There are studies of hydrogen production from by-product of raw rice like rice stalk, rice stem. In these studies, different hydrogen production methods, different pure cultures or anaerobic sludge to get good hydrogen production yields which are reported in a range of 7.4-155 H₂ mL g⁻¹ VS (Cheng et al., 2008; Chen et al., 2011; He et al., 2014; Kim et al., 2012). There is a limited study of bio-hydrogen production from rice husk compared to bio-ethanol production. For instance, after diluted acid hydrolysis and alkaline hydrolysis of rice husk, bio-ethanol were produced with saccharification and fermentation (Saha et al., 2005; Singh, 2014; Megawati, 2011). Table 2.4 summarizes some studies performed with raw rice by-products trying different pretreatment methods for biofuel production. In these studies, it can be seen that hydrogen gas produced with pure and mixed cultures with a maximum hydrogen production yield of 155 mL H₂ g⁻¹ TVS.

Table 2.4 Bio-hydrogen production from different by-products of raw rice

<i>Substrate</i>	<i>Method</i>	<i>Pre-Treatment</i>	<i>Product</i>	<i>Microorganisms</i>	<i>Maximum Production Yield</i>	<i>References</i>
Rice Straw	Combined Fermentation	Alkaline via Microwave	Hydrogen	<i>Clostridium butyricum</i> , <i>Rhodopseudomonas palustris</i>	155 mL H ₂ g ⁻¹ TVS	Cheng, et al., 2011
Rice Straw	Dark Fermentation	Acid Hydrolysis	Hydrogen	<i>Active sludge culture</i>	0.305 mmol H ₂ g ⁻¹ rice straw	Cheng, et al., 2011
Rice Straw	Dark Fermentation	-	Hydrogen	<i>Active sludge culture</i>	24.8 mL H ₂ g ⁻¹ TS	Cheng, et al., 2012
Rice Husk	Dark Fermentation	Microwave-assisted Alkaline	Ethanol	<i>Saccharomyces cerevisiae</i> , <i>Scheffersomyces stipitis</i> , and <i>co-culture</i>	0.42g ethanol g ⁻¹ rice husk	Singh et al., 2012
Rice Husk	Dark Fermentation	-	Bio-gas		382 mL biogas d ⁻¹	Oked et al., 2012

2.8 Energy and Economics of Hydrogen

Hydrogen energy, is the energy of the future which does not damage the environment due to the rapid depletion of fossil energy resources (coal, oil, natural gas). It can be carried with little energy loss in transportation. It can be used for electricity and mechanical energy. Hydrogen is not a natural fuel but can be produced in different ways from different materials. Hydrogen can easily be converted to electricity and thus will have an important role in technological advances in the future (Momirlan & Veziroglu, 2002).

There are advantages in hydrogen such as no infrastructure requirement for transport the fuel, can be stored without any loss. Hydrogen is the most plentiful element in the universe and does not emit polluting products such as carbon monoxide, carbon dioxide etc. (Tutar & Eren, 2011). The energy of hydrogen per kilogram is almost three-fold bigger than fossil fuels (Argun, 2010).

The production of hydrogen with minimum cost and environmentally friendly manner is crucial for the hydrogen economy. Conventionally, automotive and transport fuel industries are connected and have to co-operate. The absence of hydrogen re-fueling stations hinder the establishment and development of the fuel cell vehicle market (Mc Dowall & Eames, 2006). For example, the infrastructure required for energy generation, storage and distribution in a large country like the US is estimated to cost billions of dollars. Generally, energy infrastructure investments have long been dependent on a fixed energy system which cannot be changed easily to meet changing market conditions (Waegel et al., 2006).

The WE-NET project, In Japan, is the biggest and most attractive hydrogen project in the world. Japan plans to spend \$ 4 billion by 2020 within the framework of plans to convert the energy system to hydrogen (Momirlan & Veziroglu, 2002). In addition, Japan is planning to make an artificial island in the equatorial part of the Pacific Ocean and to produce hydrogen from seawater by electrolysis (Goltsov & Veziroglu, 2001).

2.9 Objectives and Scope of This Study

The objective of this study is to investigate bio-hydrogen production by dark fermentation of rice husk under consolidated and submerged conditions. The most suitable operating conditions resulting with the highest bio-hydrogen production yield and rate were determined. *Clostridium intestinale* and *Clostridium termitidis* were used for this purpose.

Objectives of the study can be summarized as follows:

- To select the most effective pre-treatment method for maximizing the bio-hydrogen production yield and bio-hydrogen production rate in dark fermentation where consolidated fermentation can take place.
- To select the most effective substrate concentration for maximizing the bio-hydrogen production yield and bio-hydrogen production rate in dark and submerged fermentation with the selected pure organisms.
- To select the most effective co-culture ratio for maximizing the bio-hydrogen production yield and bio-hydrogen production rate in dark fermentation.
- To select the most effective substrate concentration for maximizing the bio-hydrogen production yield and bio-hydrogen production rate in dark fermentation with the selected co-culture ratio.
- To select the most effective substrate particle size for maximizing the bio-hydrogen production yield and bio-hydrogen production rate in dark fermentation with the selected co-culture ratio.

CHAPTER THREE

MATERIAL AND METHODS

3.1 Organisms

Clostridium termitidis ATCC-21846 and *Clostridium intestinale* ATCC-BAA 1027 cultures were cultivated in Medium 1191 (1.5 g L⁻¹ KH₂PO₄, 3.35 g L⁻¹ Na₂HPO₄, 0.5 g L⁻¹ NH₄Cl, 0.18 g L⁻¹ MgSO₄, 2 g L⁻¹ yeast extract, 0.25 g L⁻¹ resazurin, 1 g L⁻¹ L-cysteine, 10 mg L⁻¹ piridoxin, 5 mg L⁻¹ riboflavin, 5 mg L⁻¹ thiamin, 5 mg L⁻¹ nicotinic acid, 5 mg L⁻¹ p-aminobenzoic acid, 5 mg L⁻¹ lipoic acid, 2 mg L⁻¹ biotin, 2 mg L⁻¹ folic acid, 1 mg L⁻¹ cyanocobalamin, 2.02 g L⁻¹ trisodium nitrilacetate, 0.21 g L⁻¹ FeCl₂, 1 0.2 g L⁻¹ CoC, 1 0.1 g L⁻¹ MnC, 0.1 g L⁻¹ ZnCl₂, 0.01 g L⁻¹ NiCl₂, 0.05 g L⁻¹ CaCl₂, 0.05 g L⁻¹ CuSO₄, and 0.05 g L⁻¹ Na₂MoO). pH was adjusted to 7.5 by using NaOH. 10 g L⁻¹ glucose for *Clostridium intestinale* and 10 g L⁻¹ α-cellulose for *Clostridium termitidis* were used as carbon sources. Stock cultures were stored at 4 °C. *Clostridium intestinale* and *Clostridium termitidis* were cultivated for 24 and 168 hours, respectively. After cultivation, their protein contents were adjusted to have a co-culture ratio to be used in the experimental bottles. Microorganisms were cultivated in the growth medium using an incubator under sterile conditions at pH 7.5 and 37°C.

The most important and expensive step in utilization of lignocellulosic substances for hydrogen production is hydrolysis. Enzymatic hydrolysis of lignocellulosic material was achieved through consolidated fermentation by using *C.termitidis* which can grow on cellulosic material. This culture has the capacity to produce hydrogen, as well as to promote disintegration. Since the doubling time of the *Clostridium termitidis* was high, *C. termitidis* and *C. intestinale* were used together to accelerate biohydrogen production.

3.2 Substrate Preparation

Rice husk was the lignocellulosic material provided by Birlik Çeltik A.Ş / Edirne. The rice husk was used in different particle sizes (<2000 µm, <300 µm, <74 µm). It was ground and sieved then used in the experiment. Cellulose, hemicellulose and lignin contents of rice husk used in the study were examined according to Yasar, (2002) and

they were as 34.52 % cellulose, 26 % hemicellulose, 22.68% lignin and 16.8 % extractive substances.

Since the aim of this study was to provide hydrolysis without chemical pre-treatment, the milled / non-milled particles were used in the experiments by thermal treatment only without physicochemical pre-treatment. The goal is mainly to break down hemicellulose. Thus, enzymatic degradability of cellulose is increased and inhibition products such as 5-hydroxymethylfurfur (5-HMF) and furfural are prevented.

Acid hydrolyzed substrate was tested for fermentation. The sugars in hemicellulose are converted into saccharide form (glucose, xylose, arabinose, mannose). The aim of the method is to obtain high sugar concentrations. For this purpose, sulphuric acid was used. The lignocellulosic material was broken down and the hemicellulose fraction was converted to soluble sugars, and at the second stage cellulose fraction was converted by enzymatic hydrolysis. Sulphuric acid acts as a highly active catalyst and high concentrations of sugar are obtained. 2 % of H₂SO₄ solution was applied to the substrate at 120°C for 15 min to convert cellulose and hemicelluloses into dissolved sugars.

Furthermore, during the preparation of the substrate, pure cultures of *C. termitidis* and *C. intestinale* were subjected to different substrate conditions (explained in part 3.3.1) and their hydrogen production performance was evaluated. For this purpose, acid hydrolysis, which gives the best total sugar value in substrate preparation, 5 g L⁻¹ raw and ground rice husk at an initial pH of 7.5 was added to the experiments as positive controls besides thermal treated rice husk experiments.

The furfural and 5-HMF concentrations after application of pre-treatment has a crucial importance to the fermentation performance. There were only furfurals and 5-HMF's in acid pretreated rice husk which were around 0.07 - 0.15 mg L⁻¹ and 0.07 - 0.08 mg L⁻¹, respectively. They were no furfurals or 5-HMFs in thermal treated rice husks.

3.3 Experimental Set-up and Procedure

3.3.1 Experiments Performed with Pure Cultures

Pretreated and untreated different rice husk concentrations were placed in serum bottles with growth medium. Serum bottles (Isolab-Germany Boro 3.3) with a working volume of 150 mL and a total volume of 310 mL were used. Serum bottles were autoclaved with medium 1191 and substrate concentrations for sterilization. After sterilization, anaerobic conditions were maintained by passing nitrogen gas from the head space of the bottles for 10 min at the beginning of the experiments. Silicone stoppers were used to avoid gas leakage from the bottles. Substrates with *Clostridium intestinale* ATCC-BAA 1027 were placed in serum bottles. Experiments were carried out in an incubator at 37°C at static conditions for 2 days. Pure *Clostridium intestinale* culture with 5 g L⁻¹ glucose, cellulose, acid hydrolysed rice husk was used as positive control and also a bottle of raw rice with a particle size of <2000 µm was added to the experiments. 5 g L⁻¹ rice husk was also used without culture for negative control. Pure culture experiments were conducted with different substrate concentrations (1, 2, 5, 10, 15, 20, 33 g L⁻¹) and 10% total inoculum culture (*Clostridium.intestinale*). The biomass concentration in this experiment was analyzed via suspended matter and it was set to a constant value of 1 g L⁻¹. Table 3.1 shows the experimental setup of the experiments conducted with pure *C. intestinale*.

Rice husk with *Clostridium termitidis* ATCC-21846 were placed in serum bottles. Experiment were carried out in an incubator at 37°C at static conditions for 14 days. Pure *Clostridium termitidis* culture with 5 g L⁻¹ rice husk was used as positive control. Fermentation without microorganisms with 5 g L⁻¹ rice husk was used as negative control. Pure culture experiments were conducted at different substrate concentrations (1, 2, 5, 10, 15, 20 g L⁻¹) and 10% total inoculum culture (*Clostridium termitidis*). Biomass concentration of *C. termitidis* was analyzed based on protein concentration. The initial protein concentration of microorganisms was set to 0.15 g L⁻¹. Table 3.2 shows the experimental setup of the experiments conducted with pure *C. termitidis*.

Table 3.1 Different substrate concentrations for pure *Clostridium intestinale* experiment

Substrate	Particle Sizes	Thermal Treatment	Acid Hydrolysis	Micro-Organisms
5 g L ⁻¹ Rice Husk	<300 µm	+	-	-
5 g L ⁻¹ Glucose		-	-	+
5 g L ⁻¹ Cellobiose		-	-	+
5 g L ⁻¹ Rice Husk	<300 µm	-	+	+
5 g L ⁻¹ Rice Husk	<2000 µm	-	-	+
5 g L ⁻¹ Rice Husk	<300 µm	-	-	+
1 g L ⁻¹ Rice Husk	<300 µm	+	-	+
2 g L ⁻¹ Rice Husk	<300 µm	+	-	+
5 g L ⁻¹ Rice Husk	<300 µm	+	-	+
10 g L ⁻¹ Rice Husk	<300 µm	+	-	+
15 g L ⁻¹ Rice Husk	<300 µm	+	-	+
20 g L ⁻¹ Rice Husk	<300 µm	+	-	+
33 g L ⁻¹ Rice Husk	<300 µm	+	-	+

Table 3.2 Different substrate concentrations for pure *Clostridium termitidis* experiment

Substrate	Particle Sizes	Thermal Treatment	Acid Hydrolysis	Organisms
5 g L ⁻¹ Rice Husk	<300 µm	+	-	-
5 g L ⁻¹ Glucose		-	-	+
5 g L ⁻¹ Cellulose		-	-	+
5 g L ⁻¹ Rice Husk	<2000 µm	-	-	+
5 g L ⁻¹ Rice Husk	<300 µm	-	-	+
5 g L ⁻¹ Rice Husk	<300 µm	-	+	+
1 g L ⁻¹ Rice Husk	<300 µm	+	-	+
2 g L ⁻¹ Rice Husk	<300 µm	+	-	+
5 g L ⁻¹ Rice Husk	<300 µm	+	-	+
10 g L ⁻¹ Rice Husk	<300 µm	+	-	+
15 g L ⁻¹ Rice Husk	<300 µm	+	-	+
20 g L ⁻¹ Rice Husk	<300 µm	+	-	+

3.3.2 Experiments Performed with Co-culture

Rice husks (RH) without pretreatment were placed in serum bottles with growth medium. Serum bottles (Isolab-Germany Boro 3.3) with a working volume of 150 mL and a total volume of 310 mL were used. Serum bottles were autoclaved for sterilization. After sterilization, anaerobic conditions were maintained by passing nitrogen gas from the head space of the bottles for 10 min at the beginning of the experiments. Silicone stoppers were used to avoid gas leakage from the bottles. Rice husk with different co-culture ratios (1:1, 2:1, 5:1, 10:1, 15:1, 20:1, *Clostridium termitidis* ATCC-21846: *Clostridium intestinale* ATCC-BAA 1027) were placed in serum bottles with 10% total inoculum culture. The substrate concentration was constant at 5 g L⁻¹. Experiments were carried out in an incubator at 37°C at static conditions. Pure *Clostridium intestinale* culture and pure *Clostridium termitidis* with 5 g L⁻¹ rice husk was used as positive controls. Fermentation without microorganisms with 5 g L⁻¹ rice husk was used as negative control. The co-culture ratios were subjected to raw (<2000 µm,) ground (<300 µm), ground and sieved rice husk (<74 µm). Biomass concentrations of *C. termitidis* and *C. intestinale* were analyzed based on protein concentration. The initial protein concentrations of the microorganisms were set to 2 g L⁻¹ and 4 g L⁻¹, respectively.

3.3.3 Different Particle Sizes for Biohydrogen Production

Rice husks were placed in serum bottles with growth medium. Serum bottles (Isolab-Germany Boro 3.3) with a working volume of 150 mL and a total volume of 310 mL were used. Serum bottles autoclaved for sterilization. After sterilization, anaerobic conditions were maintained by passing nitrogen gas from the head space of the bottles for 10 min at the beginning of the experiments. Silicone stoppers were used to avoid gas leakage from the bottles. Rice husk was used with 3 different particle sizes for determining the best particle size which achieve high hydrogen production performance. Rice husk was ground due to achieve particle size of under 50 mesh, 200 mesh and sieved. Particle sizes of rice husk were used as raw, under 50 mesh and under 200 mesh referring to <2000 µm, <300 µm and <74 µm. Co-culture and particle size experiments were conducted together.

3.3.4 Different Substrate Concentrations with the Co-Culture (*Clostridium termitidis*: *Clostridium intestinale*)

Rice husks were placed in serum bottles with growth medium. Serum bottles (Isolab-Germany Boro 3.3) with a working volume of 150 mL and a total volume of 310 mL were used. Serum bottles autoclaved for sterilization. After sterilization, anaerobic conditions were maintained by passing nitrogen gas from the head space of the bottles for 10 min at the beginning of the experiments. Silicone stoppers were used to avoid gas leakage from the bottles. Rice husk with a co-culture ratio of 5:1 (*Clostridium. termitidis* ATCC-21846: *Clostridium intestinale* ATCC-BAA 1027) were placed in serum bottles with 10% total inoculum culture. Different substrate concentrations were used (1, 2, 5, 10, 15, 20 g L⁻¹). Experiments were carried out in an incubator at 37°C at static conditions. Pure *Clostridium intestinale* culture and pure *Clostridium termitidis* with 5 g L⁻¹ rice husk was used as positive controls. Fermentation without microorganisms with 5 g L⁻¹ rice husk was used as negative control. Biomass concentration of *C. termitidis* and *C. intestinale* were analyzed based on protein concentration. The initial protein concentrations of the experiments were set to 2 g L⁻¹ and 4 g L⁻¹, respectively.

3.4 Analysis

At certain intervals (daily) 5 mL samples were taken and centrifuged for 15 min at 8000 rpm. After centrifugation, total sugar, TVFA analyzes were performed from the upper liquid. In addition, the mono saccharides composition and total volatile fatty acids (TVFA) analyses were carried out by HPLC (Agilent 1100) with Aminex HPX-87H column (9 µm x 300 x 7.8 mm). The mobile phase was 5 mM sulphuric acid with a flow rate of 0.6 mL min⁻¹. TVFA was detected by UV detector at 220 nm and the other metabolites such as glucose, cellobiose were detected by RID at 50°C.

Hydrogen gas was sampled from the headspace of the bottles by using gas-tight glass syringes and hydrogen concentration was determined by using a gas chromatograph (Agilent 6890 N-GC). The GC column was Alltech, Hayesep D 80/100 6 in. x 1/8 in. x 0.85 in. Nitrogen gas was used as the carrier with a flow rate of 30

mL min⁻¹ and the head pressure was 22 psi. Temperatures of the oven, injection, detector, and filament were 35 °C, 120 °C, 120 °C, 140 °C, respectively.

Sugar analysis in rice husk was analyzed by phenol-acid method (Dubois et al., 1956). 1 mL of sample, 1 mL of 5% phenol solution and 5 mL of pure H₂SO₄ were mixed. The samples prepared in the glass tube were incubated for 20 minutes at 25-30°C and the absorbance values of the samples at 480 nm wavelength were read (Dubois et al., 1956). The same procedure was carried out by using pure water instead of the sample in the blank. In forming the calibration graph, the absorbance values corresponding to the glucose solutions at the concentrations of 20-40-60-80-100-120 mg L⁻¹ were determined by the method described above.

The total gas volume produced was determined using the water displacement system based on the principle of compound containers (2% H₂SO₄, 10% NaCl). Biomass concentrations were based on protein analysis by modified Lowry method. Modified Lowry method is an analysis used for determining the total protein concentration for liquid samples. The total protein develops a color, therefore total protein concentration can be determined by spectrophotometric methods. The pH and ORP of the fermentation medium were performed with the aid of probes (WTW Scientific, Germany). In addition, the method used for characterization of rice husk is given in part 3.4.1.

Furfural and 5-HMF analyses were carried out by HPLC (Agilent 1100). C18 column (150 x 4.6 mm) at 40 °C and Diode Array Detector at 284 nm were used. Water: acetonitrile (95: 5) was used as the mobile phase at a flow rate of 0.5 mL min⁻¹ for 20 minutes. Furfural and 5-HMF standards were prepared in the range of 0.01-2 mg L⁻¹ (Garcia-Villanova et al., 1993; Gomis, 1991; Theobald et al., 1998; Rufian-Henares et al. 2001).

Rice husk conversion efficiency was determined by analyzing the residual rice husk. First the pellet taken from the centrifuged sample after fermentation period was washed with 0.9% NaCl solution and centrifuged at 7000 rpm for 15 minutes. This

procedure was carried out three times. Then the pellet was dried at 105 °C and the weight of the residual rice husk was measured.

3.4.1 Cellulose, Hemicellulose, Lignin Determination Method

2.5 g of material, which was ground to 0.5 mm, was added to 100 mL of deionized water and hot water extraction was carried out at 95 °C for three hours. 60 mL of acetone were added to the resulting hot water extraction residue and extraction was carried out for 24 hours. The acetone extraction residue was dried at room temperature and ground in ceramic mortar.

After the addition of 0.1 g of ground material, firstly 2.5 mL of 17.5% NaOH solution was added with magnetic stirrer for 1 hour at 20-25 °C and then, 3.3 mL of deionized water was added to the contents and the extraction was continued for 1 hour. The alkaline extract separated from the cellulose residue by centrifugation was diluted to 20 mL with 8.3% NaOH. 2 mL of the extract was acidified with 0.5 mL of CH₃COOH and the suspension of hemicelluloses was allowed to stand at -20 °C for 24 hours after addition of 8 mL of acetone. The precipitate collected by centrifugation was dried in the oven and the amount of hemicellulose was determined gravimetrically. The cellulose residue was also dried in the oven and the amount was determined.

For the lignin analysis, 1 g of the residue obtained as a result of the extraction was left in a solution of 30 mL of 72% H₂SO₄ for 24 hours at room temperature. Then 300 mL of distilled water was added and refluxed for 1 hour. After cooling the mixture, it was filtered through filter paper and washed with pure water to neutral pH. The lignin content was determined in the sample at a weight of 80 °C until constant weight and dried. The amount of cellulose was calculated by calculating the percentage of lignin, hemicellulose and extractable substances determined experimentally. The method was modified from the 2002 article of Yaşar (Yasar, 2002).

3.5 Theoretical Background

- Hydrogen Gas Volume

$$V_{H_2} = C_{H_2} * V_{GAS} \quad (3.1)$$

C_{H_2} = Hydrogen Gas Concentration , (% H_2)

V_{GAS} = Total Produced Gas (mL)

- mmol Hydrogen Gas (37°C)

$$PV = nRT \quad (3.2)$$

n = mmol H_2 gas, P =1 atm, V_{H_2} = Total Produced Gas (mL)

R = 0.082 (L atm mol⁻¹ °K), T = temperature (°K)

- Cumulative Hydrogen Gas Volume (CHF)

$$V_{H_2, i} = V_{H_2, i-1} + V_W * C_{H_2, i} + V_{G, i} * C_{H_2, i} - V_{G, i-1} * C_{H_2, i-1} \quad (3.3)$$

- Hydrogen Production Yield (Y)

$$Y = \frac{\Delta H_2}{\Delta S} \quad (3.4)$$

Y = mL H_2 g⁻¹ substrate or mol H_2 mol⁻¹ substrate

ΔH_2 = H_2 production (mL H_2 or mol H_2)

ΔS = Consumed Substrate (total sugar, starch) (gram or mol)

- Specific Hydrogen Production Rate (SHPR)

$$SHPR = \frac{Rm}{V_0 X_0} \quad (3.5)$$

V_0 = Initial Liquid Volume (L)

X_0 = Initial Biomass Concentration g X L⁻¹

- Volumetric Hydrogen Production Rate (VHPR)

$$VHPR = \frac{CHF}{V_0 t} \quad (3.6)$$

V_0 = Initial Liquid Volume (L)

CHF= Cumulative Hydrogen (mL H₂)

t= Fermentation Time (h)

- Rice husk conversion efficiency

$$E \% = \frac{\text{Initial Rice Husk} - \text{Residual Rice Husk}}{\text{Initial Rice Husk}} * 100 \quad (3.7)$$

Initial rice husk (g)

Residual rice husk (g)

- Hydrogen Production Rate (HPR)

$$HPR = \frac{CHF}{\Delta S * t} \quad (3.8)$$

ΔS = Consumed substrate (g)

t= Time (h)

CHF= Cumulative Hydrogen (mL H₂)

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Determination of Most Convenient Substrate Concentration and Substrate Pretreatment Method with Pure Cultures of *Clostridium termitidis* and *Clostridium intestinale*

Two different *Clostridium* types were used to evaluate biohydrogen production performance of rice husk. *Clostridium termitidis* and *Clostridium intestinale* was used as the organisms. *Clostridium intestinale* is a fast growing organism which only uses simple sugars to convert them into biohydrogen. *Clostridium termitidis* has the ability to degrade cellulosic substrates into simple sugars and convert these sugars into biohydrogen via consolidated fermentation.

The first step of the study includes evaluation of the performance of the selected organisms as pure cultures with rice husk to understand their ability to produce hydrogen. Experiments were carried out with *Clostridium intestinale*, which could easily grow on glucose but could not break the alpha cellulose. Then, *Clostridium termitidis* experiments were conducted which can grow easily on alpha cellulose. For this purpose, rice husk was prepared with control experiments and different substrate concentrations. Table 3.1 and Table 3.2 show the amount of rice husk prepared, whether they are ground or not, whether the organism has been added, or the rice husk was pretreated via thermal treatment or acid hydrolysis.

4.1.1 Growth of *Clostridium intestinale* ATCC-BAA 1027 in Rice Husk and Evaluation of Bio- Hydrogen Performance

Clostridium intestinale was subjected to different rice husk (RH) substrate concentrations varying between 1-33 g L⁻¹ for biohydrogen production. A two days incubation period was carried out at 37°C and static conditions with an initial pH of 7.5 and with a working volume of 150 mL. Different control experiments were conducted as well, to compare the hydrogen production performance of *Clostridium intestinale* with varies pretreated substrate concentrations.

The results of control experiments conduct with glucose and cellulose were not presented in the figure. Table 4.1 shows the hydrogen gas production performances of *C.intestinale* on glucose and cellobiose. The amount of hydrogen produced from glucose was 71.61 mL and it was 15.62 mL from cellulose. 95.48 mL H₂ g⁻¹ substrate of hydrogen production yield and 1.99 mL H₂ g⁻¹ substrate h⁻¹ of hydrogen production rate was obtained from 5 g L⁻¹ glucose. Hydrogen production yield and hydrogen production rate were 20.83 mL H₂ g⁻¹ substrate and 0.43 mL H₂ g⁻¹ initial substrate h⁻¹ when cellobiose was used as carbon source. The main volatile fatty acids were acetic, butyric and propionic acids and the main saccharides in the fermentation media were analyzed as cellulose and glucose. These results showed that *Clostridium intestinale* was active and converted simple sugars into bio hydrogen very fast.

Table 4.1 Hydrogen gas production performances of *C.intestinale* on glucose and cellulose

<i>Clostridium intestinale</i>	Cumulative Hydrogen Formation (mL H ₂)	pH	Final Sugar Concentration (mg L ⁻¹)	Hydrogen Production Yield (mL H ₂ g ⁻¹ substrate)	Hydrogen Production Rate (mL H ₂ g ⁻¹ substrate h ⁻¹)
Glucose	71.61	4.8	334.94	95.48	1.99
Cellobiose	15.62	6.5	123.25	20.83	0.43

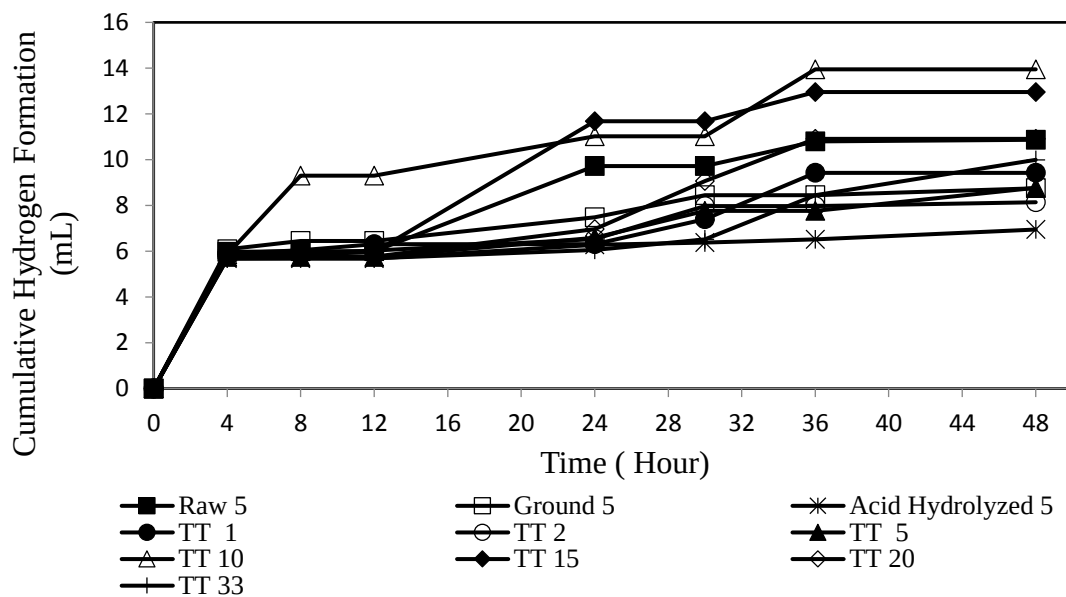


Figure 4.1 Cumulative hydrogen volumes with time in different rice husk concentrations *TT: Thermal treated rice husk in g L^{-1}

The cumulative amount of hydrogen in different substrate concentrations is shown in Figure 4.1. 10 g L^{-1} thermal treated rice husk gave the highest cumulative hydrogen production volume (14 mL H_2) at 36 hours. The acid pretreated bottle had the highest simple sugars, the cumulative amount of hydrogen was the lowest (6 mL). The probable cause of these low values may be due to the inhibition effect of the produced 5-HMF and furfural in fermentation media. The furfural and 5-HMF were analyzed after the fermentation period and they were 0.07 and 0.06 mg L^{-1} , respectively. The cumulative hydrogen amounts in the raw ($< 2000 \mu\text{m}$) and ground rice husk ($< 300 \mu\text{m}$) at 5 g L^{-1} concentration were 10 mL and 8 mL , respectively. There was just a little difference in different particle sizes when *Clostridium intestinale* was used as inoculum. On the other hand, it was observed that the increasing concentrations of rice husk up to 10 g L^{-1} increased the cumulative hydrogen volume, but decreased with 15 g L^{-1} .

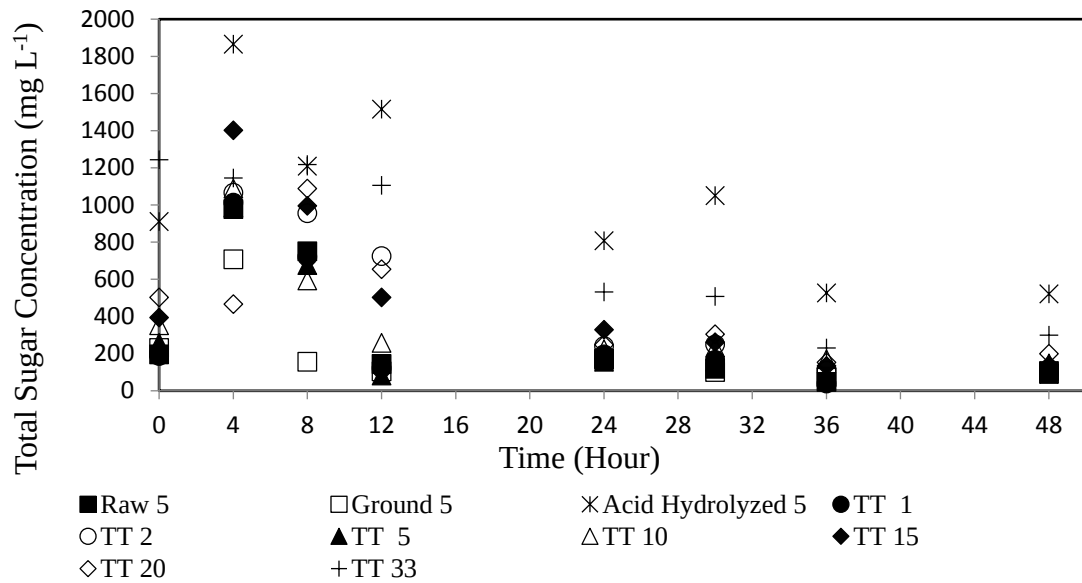


Figure 4.2 Variation of total sugar with time for different initial rice husk concentrations *TT: Thermal treated rice husk in g L⁻¹

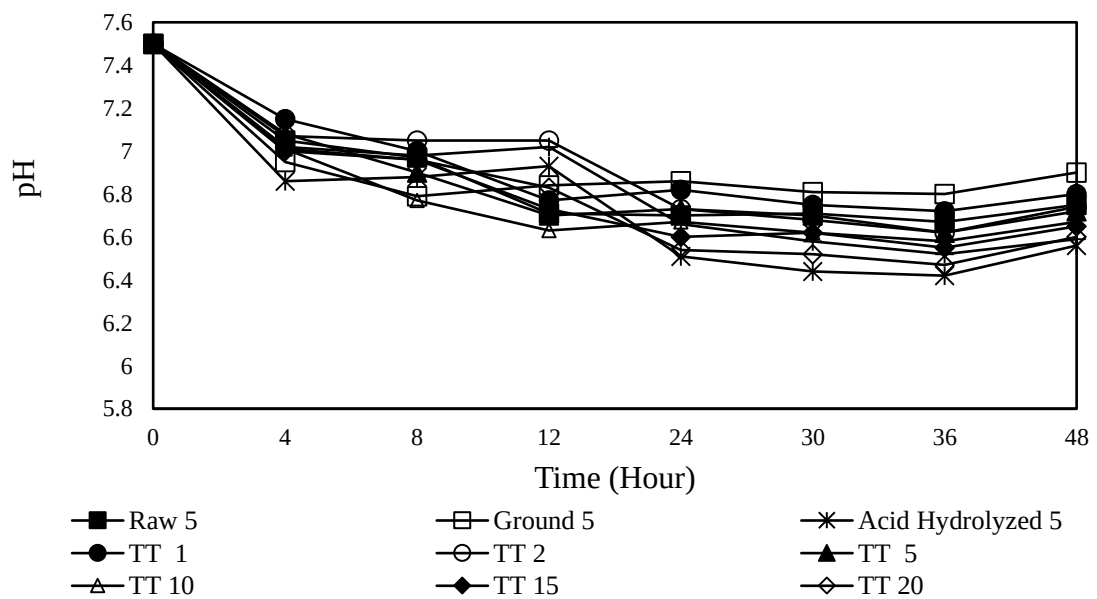


Figure 4.3 Variation of pH with time for different initial rice husk concentrations *TT: Thermal treated rice husk in g L⁻¹

Figure 4.2 shows the variation of total sugar concentrations with time in different rice husk concentrations. Figure 4.3 gives the pH values with time in different rice husk concentrations and conditions. It was observed that the highest sugar

consumption efficiency and pH change (Figure 4.3) was obtained from acid pretreated experiment. Approximately 1900 mg L⁻¹ total sugar was obtained the beginning of the fermentation period but the total sugar concentration decreased to 520 mg L⁻¹ in 36 hours. At the beginning of the fermentation period the total sugar concentrations were lower than the fourth hour. *C. intestinale* is a very fast growing organism and probably it converted the saccharides very fast and the misapplication of the analysis in the beginning of the experiments affected the total sugar concentrations at point zero. There was no increasing trend with increasing substrate concentration at the beginning of the fermentation period. At 15 g L⁻¹ thermal treated rice husk (RH) concentration, there was 1400 mg L⁻¹ total sugar concentration which decreased to 136 mg L⁻¹ after 36 hours. However, at the end of the fermentation time, sugar removal increased with increasing rice husk amounts. The pH was constant in all experimental bottles after a certain period of time and it remained in the range of 6.4-6.8. Phosphate buffer was added to the assay media at the beginning time to prevent rapid pH decreases and to remain at pH conditions that maximized hydrogen production.

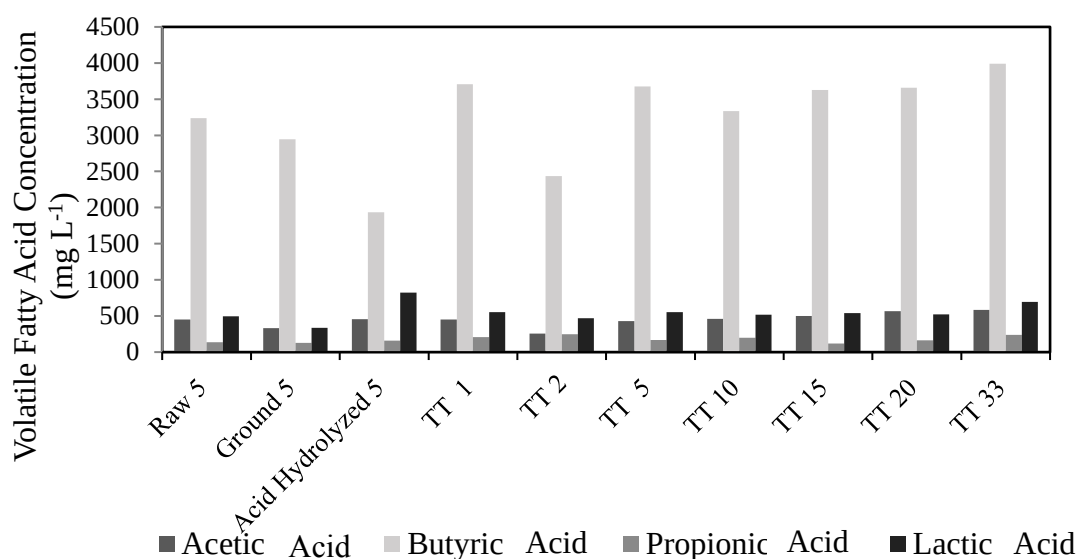


Figure 4.4 Variation of volatile fatty acid concentrations at the end of the fermentation time in different rice husk concentrations *TT: Thermal treated rice husk in g L⁻¹

Volatile fatty acid concentrations are depicted in Figure 4.4 for each experimental condition. The predominant organic acids were acetic, butyric, propionic and lactic acid. In the fermentation medium, it is observed that butyric acid was produced more

than the other VFAs by *C. intestinale* while the literature is also terming this organism as a butyrate producer (Rhamachandran, 2011). The highest amount of butyric acid (4000 mg L^{-1}) was produced at the highest substrate concentration. When thermal treatment was applied, except 2 g L^{-1} , the butyric acid concentration was nearly constant at around $3600\text{-}3900 \text{ mg L}^{-1}$ in thermal treated RH's. Furthermore; untreated RH, independent from particle size, gave a butyric acid concentration of $2900\text{-}3200 \text{ mg L}^{-1}$. The acid hydrolyzed RH produced the lowest butyric acid concentration due to possible inhibitors affecting the fermentation period. Butyric acid concentrations obtained under submerged fermentation conditions are compatible with the literature. The obtained volatile fatty acid concentrations were high, however the total sugar and saccharide concentrations taken from the fermentation media were not meeting the production of VFAs. This might be due to the substrate that was immediately converted into simple sugars by *C. intestinale* at the beginning of the fermentation period. The highest volatile fatty acid concentration was obtained at 33 g L^{-1} , but this organic acid could not be converted into hydrogen. This might be due to the produced butyric acid that may have inhibited the fermentation process.

Glucose and cellobiose were observed in the medium and the best saccharide concentrations were obtained after acid hydrolysis (not shown in the graphs). In the other experimental bottles, it was found that thermal treatment was not sufficient to produce simple sugars, but provided sufficient substrate for the survival of *C. intestinale*, which would support *C. termitidis* serving as the breakdown of lignocellulose for initial fermentation conditions in the co-culture.

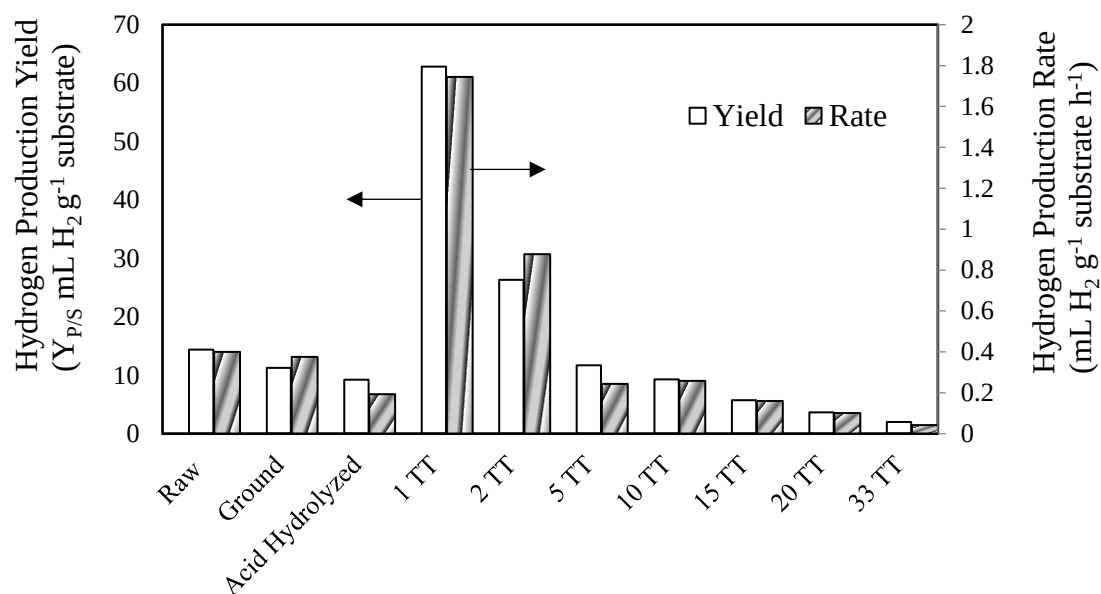


Figure 4.5 Hydrogen production yields and hydrogen production rates at different substrate concentrations and pretreatment conditions *TT: Thermal treated RH

Figure 4.5 depicts the hydrogen production yield and rate based on the initial substrate concentration at different experimental conditions. The biomass concentration at the end of the fermentation experiment was analyzed and it was found constant in all experiments as 1 g L^{-1} . The highest hydrogen production yield and hydrogen production rate was obtained with 1 g L^{-1} RH concentration when thermal treatment was applied and were $62.8 \text{ mL H}_2 \text{ g}^{-1} \text{ substrate}$ and $1.74 \text{ mL H}_2 \text{ g}^{-1} \text{ substrate h}^{-1}$, respectively. The yields and rates decreased with decreasing thermal treated substrate concentrations. This is due to the low soluble sugar concentrations in the fermentation media when compared with the initial substrate concentration. These results show that *C. intestinale* is a good soluble sugar consumer and butyric acid producer, however it can not convert cellulosic substrates into saccharide and ferment them thereafter into hydrogen.

4.1.2 Growth of *Clostridium termitidis* ATCC 21846 in Rice Husk and Evaluation of Biohydrogen Performance

The culture was grown in alpha cellulose with medium 1191 for 165 hours. The experiments were carried out in a working volume of 150 mL, at static conditions in a 37°C incubator for 14 days. Different pretreatment methods (thermal treatment, acid hydrolysis, grinding) and different substrate concentrations with thermal treated RH (1-20 g L⁻¹) were applied. The hydrogen production performance under different experimental conditions was examined and time variations of the cumulative hydrogen volumes are given in Figure 4.6. The highest cumulative hydrogen formation was 16.6 mL in the bottle containing untreated raw rice husk. In the experiments, a similar increase was observed in almost all bottles in the first 24 hours (5-5.5 mL) and in later periods, this increase was observed to pause in certain periods and then tend to increase in certain periods. This type of growth shows a growth pathern called “Diauxic Growth”. “Consolidated Growth” also can be termed for this type of growth where enzymatic hydrolysis first degrade cellulosic material into simplies forms and then converts them into hydrogen. In these conditions, the culture first converts the lignocellulosic material down to simpler structures like cellobiose via enzymatic hydrolysis and then converts them into organic acid and hydrogen.

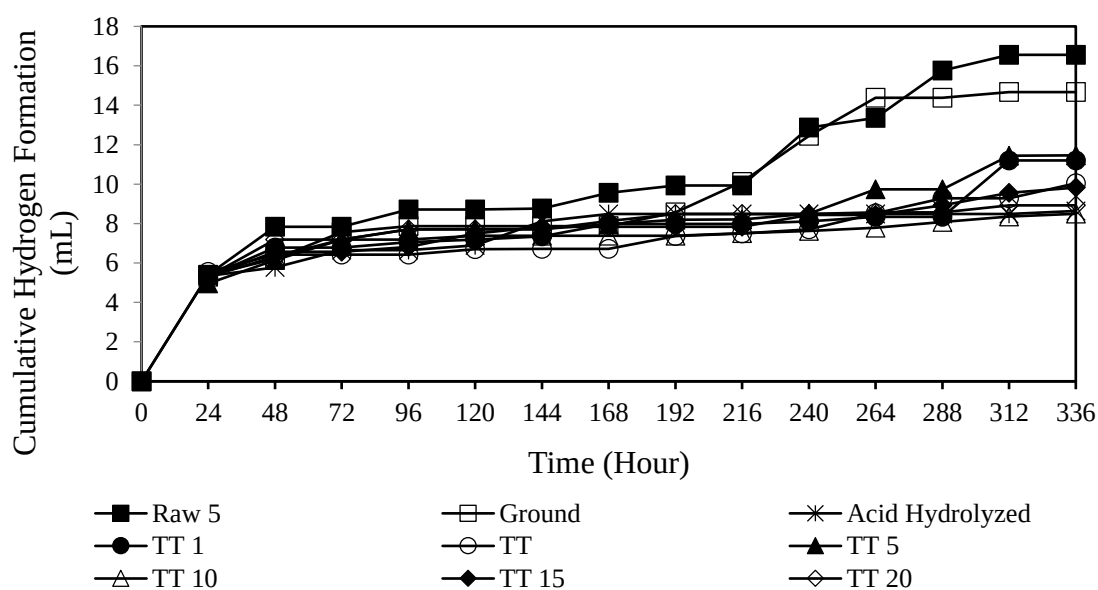


Figure 4.6 Cumulative hydrogen volumes with time in different rice husk concentrations *TT: Thermal treated rice husk in g L⁻¹

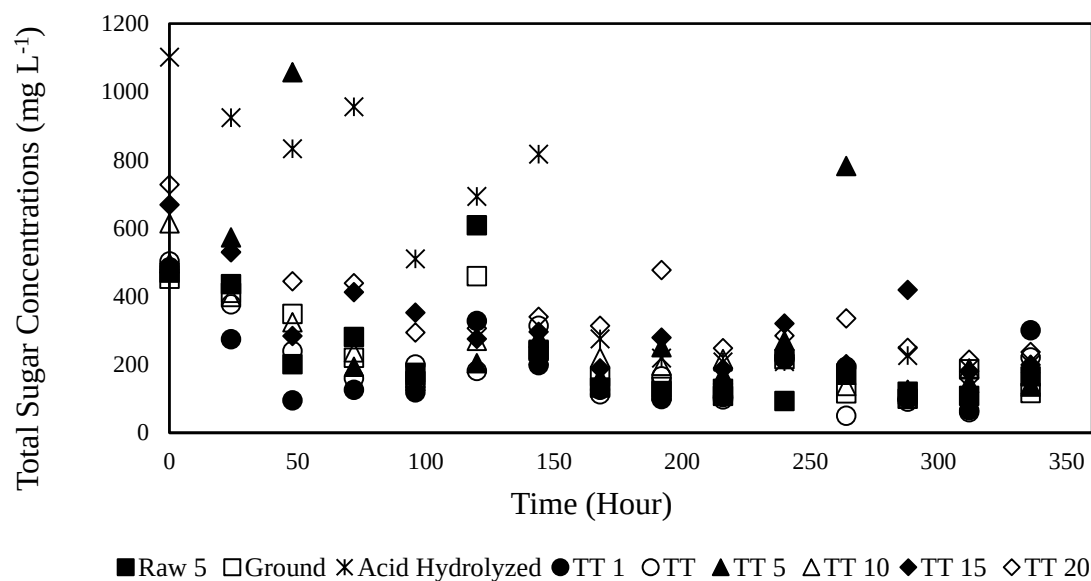


Figure 4.7 Variation of total sugar with time for different initial rice husk concentrations *TT: Thermal treated rice husk in g L⁻¹

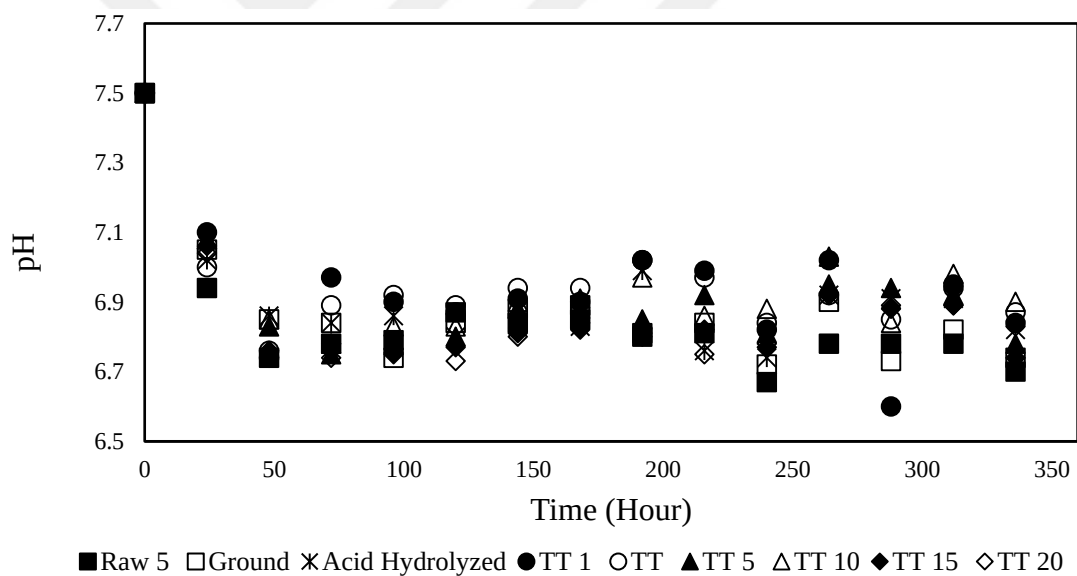


Figure 4.8 Variation of pH with time for different initial rice husk concentrations *TT: Thermal treated rice husk in g L⁻¹

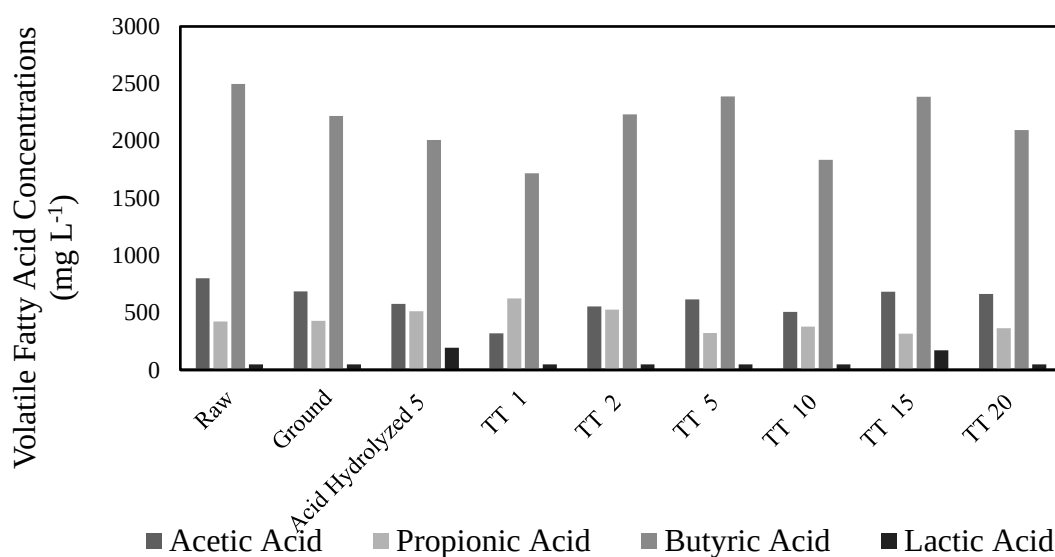


Figure 4.9 Variation of volatile fatty acid concentrations at the end of the fermentation time in different rice husk concentrations *TT: Thermal treated rice husk in g L⁻¹

The control bottles contained 5 g L⁻¹ glucose and cellulose as carbon sources not shown in the graphs. Table 4.2 shows the hydrogen gas production performances of *C. termitidis* on glucose and cellulose. The amount of hydrogen produced from glucose and cellulose were 96.53 mL and 22.94 mL, respectively. 126.04 mL H₂ g⁻¹ substrate of hydrogen production yield and 0.53 mL H₂ g⁻¹ substrate h⁻¹ of hydrogen production rate was obtained with 5 g L⁻¹ glucose containing bottles. Hydrogen production yield and rates were 30.1 mL H₂ g⁻¹ substrate and 0.13 mL H₂ g⁻¹ substrate h⁻¹ when cellulose was used as carbon source. These results show that *Clostridium termitidis* was active and converted cellulose via enzymatic hydrolysis effectively.

Table 4.2 Hydrogen gas production performances of *C. termitidis* on glucose and cellulose

<i>Clostridium termitidis</i>	Cumulative Hydrogen Formation (mL H ₂)	pH	Final Sugar Concentration (mg L ⁻¹)	Hydrogen Production Yield (mL H ₂ g ⁻¹ substrate)	Hydrogen Production Rate (mL H ₂ g ⁻¹ substrate h ⁻¹)
Glucose	96.62	5.25	259.24	126.04	0.53
Cellulose	22.96	7.0	47.56	30.10	0.13

The second best medium was obtained in the experiments with the ground rice husk and the cumulative hydrogen formation was observed as 14.6 mL. The cumulative hydrogen formation in the thermal treated experiments were less than the others and that the cumulative hydrogen volume determined in the acid pretreated RH containing 5 g L⁻¹ substrate concentration (8.5 mL).

Variation of total sugar concentrations with time at different RH concentrations and different pretreatments was evaluated in Figure 4.7. The increase and stationary periods in cumulative hydrogen formation is related with the fluctuation of sugar concentrations. It is seen that the highest initial total sugar content is obtained with acid hydrolyzed rice husk (1100 mg L⁻¹). It should be considered that this value is 20% of the initial rice husk value. The total sugar concentrations analyzed in other experimental bottles are lower than acid pretreated RH. If there is sugar in the fermentation media *Clostridium termitidis* directs to organic acid and hydrogen production, however in lignocellulosic materials first it directs to convert cellulosic substances into simpler forms via enzymatic hydrolysis and then ferments these simple sugars into biohydrogen. The pH decreased to pH 6.9-6.7 in all experimental conditions. The initial pH was set to 7.5 and due to the formation of organic acids it decreased, avoiding very hard pH changes phosphate buffer was added to the fermentation medium. During the fermentation period, the pH remained in pH range of 6.6 to 7 (Figure 4.8).

Glucose was the commonly produced saccharide and the highest glucose concentration was obtained from 10 g L⁻¹ RH (not shown in the graphs). Xylose and cellobiose was produced in various thermal treated experiments. Arabinose was only obtained in acid hydrolysed pretreated RH. Also mannose and galactose were observed in small amounts in the fermentation media.

Figure 4.9 shows the organic acid concentration in different fermentation media at the end of the fermentation period. The butyric acid concentrations in all fermentation media was the dominant organic acid produced with *Clostridium termitidis*. The highest butyric acid concentration was obtained in the 5 g L⁻¹ raw rice husk, followed

by thermal treated 5 and 15 g L⁻¹ rice husk. It is understood from the results that thermal treatment was sufficient to obtain the required butyric acid concentration. Acid hydrolysis adversely affects the organic acid production performance due to the possible inhibition effects of furfural and 5-HMF. It is observed that the second most produced organic acid is acetic acid and under certain conditions also propionic acid was seen in the fermentation media. The production of propionic acid is not desirable because it causes the consumption of hydrogen in the medium. Lactic acid production takes place at the end of the fermentation period.

Biomass concentration of *C. termitidis* was analyzed based on protein concentration. The initial protein concentration of the *C.termitidis* was set to 0.15 g L⁻¹. Hydrogen production yields and hydrogen production rates were given in Figure 4.10. The highest hydrogen production yield was obtained with 1 g L⁻¹ thermal treated RH with a value of 75 mL H₂ g⁻¹ substrate. The highest hydrogen production rate was found at 1 g L⁻¹ as well and was 0.24 mL H₂ g⁻¹ substrate h⁻¹. In thermal treated substrates, hydrogen production yield and rates were higher when compared with the acid hydrolyzed and ground RH. These values are low when compared with different substrates in the literature having hydrolysis step before fermentation. However, when rice husk is used the yield and rates are comparable which is discussed at the end.

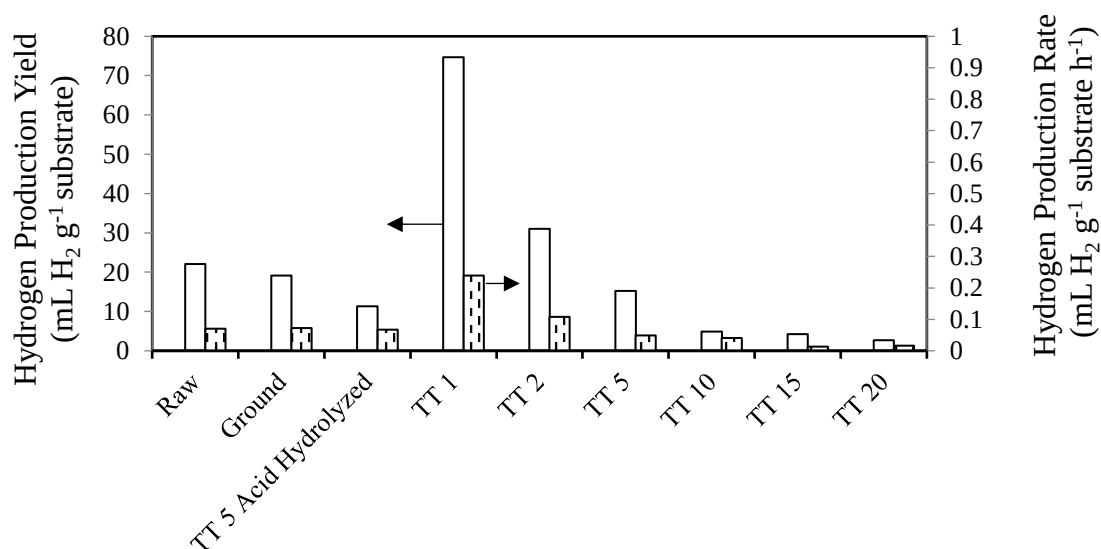


Figure 4.10 Hydrogen production yield and hydrogen production rate at different rice husk concentrations and conditions with *C. termitidis*

The residual RH concentrations at the end of the fermentation period was evaluated at Table 4.3. As can be seen from the Table, the concentrations of residual RH decreased at the end of the fermentation period. At raw RH 49% of the substrate was converted. In the ground rice husk the conversion efficiency was 82% and in the acid pretreated RH it was 88%. In thermal treated RH experiments RH conversion efficiencies ranged between 43-92%.

Table 4.3 Rice husk conversion efficiencies after fermentation with *C. termitidis*

Experiment	Initial Substrate Concentration (g L ⁻¹)	Substrate Conversion Efficiency (%)
Control	5	-
Raw	5	49.24
Ground	5	82
Acid Hydrolysed	5	88
1 Thermal Treated	1	42.8
2 Thermal Treated	2	92.3
5 Thermal Treated	5	92.6
10 Thermal Treated	10	89.5
15 Thermal Treated	15	92.5
20 Thermal Treated	20	91.8

4.2 Evaluation of Co-Culture (*Clostridium termitidis* and *Clostridium intestinale*) Ratios at different Rice Husk Particle Sizes

Clostridium termitidis and *Clostridium intestinale* cultures were subjected together to enhance the biohydrogen production potential. The ability of hydrolyzing lignocellulosic substrates of *C. termitidis* and the fast growing ability of *C. intestinale* was combined to get good amounts of biohydrogen fermentation performance from rice husk. Co-culture ratios of (*C. termitidis*: *C. intestinale*) 1:1, 2:1, 5:1, 10:1, 15:1, 20:1 were subjected to raw (<2000 µm), ground RH (<300 µm), ground and sieved

RH (<74 μm) at a constant RH concentration of 5 g L⁻¹. A 37°C incubator with an initial pH of 7.5 and 150 mL liquid volume at static conditions were used for the experiments.

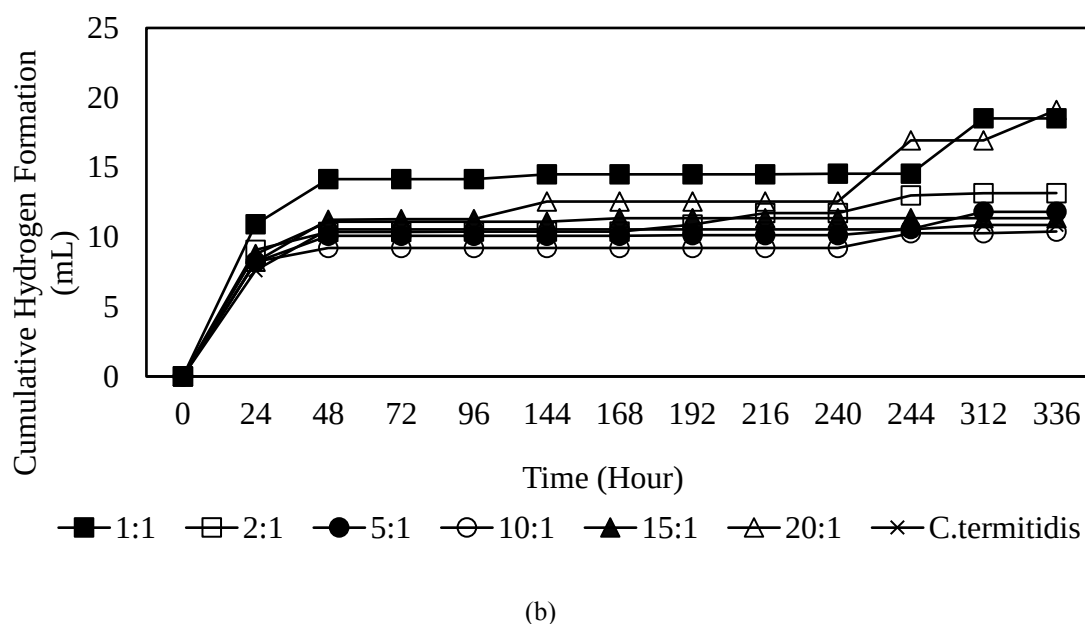
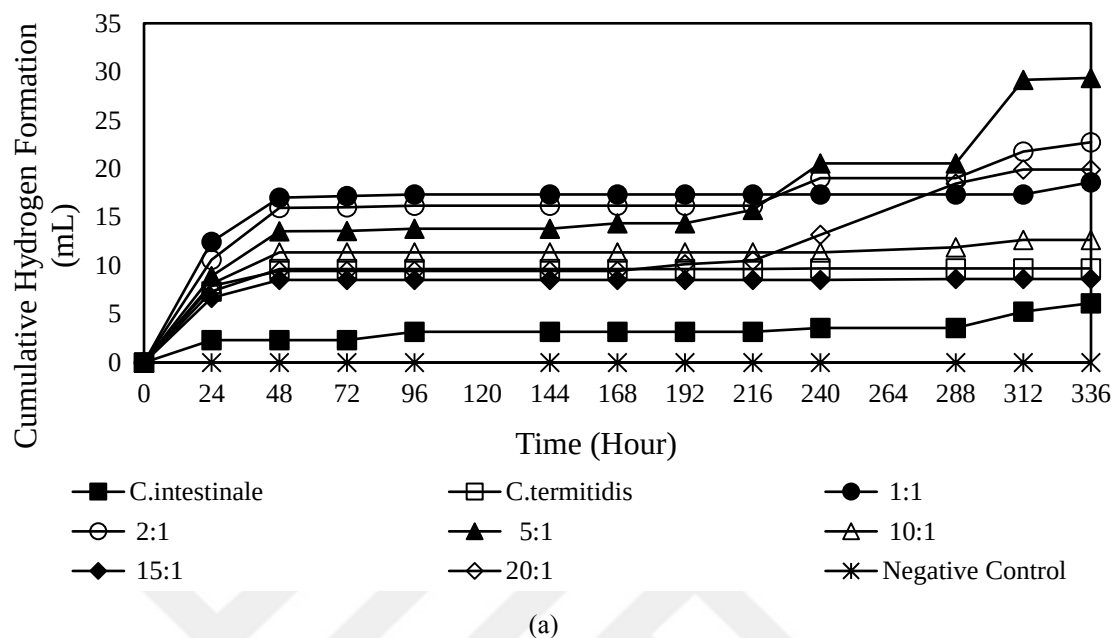
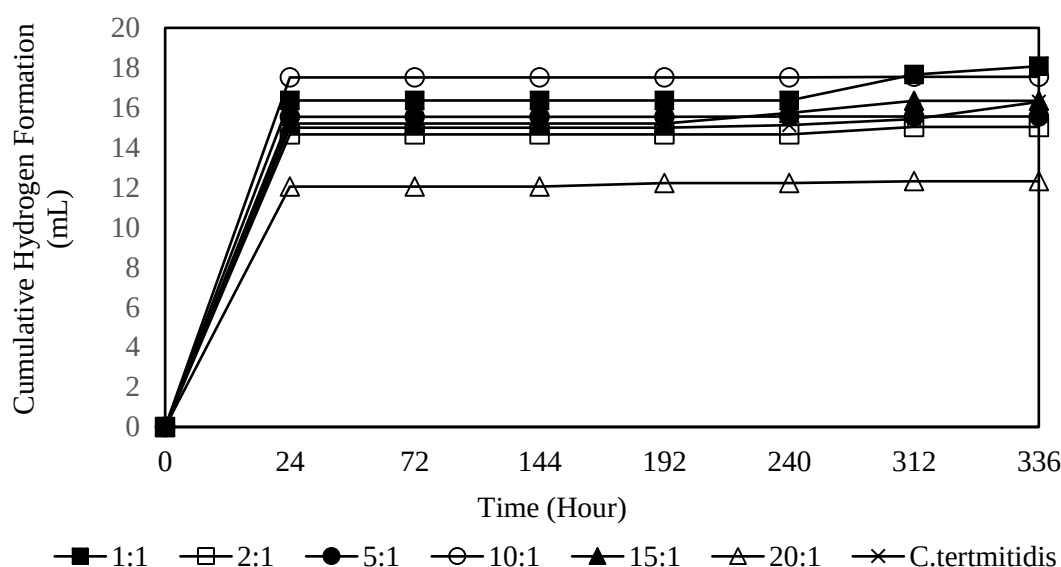


Figure 4.11 Cumulative hydrogen formation with time for different co-culture (*C. termitidis*: *C. intestinale*) ratios a. Rice Husk particle size of <2000 μm b. Rice Husk particle size of <300 μm c. Rice Husk particle size of <74 μm

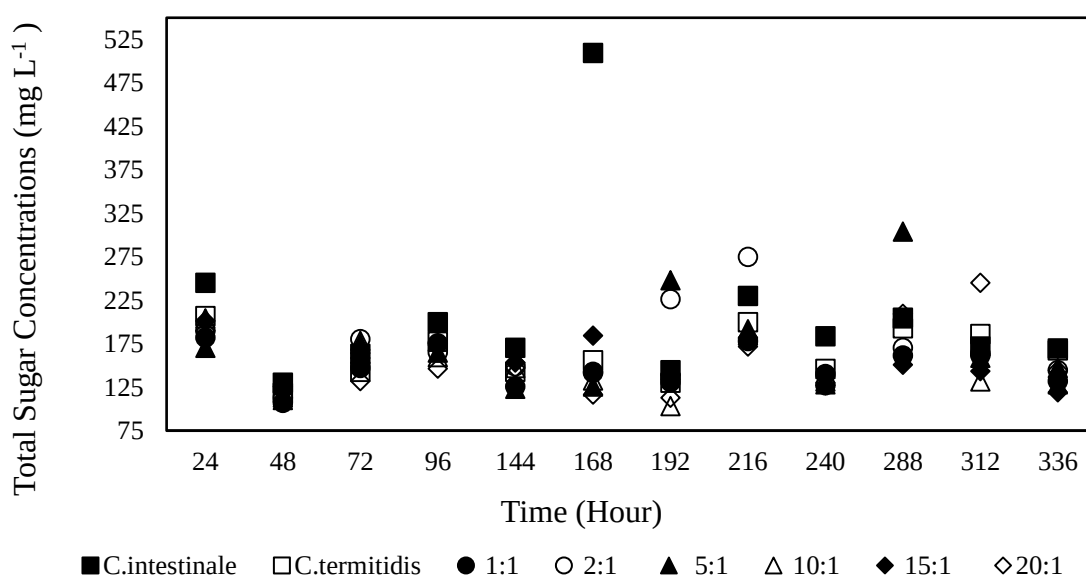


(c)

Figure 4.11 continues

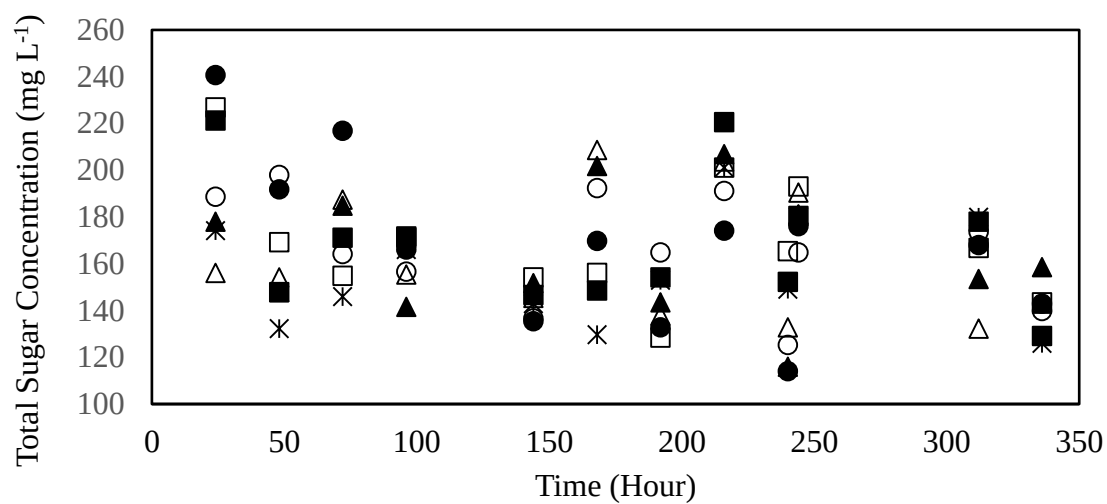
The cumulative hydrogen formations at three different particle sizes were depicted at Figure 4.11 a, b, c. The highest cumulative hydrogen formation was obtained with raw rice husk in a ratio of 5:1 at 312 hours (Figure 4.11 a). The hydrogen gas volume obtained was 29.15 mL. This ratio was followed by 2:1, however the cumulative hydrogen volume remained at 22.7 mL. The cumulative hydrogen formation obtained when *Clostridium intestinale* and *Clostridium termitidis* were used alone were much lower than the co-culture. There was no gas production in the control experiment. Figure 4.11 b showed the highest cumulative hydrogen formation in the co-culture ratio (*C. termitidis*: *C. intestinale*) of 20:1 with 19mL at the end of the fermentation period followed by 1:1 with 18.5 mL. The other ratios were lower than these values. The hydrogen was produced with long stationary periods where *C. termitidis* tried to hydrolyse RH. *C. termitidis* alone produced 10 mL hydrogen. *C. intestinale* was not shown in the graphs due to contamination of the experimental bottle. At the smallest particle size all co-culture experiments produced hydrogen at the first 24 hours and there was no further hydrogen production till the end of the fermentation period. The highest cumulative hydrogen formation at the lowest particle size under 200 mesh was obtained with a co-culture ratio of 10:1 and was 17.51 mL for the first 312 hours. A co-culture ratio of 1:1 followed this ratio giving a cumulative hydrogen volume of 16.3 mL which had an increase at the end of the fermentation period ending with 18mL.

Figure 4.12 (a, b, c) shows the total sugar concentrations with time in co-cultures (*Clostridium termitidis*: *Clostridium intestinale*). It is understood that *Clostridium termitidis*, met the expectations, breaking down lignocellulosic material by enzymatic hydrolysis and forms simple sugars and *Clostridium intestinale* transformed these sugars rapidly into organic acid and hydrogen. It is observed that the total sugar value varies between 100-300 mg L⁻¹ in raw RH (<2000µm). At the ground rice husk (<300µm, Figure 4.12 b) the total sugar varied between 120-240 mg L⁻¹ and at the particle size under (<74µm) at Figure 4.12 c. the total sugar ranged between 110-315 mg L⁻¹.

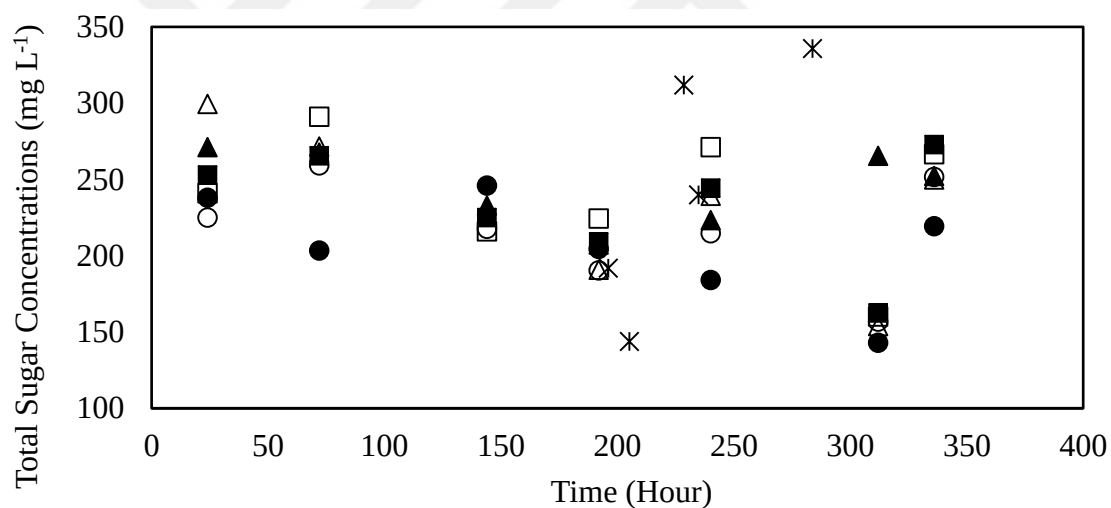


(a)

Figure 4.12 Variation of total sugar concentrations with time for different co-culture (*C. termitidis*: *C. intestinale*) ratios a. Rice Husk particle size of <2000 µm b. Rice Husk particle size of <300 µm c. Rice Husk particle size of <74 µm



(b)



(c)

Figure 4.12 continues

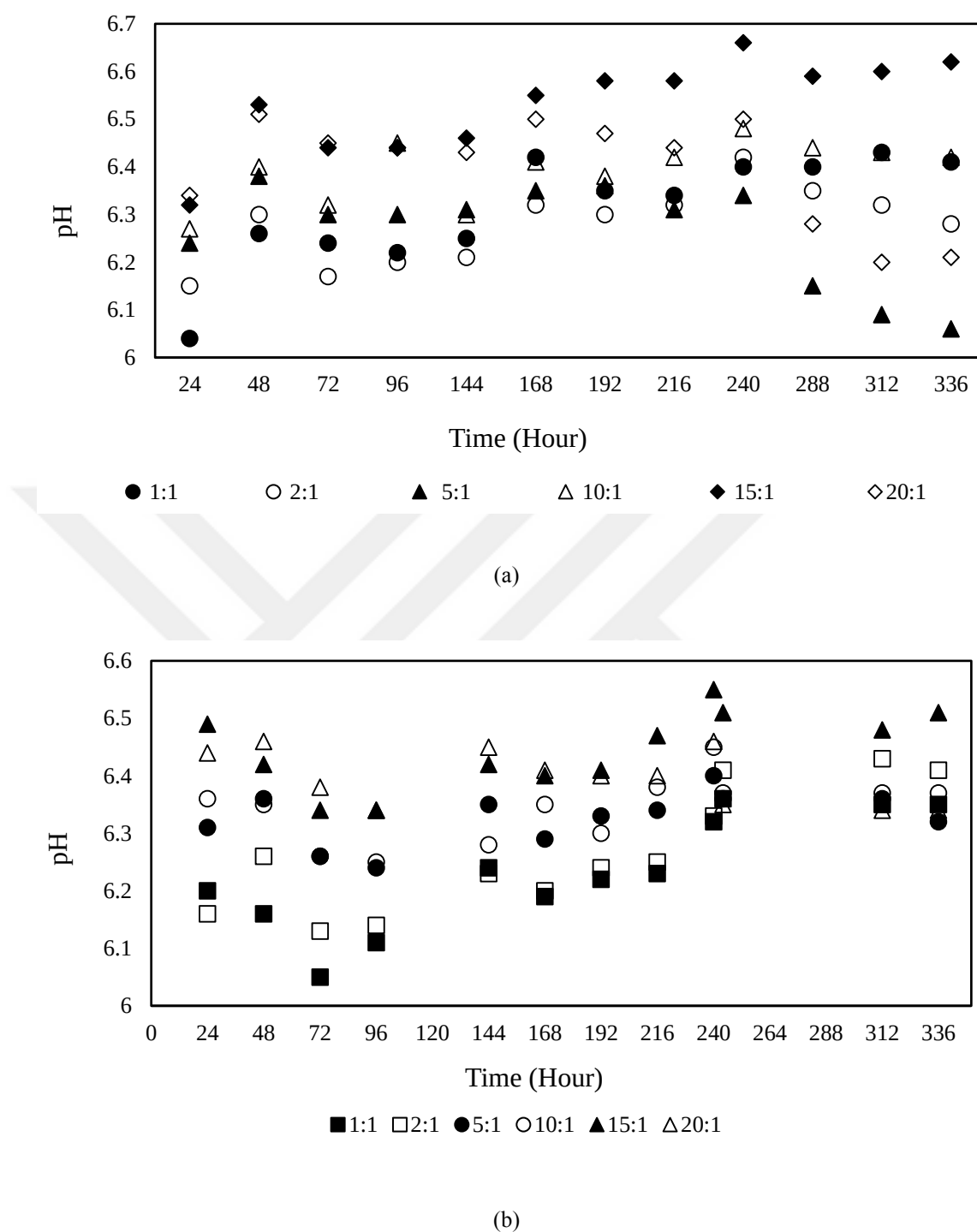
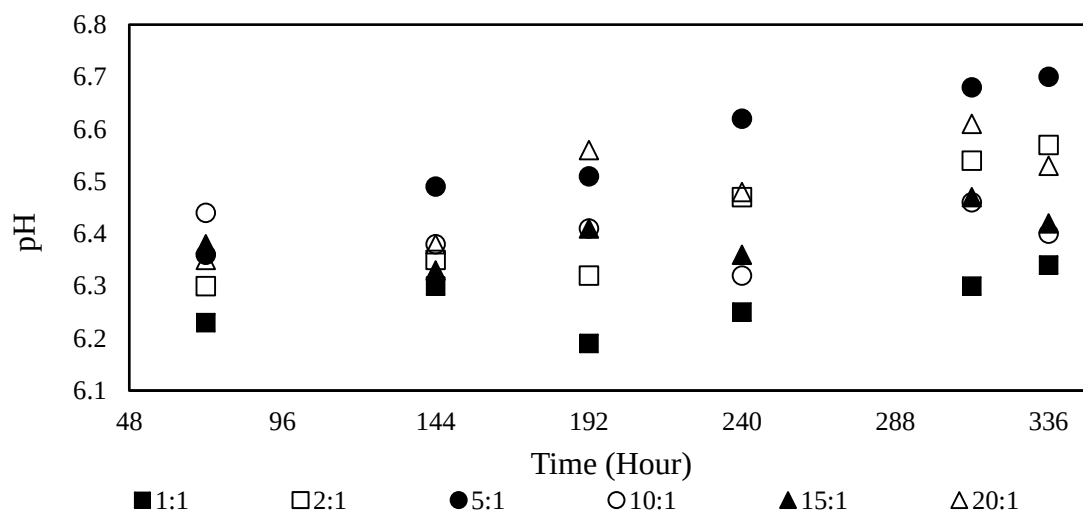


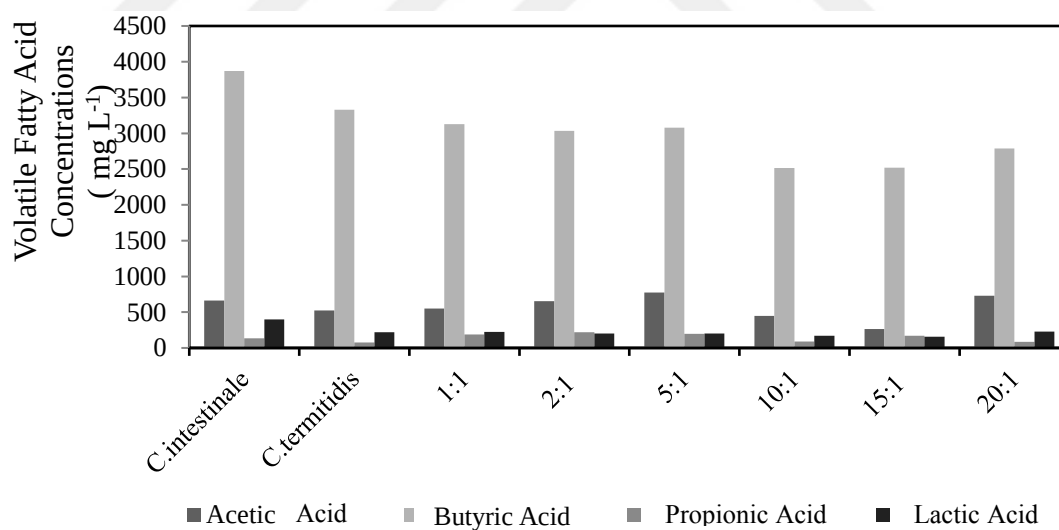
Figure 4.13 Variation of pH with time for different co-culture (*C. termitidis*: *C. intestinale*) ratios a. Rice Husk particle size of <2000 μm b. Rice Husk particle size of <300 μm c. Rice Husk particle size of <74 μm



(c)

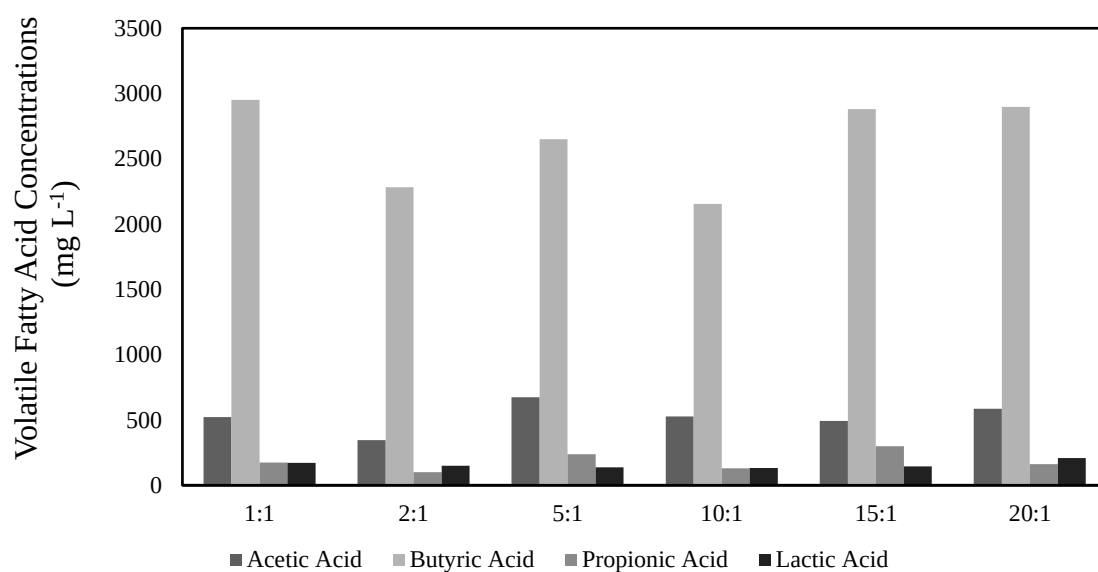
Figure 4.13 continues

Figure 4.13 a, b, c depicts the variation of pH in different co-culture ratios and different particle sizes. The pH reduced from about 7.5 to about 6.5 and after 24 hours it stood stable at around 6-6.5 in all experimental media.

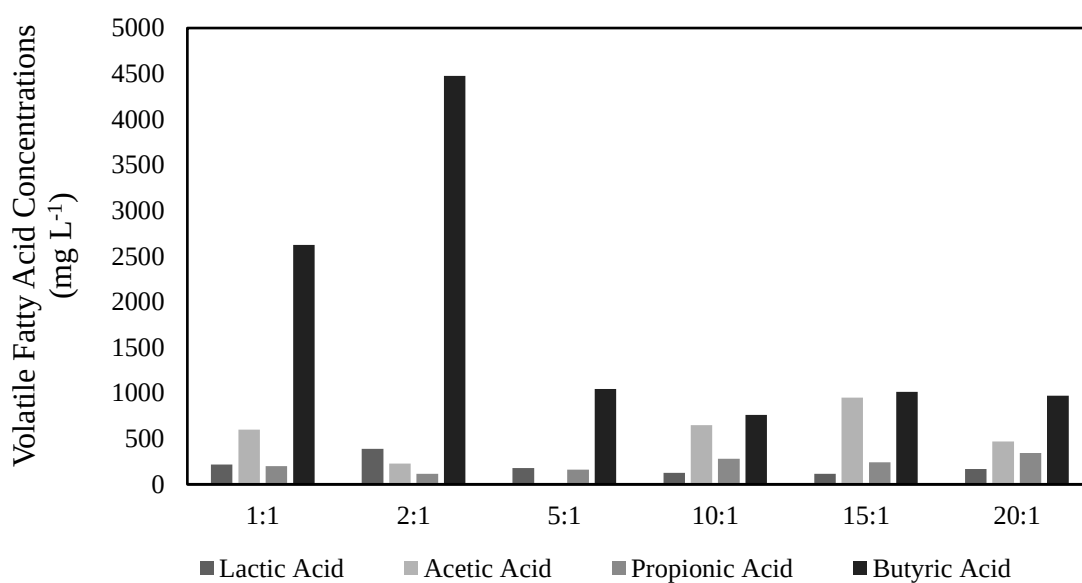


(a)

Figure 4.14 Variation of volatile fatty acids at the end of the fermentation time at different co-culture (*C. termitidis*: *C. intestinale*) ratios a. Rice Husk particle size of <2000 μm b. Rice Husk particle size of <300 μm c. Rice Husk particle size of <74 μm



(b)



(c)

Figure 4.14 continues

The volatile fatty acid concentrations were shown in Figure 4.14 a, b, c for different co-cultures of *C. termitidis*: *C. intestinalis*. The highest organic acid concentration was butyric acid in the medium in which the *Clostridium termitidis* was alone. However, the high butyric acid concentration in the fermentation media caused inhibition effects

for further hydrogen gas production. At co-cultures, the accumulation of organic acid was less than that of pure culture. Butyric acid production was followed by acetic acid in the fermentation media. The propionic acid production reached to remarkable concentrations (2990 mg / L) in the co-culture ratio of 10: 1. Propionic acid, which is known as hydrogen consumer, affects the production of hydrogen negatively. It is understood that the production of propionic acid and lactic acid occurred at the end of the fermentation period. Therefore, as the fermentation time has a great effect on the formation of these organic acids, it is certain that the undesirable organic acids will be formed if the fermentation time is kept longer. At the ground RH depicted at Figure 4.14 b the organic acid composition was composed of butyric, acetic, propionic and lactic acid varying between 2100-3300 mg L⁻¹, 350-675 mg L⁻¹, 150-300 mg L⁻¹, 130-270 mg L⁻¹, respectively. At the lowest particle size in Figure 4.14 c butyric acid dominated the fermentation media with the highest concentration at pure culture of *C. intestinale* (5376 mg L⁻¹) and followed by pure culture of *C. termitidis* (4100 mg L⁻¹). In the co-cultures the highest butyric acid concentration was observed with 2:1 (4400 mg L⁻¹). Reduction of particle size increased organic acid production. However, the high amounts of volatile fatty acids negatively affect further hydrogen gas production. Also acetic acid was produced in the fermentation media at low concentrations. At the end of the fermentation period also propionic acid and lactic acid was formed.

Cellobiose and glucose levels are the most common types of sugars in all experiments. While the presence of other sugars at *Clostridium termitidis* was observed at the end of the fermentation time, it is understood that these type of sugars were consumed by the co-culture. In ground RH also some galactose was obtained in some experiments. In the lowest particle size the cellobiose and glucose concentration in all experimental bottles were nearly constant.

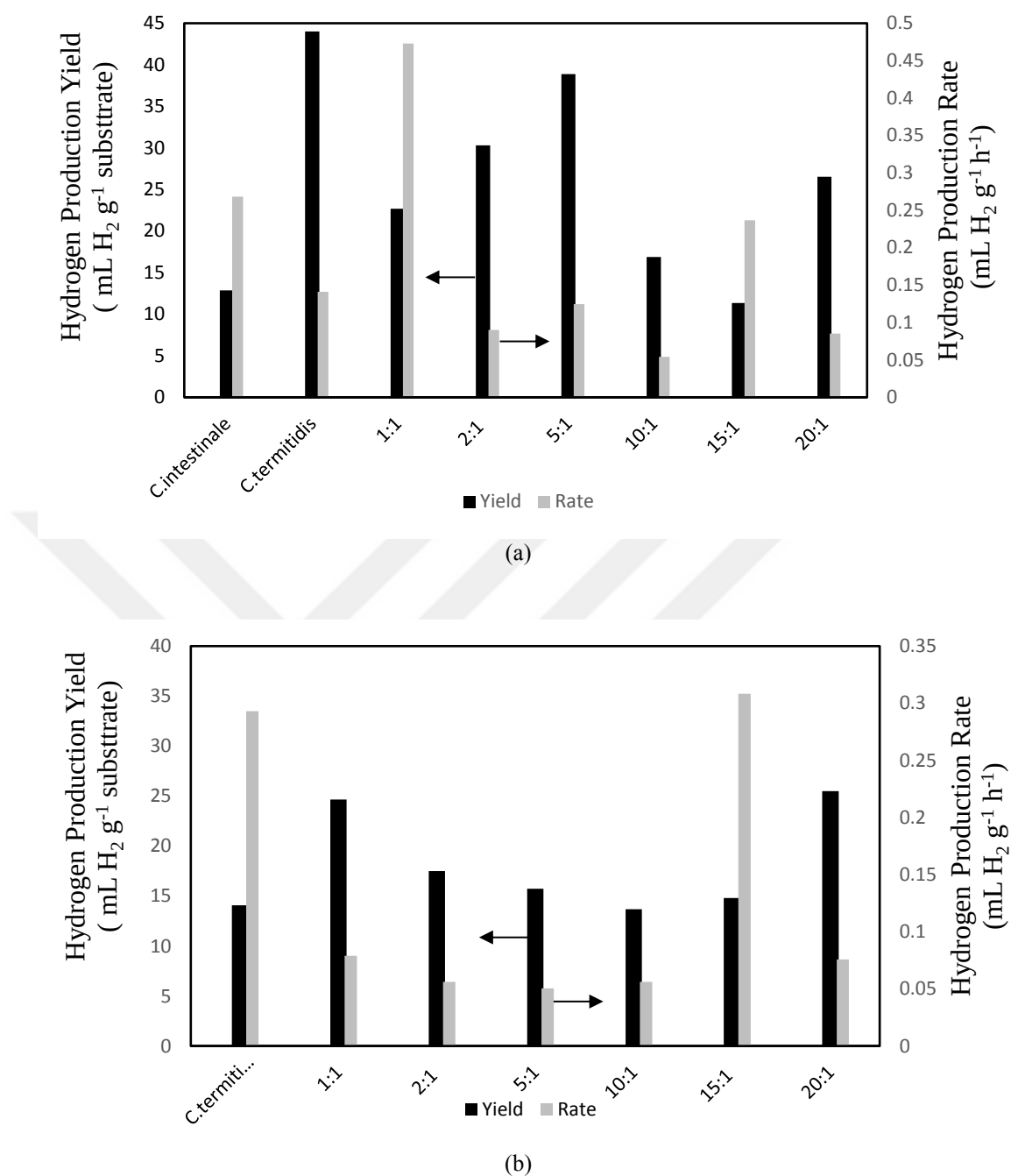
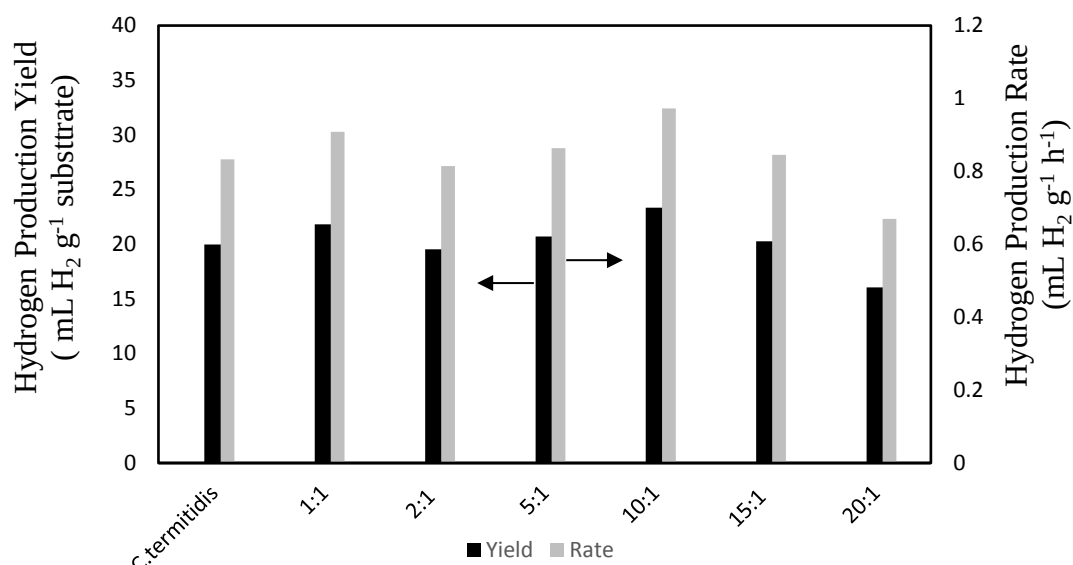


Figure 4.15 Hydrogen production yields and hydrogen production rates at different co-culture (*C. termitidis*: *C. intestinale*) ratios a. Rice Husk particle size of <2000 μm b. Rice Husk particle size of <300 μm c. Rice Husk particle size of <74 μm



(c)

Figure 4.15 continues

Figure 4.15 a, b, c shows hydrogen production yields and hydrogen production rates. The highest hydrogen production yield was obtained with the co-culture ratio of 5: 1 based on the initial rice husk concentration. It was 38.86 mL H₂ g⁻¹ substrate. The highest hydrogen production rate was obtained with a co-culture ratio of 1:1 when raw rice husk was used as substrate (Figure 4.15 a). In Figure 4.15 b with the RH having a particle size of <300µm the highest hydrogen production yield was obtained with a co-culture ratio of 20:1 and it was 25.5 mL H₂ g⁻¹ substrate. The maximum hydrogen production rate was seen in a co-culture ratio of 15:1 (0.31 mL H₂ g⁻¹ substrate h⁻¹). Figure 4.15 c depicts the results of <74 µm. The highest hydrogen production yield and hydrogen production rate was determined at a co-culture ratio of 10:1 (23.3 mL H₂ g⁻¹ substrate, 0.14 mL H₂ g⁻¹ substrate h⁻¹). The results from these experiments showed that the best co-culture ratio of *C. termitidis*: *C. intestinalis* was 5:1 and the best particle size is the raw rice giving the highest hydrogen production volume, yield and rate.

4.3 Effect of Rice Husk Substrate Concentration using a co-culture of *C. termitidis*: *C. intestinale* on Hydrogen Production Performance

After determining the appropriate rice husk particle size and the co-culture ratio of *C. termitidis*: *C. intestinale* the effects of substrate concentration on hydrogen production performance was evaluated. For this purpose, 1-20 g L⁻¹ raw rice husk was subjected to a co-culture of 5:1 (*C. termitidis*: *C. intestinale*) at 37°C for 14 days.

Variations of cumulative hydrogen formation with time in different rice husk concentrations are given in Figure 4.16. The highest substrate concentration gave the best cumulative volume of hydrogen. In the first 24 hours, approximately 10-14.5 mL of hydrogen gas was produced in all experimental bottles, followed by an increase in three days, and in some bottles (1, 5, 10, 15 g L⁻¹) the cumulative hydrogen production slightly increased in time. In 2 g L⁻¹ RH after 175 hours the cumulative hydrogen formation reached to 22.9 mL. At 20 g L⁻¹ RH concentration the cumulative hydrogen increased to 41.1 mL after 270 hours.

The changes in the total sugar concentrations with time in different rice husk concentrations are given in Figure 4.17. The total sugar concentration increased as the substrate concentration increased. Total sugar concentration in 1 g L⁻¹ rice husk was in the range of 76-150 mg L⁻¹ and this range increased to 200-278 mg L⁻¹ at 20 g L⁻¹. It can be seen from the graph that, the total sugars obtained can be effectively used and the sugars are reduced from time to time, furthermore the substrate is been hydrolyzed and sugars are been released to the fermentation media to be used again.

In the experimental bottles, the pH decreased from 7.5 to 6.1-6.3 within the first 24 hours and it remained constant at 6.2-6.4 in concentrations other than 20 g L⁻¹ rice husk. At 20 g L⁻¹ rice husk, pH decreased in time and reached 5.7 at the end of the fermentation period.

The organic acid concentrates obtained at different rice husk concentrations are given in Figure 4.18. Lactic acid concentration is minimal in all mediums. It is understood that the butyric acid concentration is around 2000-2600 mg L⁻¹. The sugar compositions in the highest cumulative hydrogen value obtained have cellobiose and glucose and their values are very close to each other.

Figure 4.19 gives the hydrogen production yields and hydrogen production rates obtained with different rice husk concentrations. As can be seen from the figure, the highest hydrogen production yield and specific hydrogen production rate were obtained with 1 g L⁻¹ rice husk. It is observed that yield and rate decreased with increasing substrate concentrations.

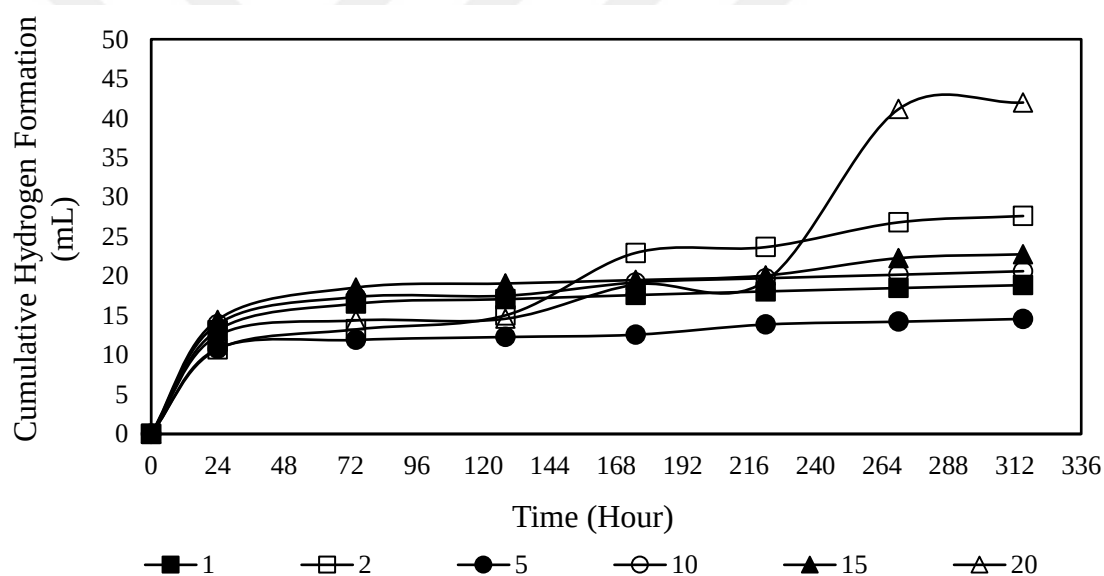


Figure 4.16 Variations of cumulative hydrogen volumes with time at different rice husk concentrations

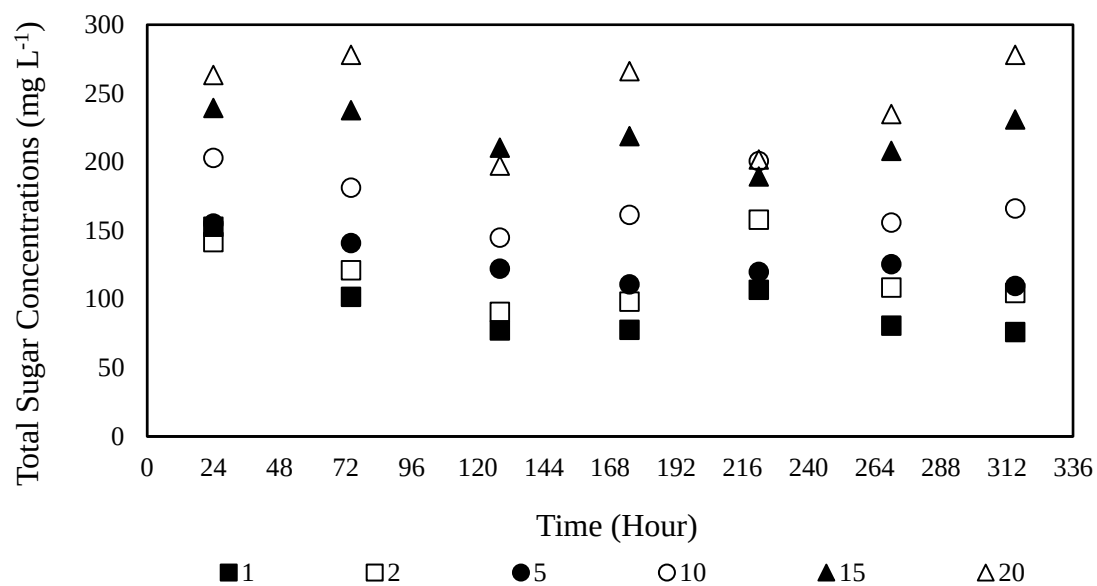


Figure 4.17 Variation of the total sugar concentrations with time at different rice husk concentrations

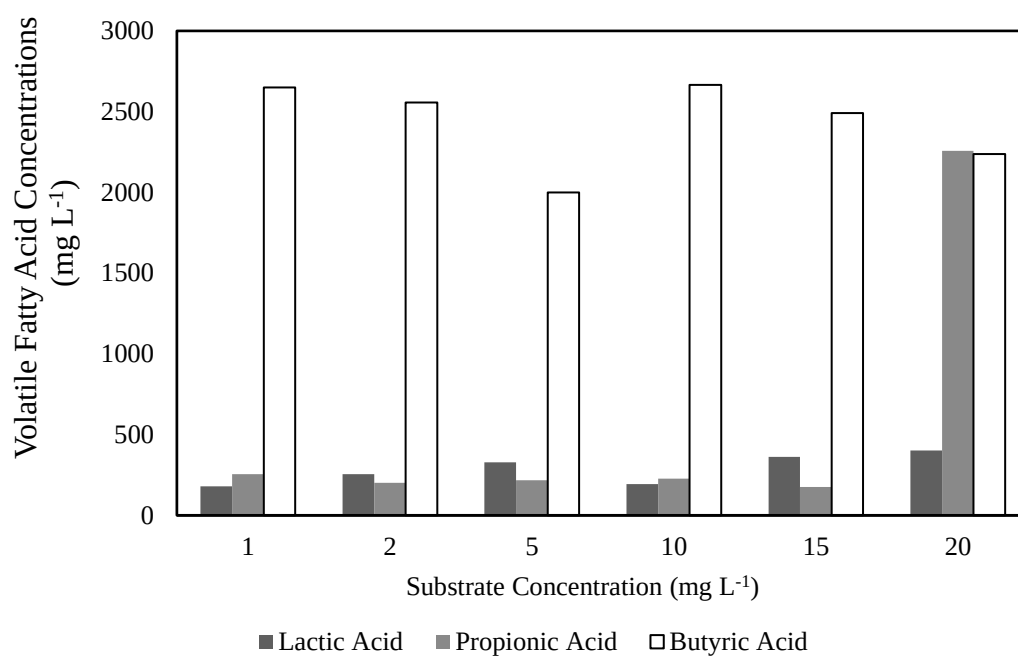


Figure 4.18 The volatile fatty acid concentrations at different rice husk concentrations

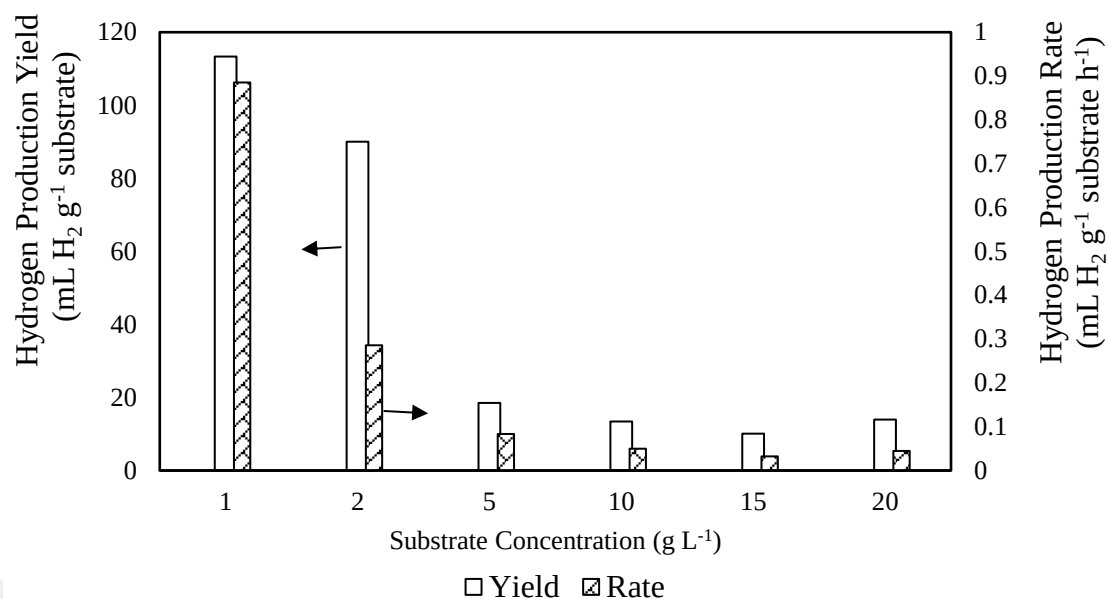


Figure 4.19 The hydrogen production yield and hydrogen production rates with different rice husk

The control bottles containing 5 g L⁻¹ glucose and cellulose as carbon sources (not shown in the graphs). Table 4.4 shows the hydrogen gas production performances of 5:1 co-culture ratio on glucose and cellulose. The amount of hydrogen produced from glucose was 114.04 mL and it was 29.04 mL from cellulose. 152.06 mL H₂ g⁻¹ glucose substrate of hydrogen production yield and 0.91 mL H₂ g⁻¹ glucose substrate h⁻¹ of hydrogen production rate was obtained with 5 g L⁻¹ glucose containing bottles. Hydrogen production yield and rates were 38.76 mL H₂ g⁻¹ cellulose substrate and 0.20 mL H₂ g⁻¹ cellulose substrate h⁻¹ when cellulose was used as carbon source.

Table 4.4 Hydrogen gas production performances of 5:1 Co-culture ratio on glucose and cellulose

<i>Clostridium termitidis:</i> <i>Clostridium intestinale</i>	Cumulative Hydrogen Formation (mL H ₂)	pH	Final Sugar Concentration (mg L ⁻¹)	Hydrogen Production Yield (mL H ₂ g ⁻¹ substrate)	Hydrogen Production Rate (mL H ₂ g ⁻¹ substrate h ⁻¹)
Glucose	114.04	5.14	310.46	152.06	0.91
Cellulose	29.04	6.91	50.98	38.76	0.20

Rice Husk as a lignocellulosic substrate is a good source of raw material for the production of biohydrogen. *Clostridium termitidis* and *Clostridium intestinale* co-culture with minimal pretreatment and consolidated fermentation showed a significant biohydrogen production performance on rice husk. Simple sugar composition revealed at the end of the final fermentation was mainly composed of cellobiose and glucose and under certain conditions mannose, galactose and arabinose could be analyzed. Generally, it was observed that total sugar concentrations oscillated with time, and that *Clostridium termitidis* reduced simple sugars and *Clostridium intestinale* transformed the reduced sugars into organic acids and hydrogen gas. It is understood from the results that the consolidated fermentation was performed and accelerated by the use of a co-culture. It was also observed that pH values were almost pH 6-6.5 in all experiments and favorable conditions for organisms were observed. The resulting organic acid composition was predominantly butyric acid, and also acetic acid, lactic acid and propionic acid was formed.

Table 4.5 gives the hydrogen production yields based on initial cell concentrations. The highest yield was obtained when 1 g L⁻¹ raw rice husk with a co-culture ratio of 5:1 was used. In all experiments with the same co-culture ratio and substrate concentration, the hydrogen production yield based on cellmass ranged between 0.11-0.14 mmol mmol⁻¹ cellmass.

Table 4.5 Hydrogen production yields at different experiments

Different Experiments	Y _{P/X} (mmol mmol ⁻¹ cellmass)
Co-culture	0.11
Particle Size	0.11
Substrate Concentration	0.14

Table 4.6 Summary of different experiments performed with *C. intestinale* and *C. termitidis*

	Y_{P/S} mL H ₂ g ⁻¹ substrate	CHF mL H ₂	HPR mL H ₂ g ⁻¹ substrate h ⁻¹	SHPR mL H ₂ g ⁻¹ cellmass h ⁻¹
Pure <i>Clostridium intestinale</i> Experiment 1 g L ⁻¹ TT, RH	62.88	9.42	1.74	2,37
Pure <i>Clostridium termitidis</i> Experiment 1 g L ⁻¹ TT, RH	75	11.2	0.24	0.80
Co-Culture Experiment				
< 2000 μm; <i>C. termitidis</i> : <i>C.intestinale</i> : 5:1	38.86	29.15	0.62	0.14
Substrate Concentration Experiment 1 g L ⁻¹ (5:1 < 2000 μ m)	113.3	18.85	0.40	0.09

*TT: Thermal Treated Rice Husk

Hydrogen Production Rate: HPR

Specific Hydrogen Production Rate: SHPR

CHF: Cumulative Hydrogen Formation

The results showed that biohydrogen production from rice husk via consolidated fermentation with the co-culture of *C. termitidis*: *C. intestinale* needs long fermentation periods. The rate limiting factor of the co-culture is the slow growing *C. termitidis* which is responsible of enzymatic hydrolysis of rice husk. The comparison of different experiments performed with *C. intestinale* and *C. termitidis* are summarized in Table 4.6. The maximum cumulative hydrogen formation was evaluated with a co-culture ratio of 5:1 and with 1 g L⁻¹ substrate concentration having a hydrogen production yield of 113.3 mL H₂ g⁻¹ substrate.

A study trying to produce biohydrogen from rice husk found a cumulative hydrogen of 237.97 mL using *Phanerochaete chrysosporium* under solid state fermentation for enzymatic hydrolysis and then fermenting it under submerged fermentation with *Clostridium beijerinckii* (Saratele et.al., 2014). They found a hydrogen production yield of 2.93 mmol H₂ g⁻¹ reducing sugar. The difference of our study is to use consolidated bioprocessing in a one step reactor. Gomez-Florez et.al.

(2017) produced 26 mL d⁻¹ biohydrogen in 45 days with a hydrogen production yield of 1.92 mol H₂ mol⁻¹ hexose using a co-culture of *C. termitidis*: *C. beijerinckii*. The hydrogen production yields and rates are lower than in the literature. However, there are very few studies focusing on consolidated bioprocessing for biohydrogen production. Furthermore, the studies in the literature include pretreatment steps. This study gives an important overview on biohydrogen production from untreated rice husk using a co-culture including consolidated bioprocessing.



CHAPTER FIVE

CONCLUSIONS

The aim of this study is to provide an sustainable solution for converting lignocellulosic substrates to biohydrogen by using *Clostridium termitidis* and *Clostridium intestinale*. From this point of view; to improve the production of biohydrogen gas from a lignocellulosic material such as rice husk, a co-culture of *Clostridium termitidis*: *Clostridium intestinale* was used with consolidated fermentation. The elimination of pretreatment step was evaluated by using consolidated bioprocessing. The experiments were carried out under mesophilic conditions to determine the best ratio of these two cultures to form a co-culture, to determine the effect of rice husk particle sizes and to determine the effects of substrate concentration on biohydrogen production yield and rate.

Rice husk used was containing, 34.52% cellulose, 26% hemicellulose, 22.68% lignin and 16.8% extractive agents. Different pre-treatments were applied to prepare the fermentation process along with pure cultures. These were grinding, thermal treatment, and acid hydrolysis. Furfural and 5-HMF values which are known to have negative effects on the fermentation process were measured.

The biohydrogen production performances of *Clostridium intestinale* and *Clostridium termitidis* cultures on the rice husk were evaluated with different pretreatments and different thermal treated rice husk concentrations. Acid pretreatment resulted in highest reducing and fermentable sugar. But hydrogen formation potential from this substrate was low.

As one one of the simplest pretreatment thermal pretreatment was conducted to RH. The highest cumulative hydrogen formation was obtained as 14 mL with 10 g L⁻¹ thermal treated rice husk, and the highest hydrogen production yield and rate was conducted with 1 g L⁻¹ thermal treated rice husk as 62.8 mL H₂ g⁻¹ substrate and 1.74 mLH₂ g⁻¹ substrate h⁻¹, respectively when *C. intestinale* was used alone in the fermentation media. The main organic acid was butyric acid in the fermentation media. *C. termitidis* under the same conditions gave the highest cumulative hydrogen with 5

g L⁻¹ ground rice husk as 14.6 mL where thermal treatment was not applied. The highest hydrogen production yield and rate was obtained with 1 g L⁻¹ thermal treated RH with a value of 75 mL H₂ g⁻¹ substrate and 0.24 mL H₂ g⁻¹ substrate h⁻¹, respectively. Glucose and cellulose was also subjected to pure cultures giving cumulative hydrogen formations of 71.61 mL and 15.61 mL for *C. intestinale* and 96.53 mL, 22.95 mL for *C. termitidis*, respectively.

Co-culture helps to obtain fermentable sugars. They enzymatic hydrolysis carried out by the co-culture. Co-culture ratios of (*C. termitidis*: *C. intestinale*) 1:1, 2:1, 5:1, 10:1, 15:1, 20:1 were subjected to raw (<2000 µm), ground RH (<300 µm), ground and sieved RH (<74 µm) at a constant RH concentration of 5 g L⁻¹. In raw RH the highest cumulative hydrogen formation was obtained with the co-culture ratio of 5:1 and was 29.15 mL. In the ground RH (<300 µm) the best cumulative hydrogen formation was at the co-culture ratio of 20:1 (19 mL) and with the smallest particle size (<74µm) the cumulative hydrogen volume was 17.51mL at a co-culture ratio of 10:1. The pH varied between 6-6.5 through the experiment. Butyric acid was the main organic acid produced. When raw RH was used the organic acid accumulated in the fermentation media which may effect further fermentation. At particle size of <300 µm butyric acid varied between 2100-3300 mg L⁻¹ and at particle size of <74 µm the butyric acid concentration in pure cultures of *C. termitidis* was 4100 mg L⁻¹, respectively. The highest hydrogen production yield with raw RH was obtained with the co-culture ratio of 5:1 (38.86 mL H₂ g⁻¹ substrate) and the highest hydrogen production rate was obtained with a co-culture ratio of 1:1. In particle size of <300µm the highest hydrogen production yield was obtained with a co-culture ratio of 20:1 (25.5 mL H₂ g⁻¹ substrate) and the maximum hydrogen production rate was seen in a co-culture ratio of 15:1 (0.31 mL H₂ g⁻¹ substrate h⁻¹). The highest hydrogen production yield and hydrogen production rate was determined at a co-culture ratio of 10:1 (23.3 mL mL H₂ g⁻¹ substrate, 0.14 mL H₂ g⁻¹ substrate h⁻¹) when the particle size was <74 µm.

After determining the appropriate rice husk particle size and the co-culture ratio of *C. termitidis*: *C. intestinale* the effects of substrate concentration on hydrogen

production performance was evaluated. The highest substrate concentration gave the best cumulative volume of hydrogen (41.1 mL). The highest hydrogen production yield (90 mL H₂ g⁻¹ substrate) and hydrogen production rate (0.29 mL H₂ g⁻¹ substrate h⁻¹) were obtained from 1 g L⁻¹ rice husk.

Clostridium termitidis and *Clostridium intestinale* co-culture with minimal pretreatment and consolidated fermentation showed a significant biohydrogen production performance on rice husk. This type of growth shows a growth path called “Diauxic Growth”. At the same time, this pathway can refer to “Consolidated Growth” where enzymatic hydrolysis first tries to form simple sugars and then convert them into hydrogen. In these conditions, the organism culture first converts the lignocellulosic material down to simpler structures like cellobiose via enzymatic hydrolysis and then converts them into organic acid and hydrogen. Simple sugar composition revealed at the end of the final fermentation was mainly composed of cellobiose and glucose in all experiments. Generally, it was observed that total sugar concentrations oscillated with time, and that *Clostridium termitidis* reduced simple sugars and *Clostridium intestinale* transformed the reduced sugars into organic acids and hydrogen gas.

The hydrogen production yields and rates are lower than in the literature. However, there are very few studies focusing on consolidated bioprocessing for biohydrogen production. Furthermore, the studies in the literature include pretreatment steps. This study gives an important overview on biohydrogen production from raw rice husk using a co-culture including consolidated bioprocessing without pretreatment.

RECOMMENDATION FOR THE FUTURE

Following recommendations may be followed in order to extend investigations on bio hydrogen production from rice husk with consolidated fermentation.

1. Longer fermentation periods, by avoiding lactic/ propionic acid production can be observed to enhance biohydrogen production from rice husk with this co-culture and consolidated fermentation.
2. Fed-batch process can be tried to show the enhancement of hydrogen gas production potential.
3. Continuous processes may be tried by avoiding accumulation of organic acids in the fermentation media to enhance bio hydrogen production.

REFERENCES

- Afsar, N., Özgür, E., Gürkan, M., Akköse, S., Yücel, M., Gündüz, U., & Eroglu, I. (2011). Hydrogen productivity of photosynthetic bacteria on dark fermenter effluent of potato steam peels hydrolysate. *International Journal of Hydrogen Energy*, 36(1), 432-438.
- Agbor, V. B., Cicek, N., Sparling, R., Berlin, A., & Levin, D. B. (2011). Biomass pretreatment: fundamentals toward application. *Biotechnology Advances*, 29(6), 675-685.
- Agbor, V., Carere, C., Cicek, N., Sparling, R., & Levin, D. (2014). *Biomass pretreatment for consolidated bioprocessing (CBP)*. In *Advances in biorefineries* (234-258). Sawston, Cambridge: Woodhead Publishing.
- Agbor, V., Zurzolo, F., Blunt, W., Dartailh, C., Cicek, N., Sparling, R., & Levin, D. B. (2014). Single-step fermentation of agricultural hemp residues for hydrogen and ethanol production. *Biomass and Bioenergy*, 64, 62-69.
- Alalwan, H. A., Alminshid, A. H., & Aljaafari, H. A. (2019). Promising evolution of biofuel generations. Subject review. *Renewable Energy Focus*, 28, 127-139.
- Alibardi, L., & Cossu, R. (2016). Effects of carbohydrate, protein and lipid content of organic waste on hydrogen production and fermentation products. *Waste management*, 47, 69-77.
- An, Q., Wang, J. L., Wang, Y. T., Lin, Z. L., & Zhu, M. J. (2018). Investigation on hydrogen production from paper sludge without inoculation and its enhancement by *Clostridium thermocellum*. *Bioresource Technology*, 263, 120-127.
- Antizar-Ladislao, B., & Turrion-Gomez, J. L. (2008). Second-generation biofuels and local bioenergy systems. *Biofuels, Bioproducts and Biorefining*, 2(5), 455-469.
- Argun, H. (2010). *Hydrogen gas production from waste ground wheat by dark and light fermentations*. Doctoral dissertation, DEÜ, İzmir.

- Argun, H., & Kargi, F. (2010). Bio-hydrogen production from ground wheat starch by continuous combined fermentation using annular-hybrid bioreactor. *International Journal of Hydrogen Energy*, 35(12), 6170-6178.
- Argun, H., & Kargi, F. (2011). Bio-hydrogen production by different operational modes of dark and photo-fermentation: an overview. *International Journal of Hydrogen Energy*, 36(13), 7443-7459.
- Armor, J. N. (1999). The multiple roles for catalysis in the production of H₂. *Applied Catalysis A: General*, 176(2), 159-176.
- Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology*, 141(2), 391-396.
- Assawamongkholsiri, T., Reungsang, A., & Pattra, S. (2013). Effect of acid, heat and combined acid-heat pretreatments of anaerobic sludge on hydrogen production by anaerobic mixed cultures. *International Journal of Hydrogen Energy*, 38(14), 6146-6153.
- Astolfi, V., Astolfi, A. L., Mazutti, M. A., Rigo, E., Di Luccio, M., Camargo, A. F., ... & Treichel, H. (2019). Cellulolytic enzyme production from agricultural residues for biofuel purpose on circular economy approach. *Bioprocess and Biosystems engineering*, 1-9.
- Azwar, M. Y., Hussain, M. A., & Abdul-Wahab, A. K. (2014). Development of biohydrogen production by photobiological, fermentation and electrochemical processes: a review. *Renewable and Sustainable Energy Reviews*, 31, 158-173.
- Banik, S. B. A. S. G. S., Bandyopadhyay, S., & Ganguly, S. (2003). Bioeffects of microwave—a brief review. *Bioresource Technology*, 87(2), 155-159.
- Bay, S. (2009). *Türk çeltik çeşitlerinin (oryza sativa l.) tohum depo proteinleri ve Random amplified polymorphic DNA (RAPD) belirleyicileri ile genetik analizi*. Phd Thesis, Kahramanmaraş Sütçü İmam University, Kahramanmaraş.

- Benjamin, C., Singh, P. K., & Dipuraj, P. S. (2014). Bio-ethanol production from banana peel by simultaneous saccharification and fermentation process using cocultures *Aspergillus niger* and *Saccharomyces cerevisiae*. *International Journal of Current Microbiology and Applied Sciences*, 3(5), 84-96.
- Bhargav, S., Panda, B. P., Ali, M., & Javed, S. (2008). Solid-state fermentation: an overview. *Chemical and Biochemical Engineering Quarterly*, 22(1), 49-70.
- Buruiana, C. T., Garrote, G., & Vizireanu, C. (2013). Bioethanol Production from Residual Lignocellulosic Materials: A Review—Part 2. *Annals of the University Dunarea de Jos of Galati Fascicle VI--Food Technology*, 37(1).
- Carere, C., Sparling, R., Cicek, N., & Levin, D. (2008). Third generation biofuels via direct cellulose fermentation. *International Journal of Molecular Sciences*, 9(7), 1342-1360.
- Carrère, H., Monlau, F., Barakat, A., Dumas, C., & Steyer, J. P. (2011). Biogas from lignocellulosic biomass: interest of pretreatments. *Progress in Biogas II-Biogas Production from Agricultural Biomass and Organic Residues* (83-88).
- Chandra, R. P., Bura, R., Mabee, W. E., Berlin, D. A., Pan, X., & Saddler, J. N. (2007). Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocellulosics?. *In Biofuels* (67-93). Berlin, Heidelberg: Springer.
- Charles, M.B., Ryan, R., Ryan, N., Oloruntoba, R. 2007. “Public Policy and Biofuels: The Way Forward”, *Energy Policy*, 35, 5737–5746.
- Chen, C. C., & Lin, C. Y. (2000, December). Using sewage sludge as seed in an anaerobic hydrogen producing reactor. *In Proceedings 25th Wastewater Treatment Technology Conference* (368-72).
- Chen, C. C., Lin, C. Y., & Chang, J. S. (2001). Kinetics of hydrogen production with continuous anaerobic cultures utilizing sucrose as the limiting substrate. *Applied Microbiology and Biotechnology*, 57(1-2), 56-64.

- Chen, C. Y., Yang, M. H., Yeh, K. L., Liu, C. H., & Chang, J. S. (2008). Biohydrogen production using sequential two-stage dark and photo fermentation processes. *International Journal of Hydrogen Energy*, 33(18), 4755-4762.
- Cheng, C. L., Lo, Y. C., Lee, K. S., Lee, D. J., Lin, C. Y., & Chang, J. S. (2011). Biohydrogen production from lignocellulosic feedstock. *Bioresource technology*, 102(18), 8514-8523.
- Chin, H. L., Chen, Z. S., & Chou, C. P. (2003). Fedbatch operation using *Clostridium acetobutylicum* suspension culture as biocatalyst for enhancing hydrogen production. *Biotechnology Progress*, 19(2), 383-388.
- Dagnino, E. P., Chamorro, E. R., Romano, S. D., Felissia, F. E., & Area, M. C. (2013). Optimization of the acid pretreatment of rice hulls to obtain fermentable sugars for bioethanol production. *Industrial Crops and Products*, 42, 363-368.
- Das, D., & Veziroğlu, T. N. (2001). Hydrogen production by biological processes: a survey of literature. *International Journal of Hydrogen Energy*, 26(1), 13-28.
- Dasgupta, C. N., Gilbert, J. J., Lindblad, P., Heidorn, T., Borgvang, S. A., Skjanes, K., & Das, D. (2010). Recent trends on the development of photobiological processes and photobioreactors for the improvement of hydrogen production. *International Journal of Hydrogen Energy*, 35(19), 10218-10238.
- Demain, A. L., Newcomb, M., & Wu, J. D. (2005). Cellulase, clostridia, and ethanol. *Microbiology and Molecular Biology Reviews*, 69(1), 124-154.
- Dincer, I. (2000). Renewable energy and sustainable development: a crucial review. *Renewable and Sustainable Energy Reviews*, 4(2), 157-175.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28(3), 350-356.

- Eroğlu, E., Gündüz, U., Yücel, M., Türker, L., & Eroğlu, İ. (2004). Photobiological hydrogen production by using olive mill wastewater as a sole substrate source. *International Journal of Hydrogen Energy*, 29(2), 163-171.
- ETKB, (2014). *Türkiye Cumhuriyeti İklim Değişikliği Eylem Planı* Retrieved June 2019 from www.csb.gov.tr/db/iklim/banner/banner591.pdf, 12.12.2014.
- Fang, H. H., & Liu, H. (2002). Effect of pH on hydrogen production from glucose by a mixed culture. *Bioresource Technology*, 82(1), 87-93.
- Fang, H. H., Liu, H., & Zhang, T. (2005). Phototrophic hydrogen production from acetate and butyrate in wastewater. *International Journal of Hydrogen Energy*, 30(7), 785-793.
- Gao, J., Chen, L., Yuan, K., Huang, H., & Yan, Z. (2013). Ionic liquid pretreatment to enhance the anaerobic digestion of lignocellulosic biomass. *Bioresource Technology*, 150, 352-358.
- Garcia-Villanova, B., Guerra-Hernandez, E., Martinez-Gomez, E., & Montilla, J. (1993). Liquid chromatography for the determination of 5-(hydroxymethyl)-2-furaldehyde in breakfast cereals. *Journal of Agricultural and Food Chemistry*, 41(8), 1254-1255.
- Garland, N. L., Papageorgopoulos, D. C., & Stanford, J. M. (2012). Hydrogen and fuel cell technology: Progress, challenges, and future directions. *Energy Procedia*, 28, 2-11.
- Goltsov, V. A., & Veziroglu, T. N. (2001). From hydrogen economy to hydrogen civilization. *International Journal of Hydrogen Energy*, 26(9), 909-915.
- Gomis, D. B., Alvarez, M. G., Naredo, L. S., & Alonso, J. M. (1991). High-performance liquid chromatographic determination of furfural and hydroxymethylfurfural in apple juices and concentrates. *Chromatographia*, 32(1-2), 45-48.

- Guo, Y. P., Fan, S. Q., Fan, Y. T., Pan, C. M., & Hou, H. W. (2010). The preparation and application of crude cellulase for cellulose-hydrogen production by anaerobic fermentation. *International Journal of Hydrogen Energy*, 35(2), 459-468.
- Gupta, P., Samant, K., & Sahu, A. (2012). Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. *International Journal of Microbiology*, 2012.
- Han, S. K., & Shin, H. S. (2004). Biohydrogen production by anaerobic fermentation of food waste. *International Journal of Hydrogen Energy*, 29(6), 569-577.
- Han, W., Ye, M., Zhu, A. J., Zhao, H. T., & Li, Y. F. (2015). Batch dark fermentation from enzymatic hydrolyzed food waste for hydrogen production. *Bioresource Technology*, 191, 24-29.
- Hawkes, F. R., Dinsdale, R., Hawkes, D. L., & Hussy, I. (2002). Sustainable fermentative hydrogen production: challenges for process optimisation. *International Journal of Hydrogen Energy*, 27(11-12), 1339-1347.
- He, L., Huang, H., Lei, Z., Liu, C., & Zhang, Z. (2014). Enhanced hydrogen production from anaerobic fermentation of rice straw pretreated by hydrothermal technology. *Bioresource Technology*, 171, 145-151.
- Hendriks, A. T. W. M., & Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology*, 100(1), 10-18.
- Hethener, P., Brauman, A., & Garcia, J. L. (1992). *Clostridium termitidis* sp. nov., a cellulolytic bacterium from the gut of the wood-feeding termite, *Nasutitermes lujae*. *Systematic and Applied Microbiology*, 15(1), 52-58.
- Hoogwijk, M., Faaij, A., Eickhout, B., de Vries, B., & Turkenburg, W. (2005). Potential of biomass energy out to 2100, for four IPCC SRES land-use scenarios. *Biomass and Bioenergy*, 29(4), 225-257.

- Hung, C. H., Chang, Y. T., & Chang, Y. J. (2011). Roles of microorganisms other than *Clostridium* and *Enterobacter* in anaerobic fermentative biohydrogen production systems—a review. *Bioresource technology*, 102(18), 8437-8444.
- Islam, R., Cicek, N., Sparling, R., & Levin, D. (2009). Influence of initial cellulose concentration on the carbon flow distribution during batch fermentation by *Clostridium thermocellum* ATCC 27405. *Applied Microbiology and Biotechnology*, 82(1), 141.
- Jamradloedluk, J., & Wiriyaumpaiwong, S. (2007). Production and characterization of rice husk based charcoal briquettes. *KKU Engineering Journal*, 34(4), 391-398.
- Jung, K. W., Cho, S. K., Yun, Y. M., Shin, H. S., & Kim, D. H. (2013). Rapid formation of hydrogen-producing granules in an up-flow anaerobic sludge blanket reactor coupled with high-rate recirculation. *International Journal of Hydrogen Energy*, 38(22), 9097-9103.
- Jung, K. W., Cho, S. K., Yun, Y. M., Shin, H. S., & Kim, D. H. (2013). Rapid formation of hydrogen-producing granules in an up-flow anaerobic sludge blanket reactor coupled with high-rate recirculation. *International Journal of Hydrogen Energy*, 38(22), 9097-9103.
- Kaparaju, P., Serrano, M., Thomsen, A. B., Kongjan, P., & Angelidaki, I. (2009). Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept. *Bioresource Technology*, 100(9), 2562-2568.
- Kapdan, I. K., & Kargi, F. (2006). Bio-hydrogen production from waste materials. *Enzyme and Microbial Technology*, 38(5), 569-582.
- Kargi, F., Eren, N. S., & Ozmihci, S. (2012). Bio-hydrogen production from cheese whey powder (CWP) solution: comparison of thermophilic and mesophilic dark fermentations. *International Journal of Hydrogen Energy*, 37(10), 8338-8342.

- Khanal, S. K., Chen, W. H., Li, L., & Sung, S. (2004). Biological hydrogen production: effects of pH and intermediate products. *International Journal of Hydrogen Energy*, 29(11), 1123-1131.
- Kotay, S. M., & Das, D. (2008). Biohydrogen as a renewable energy resource—prospects and potentials. *International Journal of Hydrogen Energy*, 33(1), 258-263.
- Kumar, A. K., & Sharma, S. (2017). Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. *Bioresources and Bioprocessing*, 4(1), 7.
- Kumar, N., & Das, D. (2000). Enhancement of hydrogen production by *Enterobacter cloacae* IIT-BT 08. *Process Biochemistry*, 35(6), 589-593.
- Lay, J. J. (2001). Biohydrogen generation by mesophilic anaerobic fermentation of microcrystalline cellulose. *Biotechnology and Bioengineering*, 74(4), 280-287.
- Leaño, E. P., & Babel, S. (2012). Effects of pretreatment methods on cassava wastewater for biohydrogen production optimization. *Renewable Energy*, 39(1), 339-346.
- Levin, D. B., & Chahine, R. (2010). Challenges for renewable hydrogen production from biomass. *International Journal of Hydrogen Energy*, 35(10), 4962-4969.
- Levin, D. B., Islam, R., Cicek, N., & Sparling, R. (2006). Hydrogen production by *Clostridium thermocellum* 27405 from cellulosic biomass substrates. *International Journal of Hydrogen Energy*, 31(11), 1496-1503.
- Levin, D. B., Pitt, L., & Love, M. (2004). Biohydrogen production: prospects and limitations to practical application. *International Journal of Hydrogen Energy*, 29(2), 173-185.
- Levin, D.B., Carere, C.R., Cicek, N., Sparling, R. 2009. “Challenges for Bio-Hydrogen Production via Direct Lignocellulose Fermentation”. *International Journal of*

Hydrogen Energy, 34, 7390-7403.

- Li, P., & Zhu, M. (2011). A consolidated bio-processing of ethanol from cassava pulp accompanied by hydrogen production. *Bioresource Technology*, 102(22), 10471-10479.
- Lim, J. S., Manan, Z. A., Alwi, S. R. W., & Hashim, H. (2012). A review on utilisation of biomass from rice industry as a source of renewable energy. *Renewable and Sustainable Energy Reviews*, 16(5), 3084-3094.
- Lin, C. Y., & Chang, R. C. (2004). Fermentative hydrogen production at ambient temperature. *International Journal of Hydrogen Energy*, 29(7), 715-720.
- Liu, D., Zhang, R., Yang, X., Wu, H., Xu, D., Tang, Z., & Shen, Q. (2011). Thermostable cellulase production of *Aspergillus fumigatus* Z5 under solid-state fermentation and its application in degradation of agricultural wastes. *International Biodeterioration & Biodegradation*, 65(5), 717-725.
- Logan, B. E., Oh, S. E., Kim, I. S., & Van Ginkel, S. (2002). Biological hydrogen production measured in batch anaerobic respirometers. *Environmental Science & Technology*, 36(11), 2530-2535.
- Lynd, L. R. (1996). Overview and evaluation of fuel ethanol from cellulosic biomass: technology, economics, the environment, and policy. *Annual Review of Energy and The Environment*, 21(1), 403-465.
- Lynd, L. R., Van Zyl, W. H., McBride, J. E., & Laser, M. (2005). Consolidated bioprocessing of cellulosic biomass: an update. *Current Opinion in Biotechnology*, 16(5), 577-583.
- Lynd, L. R., Weimer, P. J., Van Zyl, W. H., & Pretorius, I. S. (2002). Microbial cellulose utilization: fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews*, 66(3), 506-577.

- Manish, S., & Banerjee, R. (2008). Comparison of biohydrogen production processes. *International Journal of Hydrogen Energy*, 33(1), 279-286.
- McDowall, W., & Eames, M. (2006). Forecasts, scenarios, visions, backcasts and roadmaps to the hydrogen economy: A review of the hydrogen futures literature. *Energy Policy*, 34(11), 1236-1250.
- Megawati, M., Sediawan, W. B., Sulistyo, H., & Hidayat, M. (2011). Kinetics of sequential reaction of hydrolysis and sugar degradation of rice husk in ethanol production: effect of catalyst concentration. *Bioresource Technology*, 102(2), 2062-2067.
- Minnan, L., Jinli, H., Xiaobin, W., Huijuan, X., Jinzao, C., Chuannan, L., ... & Liangshu, X. (2005). Isolation and characterization of a high H₂-producing strain *Klebsiella oxytoca* HP1 from a hot spring. *Research in Microbiology*, 156(1), 76-81.
- Momirlan, M., & Veziroglu, T. N. (2002). Current status of hydrogen energy. *Renewable and Sustainable Energy Reviews*, 6(1-2), 141-179.
- Momirlan, M., & Veziroglu, T. N. (2005). The properties of hydrogen as fuel tomorrow in sustainable energy system for a cleaner planet. *International Journal of Hydrogen Energy*, 30(7), 795-802.
- Morimoto, M., Atsuko, M., Atif, A. A. Y., Ngan, M. A., Fakhru'l-Razi, A., Iyuke, S. E., & Bakir, A. M. (2004). Biological production of hydrogen from glucose by natural anaerobic microflora. *International Journal of Hydrogen Energy*, 29(7), 709-713.
- Moxley, G., Zhu, Z., & Zhang, Y. H. P. (2008). Efficient sugar release by the cellulose solvent-based lignocellulose fractionation technology and enzymatic cellulose hydrolysis. *Journal of Agricultural and Food Chemistry*, 56(17), 7885-7890.
- Naik, S. N., Goud, V. V., Rout, P. K., & Dalai, A. K. (2010). Production of first and second generation biofuels: a comprehensive review. *Renewable and Sustainable Energy Reviews*, 14(2), 578-597.

- Nikzad, M., Talebniaa, F., Movagharnejada, K., Najafpoura, G., & Esfahanianb, M. (2017). Kinetic Modeling of Enzymatic Hydrolysis of Pretreated Sorghum Bicolor and Rice Husk. *International Journal of Engineering*, 30(11), 1622-1630.
- Noike, T., Takabatake, H., Mizuno, O., & Ohba, M. (2002). Inhibition of hydrogen fermentation of organic wastes by lactic acid bacteria. *International Journal of Hydrogen Energy*, 27(11-12), 1367-1371.
- Ntaikou, I., Antonopoulou, G., & Lyberatos, G. (2010). Biohydrogen production from biomass and wastes via dark fermentation: a review. *Waste and Biomass Valorization*, 1(1), 21-39.
- Oh, Y. K., Kim, S. H., Kim, M. S., & Park, S. (2004). Thermophilic biohydrogen production from glucose with trickling biofilter. *Biotechnology and Bioengineering*, 88(6), 690-698.
- Oh, Y. K., Seol, E. H., Kim, M. S., & Park, S. (2004). Photoproduction of hydrogen from acetate by a chemoheterotrophic bacterium *Rhodospseudomonas palustris* P4. *International Journal of Hydrogen Energy*, 29(11), 1115-1121.
- Olawale, O., & Oyawale, F. A. (2012). Characterization of rice husk via atomic absorption spectrophotometer for optimal silica production. *International Journal of science and Technology*, 2(4), 210-213.
- Olson, D. G., McBride, J. E., Shaw, A. J., & Lynd, L. R. (2012). Recent progress in consolidated bioprocessing. *Current Opinion in Biotechnology*, 23(3), 396-405.
- Ozmihci, S., & Kargi, F. (2010). Bio-hydrogen production by photo-fermentation of dark fermentation effluent with intermittent feeding and effluent removal. *International Journal of Hydrogen Energy*, 35(13), 6674-6680.
- Ozmihci, S., & Kargi, F. (2010). Comparison of different mixed cultures for bio-hydrogen production from ground wheat starch by combined dark and light fermentation. *Journal of Industrial Microbiology & Biotechnology*, 37(4), 341-347.

- Ozmihei, S., & Kargi, F. (2011). Dark fermentative bio-hydrogen production from waste wheat starch using co-culture with periodic feeding: effects of substrate loading rate. *International Journal of Hydrogen Energy*, 36(12), 7089-7093.
- Pachapur, V. L., Sarma, S. J., Brar, S. K., Le Bihan, Y., Buelna, G., & Verma, M. (2015). Biohydrogen production by co-fermentation of crude glycerol and apple pomace hydrolysate using co-culture of *Enterobacter aerogenes* and *Clostridium butyricum*. *Bioresource Technology*, 193, 297-306.
- Pan, J., Zhang, R., El-Mashad, H. M., Sun, H., & Ying, Y. (2008). Effect of food to microorganism ratio on biohydrogen production from food waste via anaerobic fermentation. *International Journal of Hydrogen Energy*, 33(23), 6968-6975.
- Panwar, N. L., Kaushik, S. C., & Kothari, S. (2011). Role of renewable energy sources in environmental protection: A review. *Renewable and Sustainable Energy Reviews*, 15(3), 1513-1524.
- Perera, K. R. J., & Nirmalakhandan, N. (2010). Enhancing fermentative hydrogen production from sucrose. *Bioresource Technology*, 101(23), 9137-9143.
- Petersson, A., & Lidén, G. (2007). Fed-batch cultivation of *Saccharomyces cerevisiae* on lignocellulosic hydrolyzate. *Biotechnology Letters*, 29(2), 219-225.
- Podesta, J. J., Gutierrez-Navarro, A. M., Estrella, C. N., & Estes, M. A. (1997). Electrochemical measurement of trace concentrations of biological hydrogen produced by *Enterobacteriaceae*. *Research in Microbiology*, 148(1), 87-93.
- Rafiee, E., Khodayari, M., Kahrizi, M., & Tayebee, R. (2012). H5CoW12O40 supported on nano silica from rice husk ash: A green bifunctional catalyst for the reaction of alcohols with cyclic and acyclic 1, 3-dicarbonyl compounds. *Journal of Molecular Catalysis A: Chemical*, 358, 121-128.
- Rafieenia, R., Lavagnolo, M. C., & Pivato, A. (2018). Pre-treatment technologies for dark fermentative hydrogen production: current advances and future directions. *Waste Management*, 71, 734-748.

- Ramachandran, U., Wrana, N., Cicek, N., Sparling, R., & Levin, D. B. (2008). Hydrogen production and end-product synthesis patterns by *Clostridium termitidis* strain CT1112 in batch fermentation cultures with cellobiose or α -cellulose. *International Journal of Hydrogen Energy*, 33(23), 7006-7012.
- Ramachandran, U., Wrana, N., Cicek, N., Sparling, R., & Levin, D. B. (2011). Isolation and characterization of a hydrogen-and ethanol-producing *Clostridium* sp. strain URNW. *Canadian Journal of Microbiology*, 57(3), 236-243.
- Ren, Z., Ward, T. E., Logan, B. E., & Regan, J. M. (2007). Characterization of the cellulolytic and hydrogen-producing activities of six mesophilic *Clostridium* species. *Journal of Applied Microbiology*, 103(6), 2258-2266.
- Rufian-Henares, J. A., Garcia-Villanova, B., & Guerra-Hernandez, E. (2001). Determination of furfural compounds in enteral formula. *Journal of Liquid Chromatography & Related Technologies*, 24(19), 3049-3061.
- Sağnak, R. (2011). *Biohydrogen production by dark/light fermentation of hydrolysed wheat starch*. Master Thesis, Dokuz Eylül University, İzmir.
- Saha, B. C., Iten, L. B., Cotta, M. A., & Wu, Y. V. (2005). Dilute acid pretreatment, enzymatic saccharification, and fermentation of rice hulls to ethanol. *Biotechnology Progress*, 21(3), 816-822.
- San, S. (2005). *Türkiye’de Yetistirilen Çeltik (Oryza sativa L.) Çesitlerinde Genetik Farklılığı Oryzin Elektroforegrami Yöntemi ile Belirlenmesi*. Master Thesis, Ankara University, Ankara.
- Saratale, G. D., Chen, S. D., Lo, Y. C., Saratale, R. G., & Chang, J. S. (2008). Outlook of biohydrogen production from lignocellulosic feedstock using dark fermentation—a review. *Journal of Scientific & Industrial Research*, 962-979
- Sediawan, W. B., Sulistyo, H., & Hidayat, M. (2011). Kinetics of sequential reaction of hydrolysis and sugar degradation of rice husk in ethanol production: Effect of catalyst concentration. *Bioresource Technology*, 102(2), 2062-2067.

- Shi, X. Y., & Yu, H. Q. (2005). Response surface analysis on the effect of cell concentration and light intensity on hydrogen production by *Rhodospseudomonas capsulata*. *Process Biochemistry*, 40(7), 2475-2481.
- Shukla, S., Nidhi, S., Pooja, N., Charu, A., Silvi, M., Rizwana, A. B., ... & Tiwari, A. (2013). Preparation and characterization of cellulose derived from rice husk for drug delivery. *Advanced Materials Letters*, 4(9), 714-719.
- Sluiter, A. (2012). *Determination of structural carbohydrates and lignin in biomass: laboratory analytical procedure (LAP)*: Issue Date, April 2008, Revision Date: August 2012 (Version 08-03-2012). National Renewable Energy Laboratory.
- Soltani, N., Bahrami, A., Pech-Canul, M. I., & González, L. A. (2015). Review on the physicochemical treatments of rice husk for production of advanced materials. *Chemical Engineering Journal*, 264, 899-935.
- Sun, Y., & Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology*, 83(1), 1-11.
- Tabatabaie, S. M. H., Rafiee, S., Keyhani, A., & Ebrahimi, A. (2013). Energy and economic assessment of prune production in Tehran province of Iran. *Journal of Cleaner Production*, 39, 280-284.
- Tarım Ürünleri Piyasaları, Çeltik from (n.d). Retrieved August <https://arastirma.tarimorman.gov.tr/tepge/Belgeler/PDF%20Tar%C4%B1m%20%C3%9Cr%C3%BCnleri%20Piyasalar%C4%B1/2019Ocak%20Tar%C4%B1m%20%C3%9Cr%C3%BCnleri%20Raporu/2019-Ocak%20%C3%87eltik.pdf>
- Thauer, R. (1977). *Limitation of microbial H₂-formation via fermentation. In Microbial energy conversion* (201-204). Germany: Pergamon.
- Theobald, A., Müller, A., & Anklam, E. (1998). Determination of 5-hydroxymethylfurfural in vinegar samples by HPLC. *Journal of Agricultural and Food Chemistry*, 46(5), 1850-1854.

- Tosuner, Z. V., Taylan, G. G., & Özmihçı, S. (2019). Effects of rice husk particle size on biohydrogen production under solid state fermentation. *International Journal of Hydrogen Energy*, 44(34), 18785-18791.
- Tutar, F., & Eren, M.V. (2011). Geleceğin enerjisi: Hidrojen ekonomisi ve Türkiye. *Uluslararası İktisadi ve İdari İncelemeler Dergisi*, 3(6), 1-25.
- UHK, (2011). *Ulusal Hububat Konseyi Çeltik Raporu/Aralık (n.d.)*. Retrieved August, 2019, from http://uhk.org.tr/dosyalar/UHK_celtikraporu.pdf, son erişim tarihi: 18. Ocak 2015
- Union, E. (2009). Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. *Official Journal of the European Union*, 5, 2009.
- Urbaniec, K., & Grabarczyk, R. (2009). Raw materials for fermentative hydrogen production. *Journal of Cleaner Production*, 17(10), 959-962.
- Waegel, A., Byrne, J., Tobin, D., & Haney, B. (2006). Hydrogen highways: Lessons on the energy technology-policy interface. *Bulletin of Science, Technology & Society*, 26(4), 288-298.
- Wang, A., Ren, N., Shi, Y., Lee, D.J. 2008. “Bioaugmented Hydrogen Production from Microcrystalline Cellulose Using Co-Culture—Clostridium Acetobutylicum X9 and Ethanoigenens harbinense B49”, *International Journal of Hydrogen Energy*, 33, 912-917.
- Wang, X., Maeda, K., Thomas, A., Takanabe, K., Xin, G., Carlsson, J. M., ... & Antonietti, M. (2009). A metal-free polymeric photocatalyst for hydrogen production from water under visible light. *Nature Materials*, 8(1), 76.
- Wannapeera, J., Worasuwanarak, N., & Pipatmanomai, S. (2008). Product yields and characteristics of rice husk, rice straw and corncob during fast pyrolysis in a drop-tube/fixed-bed reactor. *Songklanakarin Journal of Science & Technology*, 30(3).

- Wei, Y., Wu, D., Wei, D., Zhao, Y., Wu, J., Xie, X., ... & Wei, Z. (2019). Improved lignocellulose-degrading performance during straw composting from diverse sources with actinomycetes inoculation by regulating the key enzyme activities. *Bioresource Technology*, 271, 66-74.
- Wong, Y. M., Juan, J. C., Ting, A., & Wu, T. Y. (2014). High efficiency bio-hydrogen production from glucose revealed in an inoculum of heat-pretreated landfill leachate sludge. *Energy*, 72, 628-635.
- Yang, B., Tao, L., & Wyman, C. E. (2018). Strengths, challenges, and opportunities for hydrothermal pretreatment in lignocellulosic biorefineries. *Biofuels, Bioproducts and Biorefining*, 12(1), 125-138.
- Yetis, M., Gündüz, U., Eroglu, I., Yücel, M., & Türker, L. (2000). Photoproduction of hydrogen from sugar refinery wastewater by *Rhodobacter sphaeroides* OU 001. *International Journal of Hydrogen Energy*, 25(11), 1035-1041.
- Yu, J., & Takahashi, P. (2007). Biophotolysis-based hydrogen production by cyanobacteria and green microalgae. *Communicating Current Research and Educational Topics and Trends in Applied Microbiology*, 1, 79-89.
- Zhu, H., Suzuki, T., Tsygankov, A. A., Asada, Y., & Miyake, J. (1999). Hydrogen production from tofu wastewater by *Rhodobacter sphaeroides* immobilized in agar gels. *International Journal of Hydrogen Energy*, 24(4), 305-310.
- Zhu, H., Ueda, S., Asada, Y., & Miyake, J. (2002). Hydrogen production as a novel process of wastewater treatment—studies on tofu wastewater with entrapped *R. sphaeroides* and mutagenesis. *International Journal of Hydrogen Energy*, 27(11-12), 1349-1357.

APPENDICES

Appendix-1: Raw Data for Pure *Clostridium intestinale* Experiments

Table A.1 Raw rice husk 5 g l⁻¹ in pure *Clostridium intestinale* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	150	160	0	0	0	0	0	0	0
4	140	170	0.0349	5.933	1	0.0349	0.0349	5.9679	0.240
8	130	180	0.0351	0.036	0	0.0351	0	6.0039	0.242
12	120	190	0.0344	0	0	0.0344	0	6.0039	0.242
24	110	200	0.053	3.72	0	0.053	0	9.7239	0.391
30	100	210	0.0432	0	0	0.0432	0	9.7239	0.391
36	90	220	0.0481	1.078	0	0.0481	0	10.8019	0.435
48	80	230	0.0484	0.069	0	0.0484	0	10.8709	0.438

Table A.2 Raw rice husk 5 g L⁻¹ in pure *Clostridium intestinale* experiment

t (h)	Total Sugar Concentration (mg L ⁻¹)	pH	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	195.13		0.00	373.03	325.41	0.00
4	977.57	7.05	305.99	86.32	336.67	191.02
8	749.95	6.97	123.20	318.30	193.94	292.68
12	143.91	6.71	318.83	98.88	247.51	591.33
24	166.68	6.7	567.50	2671.67	219.96	576.86
30	118.31	6.71	608.29	2732.12	189.76	519.80
36	47.32	6.67	515.95	2744.58	196.33	562.37
48	105.37	6.75	450.81	3235.53	137.66	493.12

Table A.3 Ground rice husk 5 g L⁻¹ in Pure *Clostridium intestinale* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	150	160	0		0	0	0	0	0
4	140	170	0.0358	6.086	0	0.0358	0	6.086	0.239
8	130	180	0.0378	0.36	0	0.0378	0	6.446	0.254
12	120	190	0.0345	0	0	0.0345	0	6.446	0.254
24	110	200	0.0397	1.04	0	0.0397	0	7.486	0.294
30	100	210	0.0443	0.966	0	0.0443	0	8.452	0.332
36	90	220	0.0349	0	0	0.0349	0	8.452	0.332
48	80	230	0.0362	0.299	0	0.0362	0	8.751	0.344

Table A.4 Ground rice husk 5 g L⁻¹ in Pure *Clostridium intestinale* experiment

t (h)	Total Sugar Concentration (mg L ⁻¹)	pH	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	229.27		413.85	365.61	406.71	290.57
4	707.27	6.95	334.97	27.01	314.62	284.22
8	155.30	6.79	325.90	494.37	217.84	301.26
12	104.08	6.84	353.17	600.30	184.51	382.02
24	172.37	6.86	554.65	1206.49	232.49	371.82
30	101.24	6.81	557.27	2643.06	222.70	408.49
36	74.64	6.8	497.84	2926.29	228.18	436.29
48	93.99	6.9	329.86	2943.81	128.81	335.71

Table A.5 5 g l⁻¹ rice husk with Acid hydrolysis in pure *Clostridium intestinale* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	150	160	0		0	0	0	0	0
4	140	170	0.033	5.61	2	0.033	0.066	5.676	0.223
8	130	180	0.033	0	0	0.033	0	5.676	0.223
12	120	190	0.03	0	0	0.03	0	5.676	0.223
24	110	200	0.033	0.6	0	0.033	0	6.276	0.246
30	100	210	0.0335	0.105	0	0.0335	0	6.381	0.251
36	90	220	0.0341	0.132	0	0.0341	0	6.513	0.256
48	80	230	0.036	0.437	0	0.036	0	6.95	0.273

Table A.6 5 g L⁻¹ rice husk with Acid hydrolysis in pure *Clostridium intestinale* experiment

t (h)	Total Sugar Concentration (mg L ⁻¹)	pH	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	909.75		286.96	325.46	368.07	224.40
4	1865.75	6.86	262.16	105.37	393.86	188.33
8	1208.50	6.88	126.73	349.44	153.62	162.52
12	1515.78	6.93	122.80	568.57	170.09	152.89
24	807.32	6.51	552.03	182.36	172.33	756.72
30		6.44	54.78	1698.54	258.21	139.76
36	526.83	6.42	458.58	1702.17	179.24	903.06
48	521.14	6.56	457.05	1934.73	160.23	821.03

Table A.7 1 g L⁻¹ rice husk with thermal treatment in pure *Clostridium intestinale* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	150	160	0		0	0	0	0	0
4	140	170	0.0341	5.797	3	0.0341	0.1023	5.8993	0.232
8	130	180	0.0349	0.144	0	0.0349	0	6.0433	0.237
12	120	190	0.0363	0.266	0	0.0363	0	6.3093	0.248
24	110	200	0.0361	0	0	0.0361	0	6.3093	0.248
30	100	210	0.0413	1.092	0	0.0413	0	7.4013	0.291
36	90	220	0.0505	2.024	0	0.0505	0	9.4253	0.370
48	80	230	0.046	0	0	0.046	0	9.4253	0.370

Table A.8 1 g L⁻¹ rice husk with thermal treatment in pure *Clostridium intestinale* experiment

t (h)	Total Sugar Concentration (mg L ⁻¹)	pH	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	186.59		442.42	233.89	189.51	0.00
4	1011.72	7.15	373.98	94.82	350.23	182.44
8	721.50	7	101.98	87.54	268.21	322.52
12	121.15	6.77	767.90	323.88	292.36	551.84
24	195.13	6.82	538.77	1714.21	278.10	494.17
30	166.68	6.75	389.24	2324.67	247.74	552.94
36	38.22	6.72	449.07	3529.74	267.20	666.50
48	95.12	6.8	450.45	3709.16	206.18	551.49

Table A.9 2 g L⁻¹ rice husk with thermal treatment in pure *Clostridium intestinale* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	150	160	0		0	0	0	0	0
4	140	170	0.0347	5.899	0	0.0347	0	5.899	0.232
8	130	180	0.0339	0	0	0.0339	0	5.899	0.232
12	120	190	0.0346	0.133	0	0.0346	0	6.032	0.237
24	110	200	0.0371	0.5	0	0.0371	0	6.532	0.256
30	100	210	0.044	1.449	0	0.044	0	7.981	0.313
36	90	220	0.0433	0	0	0.0433	0	7.981	0.313
48	80	230	0.044	0.161	0	0.044	0	8.142	0.320

Table A.10 2 g L⁻¹ rice husk with thermal treatment in pure *Clostridium intestinale* experiment

t (h)	Total Sugar Concentration (mg L ⁻¹)	pH	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	200.82		392.03	190.99	378.45	107.67
4	1065.78	7.07	334.18	55.82	356.64	223.43
8	954.81	7.05	549.08	144.46	199.17	155.50
12	724.35	7.05	354.60	189.74	255.24	200.65
24	240.65	6.73	783.87	587.26	252.60	564.41
30	249.19	6.68	552.47	2163.63	257.99	487.79
36	107.64	6.62	249.37	1819.52	268.31	607.18
48	116.75	6.74	254.19	2434.02	245.74	469.50

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Table A.11 5 g L⁻¹ rice husk with thermal treatment in pure *Clostridium intestinale* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	150	160	0		0	0	0	0	0
4	140	170	0.0338	5.746	0	0.0338	0	5.746	0.226
8	130	180	0.0336	0	0	0.0336	0	5.746	0.226
12	120	190	0.0336	0	0	0.0336	0	5.746	0.226
24	110	200	0.0378	0.84	0	0.0378	0	6.586	0.259
30	100	210	0.0434	1.176	0	0.0434	0	7.762	0.305
36	90	220	0.0399	0	0	0.0399	0	7.762	0.305
48	80	230	0.0443	1.012	0	0.0443	0	8.774	0.345

Table A.12 5 g L⁻¹ rice husk with thermal treatment in pure *Clostridium intestinale* experiment

t (h)	Total Sugar Concentration (mg L ⁻¹)	pH	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	257.72		317.06	271.23	264.69	165.63
4	986.11	7.08	358.53	296.94	365.27	156.06
8	675.98	6.9	309.09	333.65	261.49	280.62
12	81.32	6.7	464.15	1063.69	277.16	553.92
24	155.30	6.73	532.21	2452.00	251.97	604.42
30	158.14	6.7	566.15	2871.65	243.64	586.30
36	72.36	6.62	403.18	3204.33	235.18	532.95
48	90.57	6.72	427.39	3674.91	166.42	554.57

Table A.13 10 g L⁻¹ rice husk with thermal treatment in pure *Clostridium intestinale* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	150	160	0		0	0	0	0	0
4	140	170	0.0345	5.865	0	0.0345	0	5.865	0.230
8	130	180	0.0536	3.438	0	0.0536	0	9.303	0.365
12	120	190	0.0354	0	0	0.0354	0	9.303	0.365
24	110	200	0.044	1.72	0	0.044	0	11.023	0.433
30	100	210	0.0404	0	0	0.0404	0	11.023	0.433
36	90	220	0.0537	2.926	0	0.0537	0	13.949	0.548
48	80	230	0.0407	0	0	0.0407	0	13.949	0.548

Table A.14 10 g L⁻¹ rice husk with thermal treatment in pure *Clostridium intestinale* experiment

t (h)	Total Sugar Concentration (mg L ⁻¹)	pH	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	351.62		399.93	246.30	413.81	211.85
4	1088.54	7.01	350.41	69.82	401.90	182.62
8	593.46	6.77	459.57	133.99	253.39	293.25
12	257.72	6.63	515.68	602.73	254.37	592.30
24	223.58	6.67	572.53	2121.13	242.93	614.41
30	232.12	6.62	530.12	3052.70	215.38	605.78
36	169.10	6.58	457.43	3052.92	233.67	553.65
48	147.48	6.67	461.04	3336.99	196.86	518.24

Table A.15 15 g L⁻¹ rice husk with thermal treatment in pure *Clostridium intestinale* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	150	160	0		0	0	0	0	0
4	140	170	0.0343	5.831	1	0.0343	0.0343	5.8653	0.230
8	130	180	0.0334	0	0	0.0334	0	5.8653	0.230
12	120	190	0.0339	0.095	0	0.0339	0	5.9603	0.234
24	110	200	0.0625	5.72	0	0.0625	0	11.6803	0.459
30	100	210	0.0624	0	0	0.0624	0	11.6803	0.459
36	90	220	0.0682	1.276	0	0.0682	0	12.9563	0.509
48	80	230	0.0418	0	0	0.0418	0	12.9563	0.509

Table A.16 15 g L⁻¹ rice husk with thermal treatment in pure *Clostridium intestinale* experiment

t (h)	Total Sugar Concentration (mg L ⁻¹)	pH	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	394.30		388.16	350.44	397.44	194.08
4	1401.52	7.01	392.25	402.23	286.32	200.20
8	994.64	6.96	389.76	512.16	232.96	300.28
12	502.42	6.73	574.57	159.98	258.33	497.32
24	328.86	6.6	556.04	3003.12	239.71	637.37
30	260.57	6.62	524.60	3125.64	230.97	597.63
36	132.68	6.55	453.68	3430.32	202.70	596.14
48	136.09	6.65	499.19	3625.51	118.34	538.77

Table A.17 20 g L⁻¹ rice husk with thermal treatment in pure *Clostridium intestinale* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	150	160	0		0	0	0	0	0
4	140	170	0.0339	5.763	0	0.0339	0	5.763	0.226
8	130	180	0.033	0	0	0.033	0	5.763	0.226
12	120	190	0.0329	0	0	0.0329	0	5.763	0.226
24	110	200	0.0389	1.2	0	0.0389	0	6.963	0.273
30	100	210	0.0489	2.1	0	0.0489	0	9.063	0.356
36	90	220	0.0573	1.848	0	0.0573	0	10.911	0.429
48	80	230	0.0396	0	0	0.0396	0	10.911	0.429

Table A.18 20 g L⁻¹ rice husk with thermal treatment in pure *Clostridium intestinale* experiment

t (h)	Total Sugar Concentration (mg L ⁻¹)	pH	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	502.42		402.31	53.13	130.46	275.59
4	465.43	7	379.24	204.50	507.87	338.13
8	1088.54	6.96	180.44	568.04	233.04	0.00
12	653.21	6.83	340.00	1224.86	241.30	479.53
24	246.34	6.54	575.08	3765.94	223.48	770.08
30	303.25	6.52	616.31	3056.38	215.60	666.41
36	153.17	6.47	550.06	3446.36	195.46	562.65
48	198.69	6.6	564.26	3657.15	161.48	520.47

Table A.19 33 g L⁻¹ rice husk with thermal treatment in pure *Clostridium intestinale* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	150	160	0		0	0	0	0	0
4	140	170	0.0334	5.678	0	0.0765	0	5.678	0.223
8	130	180	0.0335	0.018	0	0.21	0	5.696	0.224
12	120	190	0.0331	0	0	0.261	0	5.696	0.224
24	110	200	0.0349	0.36	0	0.261	0	6.056	0.238
30	100	210	0.0371	0.462	0	0.261	0	6.518	0.256
36	90	220	0.0459	1.936	0	0.261	0	8.454	0.332
48	80	230	0.0526	1.541	0	0.261	0	9.995	0.393

Table A.20 33 g L⁻¹ rice husk with thermal treatment in pure *Clostridium intestinale* experiment

t (h)	Total Sugar Concentration (mg L ⁻¹)	pH	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	1242.18		325.01	240.91	414.65	301.41
4	1145.44	7.02	330.24	85.33	378.99	249.44
8	1216.57	6.98	460.26	259.67	262.86	362.58
12	1105.61	7.02	403.72	362.97	284.47	189.01
24	530.87	6.66	624.52	448.91	262.60	685.79
30	508.11	6.58	516.65	2027.40	252.94	721.38
36	229.42	6.52	481.45	2586.76	262.63	731.74
48	298.84	6.59	585.06	3988.94	240.45	695.39

A.2 Raw Data for Pure *Clostridium termitidis* ExperimentsTable A.21 Raw rice husk 5 g L⁻¹ in pure *Clostridium termitidis* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0338	5.408	0	0.0338	0	5.408	0.213
48	145	165	0.0485	2.4255	0	0.0485	0	7.8335	0.308
72	140	170	0.0397	0	0	0.0397	0	7.8335	0.308
96	135	175	0.0445	0.84	1	0.0445	0.0445	8.718	0.343
120	130	180	0.043	0	0	0.043	0	8.718	0.343
144	125	185	0.0368	0	1	0.0368	0.0368	8.7548	0.344
168	120	190	0.041	0.798	0	0.041	0	9.5528	0.376
192	115	195	0.0429	0.3705	0	0.0429	0	9.9233	0.390
216	110	200	0.0428	0	0	0.0428	0	9.9233	0.390
240	105	205	0.0572	2.952	0	0.0572	0	12.8753	0.507
264	100	210	0.0595	0.483	0	0.0595	0	13.3583	0.526
288	95	215	0.0707	2.408	0	0.0707	0	15.7663	0.6202
312	90	220	0.0743	0.792	0	0.0743	0	16.5583	0.651
336	85	225	0.0614	0	0	0.0614	0	16.5583	0.651

Table A.22 Raw rice husk 5 g L⁻¹ in pure *Clostridium termitidis* experiment

t (h)	pH	Total Sugar (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	7.5	469.41	0.00	771.73	686.38	0.00
24	6.94	436.00	0.00	1316.20	169.35	48.51
48	6.74	201.00	456.70	1216.67	0.00	0.00
72	6.78	281.00	419.83	2609.52	446.71	0.00
96	0	176.00	451.35	2808.84	280.45	0.00
120	6.87	608.56	501.76	3199.96	226.99	0.00
144	6.84	239.99	516.60	2764.51	423.82	48.51
168	6.85	133.00	525.82	3061.50	441.08	48.51
192	6.8	122.20	558.18	3083.23	404.40	48.51
216	6.81	128.43	574.81	3212.98	446.65	48.51
240	6.67	93.30	631.72	2853.66	411.31	48.51
264	6.78	170.00	687.96	3323.67	362.69	48.51
288	6.78	120.00	683.61	2834.51	390.74	48.51
312	6.78	108.00	771.89	2749.69	553.17	48.51
336	6.7	165.00	801.09	2496.96	424.06	48.51

Table A.23 Ground rice husk 5 g L⁻¹ in pure *Clostridium termitidis* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
24	150	160	0.0337	5.392	0	0.0337	0	5.392	0.212
48	145	165	0.0387	0.825	0	0.0387	0	6.217	0.244
72	140	170	0.0463	1.292	1	0.0463	0.0463	7.5553	0.297
96	135	175	0.0481	0.315	0	0.0481	0	7.8703	0.309
120	130	180	0.0472	0	0	0.0472	0	7.8703	0.309
144	125	185	0.0391	0	0	0.0391	0	7.8703	0.309
168	120	190	0.039	0	0	0.039	0	7.8703	0.309
192	115	195	0.0426	0.702	0	0.0426	0	8.5723	0.337
216	110	200	0.0503	1.54	0	0.0503	0	10.1123	0.397
240	105	205	0.0617	2.337	0	0.0617	0	12.4493	0.489
264	100	210	0.0709	1.932	0	0.0709	0	14.3813	0.565
288	95	215	0.0656	0	0	0.0656	0	14.3813	0.565
312	90	220	0.0669	0.286	0	0.0669	0	14.6673	0.576
336	85	225	0.0574	0	0	0.0574	0	14.6673	0.576

Table A.24 Ground rice husk 5 g L⁻¹ in pure *Clostridium termitidis* experiment

t (h)	pH	Total Sugar (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	7.5	451.86	3057.02	222.32	477.47	48.51
24	7.05	410.00	0.00	776.86	30.18	48.51
48	6.85	348.00	163.30	813.25	0.00	0.00
72	6.84	220.00	432.32	2321.10	218.62	184.54
96	6.87	151.00	486.15	2718.98	204.06	144.90
120	6.84	459.38	496.27	3201.55	161.50	160.26
144	6.88	243.76	547.75	2528.73	382.65	152.72
168	6.89	165.00	523.00	2778.15	355.43	48.51
192	6.81	134.70	576.69	2799.98	364.51	147.64
216	6.84	108.37	594.49	2782.87	351.84	157.76
240	6.72	216.00	647.86	2272.49	366.49	48.51
264	6.9	116.00	678.35	3523.91	345.72	48.51
288	6.73	99.60	602.95	3323.75	347.99	48.51
312	6.82	186.00	663.40	2342.18	447.91	129.31
336	6.74	117.00	687.05	2218.57	428.99	48.51

Table A.25 5 g L⁻¹ Rice husk with Acid hydrolysis in pure *Clostridium termitidis* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
24	150	160	0.0334	5.344	0	0.0334	0	5.344	0.210
48	145	165	0.036	0.429	0	0.036	0	5.773	0.227
72	140	170	0.0412	0.884	0	0.0412	0	6.657	0.261
96	135	175	0.036	0	0	0.036	0	6.657	0.261
120	130	180	0.0373	0.234	0	0.0373	0	6.891	0.271
144	125	185	0.0439	1.221	0	0.0439	0	8.112	0.319
168	120	190	0.0449	0.19	4	0.0449	0.1796	8.4816	0.333
192	115	195	0.0429	0	0	0.0429	0	8.4816	0.333
216	110	200	0.0412	0	0	0.0412	0	8.4816	0.333
240	105	205	0.0398	0	0	0.0398	0	8.4816	0.333
264	100	210	0.0397	0	0	0.0397	0	8.4816	0.333
288	95	215	0.0397	0	0	0.0397	0	8.4816	0.333
312	90	220	0.0364	0	0	0.0364	0	8.4816	0.333
336	85	225	0.0371	0.1575	0	0.0371	0	8.6391	0.339

Table A.26 5 g L⁻¹ Rice husk with Acid hydrolysis in pure *Clostridium termitidis* experiment

t (h)	pH	Total Sugar (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	7.5	1101.22	0.00	438.96	474.75	48.51
24	7.02	924.00	0.00	68.00	0.00	48.51
48	6.86	833.00	213.48	1808.14	0.00	155.33
72	6.84	956.00	397.47	2838.90	13.26	188.41
96	6.81	510.00	448.88	4057.67	169.62	201.72
120	6.8	692.55	443.58	4040.30	137.11	156.26
144	6.85	816.65	500.46	2977.51	327.30	48.51
168	6.83	275.00	529.82	2775.40	322.57	48.51
192	6.99	218.70	529.96	2683.63	325.89	48.51
216	6.76	207.41	548.88	4632.13	1003.66	48.51
240	6.74	209.00	576.04	2840.60	314.55	48.51
264	6.92	172.00	565.51	3128.33	317.43	217.10
288	6.91	226.00	570.60	2810.62	344.75	189.64
312	6.91	133.00	500.39	2240.26	466.49	243.88
336	6.82	201.00	577.69	2009.62	513.30	192.60

Table A.27 1 g L⁻¹ rice husk with thermal treatment in pure *Clostridium termitidis* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
24	150	160	0.0332	5.312	0	0.0332	0	5.312	0.208
48	145	165	0.0421	1.4685	0	0.0421	0	6.7805	0.266
72	140	170	0.0348	0	0	0.0348	0	6.7805	0.266
96	135	175	0.0336	0	8	0.0336	0.2688	7.0493	0.277
120	130	180	0.0344	0.144	0	0.0344	0	7.1933	0.282
144	125	185	0.0352	0.148	0	0.0352	0	7.3413	0.288
168	120	190	0.0386	0.646	0	0.0386	0	7.9873	0.314
192	115	195	0.0378	0	0	0.0378	0	7.9873	0.314
216	110	200	0.0364	0	0	0.0364	0	7.9873	0.314
240	105	205	0.037	0.123	0	0.037	0	8.1103	0.319
264	100	210	0.0381	0.231	0	0.0381	0	8.3413	0.328
288	95	215	0.0358	0	0	0.0358	0	8.3413	0.328
312	90	220	0.0488	2.86	0	0.0488	0	11.2013	0.440
336	85	225	0.037	0	0	0.037	0	11.2013	0.440

Table A.28 1 g L⁻¹ rice husk with thermal treatment in pure *Clostridium termitidis* experiment

t (h)	pH	Total Sugar (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	7.5	485.70	0.00	541.46	556.66	48.51
24	7.1	274.00	0.00	1172.65	103.48	106.45
48	6.74	94.60	0.00	1158.89	0.00	0.00
72	6.97	126.00	430.95	4306.81	121.16	0
96	0	118.00	519.27	4018.60	151.68	0
120	6.87	327.75	503.22	4065.33	139.11	0
144	6.91	198.63	485.09	4330.71	380.87	48.51
168	6.9	126.00	521.51	3188.81	336.01	48.51
192	7.02	98.34	531.04	3021.02	314.27	48.51
216	6.99	104.61	387.37	2389.66	326.17	48.51
240	6.82	216.00	406.46	3459.69	335.64	48.51
264	7.02	194.00	412.63	3502.17	371.92	48.51
288	6.6	98.30	314.84	3150.30	333.17	48.51
312	6.95	65.70	365.47	2017.49	388.79	48.51
336	6.84	301.00	319.65	1718.90	625.33	48.51

Table A.29 2 g L⁻¹ Rice husk with thermal treatment in pure *Clostridium termitidis* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0347	5.552	0	0.0347	0	5.552	0.218
48	145	165	0.04	0.8745	0	0.04	0	6.4265	0.252
72	140	170	0.0355	0	0	0.0355	0	6.4265	0.252
96	135	175	0.0351	0	0	0.0351	0	6.4265	0.252
120	130	180	0.0366	0.27	0	0.0366	0	6.6965	0.263
144	125	185	0.0367	0.0185	0	0.0367	0	6.715	0.264
168	120	190	0.0342	0	0	0.0342	0	6.715	0.264
192	115	195	0.0375	0.6435	0	0.0375	0	7.3585	0.289
216	110	200	0.0382	0.14	0	0.0382	0	7.4985	0.294
240	105	205	0.0392	0.205	0	0.0392	0	7.7035	0.303
264	100	210	0.0431	0.819	0	0.0431	0	8.5225	0.335
288	95	215	0.0466	0.7525	0	0.0466	0	9.275	0.364
312	90	220	0.0387	0	0	0.0387	0	9.275	0.364
336	85	225	0.0421	0.765	0	0.0421	0	10.04	0.394

Table A.30 2 g L⁻¹ Rice husk with thermal treatment in pure *Clostridium termitidis* experiment

t (h)	pH	Total Sugar (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	7.5	502.00	0	414.36	523.56	48.51
24	7	377.00	241.54	795.78	0	48.51
48	6.76	240	0	1366.29	0	0
72	6.89	160	459.65	3956.35	215.22	144.74
96	0	200	511.24	3837.42	207.64	0
120	6.89	181.00	508.32	3421.14	286.74	0
144	6.94	314.00	519.93	3196.76	424.71	48.51
168	6.94	113.00	557.82	2926.20	444.02	48.51
192	7.02	165.00	565.86	2907.53	439.04	48.51
216	6.97	98.00	539.02	2525.54	446.65	48.51
240	6.84	221.00	566.04	3541.56	367.26	48.51
264	6.92	50	597.79	2975.56	359.69	48.51
288	6.85	91.00	579.89	2907.53	380.38	48.51
312	6.94	60	563.14	2429.57	506.81	48.51
336	6.87	221.00	554.94	2230.91	525.28	48.51

Table A.31 5 g L⁻¹ rice husk with thermal treatment in pure *Clostridium termitidis* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.031	4.96	0	0.031	0	4.96	0.195
48	145	165	0.0382	1.188	0	0.0382	0	6.148	0.241
72	140	170	0.0441	1.003	1	0.0441	0.0441	7.1951	0.283
96	135	175	0.047	0.5075	0	0.047	0	7.7026	0.303
120	130	180	0.0471	0.018	0	0.0471	0	7.7206	0.303
144	125	185	0.0337	0	0	0.0337	0	7.7206	0.303
168	120	190	0.0347	0.19	1.5	0.0347	0.05205	7.96265	0.313
192	115	195	0.0359	0.234	0	0.0359	0	8.19665	0.322
216	110	200	0.036	0.02	0	0.036	0	8.21665	0.323
240	105	205	0.0374	0.287	0	0.0374	0	8.50365	0.334
264	100	210	0.0433	1.239	0	0.0433	0	9.74265	0.383
288	95	215	0.0371	0	0	0.0371	0	9.74265	0.383
312	90	220	0.0448	1.694	0	0.0448	0	11.43665	0.449
336	85	225	0.0449	0.0225	0	0.0449	0	11.45915	0.450

Table A.32 5 g L⁻¹ rice husk with thermal treatment in pure *Clostridium termitidis* experiment

t (h)	pH	Total Sugar (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	7.5	497.00	0	3998.38	390.09	0
24	7.08	573.00	168.81	718.60	0	0
48	6.83	1057.00	0	385.43	0	0
72	6.75	194.00	450.38	3346.41	78.12	0
96	0	170	516.70	3675.17	105.88	0
120	6.8	204.00	495.86	3701.06	95.44	129.34
144	6.88	278.00	533.68	4212.02	258.32	48.51
168	6.88	162.00	508.81	3155.52	257.14	48.51
192	6.85	251.00	550.54	3134.51	0	48.51
216	6.92	170	563.26	2661.76	342.20	134.28
240	6.81	270	612.84	3787.18	261.88	48.51
264	6.95	782.00	643.95	3860.61	254.47	48.51
288	6.94	126.00	622.95	3514.92	298.80	48.51
312	6.92	146.00	601.51	2566.92	391.64	140.22
336	6.78	135.00	617.28	2389.89	322.00	48.51

Table A.33 10 g L⁻¹ Rice husk with thermal treatment in pure *Clostridium termitidis* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0331	5.296	0	0.0331	0	5.296	0.208
48	145	165	0.0393	1.023	0	0.0393	0	6.319	0.248
72	140	170	0.0444	0.867	0	0.0444	0	7.186	0.282
96	135	175	0.037	0	0	0.037	0	7.186	0.282
120	130	180	0.0381	0.198	0	0.0381	0	7.384	0.290
144	125	185	0.0336	0	0	0.0336	0	7.384	0.290
168	120	190	0.0334	0	0	0.0334	0	7.384	0.290
192	115	195	0.0332	0	0	0.0332	0	7.384	0.290
216	110	200	0.0338	0.12	0	0.0338	0	7.504	0.295
240	105	205	0.0344	0.123	0	0.0344	0	7.627	0.300
264	100	210	0.0352	0.168	0	0.0352	0	7.795	0.306
288	95	215	0.0365	0.2795	0	0.0365	0	8.0745	0.317
312	90	220	0.0378	0.286	0	0.0378	0	8.3605	0.328
336	85	225	0.0384	0.135	0	0.0384	0	8.4955	0.334

Table A.34 10 g L⁻¹ Rice husk with thermal treatment in pure *Clostridium termitidis* experiment

t (h)	pH	Total Sugar (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	7.5	614.00	0	474.57	522.85	0
24	7.08	398.00	167.57	699.16	0	0
48	6.85	324.00	204.44	1115.16	0	0
72	6.78	236.00	484.33	3635.75	146.98	175.79
96	6.82	161.00	542.43	3565.98	175.46	186.28
120	6.83	270	614.84	4368.13	150.98	168.87
144	6.9	226.00	578.70	4150.22	321.03	163.33
168	6.91	220	603.12	3459.25	303.62	48.51
192	6.97	196.00	610.13	3231.91	390.98	48.51
216	6.86	216.00	541.79	2468.55	444.31	161.85
240	6.88	255.00	583.89	3853.35	344.71	48.51
264	7.03	137.00	570.67	3061.46	340.79	143.22
288	6.84	125.00	437.89	3446.76	295.44	48.51
312	6.98	189.00	512.14	2255.48	395.68	138.99
336	6.9	141.00	505.69	1836.53	378.45	48.51

Table A.35 15 g L⁻¹ Rice husk with thermal treatment in pure *Clostridium termitidis* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.033	5.28	0	0.033	0	5.28	0.207
48	145	165	0.0408	1.287	0	0.0408	0	6.567	0.258
72	140	170	0.0343	0	0	0.0343	0	6.567	0.258
96	135	175	0.0353	0.175	2	0.0353	0.0706	6.8126	0.268
120	130	180	0.0391	0.684	0	0.0391	0	7.4966	0.294
144	125	185	0.0409	0.333	0	0.0409	0	7.8296	0.308
168	120	190	0.0372	0	0	0.0372	0	7.8296	0.308
192	115	195	0.034	0	0	0.034	0	7.8296	0.308
216	110	200	0.0337	0	0	0.0337	0	7.8296	0.308
240	105	205	0.0367	0.615	0	0.0367	0	8.4446	0.332
264	100	210	0.0352	0	0	0.0352	0	8.4446	0.332
288	95	215	0.0374	0.473	0	0.0374	0	8.9176	0.350
312	90	220	0.0403	0.638	0	0.0403	0	9.5556	0.375
336	85	225	0.0415	0.27	0	0.0415	0	9.8256	0.386

Table A.36 15 g L⁻¹ Rice husk with thermal treatment in pure *Clostridium termitidis* experiment

t (h)	pH	Total Sugar (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	7.5	669.00	0	321.32	353.57	0
24	7.06	530	286.94	763.95	0	0
48	6.76	284.00	151.76	570.33	0	0
72	6.78	412.00	163.42	3420.25	124.43	163.42
96	0	352.00	487.29	3606.07	108.72	179.87
120	6.77	275.00	494.30	3771.51	116.22	195.64
144	6.81	296.00	519.44	3739.61	265.40	144.17
168	6.82	189.00	530.08	2993.28	267.71	139.64
192	6.8	279.00	597.16	3003.20	274.01	48.51
216	6.82	187.00	595.47	2440.83	495.55	219.17
240	6.77	320	611.58	3565.80	286.62	48.51
264	6.92	201.00	610.49	3588.73	287.67	175.29
288	6.88	419.00	620.04	3760.86	335.85	164.86
312	6.89	180	626.88	2457.29	398.28	225.07
336	6.74	199.00	684.18	2386.78	316.12	171.10

Table A.37 20 g L⁻¹ Rice husk with thermal treatment in pure *Clostridium termitidis* experiment

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0332	5.312	0	0.0332	0	5.312	0.208
48	145	165	0.0446	1.881	0	0.0446	0	7.193	0.282
72	140	170	0.0391	0	0	0.0391	0	7.193	0.282
96	135	175	0.042	0.5075	0	0.042	0	7.7005	0.302
120	130	180	0.042	0	0	0.042	0	7.7005	0.302
144	125	185	0.0337	0	0	0.0337	0	7.7005	0.302
168	120	190	0.0335	0	13	0.0335	0.4355	8.136	0.320
192	115	195	0.0353	0.351	0	0.0353	0	8.487	0.333
216	110	200	0.0346	0	0	0.0346	0	8.487	0.333
240	105	205	0.0341	0	0	0.0341	0	8.487	0.333
264	100	210	0.0345	0.084	0	0.0345	0	8.571	0.337
288	95	215	0.0344	0	0	0.0344	0	8.571	0.337
312	90	220	0.036	0.352	0	0.036	0	8.923	0.351
336	85	225	0.035	0	0	0.035	0	8.923	0.351

Table A.38 20 g L⁻¹ Rice husk with thermal treatment in pure *Clostridium termitidis* experiment

t (h)	pH	Total Sugar (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
0	7.5	728.00	0	705.20	448.28	48.51
24	7.04	530	242.52	696.91	0	48.51
48	6.74	444.00	216.64	211.70	0	0
72	6.74	439.00	561.33	3653.47	111.96	232.05
96	5.87	294.00	675.97	4094.12	130.47	202.77
120	6.73	306.00	702.25	3167.46	93.35	170.11
144	6.8	340	743.13	4555.40	279.41	48.51
168	6.84	314.00	763.26	3158.86	350.26	48.51
192	6.81	477.00	757.35	3200.59	371.81	48.51
216	6.75	248.00	878.91	2525.00	739.21	48.51
240	6.78	285.00	848.42	4082.39	273.58	48.51
264	6.92	335.00	851.23	4184.07	277.14	48.51
288	6.89	249.00	701.51	2731.98	275.60	48.51
312	6.89	214.00	761.19	2506.48	405.97	48.51
336	6.83	237.00	663.09	2095.05	364.29	48.51

Table A.39 Raw data for *Clostridium intestinale* in <74 µm particle size

Hydrogen from head space						Hydrogen from total gas				
t (d)	t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	0			0		0	0	0	0	
1	24	150	160	0.159	25.44	12	0.159	1.908	27.348	1.075
3	72	145	165	0.0611	0	0	0.0611	0	27.348	1.075
6	144	140	170	0.0356	0	0	0.0356	0	27.348	1.075
8	192	135	175	0.033	0	0	0.033	0	27.348	1.075
10	240	130	180	0.0368	0.684	0	0.0368	0	28.032	1.103
13	312	125	185	0.0328	0	0	0.0328	0	28.032	1.103
14	336	120	190	0	0	0	0	0	28.032	1.103

Table A.40 Raw data for *Clostridium intestinale* in <74 µm particle size

t (h)	pH	Total Sugar (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
0	7.5					
24	6.5	310.4627	185.51	214.45	346.18	1545.29
72	6.02	318.9985	141.6	375.3	377.7	2404.66
144	6.23	274.6126	197.2	381.8	383.9	3513.7
192	6.35	262.6625	203.5	379.12	259.4	3686.4
240	6.56	268.353	266.7	374.8	289.9	3732.1
312	6.59	226.8124	474.9	171	243.5	4763.9
336	6.64	320.7056	478.5	187.2	238.7	5376.2

Table A.41 Raw data for *Clostridium termitidis* in <74 μm particle size

Hydrogen from head space						Hydrogen from total gas				
t (d)	t (h)	V _s (ml)	V _g (ml)	H ₂₋₁ (%)	VH _{2-head space} (ml)	V _{total gas} (ml)	H ₂₋₁ (%)	V _t x H ₂₋₁	V _{cum.} (ml)	Cumulative H ₂ (mmol)
0	0			0		0	0	0	0	
1	24	150	160	0.0882	14.112	10	0.0882	0.882	14.994	0.589
3	72	145	165	0.0496	0	0	0.0496	0	14.994	0.589
6	144	140	170	0.0404	0	0	0.0404	0	14.994	0.589
8	192	135	175	0.0361	0	0	0.0361	0	14.994	0.589
10	240	130	180	0.0368	0.126	0	0.0368	0	15.12	0.594
13	312	125	185	0.0385	0.3145	0	0.0385	0	15.4345	0.607
14	336	120	190	0.043	0.855	0	0.043	0	16.2895	0.640

Table A.42 Raw data for *Clostridium termitidis* in <74 μm particle size

t (h)	pH	Total Sugar (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
0	7.5					
24	6.4	201.7742	176.98	472.66	387.29	2086.56
72	6.42	242.7458	161.44	448.9	148.1	1902.4
144	6.46	205.1885	162.6	328.5	108.2	1321.1
192	6.62	196.0837	153.8	442.4	42.33	2066.3
240	6.68	234.7791	228.8	408.6	0	3197.6
312	6.69	228.5195	283	278	0	3682.9
336	6.71	283.7174	296.9	480.2	0	4108.3

Table A.43 Raw data for 1:1 co-culture ratio of in <74 µm particle size

Hydrogen from head space						Hydrogen from total gas				
t (d)	t (h)	V _s (ml)	V _g (ml)	H ₂₋₁ (%)	VH _{2-head space} (ml)	V _{total gas} (ml)	H ₂₋₁ (%)	V _t x H ₂₋₁	V _{cum.} (ml)	Cumulative H ₂ (mmol)
0	0			0		0	0	0	0	
1	24	150	160	0.0957	15.312	11	0.0957	1.0527	16.3647	0.643
3	72	145	165	0.072	0	0	0.072	0	16.3647	0.643
6	144	140	170	0.0494	0	0	0.0494	0	16.3647	0.643
8	192	135	175	0.0464	0	0	0.0464	0	16.3647	0.643
10	240	130	180	0.0441	0	0	0.0441	0	16.3647	0.643
13	312	125	185	0.0511	1.295	0	0.0511	0	17.6597	0.694
14	336	120	190	0.0533	0.418	0	0.0533	0	18.0777	0.711

Table A.44 Raw data for 1:1 co-culture ratio of in <74 µm particle size

t (h)	pH	Total Sugar (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
0	7.5					
24	6.23	252.9887	238.03	341.29	389.24	1820.89
72	6.3	265.5078	173.15	522.14	248.6	1334.3
144	6.19	225.1052	178.4	396.5	218.5	1030.1
192	6.25	209.1718	105.3	517.1	222.6	1470.9
240	6.3	244.4529	129.5	553.9	211.4	2132.7
312	6.34	162.5097	218.2	633	198	2388.4
336	6.36	272.9054	215.9	598.3	200.1	2621.6

Table A.45 Raw data for 2:1 co-culture ratio of in <74 µm particle size

Hydrogen from head space						Hydrogen from total gas				
t (d)	t (h)	V _s (ml)	V _g (ml)	H ₂₋₁ (%)	VH _{2-head space} (ml)	V _{total gas} (ml)	H ₂₋₁ (%)	V _t x H ₂₋₁	V _{cum.} (ml)	Cumulative H ₂ (mmol)
0	0			0		0	0	0	0	
1	24	150	160	0.0873	13.968	8	0.0873	0.6984	14.6664	0.576
3	72	145	165	0.0407	0	0	0.0407	0	14.6664	0.576
6	144	140	170	0.0356	0	0	0.0356	0	14.6664	0.576
8	192	135	175	0.0333	0	0	0.0333	0	14.6664	0.576
10	240	130	180	0.031	0	0	0.031	0	14.6664	0.576
13	312	125	185	0.033	0.37	0	0.033	0	15.0364	0.591
14	336	120	190	0.0328	0	0	0.0328	0	15.0364	0.591

Table A.46 Raw data for 2:1 co-culture ratio of in <74 µm particle size

t (h)	pH	Total Sugar (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
0	7.5					
24	6.3	241.0386	244.88	333.92	280.92	1985.47
72	6.35	291.115	221.8	383.9	241.7	2530.2
144	6.32	216.0004	182.5	366.8	214.8	2265.5
192	6.47	224.5362	152.03	417.31	208.1	2680.8
240	6.54	271.1983	205.1	383.7	170.8	3318.5
312	6.57	160.2335	360.3	263.4	132.2	4257.4
336	6.59	266.6459	389.71	225.52	113.1	4473.97

Table A.47 Raw data for 5:1 co-culture ratio of in <74 μm particle size

Hydrogen from head space						Hydrogen from total gas				
t (d)	t (h)	V _s (ml)	V _g (ml)	H ₂₋₁ (%)	VH _{2-head space} (ml)	V _{total gas} (ml)	H ₂₋₁ (%)	V _t x H ₂₋₁	V _{cum.} (ml)	Cumulative H ₂ (mmol)
0	0			0		0	0	0	0	
1	24	150	160	0.0925	14.8	8	0.0925	0.74	15.54	0.611
3	72	145	165	0.0518	0	0	0.0518	0	15.54	0.611
6	144	140	170	0.0343	0	0	0.0343	0	15.54	0.611
8	192	135	175	0.0339	0	0	0.0339	0	15.54	0.611
10	240	130	180	0.034	0.018	0	0.034	0	15.558	0.612
13	312	125	185	0.0336	0	0	0.0336	0	15.558	0.612
14	336	120	190	0.0331	0	0	0.0331	0	15.558	0.612

Table A.48 Raw data for 5:1 co-culture ratio of in <74 μm particle size

t (h)	pH	Total Sugar (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
24	6.36	238.1934	220.34	395.46	267.72	1770.95
72	6.49	203.4813	89.1	406.4	252.1	1138.8
144	6.51	246.1601	118.7	255.4	260.7	569.6
192	6.62	204.6194	99.9	205.9	349.4	566.1
240	6.68	184.1336	114.9	169.2	338.3	749.2
312	6.7	143.162	136.73	112.5	354.8	861.2
336	6.75	219.4147	176.5	0	161.44	1044.31

Table A.49 Raw data for 10:1 co-culture ratio of in <74 μm particle size

Hydrogen from head space						Hydrogen from total gas				
t (d)	t (h)	Vs (ml)	Vg (ml)	H ₂₋₁ (%)	VH _{2-head space} (ml)	Vtotal gas (ml)	H ₂₋₁ (%)	Vt x H ₂₋₁	Vcum. (ml)	Cumulative H ₂ (mmol)
0	0			0		0	0	0	0	
1	24	150	160	0.103	16.48	10	0.103	1.03	17.51	0.688
3	72	145	165	0.0988	0	0	0.0988	0	17.51	0.688
6	144	140	170	0.0814	0	0	0.0814	0	17.51	0.688
8	192	135	175	0.0614	0	0	0.0614	0	17.51	0.688
10	240	130	180	0.0563	0	0	0.0563	0	17.51	0.688
13	312	125	185	0.0565	0.037	0	0.0565	0	17.547	0.690
14	336	120	190	0.0539	0	0	0.0539	0	17.547	0.690

Table A.50 Raw data for 10:1 co-culture ratio of in <74 μm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
24	6.44	225.1052	188.32	274.6	264.12	1769.84
72	6.38	259.2482	159.97	211.9	254.3	376.8
144	6.41	217.7076	179.7	324.9	258.1	428.6
192	6.32	190.3932	108.3	390.7	296	517.9
240	6.46	214.8623	101.7	428.6	305.9	422.3
312	6.4	156.8192	114.4	607.1	287.3	529.7
336	6.43	251.8506	125.9	648.1	280.7	760.3

Table A.51 Raw data for 15:1 co-culture ratio of in <74 μm particle size

Hydrogen from head space						Hydrogen from total gas				
t (d)	t (h)	V _s (ml)	V _g (ml)	H ₂₋₁ (%)	VH _{2-head space} (ml)	V _{total gas} (ml)	H ₂₋₁ (%)	V _t x H ₂₋₁	V _{cum.} (ml)	Cumulative H ₂ (mmol)
0	0			0		0	0	0	0	
1	24	150	160	0.0922	14.752	5	0.0922	0.461	15.213	0.598
3	72	145	165	0.0846	0	0	0.0846	0	15.213	0.598
6	144	140	170	0.0614	0	0	0.0614	0	15.213	0.598
8	192	135	175	0.0564	0	0	0.0564	0	15.213	0.598
10	240	130	180	0.0593	0.522	0	0.0593	0	15.735	0.619
13	312	125	185	0.0626	0.6105	0	0.0626	0	16.3455	0.643
14	336	120	190	0.0583	0	0	0.0583	0	16.3455	0.643

Table A.52 Raw data for 15:1 co-culture ratio of in <74 μm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
24	6.38	271.1983	176.53	244.53	264.06	1888.5
72	6.33	267.784	139.2	460.6	190.2	904.3
144	6.41	233.0719	159.1	417.8	193.1	392.5
192	6.36	207.4647	97.3	534.4	237.4	323.8
240	6.47	223.3981	104.2	521.4	240.6	658.2
312	6.42	265.5078	115.5	847.3	238.9	807.7
336	6.45	252.4196	114.1	951.3	242.7	1011.4

Table A.53 Raw data for 20:1 co-culture ratio of in <74 µm particle size

Hydrogen from head space						Hydrogen from total gas				
t (d)	t (h)	V _s (ml)	V _g (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	V _{total gas} (ml)	H ₂ -1 (%)	V _t x H ₂ -1	V _{cum.} (ml)	Cumulative H ₂ (mmol)
0	0			0		0	0	0	0	
1	24	150	160	0.0739	11.824	3	0.0739	0.2217	12.0457	0.473
3	72	145	165	0.0382	0	0	0.0382	0	12.0457	0.473
6	144	140	170	0.0364	0	0	0.0364	0	12.0457	0.473
8	192	135	175	0.0374	0.175	0	0.0374	0	12.2207	0.480
10	240	130	180	0.0434	0	0	0.0434	0	12.2207	0.480
13	312	125	185	0.0439	0.0925	0	0.0439	0	12.3132	0.484
14	336	120	190	0.0425	0	0	0.0425	0	12.3132	0.484

Table A.54 Raw data for 20:1 co-culture ratio of in <74 µm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
24	6.35	299.6508	192.8	173.9	259.3	2045.2
72	6.38	271.7673	141.4	442.5	250.2	2321.1
144	6.56	225.6743	99	314.2	285.7	722.4
192	6.48	190.9622	118.05	260.83	349.2	852.3
240	6.61	239.3315	126.4	133.7	328.7	673.6
312	6.53	153.974	155.8	51.4	319.8	815.8
336	6.57	250.1434	166.3	467.8	342.9	969.9

Table A.55 Raw data for *Clostridium intestinale* of in <300 µm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	V _s (ml)	V _g (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	V _{total gas} (ml)	H ₂ -1 (%)	V _t x H ₂ -1	V _{cum.} (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0955	15.28	0	0.0955	0	15.28	0.601
48	145	165	0.181	14.1075	0	0.181	0	29.3875	1.156
72	140	170	0.114	0	0	0.114	0	29.3875	1.156
96	135	175	0.111	0	0	0.111	0	29.3875	1.156
144	130	180	0.0754	0	0	0.0754	0	29.3875	1.156
168	125	185	0.0858	1.924	0	0.0858	0	31.3115	1.231
192	120	190	0.0864	0.114	0	0.0864	0	31.4255	1.236
216	115	195	0.0613	0	0	0.0613	0	31.4255	1.236
240	110	200	0.0592	0	0	0.0592	0	31.4255	1.236
244	105	205	0.0509	0	0	0.0509	0	31.4255	1.236
312	100	210	0.0385	0	0	0.0385	0	31.4255	1.236
336	95	215	0.0353	0	0	0.0353	0	31.4255	1.236

Table A.56 Raw data for *Clostridium intestinale* of in <300 µm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
24	5.99	242.506	524.24	1178.84	421.81	420.64
48	6.08	176.6924	463.89	3099.97	236.45	408.54
72	6.04	221.822	927.72	2681.73	268.69	378.11
96	6.06	203.018	658.14	3437.17	318.59	391.06
144	6.13	164.156	593.78	2919.82	264.42	408.63
168	6.14	239.9992	519.35	3215.29	96.21	397.33
192	6.12	145.979	624.97	3281.07	249.69	390.64
216	6.16	203.018	603.16	2970.88	370.61	369.34
240	6.19	149.74	535.42	2855.6	98.92	342.11
244	6.19	193.616	728.96	2870.54	353.67	377.22
312	6.2	170.424	724.74	2782.31	267.67	290.36
336	6.28	130.936	657.45	3294.54	246.13	269.3

Table A.57 Raw data for *Clostridium termitidis* of in <300 µm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0476	7.616	0	0.0476	0	7.616	0.299
48	145	165	0.0654	2.937	0	0.0654	0	10.553	0.415
72	140	170	0.0639	0	0	0.0639	0	10.553	0.415
96	135	175	0.0632	0	0	0.0632	0	10.553	0.415
144	130	180	0.0615	0	0	0.0615	0	10.553	0.415
168	125	185	0.0539	0	0	0.0539	0	10.553	0.415
192	120	190	0.0537	0	0	0.0537	0	10.553	0.415
216	115	195	0.0489	0	0	0.0489	0	10.553	0.415
240	110	200	0.0468	0	0	0.0468	0	10.553	0.415
244	105	205	0.0409	0	0	0.0409	0	10.553	0.415
312	100	210	0.0424	0.315	0	0.0424	0	10.868	0.427
336	95	215	0.0401	0	0	0.0401	0	10.868	0.427

Table A.58 Raw data for *Clostridium termitidis* of in <300 µm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
24	6.15	174.185	417.81	1078.06	444.04	258.76
48	6.46	132.1896	458.91	4298.89	328.42	254.34
72	6.4	145.979	521.04	2609.99	466.65	250.11
96	6.41	166.0368	523.7	4686.85	354.96	250.88
144	6.49	142.845	1490.88	811.23	2163.25	501.14
168	6.48	129.6824	554.44	3186.51	257.18	223.75
192	6.48	152.874	507.35	2880.15	175.63	223.64
216	6.52	201.1376	446.33	2627.93	93.17	248.87
240	6.55	149.113	420.82	3020.03	170.52	191.68
244	6.54	179.8264	511.46	2520.05	201.73	183.72
312	6.57	179.826	393.53	2432.38	0	174.46
336	6.52	125.9216	475.02	3109.4	191.91	178.51

Table A.59 Raw data for 1:1 Co-culture ratio of in <300 μm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0637	10.192	11.5	0.0637	0.73255	10.92455	0.429
48	145	165	0.0828	3.1515	1	0.0828	0.0828	14.15885	0.556
72	140	170	0.0815	0	0	0.0815	0	14.15885	0.556
96	135	175	0.0788	0	0	0.0788	0	14.15885	0.556
144	130	180	0.0807	0.342	0	0.0807	0	14.50085	0.570
168	125	185	0.0733	0	0	0.0733	0	14.50085	0.570
192	120	190	0.0683	0	0	0.0683	0	14.50085	0.570
216	115	195	0.0659	0	0	0.0659	0	14.50085	0.570
240	110	200	0.0661	0.04	0	0.0661	0	14.54085	0.572
244	105	205	0.0362	0	0	0.0362	0	14.54085	0.572
312	100	210	0.0551	3.969	0	0.0551	0	18.50985	0.728
336	95	215	0.0493	0	0	0.0493	0	18.50985	0.728

Table A.60 Raw data for 1:1 co-culture ratio of in <300 μm particle size

t (h)	pH	Total Sugar Concentration (mg L^{-1})	Acetic Acid (mg L^{-1})	Butyric Acid (mg L^{-1})	Propionic Acid (mg L^{-1})	Lactic Acid (mg L^{-1})
24	6.2	221.195	530.53	1266.75	316.35	336.03
48	6.16	147.8596	801.29	2681.83	333.28	311.99
72	6.05	171.051	532.55	2762.07	286.22	322.57
96	6.11	171.0512	588.6	3172.52	344.92	330.7
144	6.24	146.606	583.64	2608.51	277.27	298.03
168	6.19	148.4864	616.23	2949.72	268.4	297.81
192	6.22	154.128	411.91	2862.57	20.43	300.62
216	6.23	220.5684	429.88	3256.72	137.11	1006.42
240	6.32	152.247	499.85	2709.84	22.88	255.08
244	6.36	180.4532	405.02	2940.94	184.18	238.56
312	6.35	177.946	400.43	2846.9	102.98	174.65
336	6.35	129.0556	521.09	2950.77	174.41	171.03

Table A.61 Raw data for 2:1 co-culture ratio of in <300 µm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0557	8.912	3	0.0557	0.1671	9.0791	0.357
48	145	165	0.0635	1.287	0	0.0635	0	10.3661	0.407
72	140	170	0.0591	0	0	0.0591	0	10.3661	0.407
96	135	175	0.0592	0.0175	0	0.0592	0	10.3836	0.408
144	130	180	0.0468	0	0	0.0468	0	10.3836	0.408
168	125	185	0.0387	0	0	0.0387	0	10.3836	0.408
192	120	190	0.0414	0.513	0	0.0414	0	10.8966	0.428
216	115	195	0.0456	0.819	0	0.0456	0	11.7156	0.460
240	110	200	0.0351	0	0	0.0351	0	11.7156	0.460
244	105	205	0.0413	1.271	0	0.0413	0	12.9866	0.510
312	100	210	0.042	0.147	0	0.042	0	13.1336	0.516
336	95	215	0.0421	0.0215	0	0.0421	0	13.1551	0.517

Table A.62 Raw data for 2:1 co-culture ratio of in <300 μm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
24	6.16	226.836	557.37	2426.2	322.15	294.98
48	6.26	169.1708	697.13	3949.97	314.41	284.35
72	6.13	154.754	802.89	4351.23	338.84	287.34
96	6.14	171.678	775.73	4352.9	310.47	286.01
144	6.23	154.128	632.75	2058.97	171.34	232.75
168	6.2	156.008	801.31	4329.91	289.9	235.18
192	6.24	128.429	598.18	2514.03	23.93	224.13
216	6.25	201.1376	511.03	2869.33	108.14	272.04
240	6.33	165.41	558.88	2119.81	128.81	186.72
244	6.41	192.9892	538.27	2512.95	164.71	370.22
312	6.43	166.664	394.56	2410.62	86.39	0
336	6.41	143.472	345.83	2282.61	100.7	148.88

Table A.63 Raw data for 5:1 co-culture ratio of in <300 μm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0495	7.92	6	0.0495	0.297	8.217	0.323
48	145	165	0.0608	1.8645	0	0.0608	0	10.0815	0.396
72	140	170	0.0561	0	0	0.0561	0	10.0815	0.396
96	135	175	0.051	0	0	0.051	0	10.0815	0.396
144	130	180	0.0483	0	0	0.0483	0	10.0815	0.396
168	125	185	0.0393	0	0	0.0393	0	10.0815	0.396
192	120	190	0.0396	0.057	0	0.0396	0	10.1385	0.398
216	115	195	0.0379	0	0	0.0379	0	10.1385	0.398
240	110	200	0.0366	0	0	0.0366	0	10.1385	0.398
244	105	205	0.0387	0.4305	0	0.0387	0	10.569	0.415
312	100	210	0.0446	1.239	0	0.0446	0	11.808	0.464
336	95	215	0.0411	0	0	0.0411	0	11.808	0.464

Table A.64 Raw data for 5:1 co-culture ratio of in <300 μm particle size

t (h)	pH	Total Sugar Concentration (mg L^{-1})	Acetic Acid (mg L^{-1})	Butyric Acid (mg L^{-1})	Propionic Acid (mg L^{-1})	Lactic Acid (mg L^{-1})
24	6.31	240.626	411.31	2058.05	219.93	265.49
48	6.36	191.7356	693.49	3644.48	205.37	255.75
72	6.26	216.808	622.59	2795.59	374.38	248.97
96	6.24	166.0368	643.32	3194.91	350.06	252.02
144	6.35	135.324	492.8	2479.29	0	223.5
168	6.29	169.7976	631.22	2893.49	217.99	229.24
192	6.33	132.816	530.91	2518.23	0	214.2
216	6.34	174.1852	394.79	2944.61	87.22	174.43
240	6.4	114.012	381.42	2313.62	0	172.05
244	6.36	176.0656	483.9	2755.01	115.59	191.07
312	6.36	167.917	481.05	2605.4	0	253.52
336	6.32	142.8452	674.06	2650.26	238.18	136.71

Table A.65 Raw data for 10:1 co-culture ratio of in <300 μm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0499	7.984	5	0.0499	0.2495	8.2335	0.323
48	145	165	0.0559	0.99	0	0.0559	0	9.2235	0.362
72	140	170	0.0501	0	0	0.0501	0	9.2235	0.362
96	135	175	0.0481	0	0	0.0481	0	9.2235	0.362
144	130	180	0.0415	0	0	0.0415	0	9.2235	0.362
168	125	185	0.0376	0	0	0.0376	0	9.2235	0.362
192	120	190	0.0364	0	0	0.0364	0	9.2235	0.362
216	115	195	0.0358	0	0	0.0358	0	9.2235	0.362
240	110	200	0.0342	0	0	0.0342	0	9.2235	0.362
244	105	205	0.0393	1.0455	0	0.0393	0	10.269	0.403
312	100	210	0.0336	0	0	0.0336	0	10.269	0.403
336	95	215	0.0342	0.129	0	0.0342	0	10.398	0.409

Table A.66 Raw data for 10:1 co-culture ratio of in <300 μm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
24	6.36	188.602	472.73	2330.63	333.85	237.3
48	6.35	198.0036	780.69	3504.38	337.96	228.56
72	6.26	164.156	609.69	3552.36	315.49	217.44
96	6.25	156.6348	663.03	3629.15	366.16	221.75
144	6.28	136.577	687.77	2387.94	288.92	204.45
168	6.35	192.3624	585.86	2410.03	0	204.86
192	6.3	164.783	589.35	2139.3	50.03	184.14
216	6.38	191.1088	417.51	2435.87	103.47	175.35
240	6.45	125.295	523.68	2347.76	236.55	145.61
244	6.37	164.7832	579.66	2343.7	249.98	324.5
312	6.37	173.558	645.42	2045.27	220.5	176.72
336	6.37	139.7112	526.21	2154.78	130.73	132.93

Table A.67 Raw data for 15:1 co-culture ratio of in <300 µm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0549	8.784	3	0.0549	0	8.784	0.345
48	145	165	0.0689	2.31	0	0.0689	0	11.094	0.436
72	140	170	0.0667	0	0	0.0667	0	11.094	0.436
96	135	175	0.0629	0	0	0.0629	0	11.094	0.436
144	130	180	0.0563	0	0	0.0563	0	11.094	0.436
168	125	185	0.0577	0.259	0	0.0577	0	11.353	0.446
192	120	190	0.0514	0	0	0.0514	0	11.353	0.446
216	115	195	0.0496	0	0	0.0496	0	11.353	0.446
240	110	200	0.0491	0	0	0.0491	0	11.353	0.446
244	105	205	0.0428	0	0	0.0428	0	11.353	0.446
312	100	210	0.0406	0	0	0.0406	0	11.353	0.446
336	95	215	0.0371	0	0	0.0371	0	11.353	0.446

Table A.68 Raw data for 15:1 co-culture ratio of in <300 μm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
24	6.49	177.946	433.25	2503.4	343.21	242.99
48	6.42	147.8596	606.76	3077.54	346.46	219.18
72	6.34	184.841	465.31	3182.07	266.85	204.72
96	6.34	141.5916	564.28	3466.91	353.63	198.35
144	6.42	151.62	591.01	2807.21	294.33	186.67
168	6.4	201.7644	335.37	2761.74	0	185.8
192	6.41	143.472	496.97	2649.1	31.12	185.46
216	6.47	206.7788	307.61	2919.6	137.52	134.44
240	6.55	115.893	394.15	2744.31	171.04	124.36
244	6.51	181.08	357.17	2903.15	202.18	153.69
312	6.48	153.501	530.92	2843.02	134.18	117.1
336	6.51	158.5152	493.16	2879.36	299.31	144.92

Table A.69 Raw data for 20:1 co-culture ratio of in <300 μm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0515	8.24	6	0.0515	0	8.24	0.324
48	145	165	0.0697	3.003	0	0.0697	0	11.243	0.442
72	140	170	0.0699	0.034	0	0.0699	0	11.277	0.443
96	135	175	0.0541	0	0	0.0541	0	11.277	0.443
144	130	180	0.0612	1.278	0	0.0612	0	12.555	0.493
168	125	185	0.0564	0	0	0.0564	0	12.555	0.493
192	120	190	0.055	0	0	0.055	0	12.555	0.493
216	115	195	0.0454	0	0	0.0454	0	12.555	0.493
240	110	200	0.0388	0	0	0.0388	0	12.555	0.493
244	105	205	0.0602	4.387	0	0.0602	0	16.942	0.666
312	100	210	0.0575	0	0	0.0575	0	16.942	0.666
336	95	215	0.0676	2.1715	0	0.0676	0	19.1135	0.751

Table A.70 Raw data for 20:1 co-culture ratio of in <300 μm particle size

t (h)	pH	Total Sugar Concentration (mg L^{-1})	Acetic Acid (mg L^{-1})	Butyric Acid (mg L^{-1})	Propionic Acid (mg L^{-1})	Lactic Acid (mg L^{-1})
24	6.44	156.008	364.99	2656.59	116.34	245.53
48	6.46	154.1276	605.61	3368.94	351.7	240.03
72	6.38	187.348	505.36	3165.51	326.52	232.18
96	6.34	155.3812	541.25	3427.73	364.24	226.96
144	6.45	145.352	564.6	2950.93	292.25	217.45
168	6.41	208.6592	464.95	2918.82	89.35	202.41
192	6.4	137.831	435.18	2757.8	96.98	207.39
216	6.4	203.6448	316.9	3096.99	37.34	225.82
240	6.46	132.816	613.99	3169.33	318.63	188.25
244	6.35	190.482	574.12	3100.53	287.47	223
312	6.34	132.19	670.94	2980.46	178.88	181.26
336	6.34	142.8452	584.86	2896.93	161.99	207.64

Table A.71 Raw data for *Clostridium intestinale* of in <2000 μm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.01	1.6	7.5	0.0946	0.7095	2.3095	0.090
48	145	165	0.012	0	0.5	0.0121	0.00605	2.31555	0.091
72	140	170	0.01	0	0	0.0108	0	2.31555	0.091
96	135	175	0.015	0.875	0	0.0981	0	3.19055	0.125
144	130	180	0.015	0	0	0.0916	0	3.19055	0.125
168	125	185	0.015	0	0	0.0746	0	3.19055	0.125
192	120	190	0.015	0	0	0.0657	0	3.19055	0.125
216	115	195	0.015	0	0	0.0619	0	3.19055	0.125
240	110	200	0.017	0.4	0	0.0648	0	3.59055	0.141
288	105	205	0.022	0	0	0.0449	0	3.59055	0.141
312	100	210	0.03	1.68	0	0.0521	0	5.27055	0.207
336	95	215	0.034	0.86	0	0.0544	0	6.13055	0.241

Table A.72 Raw data for *Clostridium intestinale* of in <2000 µm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
24	6.03	245.01	568.32	1003.49	416.59	408.55
48	6.08	130.31	575.77	3274.11	255.39	402.21
72	6.02	163.53	775.94	3515.69	326.96	396.82
96	5.99	199.88	598.69	3718.47	152.24	380.19
144	6.03	170.42	942.37	3400.35	248.19	387.68
168	6.24	509.52	736.54	3291.59	87.18	381.45
192	6.13	144.73	535.01	3367.72	0	375.47
216	6.15	229.97	536.41	3787.62	78.21	286.92
240	6.22	183.59	572.58	3188.32	57.21	338.58
288	6.15	204.90	484.34	2923.30	279.65	119.23
312	6.19	171.68	641.82	3731.99	76.96	352.16
336	6.22	169.80	664.82	3869.94	136.02	399.61

Table A.73 Raw data for *Clostridium termitidis* of in <2000 µm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0452	7.232	2.5	0.0452	0.113	7.345	0.288
48	145	165	0.0592	2.31	0	0.0592	0	9.655	0.379
72	140	170	0.0551	0	0	0.0551	0	9.655	0.379
96	135	175	0.0519	0	0	0.0519	0	9.655	0.379
144	130	180	0.0485	0	0	0.0485	0	9.655	0.379
168	125	185	0.0439	0	0	0.0439	0	9.655	0.379
192	120	190	0.0417	0	0	0.0417	0	9.655	0.379
216	115	195	0.0391	0	0	0.0391	0	9.655	0.379
240	110	200	0.0394	0.06	0	0.0394	0	9.715	0.382
288	105	205	0.0357	0	0	0.0357	0	9.715	0.382
312	100	210	0.0377	0	0	0.0377	0	9.715	0.382
336	95	215	0.0356	0	0	0.0356	0	9.715	0.382

Table A.74 Raw data for *Clostridium termitidis* of in <2000 µm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
24	6.43	206.78	451.77	990.75	455.58	225.70
48	6.43	118.40	555.24	4306.68	300.51	214.08
72	6.41	142.85	544.72	4352.13	223.95	222.20
96	6.33	177.32	412.45	4479.87	13.09	216.65
144	6.38	142.85	577.09	4990.51	228.21	214.56
168	6.5	156.01	510.46	4286.36	94.92	133.63
192	6.43	130.31	368.73	4332.97	0	197.25
216	6.45	199.88	334.99	3070.49	0	168.36
240	6.53	145.98	452.23	3304.65	44.45	138.65
288	6.44	192.99	351.46	3244.47	56.52	240.27
312	6.47	186.09	305.75	3098.62	0	155.87
336	6.5	167.29	526.03	3330.13	78.24	218.84

Table A.75 Raw data for 1:1 co-culture ratio of in <2000 μm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0744	11.904	7.5	0.0744	0.558	12.462	0.490
48	145	165	0.102	4.554	0	0.102	0	17.016	0.669
72	140	170	0.103	0.17	0	0.103	0	17.186	0.676
96	135	175	0.104	0.175	0	0.104	0	17.361	0.682
144	130	180	0.0938	0	0	0.0938	0	17.361	0.682
168	125	185	0.0843	0	0	0.0843	0	17.361	0.682
192	120	190	0.075	0	0	0.075	0	17.361	0.682
216	115	195	0.0586	0	0	0.0586	0	17.361	0.682
240	110	200	0.0583	0	0	0.0583	0	17.361	0.682
288	105	205	0.0425	0	0	0.0425	0	17.361	0.682
312	100	210	0.0343	0	0	0.0343	0	17.361	0.682
336	95	215	0.0401	1.247	0	0.0401	0	18.608	0.732

Table A.76 Raw data for 1:1 co-culture ratio of in <2000 μm particle size

t (h)	pH	Total Sugar Concentration (mg L^{-1})	Acetic Acid (mg L^{-1})	Butyric Acid (mg L^{-1})	Propionic Acid (mg L^{-1})	Lactic Acid (mg L^{-1})
24	6.04	182.33	417.15	1296.06	317.71	324.93
48	6.26	107.12	488.39	2915.88	362.21	297.13
72	6.24	147.23	387.08	3115.62	201.22	300.70
96	6.22	175.44	328.23	2883.58	235.56	291.80
144	6.25	125.92	436.16	2974.56	127.99	274.60
168	6.42	142.85	487.14	2846.06	100.62	248.21
192	6.35	130.94	401.47	2837.60	52.83	203.78
216	6.34	177.95	488.06	2857.38	86.28	196.74
240	6.4	140.34	417.58	3062.04	0	219.08
288	6.4	161.65	383.31	2938.46	67.39	255.85
312	6.43	161.65	528.28	2816.89	112.52	231.99
336	6.41	132.19	550.48	3125.62	187.42	226.10

Table A.77 Raw data for 2:1 co-culture ratio of in <2000 μm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0637	10.192	6.5	0.0637	0.41405	10.60605	0.417
48	145	165	0.0962	5.3625	0	0.0962	0	15.96855	0.628
72	140	170	0.0966	0.068	0	0.0966	0	16.03655	0.630
96	135	175	0.0975	0.1575	0	0.0975	0	16.19405	0.637
144	130	180	0.0952	0	0	0.0952	0	16.19405	0.637
168	125	185	0.0843	0	0	0.0843	0	16.19405	0.637
192	120	190	0.0789	0	0	0.0789	0	16.19405	0.637
216	115	195	0.0484	0	0	0.0484	0	16.19405	0.637
240	110	200	0.0626	2.84	0	0.0626	0	19.03405	0.748
288	105	205	0.044	0	0	0.044	0	19.03405	0.748
312	100	210	0.0571	2.751	0	0.0571	0	21.78505	0.857
336	95	215	0.0615	0.946	0	0.0615	0	22.73105	0.894

Table A.78 Raw data for 2:1 co-culture ratio of in <2000 μm particle size

t (h)	pH	Total Sugar Concentration (mg L^{-1})	Acetic Acid (mg L^{-1})	Butyric Acid (mg L^{-1})	Propionic Acid (mg L^{-1})	Lactic Acid (mg L^{-1})
24	6.15	189.86	440.26	1729.05	324.86	316.61
48	6.3	112.76	524.85	3354.72	370.03	319.93
72	6.17	180.45	417.41	3121.96	250.01	278.69
96	6.2	165.41	381.28	3113.83	202.86	275.12
144	6.21	149.11	617.47	3026.15	315.06	259.79
168	6.32	141.59	479.56	2970.90	140.31	238.16
192	6.3	226.21	428.69	2616.95	21.24	182.47
216	6.32	275.10	477.33	3168.54	96.02	477.33
240	6.42	127.18	471.72	3138.14	67.82	217.26
288	6.35	170.42	475.99	3092.50	140.33	205.76
312	6.32	164.16	575.39	2900.61	42.80	177.13
336	6.28	144.73	655.11	3032.96	217.60	200.43

Table A.79 Raw data for 5:1 co-culture ratio of in <2000 μm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0537	8.592	6	0.0537	0.3222	8.9142	0.350
48	145	165	0.0815	4.587	0.5	0.0815	0.04075	13.54195	0.532
72	140	170	0.0817	0.034	0	0.0817	0	13.57595	0.534
96	135	175	0.083	0.2275	0	0.083	0	13.80345	0.543
144	130	180	0.0795	0	0	0.0795	0	13.80345	0.543
168	125	185	0.0825	0.555	0	0.0825	0	14.35845	0.564
192	120	190	0.0605	0	0	0.0605	0	14.35845	0.564
216	115	195	0.0677	1.404	0	0.0677	0	15.76245	0.620
240	110	200	0.0916	4.78	0	0.0916	0	20.54245	0.808
288	105	205	0.073	0	0	0.073	0	20.54245	0.808
312	100	210	0.114	8.61	0	0.114	0	29.15245	1.146
336	95	215	0.115	0.215	0	0.115	0	29.36745	1.155

Table A.80 Raw data for 5:1 co-culture ratio of in <2000 μm particle size

t (h)	pH	Total Sugar Concentration (mg L^{-1})	Acetic Acid (mg L^{-1})	Butyric Acid (mg L^{-1})	Propionic Acid (mg L^{-1})	Lactic Acid (mg L^{-1})
24	6.24	170.42	439.33	2306.08	328.18	301.21
48	6.38	110.25	415.56	4163.46	310.74	266.81
72	6.3	174.81	503.14	4212.12	339.72	254.87
96	6.3	164.78	558.84	3429.34	374.84	280.31
144	6.31	123.41	562.07	2976.24	252.84	242.45
168	6.35	125.92	598.86	3111.93	267.61	173.63
192	6.36	248.15	451.68	2982.15	178.32	186.11
216	6.31	191.74	463.18	3231.12	203.88	247.29
240	6.34	128.43	525.36	3049.86	0	199.44
288	6.15	303.93	693.27	3253.75	300.14	220.48
312	6.09	159.14	765.12	3158.51	128.63	236.72
336	6.06	147.23	775.28	3077.73	197.21	202.37

Table A.81 Raw data for 10:1 co-culture ratio of in <2000 μm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0498	7.968	3	0.0498	0.1494	8.1174	0.319
48	145	165	0.0695	3.2505	0	0.0695	0	11.3679	0.447
72	140	170	0.0682	0	0	0.0682	0	11.3679	0.447
96	135	175	0.0611	0	0	0.0611	0	11.3679	0.447
144	130	180	0.0596	0	0	0.0596	0	11.3679	0.447
168	125	185	0.0541	0	0	0.0541	0	11.3679	0.447
192	120	190	0.048	0	0	0.048	0	11.3679	0.447
216	115	195	0.0477	0	0	0.0477	0	11.3679	0.447
240	110	200	0.0372	0	0	0.0372	0	11.3679	0.447
288	105	205	0.0397	0.5125	0	0.0397	0	11.8804	0.467
312	100	210	0.0434	0.777	0	0.0434	0	12.6574	0.497
336	95	215	0.0421	0	0	0.0421	0	12.6574	0.497

Table A.82 Raw data for 10:1 co-culture ratio of in <2000 μm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
24	6.27	204.27	209.22	2509.94	123.05	248.36
48	6.4	113.39	565.11	4563.98	309.89	244.88
72	6.32	178.57	497.30	4094.47	206.65	241.97
96	6.45	159.77	386.53	4019.88	107.75	240.09
144	6.3	147.23	482.99	3041.70	113.41	250.45
168	6.41	132.82	524.29	3004.84	176.13	117.17
192	6.38	103.36	377.03	3127.11	0	226.40
216	6.42	184.84	438.88	3095.12	29.58	186.09
240	6.48	131.56	534.68	2710.97	289.99	164.07
288	6.44	204.27	569.16	3274.37	265.98	162.44
312	6.43	131.56	428.86	2990.94	46.09	210.81
336	6.42	128.43	446.58	2516.02	88.05	169.73

Table A.83 Raw data for 15:1 co-culture ratio of in <2000 μm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0403	6.448	6	0.0403	0.2418	6.6898	0.263
48	145	165	0.0514	1.8315	0	0.0514	0	8.5213	0.335
72	140	170	0.0478	0	0	0.0478	0	8.5213	0.335
96	135	175	0.0456	0	0	0.0456	0	8.5213	0.335
144	130	180	0.0416	0	0	0.0416	0	8.5213	0.335
168	125	185	0.0413	0	0	0.0413	0	8.5213	0.335
192	120	190	0.041	0	0	0.041	0	8.5213	0.335
216	115	195	0.0402	0	0	0.0402	0	8.5213	0.335
240	110	200	0.0367	0	0	0.0367	0	8.5213	0.335
288	105	205	0.0372	0.1025	0	0.0372	0	8.6238	0.339
312	100	210	0.0354	0	0	0.0354	0	8.6238	0.339
336	95	215	0.0344	0	0	0.0344	0	8.6238	0.339

Table A.84 Raw data for 15:1 co-culture ratio of in <2000 μm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)
24	6.32	201.14	447.91	2421.10	286.56	261.39
48	6.53	119.03	546.86	4073.32	328.92	218.19
72	6.44	158.52	546.77	4829.93	214.09	262.04
96	6.44	176.07	475.95	4172.43	90.57	247.34
144	6.46	153.50	593.67	4896.80	265.48	240.49
168	6.55	184.21	550.30	4203.81	251.39	160.43
192	6.58	139.08	528.97	3767.84	0	127.33
216	6.58	182.33	228.72	2999.55	0	294.15
240	6.66	127.18	241.64	1994.19	43.51	134.59
288	6.59	150.99	368.48	3129.22	266.14	269.19
312	6.6	143.47	378.16	3100.71	90.02	170.38
336	6.62	119.03	265.41	2520.06	171.23	155.60

Table A.85 Raw data for 20:1 co-culture ratio of in <2000 μm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0481	7.696	4.5	0.0481	0.21645	7.91245	0.311
48	145	165	0.0575	1.551	0	0.0575	0	9.46345	0.372
72	140	170	0.0496	0	0	0.0496	0	9.46345	0.372
96	135	175	0.0495	0	0	0.0495	0	9.46345	0.372
144	130	180	0.0426	0	0	0.0426	0	9.46345	0.372
168	125	185	0.0384	0	0	0.0384	0	9.46345	0.372
192	120	190	0.042	0.684	0	0.042	0	10.14745	0.399
216	115	195	0.0439	0.3705	0	0.0439	0	10.51795	0.413
240	110	200	0.0572	2.66	0	0.0572	0	13.17795	0.518
288	105	205	0.083	5.289	0	0.083	0	18.46695	0.726
312	100	210	0.0899	1.449	0	0.0899	0	19.91595	0.783
336	95	215	0.0632	0	0	0.0632	0	19.91595	0.783

Table A.86 Raw data for 20:1 co-culture ratio of in <2000 μm particle size

t (h)	pH	Total Sugar Concentration (mg L^{-1})	Acetic Acid (mg L^{-1})	Butyric Acid (mg L^{-1})	Propionic Acid (mg L^{-1})	Lactic Acid (mg L^{-1})
24	6.34	189.86	394.36	2142.14	323.13	243.62
48	6.51	125.92	322.72	4349.53	249.45	226.07
72	6.45	132.19	602.19	4357.14	426.34	256.80
96	6.44	146.61	380.76	3694.29	94.65	231.84
144	6.43	147.23	545.59	4362.76	287.35	223.74
168	6.5	116.52	413.65	4186.76	56.78	0
192	6.47	112.76	460.17	2881.52	23.89	200.19
216	6.44	171.68	411.81	2823.74	48.17	232.46
240	6.5	130.31	471.20	3243.70	0	162.22
288	6.28	209.29	687.82	3144.41	251.26	213.72
312	6.2	245.01	784.95	2910.09	162.48	190.70
336	6.21	144.73	731.64	2789.90	86.74	230.16

Table A.87 Raw data for 1 g L⁻¹ substrate concentrations of 5:1 in < 2000 µm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0759	12.144	13	0.0759	0.9867	13.1307	0.516
74	145	165	0.0908	2.4585	10	0.0908	0.908	16.4972	0.648
128	140	170	0.0817	0	7	0.0817	0.5719	17.0691	0.671
175	135	175	0.0819	0.035	6	0.0819	0.4914	17.5955	0.692
222	130	180	0.0768	0	6	0.0768	0.4608	18.0563	0.710
270	125	185	0.0611	0	7	0.0611	0.4277	18.484	0.727
315	120	190	0.053	0	6	0.0611	0.3666	18.8506	0.741

Table A.88 Raw data for 1 g L⁻¹ substrate concentrations of 5:1 in < 2000 µm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
0	7.5					
24	6.39	152.83585	197.52	10700	0	1648.64
74	6.48	101.62135	696.12	10900	184.96	2571.57
128	6.51	77.1522	444.39	11100	294.52	2894.08
175	6.45	77.72125	0	11200	325.15	2920.84
222	6.48	106.7428	203.08	10500	300.24	2774.03
270	6.43	80.5665	211.4	10700	251.79	2665.99
315	6.44	76.0141	179.49	10500	255.7	2649.29

Table A.89 Raw data for 2 g L⁻¹ substrate concentrations of 5:1 in < 2000 µm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0638	10.208	8	0.0638	0.5104	10.7184	0.421
74	145	165	0.0746	1.782	10	0.0746	0.746	13.2464	0.521
128	140	170	0.0817	1.207	7	0.0817	0.5719	15.0253	0.591
175	135	175	0.122	7.0525	7	0.122	0.854	22.9318	0.902
222	130	180	0.122	0	6	0.122	0.732	23.6638	0.930
270	125	185	0.1339	2.2015	7	0.1339	0.9373	26.8026	1.054
315	120	190	0.1339	0	6	0.1339	0.8034	27.606	1.085

Table A.90 Raw data for 2 g L⁻¹ substrate concentrations of 5:1 in < 2000 µm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
0	7.5					
24	6.34	141.45485	1164.38	10700	0	1721.17
74	6.4	120.96905	1115.05	10700	135.02	2410.25
128	6.51	90.8094	312.6	10400	241.91	2669.97
175	6.23	98.20705	316.52	11100	264.19	2699.84
222	6.21	157.9573	579.25	10600	284.52	2768.81
270	6.2	108.44995	450.12	10300	184.63	2465.55
315	6.19	104.4666	254.52	10500	200.47	2557.48

Table A.91 Raw data for 5 g L⁻¹ substrate concentrations of 5:1 in < 2000 µm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0663	10.608	3	0.0663	0.1989	10.8069	0.425
74	145	165	0.0688	0.4125	10	0.0688	0.688	11.9074	0.468
128	140	170	0.0619	0	6	0.0619	0.3714	12.2788	0.483
175	135	175	0.0585	0	5	0.0585	0.2925	12.5713	0.494
222	130	180	0.0639	0.972	5	0.0639	0.3195	13.8628	0.545
270	125	185	0.0593	0	6	0.0593	0.3558	14.2186	0.559
315	120	190	0.0593	0	6	0.0593	0.3558	14.5744	0.573

Table A.92 Raw data for 5 g L⁻¹ substrate concentrations of 5:1 in < 2000 µm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
0	7.5					
24	6.37	155.11205	324.21	10700	247.79	2289.35
74	6.42	140.8858	351.82	10500	89.06	2592.77
128	6.41	122.10715	465.45	10400	277.54	2671.04
175	6.39	110.72615	431.36	10300	283.36	2626.37
222	6.38	119.83095	369.07	9931.4	262.13	2467.86
270	6.36	125.52145	471.13	9932.11	242.84	2446.95
315	6.38	109.58805	327.26	9949.25	217.31	2000.06

Table A.93 Raw data for 10 g L⁻¹ substrate concentrations of 5:1 in < 2000 µm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	V _s (ml)	V _g (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	V _{total} gas (ml)	H ₂ -1 (%)	V _t x H ₂ -1	V _{cum.} (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0827	13.232	8	0.0827	0.6616	13.8936	0.546
74	145	165	0.0977	2.475	10	0.0977	0.977	17.3456	0.682
128	140	170	0.0795	0	2	0.0795	0.159	17.5046	0.688
175	135	175	0.0871	1.33	4	0.0871	0.3484	19.183	0.754
222	130	180	0.0774	0	7	0.0774	0.5418	19.7248	0.775
270	125	185	0.0745	0	6	0.0745	0.447	20.1718	0.793
315	120	190	0.0745	0	6	0.0745	0.447	20.6188	0.811

Table A.94 Raw data for 10 g L⁻¹ substrate concentrations of 5:1 in < 2000 µm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
0	7.5					
24	6.32	202.91225	381.18	10400	307.09	2106.44
74	6.38	181.28835	529.97	10400	242.52	2602.5
128	6.39	144.86915	383.06	10500	251.59	2888.77
175	6.39	161.3716	400.09	10200	297.24	2832.75
222	6.36	200.63605	263.58	10200	242.25	2706.44
270	6.37	155.6811	477.86	9854.43	236.94	2686.75
315	6.36	165.924	192.72	10000	227.65	2665.22

Table A.95 Raw data for 15 g L⁻¹ substrate concentrations of 5:1 in < 2000 µm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0			0		0	0	0	0	
24	150	160	0.0876	14.016	5	0.0876	0.438	14.454	0.568
74	145	165	0.106	3.036	10	0.106	1.06	18.55	0.729
128	140	170	0.1023	0	5	0.1023	0.5115	19.0615	0.749
175	135	175	0.0718	0	6	0.0718	0.4308	19.4923	0.766
222	130	180	0.0726	0.144	6	0.0726	0.4356	20.0719	0.789
270	125	185	0.0814	1.628	7	0.0814	0.5698	22.2697	0.876
315	120	190	0.0814	0	6	0.0814	0.4884	22.7581	0.895

Table A.96 Raw data for 15 g L⁻¹ substrate concentrations of 5:1 in < 2000 µm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
0	7.5					
24	6.24	239.33145	459.19	10200	276.28	2468.03
74	6.3	237.6243	405.8	10400	227.92	2769.81
128	6.3	210.3099	818.51	10100	262.36	2880.68
175	6.29	218.84565	388.79	10300	260.85	2798.52
222	6.27	189.25505	264.05	10200	191.42	2631.55
270	6.27	208.0337	380.24	10200	97.53	2494.33
315	6.23	230.7957	362.31	9946.63	175.12	2491.04

Table A.97 Raw data for 20 g L⁻¹ substrate concentrations of 5:1 in < 2000 µm particle size

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head (ml) space	Vtotal (ml) gas	H ₂ -1 (%)	Vt x H ₂ - 1	Vcum. (ml)	Cumulative (mmol) H ₂
0			0		0	0	0	0	
24	150	160	0.0768	12.288	2	0.0768	0.1536	12.4416	0.489
74	145	165	0.0835	1.1055	10	0.0835	0.835	14.3821	0.565
128	140	170	0.0707	0	3	0.0707	0.2121	14.5942	0.574
175	135	175	0.0923	3.78	6	0.0923	0.5538	18.928	0.744
222	130	180	0.0754	0	6	0.0754	0.4524	19.3804	0.762
270	125	185	0.186	20.461	7	0.186	1.302	41.1434	1.618
315	120	190	0.139	0	6	0.139	0.834	41.9774	1.651

Table A.98 Raw data for 20 g L⁻¹ substrate concentrations of 5:1 in < 2000 µm particle size

t (h)	pH	Total Sugar Concentration (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
0	7.5				
24	6.18	263.23155	370.51	188.95	1988.98
74	6.2	278.02685	296.63	207.23	2479.7
128	6.11	197.22175	628.3	280.18	2491.56
175	5.99	266.0768	581.04	289.51	2439.09
222	5.85	201.77415	408.92	218.86	2216.43
270	5.7	234.77905	292.46	219.64	2247.16
315	5.68	278.02685	401.68	2258.15	2237.99

Table A.99 Raw data for *Clostridium intestinale* for cellulose as substrate

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	150	160		0			0	0	0
24	140	170	0.0359	6.103	0	0.0359	0	6.103	0.240
48	130	180	0.0883	9.432	1	0.0883	0.0883	15.6233	0.615
72	120	190	0.0724	0	0	0.0724	0	15.6233	0.615

Table A.100 Raw data for *Clostridium intestinale* for cellulose as substrate

t (h)	pH	Protein Concentration (mg L ⁻¹)	Total Sugar Concentration (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
0	7.5						
24	6.3	1821.2068	170.4764	514	175		1127.58
48	6.28	1390.4072	162.5097	68	168	260	374
72	6.5	1469.286	123.24525		350	265	347

Table A.101 Raw data for *Clostridium intestinale* for glucose as substrate

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	150	160		0	0		0	0	0
24	140	170	0.345	58.65	2	0.345	0.69	59.34	2.334
48	130	180	0.402	10.26	5	0.402	2.01	71.61	2.817
72	120	190	0.103	0	0	0.103	0	71.61	2.817

Table A.102 Raw data for *Clostridium intestinale* for glucose as substrate

t (h)	pH	Protein Concentration (mg L ⁻¹)	Total Sugar Concentration (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
0	7.5						
24	4.75	1742.328	980.8036	636	400	260	1555
48	4.65	1845.4772	297.9436	225	173	252	2070
72	4.8	1875.8152	334.93185	297	73	267	2055

Table A.103 Raw data for *Clostridium termitidis* for cellulose as substrate

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	150	160		0	0		0	0	0
24	140	170	0.0425	7.225	0	0.0425	0	7.225	0.284
48	130	180	0.0932	9.126	0.5	0.0932	0.0466	16.3976	0.645
72	120	190	0.089	0	0	0.089	0	16.3976	0.645
96	110	200	0.078	0	0	0.078	0	16.3976	0.645
120	100	210	0.0541	0	0	0.0541	0	16.3976	0.645
144	90	220	0.0413	0	0	0.0413	0	16.3976	0.645
168	80	230	0.0549	3.128	0	0.0549	0	19.5256	0.768
192	70	240	0.0391	0	0	0.0391	0	19.5256	0.768
216	60	250	0.0365	0	0	0.0365	0	19.5256	0.768
240	50	260	0.0482	3.042	0	0.0482	0	22.5676	0.887
264	40	270	0.0472	0	0	0.0472	0	22.5676	0.887
288	30	280	0.0463	0	0	0.0463	0	22.5676	0.887
312	20	290	0.0454	0	0	0.0454	0	22.5676	0.887
336	10	300	0.0467	0.39	0	0.0467	0	22.9576	0.903

Table A.104 Raw data for *Clostridium termitidis* for cellulose as substrate

t (h)	pH	Protein Concentration (mg L ⁻¹)	Total Sugar Concentration (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
0	7.50						
24	6.55	1966.8292	119.83095	110			465
48	6.78	1020.2836	44.1473		560	335	64
72	6.81	1560.3	78.85935		548	260	102
96	6.85	1200.9635	82.65412		550	250	110
120	6.95	968.8521	96.875		568	270	145
144	6.98	783.6472	114.7095		510	295	166
168	6.67	1026.3512	89.10225		527	287	153
192	6.86	1208.3792	115.8476		513	268	186
216	6.90	1208.3792	61.2188		465	155	156
240	6.92	1080.9596	82.8427		564	289	128
264	6.95	1045.9874	65.98745		545	180	142
288	6.98	1200.6589	69.3658		585	165	68
312	7.01	1135.568	44.71635		554	98	112
336	7.00	1208.3792	47.5616		530	21	162

Table A.105 Raw data for *Clostridium termitidis* for glucose as substrate

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	150	160		0	0		0	0	0
24	140	170	0.327	55.59	1	0.327	0.327	55.917	2.199
48	130	180	0.201	0	4	0.201	0.804	56.721	2.231
72	120	190	0.362	30.59	2	0.362	0.724	88.035	3.463
96	110	200	0.355	0	0	0.355	0	88.035	3.463
120	100	210	0.35	0	1	0.35	0.35	88.385	3.476
144	90	220	0.309	0	0	0.309	0	88.385	3.476
168	80	230	0.263	0	0	0.263	0	88.385	3.476
192	70	240	0.264	0.24	0	0.264	0	88.625	3.486
216	60	250	0.1933	0	0	0.1933	0	88.625	3.486
240	50	260	0.216	5.902	0	0.216	0	94.527	3.718
264	40	270	0.214	0	0	0.214	0	94.527	3.718
288	30	280	0.204	0	0	0.204	0	94.527	3.718
312	20	290	0.191	0	0	0.191		94.527	3.718
336	10	300	0.198	2.1	0	0.198		96.627	3.801

Table A.106 Raw data for *Clostridium termitidis* for glucose as substrate

t (h)	pH	Protein Concentration (mg L ⁻¹)	Total Sugar Concentration (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
0	7.50						
24	4.80	1608.8408	697.98575	577	221	254	1412.5
48	5.12	1657.3816	159.0954	373	77	229	1880
72	4.82	2136.722	193.80745	393	69	244	2033
96	4.85	2014.6524	200.45632	402	71	232	2120
120	4.90	2001.6325	245.16005	395	68	234	2145
144	4.89	1954.694	218.84565	381	72	236	2277
168	4.76	1966.8292	246.16005	420	48	240	2275
192	4.91	1815.1392	292.2531	426	83	341	2186
216	4.97	2264.1416	288.8388	421	63	243	2540
240	5.15	1608.8408	224.53615	423	65	188	2172
264	5.17	1845.6852	245.6124	421	61	245	2200
288	5.20	1658.78	268.98741	418	65	225	1875
312	5.21	1724.1252	276.88875	402	63	230	2216
336	5.25	1317.596	259.2482	378	88	229	1894

Table A.107 Raw data for co culture ratio 5:1 with cellulose as substrate

Hydrogen from head space					Hydrogen from total gas				
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	150	160		0	0		0	0	0
24	140	170	0.0514	8.738	0	0.0514	0	8.738	0.343
48	130	180	0.0805	5.238	0	0.0805	0	13.976	0.549
72	120	190	0.113	6.175	0	0.113	0	20.151	0.792
96	110	200	0.0741	0	0	0.0741	0	20.151	0.792
120	100	210	0.0495	0	0	0.0495	0	20.151	0.792
144	90	220	0.0404	0	0	0.0404	0	20.151	0.792
168	80	230	0.0491	2.001	0	0.0491	0	22.152	0.871
192	70	240	0.0778	6.888	0	0.0778	0	29.04	1.142
216	60	250	0.0776	0	0	0.0776	0	29.04	1.142
240	50	260	0.0621	0	0	0.0621	0	29.04	1.142
264	40	270	0.0612	0	0	0.0612	0	29.04	1.142
288	30	280	0.06	0	0	0.06	0	29.04	1.142
312	20	290	0.0588	0	0	0.0588	0	29.04	1.142
336	10	300	0.0483	0	0	0.0483	0	29.04	1.142

Table A.108 Raw data for co culture ratio 5:1 with cellulose as substrate

t (h)	pH	Protein Concentration (mg L ⁻¹)	Total Sugar Concentration (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
0	7.50						
24	6.36	1784.8012	128.93575	192	368	420	196.5
48	6.62	1487.4888	71.4617		462	308	173
72	6.55	1663.4492	68.61645		523	305	185
96	6.60	1598.7896	75.658		530	341	195
120	6.61	1365.9632	102.3648		574	285	201
144	6.65	1184.1088	129.5048		564	316	211
168	6.62	1008.1484	179.5812		566	401	178
192	6.80	1299.3932	134.62625		406	272	193
216	6.78	868.5936	92.51655		431	106	131
240	6.69	801.85	43.0092		203	98	115
264	6.72	850.4264	55.469874		185	65	125
288	6.72	968.7	106.5474		175	102	165
312	6.91	989.9456	171.04545		152	92	150
336	6.91	1244.7848	50.9759		105	114	269

Table A.109 Raw data for co culture ratio 5:1 with glucose as substrate

Hydrogen from head space						Hydrogen from total gas			
t (h)	Vs (ml)	Vg (ml)	H ₂ -1 (%)	VH ₂ -head space (ml)	Vtotal gas (ml)	H ₂ -1 (%)	Vt x H ₂ -1	Vcum. (ml)	Cumulative H ₂ (mmol)
0	150	160		0	0		0	0	0
24	140	170	0.275	46.75	3	0.275	0.825	47.575	1.871
48	130	180	0.294	3.42	3	0.294	0.882	51.877	2.040
72	120	190	0.355	11.59	2	0.355	0.71	64.177	2.524
96	110	200	0.31	0	0	0.31	0	64.177	2.524
120	100	210	0.215	0	2	0.215	0.43	64.607	2.541
144	90	220	0.0865	0	0	0.0865	0	64.607	2.541
168	80	230	0.246	36.685	0	0.246	0	101.292	3.984
192	70	240	0.175	0	0	0.175	0	101.292	3.984
216	60	250	0.226	12.75	0	0.226	0	114.042	4.486
240	50	260	0.223	0	0	0.223	0	114.042	4.486
264	40	270	0.198	0	0	0.198	0	114.042	4.486
288	30	280	0.175	0	0	0.175	0	114.042	4.486
312	20	290	0.171	0	0	0.171	0	114.042	4.486
336	10	300	0.0684	0	0	0.0684	0	114.042	4.486

Table A.110 Raw data for co culture ratio 5:1 with glucose as substrate

t (h)	pH	Protein Concentration (mg L ⁻¹)	Total Sugar Concentration (mg L ⁻¹)	Lactic Acid (mg L ⁻¹)	Acetic Acid (mg L ⁻¹)	Propionic Acid (mg L ⁻¹)	Butyric Acid (mg L ⁻¹)
0	7.50						
24	4.65	1803.004	956.33445	818	283	309	1062
48	5.04	1906.1532	190.9622	357	135	274	1845
72	4.83	2464.3724	221.12185	353	131	286	2053
96	4.85	1895.3798	226.894662	355	140	290	2045
120	4.93	1715.23654	248.7985	387	98	266	2100
144	4.95	1766.5984	284.85545	366	66	299	2013
168	4.78	1778.7336	283.71735	371	74	282	1989
192	4.92	1972.8968	288.8388	430	114	276	2131
216	4.86	1839.4096	321.27465	386	154	296	2118
240	5.20	1529.962	189.25505	400	100	259	2081
264	5.15	1620.45	175.48	410	102	265	1890
288	5.20	1723.655	196.98	415	119	178	1789
312	5.10	1887.9504	260.95535	403	112		1783
336	5.14	1586.39068	310.4627	360	193	269	1762

Table A.111 Remain substrate for pure *Clostridium termitidis* experiment

	Initial Concentration	Remain Substrate
Raw RH	5 g L ⁻¹	2.538 g L ⁻¹
Ground RH	5 g L ⁻¹	0.91 g L ⁻¹
Acid Hydrolyzed RH	5 g L ⁻¹	0.572 g L ⁻¹
TT RH	1 g L ⁻¹	0.154 g L ⁻¹
TT RH	2 g L ⁻¹	0.367 g L ⁻¹
TT RH	5 g L ⁻¹	1.047 g L ⁻¹
TT RH	10 g L ⁻¹	1.126 g L ⁻¹
TT RH	15 g L ⁻¹	1.632 g L ⁻¹
TT RH	20 g L ⁻¹	2.244 g L ⁻¹