PRODUCT ANALYSIS FOR PHOTOBIOLOGICAL PRODUCTION OF HYDROGEN

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ABSTRACT

PRODUCT ANALYSIS FOR PHOTOBIOLOGICAL PRODUCTION OF HYDROGEN

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Hydrogen (H_2) energy has an important place in terms of both reducing environmental pollution problems and having the highest energy content compared to fossil fuels. There are many ways to produce H_2, and photofermentation, which is one of the subheadings of biological H_2 production, was used in this thesis. Two different strains of *Rhodobacter capsulatus* strains of photosynthetic bacteria were used: *R. capsulatus* wild (WT) and uptake hydrogenase deficient mutant YO3 (hup^-). In addition to the H_2 production, *R.capsulatus* can synthesize some valuable byproducts such as poly-β-hydroxy butyric acid (PHB). PHB stands out due to its economic value and biodegradable polymer structure.

The main objective of this study was to compare of WT and YO3 (hup^-) strains of *R.capsulatus* in terms of H_2 and PHB synthesis efficiency. Since PHB accumulates under excess carbon source and stress conditions, 65 mM acetate and 2 mM glutamate were used as carbon and nitrogen sources, respectively. In total, 6 experiments were designed and operated with respect to PHB analysis methods,

reactor design and gas collection systems. Anaerobic photobioreactors with 50-350 mL capacity were operated in batch and fed-batch modes.

The results revealed that the relationship between H_2 and PHB production was directly proportional for the WT strain under the availability of acetate concentration (higher than 20 mM acetate concentration for this study). For YO3 strain, there was generally a reverse relationship between H_2 and PHB production.

Keywords: Hydrogen, Photofermentation, Poly-β-hydroxybutyrate (PHB), *Rhodobacter capsulatus*, Acetate

ÖZ

HİDROJENİN FOTOBİYOLOJİK ÜRETİMİ İÇİN ÜRÜN ANALİZİ

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Hidrojen (H_2) enerjisi hem çevre kirliliği sorunlarının azaltılması hem de fosil yakıtlara göre en yüksek enerji içeriği olması açısından önemli bir yere sahiptir. H_2 üretmenin birçok yolu vardır ve bu tezde biyolojik H_2 üretiminin alt başlıklarından biri olan fotofermentaston kullanılmıştır. Fotosentetik bakterilerin en popüler *Rhodobacter capsulatus* türünün iki farklı suşu kullanılmıştır: *Rhodobacter capsulatus* yabanıl suş (WT) ve hidrojen tüketen hidrojenaz suşunun silindiği (hup^-) YO3 suşu. H_2 üretimine ek olarak, *R.capsulatus* poli-beta-hidroksi bütirik asit (PHB) gibi bazı değerli yan ürünleri sentezleyebilir. PHB, ekonomik değeri olan ve biyolojik olarak parçalanabilen polimer yapısı ile öne çıkmaktadır.

Bu çalışmanın ana amacı, *R. capsulatus*'un WT ve YO3 (hup^-) suşlarının H_2 ve PHB sentezi verimlilikleri açısından karşılaştırılmasıdır. PHB, fazlaca karbon kaynağı ve stres koşulları altında biriktiğinden, karbon ve azot kaynakları olarak sırasıyla 65 mM asetat ve 2 mM glutamat kullanılmıştır. Toplamda 6 deney, PHB analiz yöntemleri, reaktör tasarımı ve gaz toplama sistemleri ile ilgili olarak tasarlanmış ve çalıştırılmıştır. 50-350 mL kapasiteli anaerobik fotobioreaktörler kesikli ve kesikli beslemeli modlarda çalıştırılmıştır.

Sonuçlar, asetat konsantrasyonunun (bu çalışma için 20 mM'den yüksek asetat konsantrasyonu) mevcudiyeti altında WT suşu için H_2 ve PHB üretimi arasındaki ilişkinin doğru orantılı olduğunu ortaya koydu. YO3 suşu için, H_2 ve PHB üretimi arasında genel olarak ters bir ilişki görülmüştür.

Anahtar Kelimeler: Hidrojen, Fotofermentasyon, Poli-beta-hidroksi bütirik asit (PHB), *Rhodobacter capsulatus*, Asetat

To Mehmet Cem Elik,

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TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

LIST OF ABBREVIATIONS

ABBREVIATIONS	
PNS	Purple non-sulfur
PHA	Polyhydroxyalkanoates
PHB	Poly-β-hydroxy butyric acid
PHB/HV	Poly (3-hydroxybutyrate/3-hydroxyvalerate)
H_2	Hydrogen
CO_2	Carbon dioxide
N_2	Nitrogen
R.capsulatus	*Rhodobacter capsulatus*
R. palustris	*Rhodopseudomonas palustris*
R. sphaeroides	*Rhodobacter sphaeroides*
WT	Wild type
C/N	Carbon to nitrogen ratio
hup^-	Uptake hydrogenase deficient mutant
VFA	Volatile fatty acids
HPLC	High Performance Liquid Chromatography
GC	Gas Chromatography
MPYE	Mineral-peptone-yeast extract
Biebl and Pfennig	BP
mM	Milimolar
phaA	β-ketothiolase

phaB	Acetoacetyl-coenzyme A [CoA] reductase
phaC	PHA synthase
TCA	tricarboxylic acid
Acetyl-CoA	Acetyl Coenzyme A
ATP	Adenosine triphosphate
Fd	Ferredoxin
GC	Gas Chromatography
L	Liter
DCW	Dry Cell Weight

CHAPTER 1

INTRODUCTION

Energy is one of the most crucial requirements for a multitude of industries and energy consumption all over the world has nearly doubled every 30 years (Filippov & Yaroslavtsev, 2021). Throughout history, the energy need, which was met with wood first, has been replaced by fossil fuels such as coal, oil, and natural gas (Filippov & Yaroslavtsev, 2021). About 80% of the present world energy demand is met with fossil fuels, which are non-renewable sources containing carbon (Yeong et al., 2012). However, their combustion causes the release of NO_x, SO_x, greenhouse gases such as CO_2 and other toxic gases. These gases lead to environmental pollution, global warming, and acid rain (Ni et al., 2006). Additionally, depletion of fossil fuel resources is currently occurring more quickly than resource replenishment (Ali et al., 2021).

Nowadays, hydrogen (H_2) has become an attractive topic considering the increasing environmental pollution and depletion risk of fossil fuels due to their use (Ni et al., 2006). H_2 is accepted as a clean non-polluting energy carrier, as water is produced as the major product of its combustion (Das & Veziroglu, 2001). Moreover, it is advantageous to use H_2 due to having the highest energy amount per mass, being 2.75 times higher than the other hydrocarbon fuels (Das et al., 2008).

H_2 can be produced by several techniques from conventional processes and renewable sources. Steam reforming, partial oxidation, autothermal reforming, and hydrocarbon pyrolysis are the conventional processes for H_2 production, which depend on fossil fuels (Nikolaidis & Poullikkas, 2017). About 80% of the produced hydrogen is produced from these techniques. Steam methane reforming (SMR) is commonly used due to the lower cost compared to other commercial methods. On the other hand, SMR has a high potential for global warming as a drawback (Safari

& Dincer, 2020). While partial oxidation (POX) is used as an alternative to SMR and could be operated with a wide range of feedstocks, the application of POX in the industry faces challenges. For instance, the POX unit is damaged in a short time as it operates at high temperatures (Megia et al., 2021). Autothermal reforming (ATR) process, exothermic partial oxidation is employed to generate heat, and endothermic steam reforming is used to increase hydrogen generation efficiency (Nikolaidis & Poullikkas, 2017). Lastly, in addition to methods of SMR, POX, or ATR, hydrocarbon pyrolysis is an alternative method due to the production of clean carbon side-product (Muradov, 2003).

Compared to commercial techniques, biological H_2 production processes, which are bio-photolysis, dark fermentation, and photofermentation, are advantageous due to being environmentally friendly and sustainable (Das et al., 2008). In the bio-photolysis process, both hydrogen and oxygen can be produced by cyanobacteria and green algae. However, produced oxygen inhibits the metabolism of these microorganisms and leads to lower hydrogen yields. In dark fermentation, fermentative bacteria can convert organic acids to hydrogen but at lower yields (Basak & Das, 2007). In the photofermentation process (used in this study), organic compounds are used for producing hydrogen under light and without ambient oxygen. Nitrogenase and hydrogenase enzymes are responsible for H_2 production during photofermentation (Nikolaidis & Poullikkas, 2017). Due to allowing a high substrate efficiency and its capability to use a wide range of substances, photofermentation is an advantageous process in large-scale production (Yeong et al., 2012).

Purple non-sulfur (PNS) bacteria can produce H_2 by photofermentation. Additionally, they can grow in chemoheterotrophic, chemoautotrophic, photoheterotrophic, and photoautotrophic modes and utilize a wide range of the solar spectrum (Koku et al., 2002). *Rhodobacter capsulatus,* which is one of the species of the PNS bacteria, was used in this study thanks to its advantages.

Poly-β-hydroxy butyric acid (PHB), which is a valuable biodegradable plastic is accumulated via the bacterial fermentation process. Under excess carbon sources and stress conditions, PHB is accumulated as a storage compound in the form of intracellular granules (Luongo et al., 2017). The limited amount of nutrients like phosphorus, nitrogen, oxygen, or sulfur are examples of stress conditions (Vaishnav & Choudhary, 2021). Different bacterial types of microorganisms can accumulate PHB as reserve compound. Like other bacterium types, *Rhodobacter capsulatus*, which is used in this study, can produce large amounts of PHB under excess carbon source and nutrient-deprived conditions (Kranz et al., 1997).

Synthetic plastics such as polypropylene and polyester lead to environmental pollution due to their non-biodegradability. Unlike synthetic plastics, PHB is an environmentally beneficial biopolymer due to its 100% biodegradability (Reddy et al., 2003). In addition to its biodegradability, the thermoplastic processibility and biocompatibility of PHB give the advantage to compete with synthetic plastics (Hu et al., 2013). The application areas of PHB are a wide range of several industries such as packaging, pharmaceutical, agricultural, biomedical, and coating. However, the major problem of PHB production on the large scale is the high cost of production. There is still research on reducing the PHB production cost (Vaishnav & Choudhary, 2021).

Although the H_2 production by photofermentation using PNS bacteria has been investigated for many years, the exact metabolic route for production of H_2 as well as other side products is still quite complex (Adessi & Philippis, 2014).

There are many studies performed to understand the metabolic process related to H_2 and PHB under stress conditions. The redox power released by the breakdown of the substrates can be used by metabolic pathways of H_2 and PHB production (Hustede et al., 1993). In many studies, H_2 and PHB production generally compete with each other by sharing the redox power (Luongo et al., 2017; Wu et al., 2012; Yiğit et al., 1999). However, other research has demonstrated that H_2 production and PHB production can coexist in different conditions such as light intensity, pH fluctuation,

and nitrogen source (Corona et al., 2017; Hustede et al., 1993; Policastro et al., 2020; Uyar et al., 2009).

In this study, the main goal was to investigate the comparison of wild type (WT) and uptake hydrogenase deficient mutant (hup$^-$) strains of *Rhodobacter capsulatus* for H_2 production and PHB accumulation. For this purpose, the medium, which contains excess carbon source and limited nitrogen source, was used to enhance the PHB accumulation for both strains. This study is the first to investigate PHB production for *R.capsulatus* YO3 and compare with the WT strain. To this purpose, PHB analysis was carried out daily for both WT and YO3 strains. Depending on the main purpose of this thesis, the aims of study were developed by working on gas collection methods and reactor design.

The structure of this thesis is organized as follows. Chapter 2 outlines hydrogen energy and methods of hydrogen production, properties of PHB, and the relationship between hydrogen and PHB production, respectively. The experimental materials, techniques, and analyses are given in Chapter 3. Next, experimental results are presented and discussed in Chapter 4. Lastly, Chapter 5 covers the conclusion and recommendations.

CHAPTER 2

LITERATURE REVIEW

2.1 Hydrogen Energy

The energy consumption of the world has nearly doubled every 30 years, and around 80% of it is based on fossil fuels (Filippov & Yaroslavtsev, 2021). Some disadvantages occur with the increasing demand for these non-renewable energy sources. First, for years, the environmental pollution caused by carbon-based fossil fuels has become a topic of investigation. As a result of their combustion, some emission gases and pollutants such as C_xH_x, SO_x, NO_x, CO_x, ash, and soot are released into the atmosphere, and they cause climate change over time (Assawamongkholsiri et al., 2018; Das & Veziroglu, 2001; Dincer, 2012). Moreover, these toxic gases have a negative effect on human life specific (Ali et al., 2021). In addition to this drawback, fossil fuels are finite resources, and they are becoming increasingly limited in direct proportion to the increase in energy needs (Keskin & Hallenbeck, 2012). Hence, humankind looks forward to a clean and reliable renewable energy source (Lubitz & Tumas, 2007). Considering the environmental damage of non-renewable energy sources and their scarcity, hydrogen is a promising alternative energy carrier due to its sustainable and environmental advantages (Das & Veziroglu, 2008; Sagir et al., 2018).

Hydrogen has several advantages as an alternative energy carrier. After the combustion of the hydrogen, no CO_2 is released, and only water is produced as a major product (Sagir et al., 2018). Furthermore, the energy content per gram of hydrogen is 142 kJ/g. This energy value is the highest among all fuels (Das & Veziroglu, 2008). Thus, it has been accepted as an alternative environmentally friendly candidate for a global energy system dependent on fossil fuels (Sagir et al., 2018).

According to the report of the International Energy Agency, hydrogen is a much cleaner energy source compared to other sources. Thus, it is expected that its share of energy consumption will increase from 0.1% (2020) up to 10% in 2050 as seen in Figure 2.1(International Energy Agency, 2021).

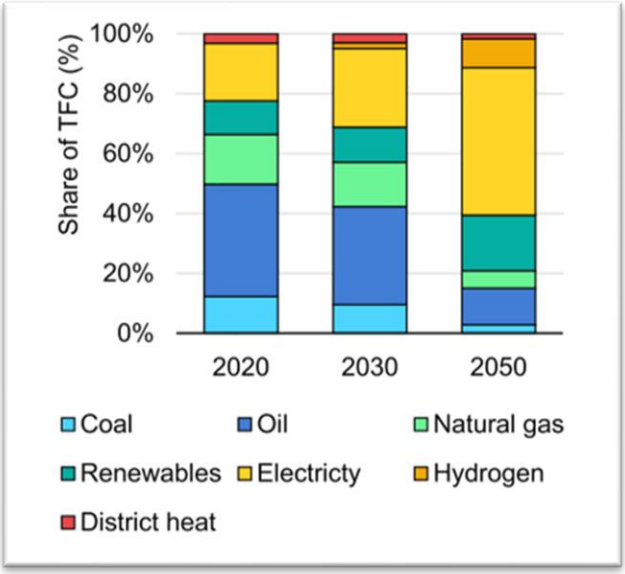

Figure 2.1 Share of total final energy consumption by fuel in the NZE, 2020-2050 (TFC = Total Final Energy Consumption; NZE = Net Zero Emissions Scenario) (International Energy Agency, 2021)

Hydrogen, which is a colorless, odorless, light, and highly flammable diatomic gas at a standard temperature and pressure, covers three-quarters of the universe, making it the most plentiful element in the universe. On the other hand, in the atmosphere of the Earth, the percentage of hydrogen is less than 0.14% (Das & Veziroglu, 2001). Therefore, there are various methods to produce hydrogen. For instance, thermochemical, electrochemical, and biological methods are techniques that are widely covered in the literature (Wang & Zhang, 2017). In the following section, an overview of hydrogen production techniques is given.

2.2 Hydrogen Production Techniques

Today, most of the hydrogen demand is met by fossil fuels, with natural gas accounting for 71.3% of all fossil fuel use. Moreover, coal and oil are utilized for producing hydrogen with 27.3% and 0.7% of shares, respectively (International Energy Agency, 2019). The rest of the hydrogen (0.7%) is supplied from water electrolysis and renewable energy sources. Besides, renewables with a share of 0.1% produce "by-product hydrogen" which means that hydrogen comes from other processes (International Energy Agency, 2019).

A broad range of raw materials are used to produce hydrogen energy and production processes can be divided into two main groups, namely conventional and renewable. Sub-methods covered by these two processes are depicted in Figure 2.2 (Nikolaidis & Poullikkas, 2017). In the conventional processes, hydrocarbon reforming and hydrocarbon pyrolysis are the two methods for producing hydrogen. Hydrocarbon reforming also has several subdivisions, namely steam reforming, partial oxidation, and autothermal reforming. The renewable processes include the production of hydrogen from water splitting and biomass. Water splitting can be obtained in three ways, which are electrolysis, thermolysis, and photolysis. The biomass process, on the other hand, is divided into two processes, which are biological and thermochemical. While the biological methods involve bio-photolysis, dark fermentation, and photofermentation (which is also used in this study), major thermochemical processes include pyrolysis, gasification, combustion, and liquefaction. The details of the mentioned processes are presented in the following sections: 2.2.1 and 2.2.2.

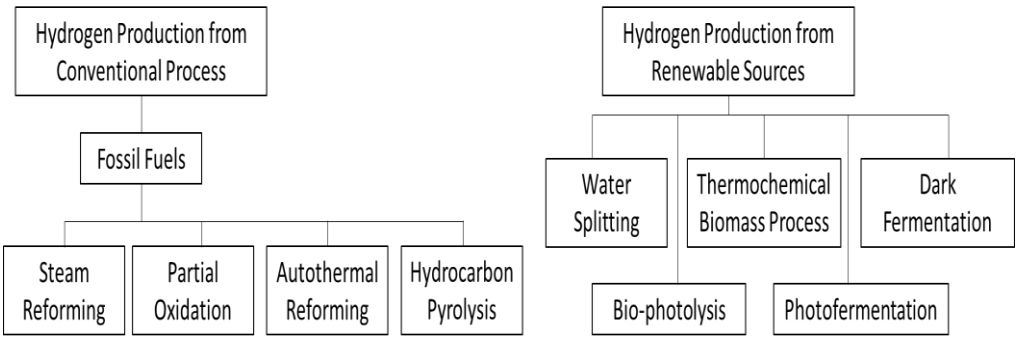

Figure 2.2 Hydrogen production methods from fossil fuels and renewable sources (Nikolaidis & Poullikkas, 2017)

2.2.1 Hydrogen Production from Conventional Processes

2.2.1.1 Steam Reforming

The majority of the world's hydrogen production is supplied via the steam methane reforming (SMR) method, which has a high hydrogen yield efficiency of about 74 % (Abdalla et al., 2018; Safari & Dincer, 2020). Impurity removal, synthesis gas (syngas) generation, water gas shifting, and methanation or gas purification are the parts of this process. The operating settings for SMR are set at temperatures of 700-850 ^{o}C, a pressure of 3-25 bars, and a steam to carbon ratio of 3.5 to achieve the targeted pure hydrogen and avoid coke formation on the catalyst surface (Ersöz, 2008). Both non-precious and precious metals can be used as catalysts for this technique. On the other hand, considering the intense limitations on both mass and heat transfer, it is found that the effectiveness of the catalyst is often no more than 5%. For this reason, non-precious metals are commonly preferred and used in the industry. At last, the produced hydrogen is sent to a pressure swing adsorption unit to purify it.

The main reaction steps of the SMR method in the reformer, water gas shift reactor, and methanator are given as follows in Equations 2.1, 2.2, and 2.3, respectively (Abdalla et al., 2018):

$$C_nH_m + nH_2O \leftrightarrow nCO + \left(n + \frac{m}{2}\right)H_2 \quad (2.1)$$

$$CO + H_2O \leftrightarrow CO_2 + H_2 \quad (2.2)$$

$$CH_4 + H_2O \leftrightarrow CO + 3H_2 \quad (2.3)$$

2.2.1.2 Partial Oxidation

The main aim of the partial oxidation method (POX) is to convert steam, oxygen, and hydrocarbons to hydrogen and carbon monoxide. Compared to steam reforming, a higher temperature is required to operate this process. As this method in the presence of catalysts works at approximately 950 ^{o}C with methane and naphtha, methane, heavy oil, and coal are preferred in the non-catalytic process, which operates at 1150 to 1315 ^{o}C (Nikolaidis & Poullikkas, 2017). The application of POX has several benefits, such as easy operation and using a broad range of feedstock. However, the lifetime of this plant is not long because of the high-temperature needs, and also, the capital cost of this plant is extremely high because of the oxygen plant and desulfurization steps that cause additional costs (Li et al., 2020; Megia et al., 2021).

The following equations (2.4 to 2.7) are the major partial oxidation reaction steps(Abdalla et al., 2018):

$$C_nH_m + \frac{1}{2}nO_2 \leftrightarrow nCO + \frac{1}{2}mH_2 \quad \text{(catalytic)} \quad (2.4)$$

$$C_nH_m + nH_2O \leftrightarrow nCO + \left(n + \frac{1}{2}m\right)H_2 \quad \text{(non-catalytic)} \quad (2.5)$$

$$CO + H_2O \leftrightarrow CO_2 + H_2 \quad \text{(water gas shifting reactor)} \quad (2.6)$$

$$CO + 3H_2 \leftrightarrow CH_4 + H_2O \quad \text{(methanator)} \quad (2.7)$$

2.2.1.3 Autothermal Reforming

Exothermic partial oxidation is utilized in the autothermal reforming (ATR) process to generate heat, while endothermic steam reforming is used to raise the hydrogen production efficiency (Nikolaidis & Poullikkas, 2017). This method involves both the techniques of SMR and POX. For instance, the optimum operating temperature has been obtained at 700 ^{o}C. Moreover, S/C and O_2/C ratios are calculated as 1.5 and 0.45, respectively (Holladay et al., 2009). As shown in equation 2.8, steam and oxygen or air react simultaneously (Nikolaidis & Poullikkas, 2017). After that, reforming and oxidation reactions take place.

$$C_nH_m + \frac{1}{2}nH_2O + \frac{1}{4}nO_2 \leftrightarrow nCO + (\frac{1}{2}n + \frac{1}{2}m)H_2 \quad (2.8)$$

Finally, operating at a low process temperature is an advantage of this method. On the other hand, there are still limitations to the experiments since they require air or oxygen.

2.2.1.4 Hydrocarbon Pyrolysis

In addition to methods of SMR, POX, or ATR, hydrocarbon pyrolysis is an alternative or preferable conventional fuel reforming technology for hydrogen production. In this process, hydrocarbons crack or decompose into carbon, which is a valuable by-product, and hydrogen without air or water. The equation for it is shown below (Muradov, 2003).

$$C_nH_m \rightarrow nC + \frac{1}{2}mH_2 \quad (2.9)$$

Moreover, as shown in equation 2.9, no carbon dioxide or carbon monoxide is produced since there is no oxidant in the reactor. Thus, this process has an important advantage due to reducing CO_2 emissions (Holladay et al., 2009).

2.2.2 Hydrogen Production from Renewable Energy Sources

Among the green hydrogen production pathways, hydrogen production technologies from renewables that have fewer negative effects on the environment, like photoelectrochemical water splitting (PEWS) or photobiological hydrogen production, have increasingly drawn attention for their long-term advantages (Safari & Dincer, 2020). In this manner, water or biomass-derived compounds can be used to produce green hydrogen.

2.2.2.1 Water Splitting

The most plentiful raw material of hydrogen is water, which can be split into hydrogen and oxygen with the required amount of energy without emitting any harmful byproducts (Megia et al., 2021; Nikolaidis & Poullikkas, 2017; Safari & Dincer, 2020). The simplest method of splitting water into hydrogen and oxygen is electrolysis, which involves an electrical current running across the two electrodes: anode and cathode. The equation for it is as follows (Li et al., 2020):

$$2H_2O\ (l) \rightarrow 2H_2(g) + O_2(g),\ \mathrm{E}_0 = -1.229\ \mathrm{V} \tag{2.10}$$

In addition to this method, water can be split by other technologies such as thermolysis, photo electrolysis, and biophotolysis (Agyekum et al., 2022). The energy source and operating conditions are the critical parameters to determine the methods. For example, heat is used during the thermolysis process at higher temperatures up to 2500 ^{o}C, whereas solar energy is used for photo electrolysis under ambient conditions. Moreover, biophotolysis utilizes microorganism metabolism as an energy source (Megia et al., 2021).

2.2.2.2 Thermochemical Biomass Process

Pyrolysis, gasification, combustion, and liquefaction are part of thermochemical biomass technologies. First, in the pyrolysis of biomass processes, bio-oil, a hydrocarbon-rich gas mixture, charcoal, and non-condensable gases are obtained as products by using biomass. In other words, more valuable and useful fuels are produced via the pyrolysis of biomass. During this process, the optimum temperature is approximately 477 ^{o}C, and there must be no or a limited amount of oxygen (Demirbas, 2004).

The most widely used and well-established process for hydrogen technology with biomass is biomass gasification. In the gasifier, there is a conversion using biomass to produce a gaseous mixture that includes hydrogen, carbon monoxide, carbon dioxide, and synthesis gas, which is commonly called syngas. Additionally, in the gasifier, the reaction occurs under heat, pressure, steam, and limited oxygen (Mohapatra, 2012).

Among the thermochemical biomass processes, the combustion and liquefaction of the biomass methods are not much preferable to others. The reason is that low hydrogen yields are obtained for both methods as well, and polluting byproducts are produced. Additionally, the optimum operating pressure is 5-20 MPa without air. Since it is difficult to achieve this pressure value, these methods are not much preferred compared to other methods (Nikolaidis & Poullikkas, 2017).

2.2.2.3 Bio-photolysis

One of the environmentally benign hydrogen production techniques is bio-photolysis, which can be done in two different ways: directly and indirectly. In direct bio-photolysis, water is converted to oxygen and hydrogen by solar energy via cyanobacteria and blue-green algae (Martino et al., 2021). Microalgae C*hlamydomonas reinhardtii* is the most utilized species during this process (Agyekum et al., 2022).

The scheme of the direct bio-photolysis process is depicted in Figure 2.3. The photosystems (PSI and PSII) and hydrogenase play an important role in producing hydrogen. At this point, it is crucial to mention that hydrogenase is overly sensitive to oxygen. Thus, this method takes place in anaerobic conditions, in which should oxygen level of less than 0.1 % (Agyekum et al., 2022).

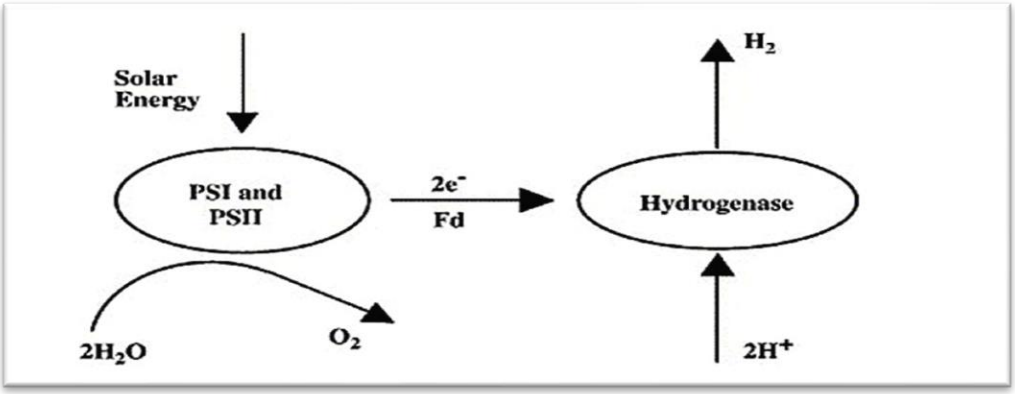

Figure 2.3 Direct bio-photolysis process. Fd: ferredoxin (Agyekum et al., 2022)

In indirect bio-photolysis, there are two stages, which are shown in Figure 2.4. This process starts with the production of cell material by photofermentation using water and CO_2. In other words, CO_2 is converted into a reserve carbohydrate like glucose. The equation of the first stage is given in equation 2.11. After that, in the second stage, produced cell material can be converted to hydrogen by the hydrogenase enzyme. As a result, 12 mol of H_2 can be produced from 1 mol of glucose, which is represented in equation 2.12.

$$12H_2O + 6CO_2 \rightarrow C_6H_{12}O_6 + 6O_2 + C_6H_{12}O_6 \quad (2.11)$$

$$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2 \quad (2.12)$$

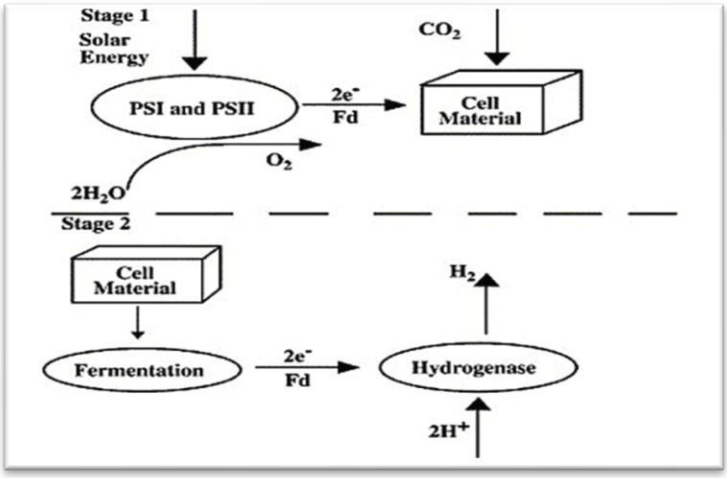

Figure 2.4 Indirect bio-photolysis (Agyekum et al., 2022)

Lastly, compared to conventional hydrogen production approaches, this biological process is more environmentally benign, and these methods are more energy efficient since they are operated under normal temperature and pressure (Basak & Das, 2007; Megia et al., 2021). However, there are some disadvantages to it, such as requiring a critical and large surface area for light penetration and low hydrogen production potential (Nikolaidis & Poullikkas, 2017).

2.2.2.4 Dark Fermentation

Dark fermentation is accepted as the most promising technology for biomass hydrogen production (Das et al., 2008; Afsar, et al., 2010). By using anaerobic organisms, organic materials, which are listed as sugars, waste materials, wastewater, and amino acids, can be utilized to achieve hydrogen as a major product in the dark (Agyekum et al., 2022). For instance, by utilizing sugarcane juice as the carbon source, it is found that the net energy ratio might be 2.9 times higher than the methane reforming technique results (Manish & Banerjee, 2008). The most dominant spore-forming obligate anaerobic microorganism has been accepted as

Clostridium due to the highest hydrogen production performance in the fermentation system (Castelló et al., 2020; Palomo-Briones et al., 2017).

In the following equations 2.13 and 2.14, the theoretical hydrogen yields for acetate and butyrate fermentations are given, respectively.

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2 \quad (2.13)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2H_2 + 2CO_2 \quad (2.14)$$

While the metabolic rate of this method is high and additional operational needs such as a light source are not needed, H_2 yield for dark fermentation can only just achieve 20% of the theoretical maximum value of 12 mol H_2/mol glucose because of incomplete substrate decomposition (D. H. Kim & Kim, 2013).

Furthermore, various parameters affect the efficiency of the system critically. These can be listed as follows: the species used, the substrate concentration, and the environmental conditions like pH, temperature, and pressure (Martino et al., 2021). According to previous studies, dark fermentation can be obtained mesophilically, thermophilically, and hyperthermophilically (30-39 ^oC, 50-64 ^oC, and higher than 65 ^oC, respectively) (Lee et al., 2011; Sinha & Pandey, 2011; Xiao et al., 2013). Moreover, pH has a crucial effect on both the hydrogenase enzyme and the metabolic pathway. Thus, the optimum initial pH range varies for different types of conditions and organisms for fermentative hydrogen production (Sinha & Pandey, 2011).

Finally, novel technologies have been offered to improve the dark fermentation system. For this purpose, there are novel studies based on nanotechnological materials and electro-fermentation methods. However, it is still progressing, and so there should be so much effort to apply a real system (Castelló et al., 2020).

2.2.2.5 Photofermentation

One of the most preferable processes for hydrogen production is photofermentation via anoxygenic photosynthetic bacteria, which is also investigated in this thesis. The scheme of this process is illustrated in Figure 2.5. With solar energy, the bacterial photosystem converts organic acids into hydrogen, and for this process, nitrogenase is a key enzyme (Ni et al., 2006). Additionally, it is critical to use high conversion yielding bacteria during the process. For this reason, the most favorable kind of photosynthetic bacteria is purple non-sulfur (PNS) bacteria, such as *Rhodobium*, *Rhodosprillium*, *Rhodobacter*, and *Rhodopseudomonas* (Martino et al., 2021). Additionally, among them, *Rhodobacter* is the most popular PNS bacterial genus (Agyekum et al., 2022). The details of them are given in Section 2.3.

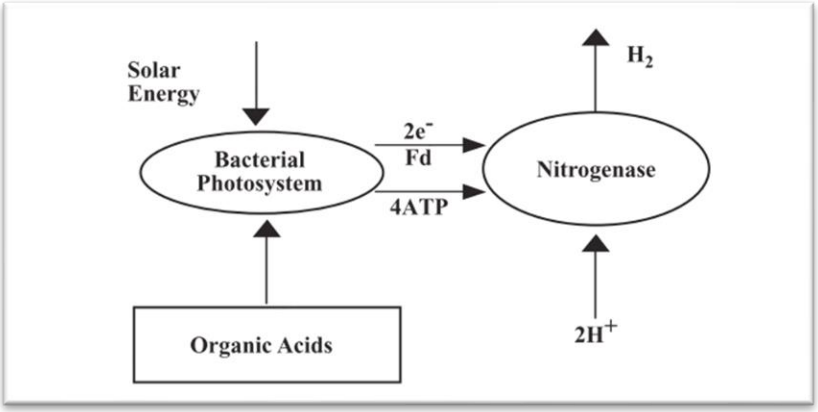

Figure 2.5 The hydrogen production system by photofermentation (Ni et al., 2006)

This method has the advantage of utilizing a wide range of substrates, such as small organic molecules as carbon sources (Megia et al., 2021). Equation (2.15) describes a reaction step for hydrogen and CO_2 production by a photofermentative mechanism from acetic acid. Furthermore, the standard Gibb free energy of it is 104 kJ. This reaction is non-spontaneous, according to this positive value. In other words, energy is needed to obtain a reaction. In this case, either natural or artificial light is necessary (Agyekum et al., 2022).

$$C_2H_4O_2 + 2H_2O \xrightarrow{light\ energy} 4H_2 + 2CO_2 \quad \text{(Acetate)} \qquad (2.15)$$

In addition to acetate, propionate and butyrate are examples of other common organic acids whose mechanisms are illustrated in equations (2.16) and (2.17), respectively (Uyar et al., 2009).

$$C_3H_6O_2 + 4H_2O \rightarrow 7H_2 + 3CO_2 \quad \text{(Propionate)} \qquad (2.16)$$

$$C_4H_8O_2 + 6H_2O \rightarrow 10H_2 + 4CO_2 \quad \text{(Butyrate)} \qquad (2.17)$$

2.3 General Characteristics of PNS Bacteria

Purple non-sulfur (PNS) bacteria are named as such due to their relative intolerance to sulfide (Hunter et al., 2009). Their optimum growth temperature range is 30-35 ^{o}C. Temperature fluctuations can affect the hydrogen production, especially in the day-night cycle or other climatic conditions. On the other hand, there are also thermo-resistant strains of them such as *Rhodospirillum centenum* which can grow at higher temperatures, 40-45 ^{o}C (Eroglu et al., 2014).

In comparison to algae and cyanobacteria, PNS bacteria are recognized as promising candidates for biological hydrogen production. (Doğan, 2016). There are several remarkable advantages to utilizing them during the photofermentation process. The first advantage is that PNS bacteria can use a multiplicity of carbon sources such as acetate, butyrate, propionate, lactate, glucose, sucrose, and a mixture of these either for growth or hydrogen production (Eroglu et al., 2014; Mirza et al., 2019; Tao et al., 2008). They have high substrate conversion efficiency, so they are appropriate for large-scale production (Koku et al., 2002). Furthermore, they can use organic substrates that are derived from agricultural or industrial wastes to produce hydrogen (Eroglu et al., 2014).

Secondly, they can grow in various modes, which are chemoheterotrophic, chemoautotrophic, photoheterotrophic, and photoautotrophic. In other words, they

are suitable and versatile organisms for growing under various conditions that are based on light, oxygen, and organic or inorganic carbon sources (Koku et al., 2002). In the mode of chemoheterotrophy, organic carbon is used as a carbon and energy source, and the growth can be observed in either the presence of oxygen or not (Koku et al., 2002). In the chemoautotrophic growth mode, CO_2 and inorganic compounds are used as carbon and energy sources, respectively. Lastly, energy is supplied from light in both modes of photoheterotrophy and photoautotrophy. However, while organic carbon is used as a carbon source in the photoheterotrophy mode, inorganic carbon (CO_2) is used in the mode of photoautotrophy (Larimer et al., 2004).

PNS bacteria contain antenna pigments such as carotenoids and chlorophyll. Due to their specific color, the absorbed wavelength scales change broadly. For instance, the absorbed wavelength range of carotenoids is between 450 nm and 516 nm (Boran, 2011). On the other hand, the range of chlorophyll light absorption is on a higher scale, which is 375 nm, 590 nm, and 830-890 nm (Eroglu et al., 2014). In summary, as they can utilize light intensity widely, it is advantageous to use these kinds of bacteria.

Finally, *Rhodobacter capsulatus,* which is one of the species of the PNS bacteria, was used in this thesis due to its benefits such as high substrate conversion efficiency and utilizing wide range of light. Moreover, wild and mutant strains of *R.capsulatus* were studied. Since the mutant strain lacks uptake hydrogenase, it is called *R.capsulatus YO3* (*hup*$^-$). More information on its mechanism is given in the following sections.

2.4 Hydrogen Production by Photosynthetic PNS Bacteria

Although there are several different modes for growth mentioned in section 2.3, PNS bacteria such as *R. capsulatus* and *R.sphaeroides* produce hydrogen and side products through the breakdown of organic substrates like acetate, malate, and lactate when there is a light and anaerobic environment present (without oxygen) (Basak & Das, 2007b; Koku et al., 2002).

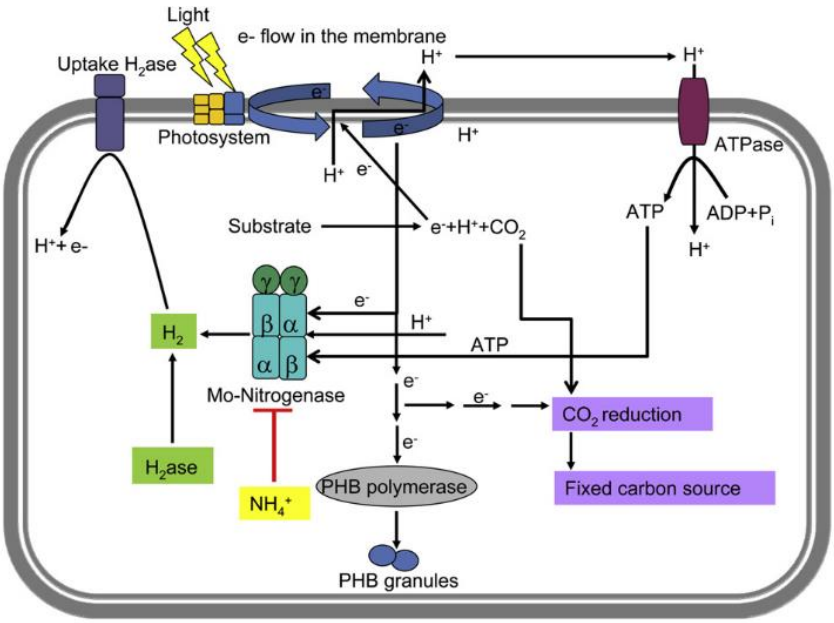

Figure 2.6 The overall scheme of metabolic pathways in a PNS bacterium (Kars & Gündüz, 2010)

Figure 2.6 depicts the overall process throughout the hydrogen and other by products produced with the activity of enzymes and electron flow to these enzymes. First, organic acids are used as energy and electron substrates to produce carbon dioxide and electrons by the metabolism of PNS bacteria. After feeding carbon substrates into the TCA (tricarboxylic acid) cycle, carbon dioxide and electrons are produced. Meanwhile, the photosynthetic membrane apparatus, activated by light energy,

converts protons into ATP form by ATPase. After that, electrons carried by ferredoxin/flavodoxin (Fd/Fn) and nitrogenase produce the molecular hydrogen by reducing electrons and consuming ATP (Kars & Gündüz, 2010). Moreover, the hydrogenase enzyme pathway illustrated in Figure 2.6 and Section 2.4.1 contains the details of these enzymes.

PNS bacteria may also use reducing equivalents and ATP to produce, PHB granules, which are one of the by-products obtained. In this thesis, the details of PHB biosynthesis and the relationship between H_2 and PHB are mentioned in the following sections.

2.4.1 Primary Enzymes involved in Hydrogen Production

2.4.1.1 Nitrogenase

The major responsible enzyme for hydrogen production is nitrogenase, and it needs ATP and reducing power. Equation (2.18) shows the stoichiometry for H_2 production (Koku et al., 2002). Moreover, light energy activates it (Zabut et al., 2002). For instance, Uyar et al. (2007) studied the effect of light intensity on hydrogen production via *Rhodobacter sphaeroides* O.U.001. According to the results, increasing light intensity up to 270 W/m^2 boosts hydrogen production and further light intensity could cause the photoinhibition (Uyar et al., 2007). Moreover, since there is no hydrogen production during the dark periods, it can be concluded that light has an important effect on photosynthesis (Basar Uyar et al., 2007).

$$2\,\mathrm{H}^+ + 2\,e^- + 4\,\mathrm{ATP} \rightarrow \mathrm{H}_2 + 4\,\mathrm{ADP} + 4\,P_\mathrm{i} \qquad (2.18)$$

Nitrogenase is an enzyme that activates and catalyzes under non-molecular nitrogen environments. For this reason, it can be repressed by ammonium, which is a salt of

nitrogen sources during its activity (Hillmer & Gest, 1977; Jones & Monty, 1979). This inhibition has been accepted as a reversible reaction since when the ammonium is removed, the activity of the nitrogenase enzyme starts to take effect again. In addition to molecular nitrogen or ammonium, oxygen is another repressor of this enzyme irreversibly (Koku et al., 2002).

The center of nitrogenase contains some metals such as molybdenum (Mo) and iron (Fe) for phototrophic bacteria. Furthermore, there are currently only three species which are *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, and *Rhodopseudomonas palustris* known to have extra nitrogenases with cofactors comprised of FeFe and FeV (vanadium). The concentration of Mo is especially critical to hydrogen production via *Rhodobacter sphaeroides* (Fang et al., 2011).

2.4.1.2 Hydrogenase

Hydrogenase or uptake hydrogenase enzyme (hup) is another enzyme involved in hydrogen production. However, although the reversible reaction occurs in the production and oxidizing of hydrogen by this enzyme, it generally consumes hydrogen. In other words, the mechanism of hydrogenase is antagonistic to that of nitrogenase. The relationship between nitrogen and hydrogenase enzymes is illustrated in Figure 2.7 (Vignais et al., 1985).

Oxygen is an inhibitor for this enzyme, the same as nitrogenase, but carbon monoxide is also an inhibitor for this enzyme. Moreover, since the center of this enzyme contains nickel, the concentration of nickel might be limited to decrease the hydrogenase synthesis (Koku et al., 2002).

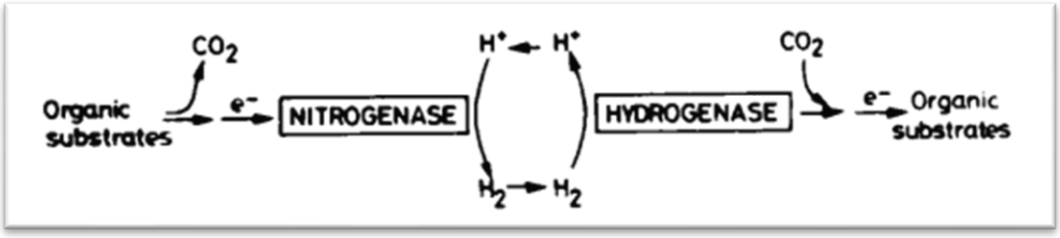

Figure 2.7 The scheme of responsible enzymes in the recycling of H_2 (Vignais et al., 1985)

Many studies have studied increasing the hydrogen production by deleting the uptake hydrogenase enzyme (hup^-) (Kars et al., 2008; Öztürk et al., 2006; Sato et al., 2017). For instance, Kars et al. (2008) studied the effects of lacking uptake hydrogenase enzyme on H_2 production by *R.sphaeroides* O.U.001 and showed that the mutant strain produced 20% more hydrogen gas overall than the wild type strain. Furthermore, Öztürk et al. (2008) found that compared to *R.capsulatus* MT1131 wild type strain, total hydrogen (mL/mL culture) and substrate conversion efficiency (%) increased 1.4 fold for *R.capsulatus* YO3 (hup^-).

2.5 Poly-β-hydroxy butyric acid (PHB)

As intercellular carbon and energy storage, polyhydroxyalkanoates (PHA) can be accumulated by a wide variety of microorganisms (Durner et al., 2000). PHAs, which are biodegradable hydrophobic polyesters, have been suggested as alternatives for petrochemical-based plastics in terms of decreasing environmental pollution. Moreover, PHA and other kinds of PHA generated by bacteria have a high molecular mass which is in the range of 50,000 to 1,000,000 Da concerning microorganism and growth conditions. This molecular weight is high enough to have polymer properties that are comparable to traditional plastics (Reddy et al., 2003).

On the other hand, the production cost of these biopolymers is still higher than petrochemical-based plastics. Therefore, as the properties of PHA are combined with

low production costs, they can be compatible with commercial plastics. On the other hand, to obtain the proper PHAs at a reasonable price, their production needs a full understanding of the relevant biosynthetic processes (Kranz et al., 1997).

Poly-β-hydroxy butyric acid (PHB) and poly(3-hydroxybutyrate/3-hydroxyvalerate) (PHB/HV) are two major kinds of microbial PHA that have been reported. The best characterized or known PHA is PHB and PHB/HV is a copolymer of PHB by co-feeding of substrates. It is composed of 3-hydroxybutyric acid and 3-hydroxyvaleric acid (Durner et al., 2000).

In Figure 2.8, an electron microscopy image is given for *R.capsulatus* grown on acetone as a carbon source. PHA accumulates in the cell cytoplasm and generally has a diameter of 0.2-0.5 μm (Sudesh et al., 2000).

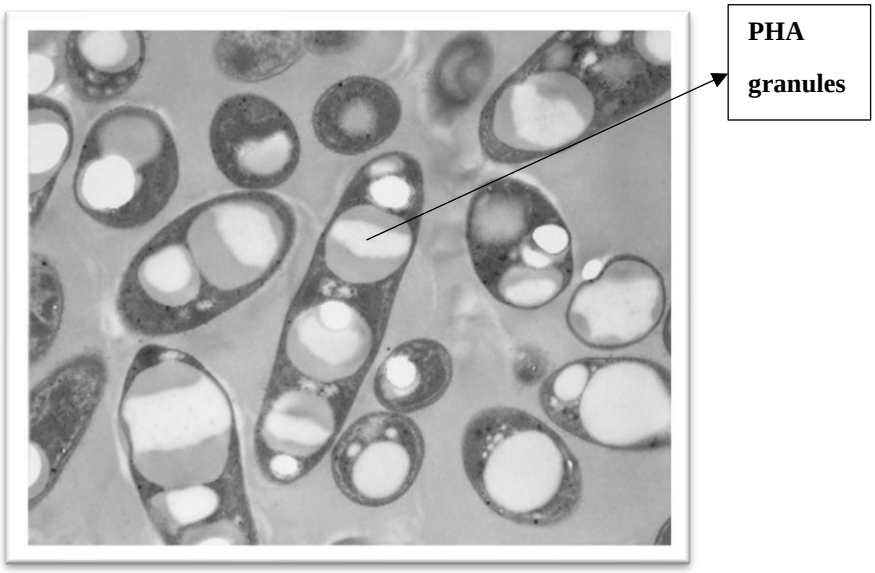

Figure 2.8 The electron microscopy image for *R.capsulatus* (Kranz et al., 1997)

PHB is accumulated as an intercellular reserve compound under limited conditions by a wide variety of microorganisms. Additionally, these limitations are excess

carbon and limited one of the nutrients like phosphorus, nitrogen, oxygen, or sulfur (Vaishnav & Choudhary, 2021). Examples of these microorganism are *Pseudomonas oleovorans, Bacillus megaterium, Alcaligenes eıtrophus, Rhodobacter capsulatus, Rhodopseudomonas palustris, Rhodocobacter sphaeroides,* and *Rhodospirillum rubrum* (Durner et al., 2000; Kranz et al., 1997; Merugu et al., 2012; Sudesh et al., 2000; Vaishnav & Choudhary, 2021).

Table 2.1 depicts the comparison of studies in terms of bacterial strain, carbon sources and concentrations, and PHB accumulation. According to the previous studies, the concentration of carbon substrate has an important effect on PHB accumulation.

Brandl et al. (1991) investigated the influence of different acetate concentrations (10 mM – 250 mM) on the PHA accumulation in *R.sphaeroides* at 30^{o}C temperature, under a continuous light source, and under nitrogen limitation. As a result, between 90 mM and 120 mM was the most optimum acetate range to achieve the maximum PHA accumulation which was 50% dry weight beyond 120 mM acetate concentration. The inhibition effect of the carbon source can be obtained (Brandl et al., 1991).

Another study, which was also based on the effects of different acetate concentrations (10 mM-65 mM) on PHB in *R.capsulatus,* was carried out and the maximum PHB amount, which was 20% of dry cell weight was obtained at 65 mM acetate concentration (Özsoy Demiriz et al., 2019). Moreover, a previous study found that acetate is the most appropriate carbon source for *R.sphaerodies* compared to other organic acids (Hustede et al., 1993). The results showed that the maximum PHB amount was achieved at 30 mM acetate as 70% of cellular dry weight. Even if less acetate concentration (10 mM) was used, the maximum amount of PHB obtained from acetate was still higher than results using other organic acids at higher concentration (30 mM) (Hustede et al., 1993).

Table 2.1 Comparison of PHB amount produced for some strains of bacteria

Bacterial Strains	Source of Carbon with Concentrations	PHB Amount % of DCW	Reference
Rhodobacter capsulatus DSM 1710	65 mM acetate	20.0	Özsoy Demiriz et al., (2019)
Rhodopseudomonas palustris WP3-5	20 mM acetate	10.2	Wu et al., (2012)
	20mM propionate	4.2	
Rhodobacter sphaeroides WT	10 mM acetate	38.0	Hustede et al., (1993)
	30mM acetate	70.0	
	30mM glucose	31.0	
	30mM fructose	27.0	
	30mM succinate	4.0	
Rhodospirillum rubrum	30 mM acetate	53.0	
Rhodobacter sphaeroides O.U.001	30% wastewater	70.4	Yiğit et al., (1999)
Rhodobacter sphaeroides	122 mM acetate	50.8	Brandl et al., (1991)
	7.5 mM malate	1.6	
	82 mM malate	6.4	
	120 mM crotonate	47.5	
Rhodobacter sphaeroides KD 131	40 mM acetate	51.0	Kim et al., (2012)
	60 mM acetate	54.1	

2.5.1 Biosynthetic Pathway of PHB

In the middle of the 1950s and 1960s, preliminary studies on photosynthetic assimilation of organic compounds and PHB metabolism in bacteria has been done first time (Doudoroff & Stainer, 1959). In an excess of carbon sources and a limited growth environment with a low nitrogen source, PHB is synthesized as an intercellular storage compound in the cytoplasm of a cell (Merugu et al., 2012).

There are a minimum of three fundamental enzymes, which are phaA (β-ketothiolase), phaB (Acetoacetyl-coenzyme A [CoA] reductase), and phaC (PHA synthase) during the biosynthesis progress. The scheme of which steps are involved for these PHB enzymes in bacteria is shown in Figure 2.9. On the contrary, phaZ (PHA depolymerase) is responsible for converting PHB to (R)-3-hydroxybutyric acid.

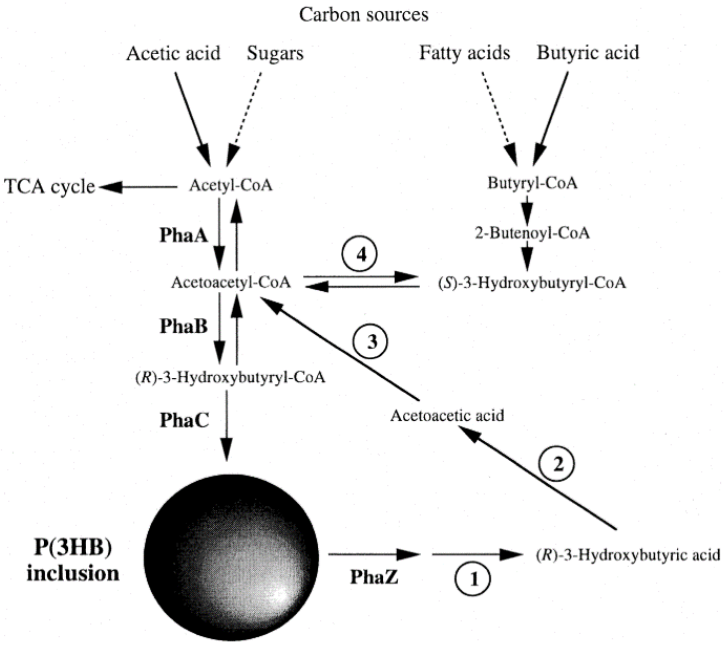

Figure 2.9 PHB synthesis pathways from carbon source in bacteria (Sudesh et al., 2000)

First, PHB can be produced by microorganisms from a variety of carbon sources, including simple carbohydrates, acetic acid, fatty acids, and butyric acid as shown in Figure 2.9. Due to the type of substrates, acetyl-CoA or butyryl-CoA are obtained. Acetyl-CoA can be converted to either acetoacetyl-CoA by phaA reversibly or can be the substrate for the TCA cycle. After that, phaB is responsible for the production step of (R)-3-hydroxybutyryl-CoA. As a final product, PHB accumulates via phaC.

If fatty acids or butyric acids are used as a substrate, butyryl-CoA, 2-butenol-CoA, and (S)-3-hydroxybutyryl-CoA are produced, respectively. After that, (S)-3-hydroxybutyryl-CoA converts acetoacetyl-CoA by NADH-dependent acetoacetyl-CoA reductase (denoted by 4 in Figure 2.9) to achieve PHB accumulation. However, this reaction occurs reversibly.

On the other hand, in addition to the PHB synthesis, PHB can be degraded into (R)-3-hydroxybutyric acid via PHB depolymerase and dimer hydrolase (denoted by 1 in Figure 2.9). After then, 3-hydroxybutyric can be converted to acetoacetic acid by (R)-3-hydroxybutyrate dehydrogenase (denoted by 2 in Figure 2.9) and acetoacetyl-CoA by acetoacetyl-CoA synthetase (denoted by 3 in Figure 2.9) (Sudesh et al., 2000).

Kranz et al. (1997) studied that since PNS bacteria can utilize a wide range of carbon substrates, three genera which are *Rhodopseudomonas, Rhodospirillum, and Rhodospirillum* are to be the starting point for investigating the PHB metabolism (Kranz et al., 1997). According to the results, even if the phaA and phaB enzymes were deleted from the strains, it was found that PHA was accumulated. On the other hand, in the case of phaC deleting strain, PHA was not observed. Consequently, although there are alternative ways for accumulating PHAs without phaA and phaB, phaC has a critical role in producing them (Higuchi-Takeuchi & Numata, 2019). Furthermore, phaC is an active dimer even if the substrate is not available and the activity of phaC increases with the increase in substrate concentration (Higuchi-Takeuchi & Numata, 2019).

2.5.2 The General Properties and Applications of PHB

Despite synthetic polymers such as polyethylene, polyvinyl chloride, and polystyrene being advantageous to use in almost every industry, the damage to the environment is quite high because of difficulties in their recycling and disposal. For this reason, the 100% biodegradability of PHB, which is the most attractive property compared to other plastics, has led to its use in many potential application areas (Reddy et al., 2003). Examples of these areas are listed below (Vaishnav & Choudhary, 2021):

- Agricultural industry
- Packaging industry
- Pharmaceutical fields
- Biomedical
- Bottles
- Coating

Lastly, in addition to the biodegradability property of PHB, it is extremely biocompatible and non-toxic to mammalian tissues. Therefore, PHB can be used as a biomaterial in tissue engineering for surgical implants, wound dressing, biodegradable screws, and staples (Pleissner et al., 2014).

2.5.3 PHB Extraction and Purification

The microorganism used and the extraction method used to separate or extract the biopolymer from the bacterial cells have a significant impact on the cost effectiveness of PHB when PHB is produced on a large scale. Most separation methods for PHB recovery that have been proposed include solvent extraction. Methylene chloride, propylene carbonate, dichloroethane and chloroform are examples for solvents during the solvent extraction (Valappil et al., 2007).

Many researchers consider the use of green solvents for toxicity reduction and environmental benefit. Compared to chloroform and dichloromethane, dimethyl carbonate (DMC), which is one of the non-halogenated solvents, has been accepted as a promising green solvent due to its low toxicity. On the other hand, chloroform and other non-halogenated solvents have higher efficiency (Reis et al., 2020; Manangan et al., 2010).

2.6 Relationship between Hydrogen and PHB Production

The redox power released by the breakdown of the substrate can be shared by many different metabolic pathways. For instance, a high amount of carbon sources and stress conditions such as limited nitrogen sources promote PHB production as well as hydrogen (Özsoy Demiriz et al., 2019). Thus, this relationship between H_2 and PHB has been the topic of previous studies and is still being investigated. This thesis contains work that is essentially a follow up on the results and analysis developed by Özsoy Demiriz (2012). Consequently, her thesis study will be frequently referred to compare and discuss the results in this study.

According to several studies, H_2 and PHB production pathways are competitive with each other as both processes are necessary for dissipating the extra reducing power (Luongo et al., 2017; Yiğit et al., 1999). On the other hand, concomitant H_2 and PHB production has also been obtained in the literature (Corona et al., 2017; Hustede et al., 1993; Policastro et al., 2020; Uyar et al., 2009). Moreover, the relationship

between H_2 and PHB with genetic manipulations has been studied (Kim et al., 2006; Kobayashi & Kondo, 2019; Kranz et al., 1997; Yang & Lee, 2011).

Hustede et al. (1992) studied two bacterial strains (*Rhodobacter sphaeroides* and *Rhodospirillum rubrum*) and their PHB synthesis mutant types of *Rhodobacter sphaeroides* and *Rhodospirillum rubrum* to observe the competition between produced H_2 and PHB accumulation. Results revealed that while competition between H_2 and PHB occurred in *R.sphaeroides* for reducing power, there was no competition between them in *R.rubrum*. It was interpreted that different strains may have different tendencies to accumulate PHB (Hustede et al., 1993). Another similar study also showed the synergetic behavior between H_2 and PHB production (Yiğit et al., 1999).

The experimental study carried out by Polcastro et al. (2020) showed that the relationship between H_2 and PHB production is more complex than just competing for electrons and energy distribution under several operating conditions such as light intensity, pH fluctuation, and nitrogen source (Policastro et al., 2020b). Similarly, Wu et al. (2012) investigated the correlation between biohydrogen and PHB synthesis under suboptimal pH values on acetate by *R.palustris*. Since PHB synthesis provides protection of bacteria from stress, stored PHB led to producing hydrogen in a pH-stress condition. It indicated that bacteria have a complex correlation between H_2 and PHB (Wu et al., 2012). Lastly, 30:30 minutes light-dark cycle promotes both H_2 and PHB production for *R.capsulatus* (Corona et al., 2017).

By genetic manipulations, the comparison of H_2 and PHB accumulation for different strains and their PHB synthase deficient mutant can be interpreted. The study of Kim et al. (2006) worked on *R.sphaeroides* and its PHB synthase deleted (Phb^-) mutant strain. Based on the results, the produced H_2 amount just increased 1.3 times compared to the wild strain. Thus, it can be interpreted that H_2 and PHB metabolism pathways share electrons by considering the slight increase in the amount of H_2. As PHB production stopped, H_2 production increased (Kim et al., 2006). A similar result was found by Yang et al. (2011). phbC gene deleted *Rhodopseudomonas palustris*

was used to enhance the H_2 production and as intended, 1.7 times higher total biogas was collected (Yang & Lee, 2011).

Although there are studies on the production of H_2 by using genetic engineering are in the literature, studies on PHB production by using genetic manipulations are limited in the literature. The first study using genetic manipulation for PHB production used a PHB depolymerase gene-disrupted *R.sphaeroides* strain. The results showed that the mutant strain produced approximately 2.9 times higher volumetric PHB production than the wild strain of it (Kobayashi & Kondo, 2019).

Comparison of wild type (WT) and uptake hydrogenase deficient mutant YO3 (hup^-) strains of *Rhodobacter capsulatus* for H_2 and PHB production was the main objective of this study.

CHAPTER 3

MATERIALS AND METHODS

3.1 The Bacterial Strain

In this study, two strains of *Rhodobacter capsulatus* were used. The wild-type strain of *Rhodobacter capsulatus* (DSM1710) was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany, while the other strain was its mutant *Rhodobacter capsulatus* YO3 (*hup*$^-$) that lacks the uptake hydrogenase enzyme, obtained by genetic modification by Dr. Yavuz Öztürk (GMBE, TÜBİTAK-MAM, GEBZE) from *Rhodobacter capsulatus* MT 1131 (Öztürk et al., 2006).

3.2 Storage and Activation of Bacteria

The bacteria were stored in 30% glycerol in the deep freezer at -80 ^{o}C. To activate the bacteria, the inoculum of bacteria was done in a solid mineral-peptone-yeast extract (MPYE) medium with agar (1.5% w/v, for details, see section 3.3.1) by using a sterile loop. Inoculum on this MPYE agar plates was not only used for activation but also to check for contamination.

3.3 Culture Media

In this study, three different media, MPYE solid medium, growth medium, and hydrogen and PHB production medium were used for different purposes.

3.3.1 Solid Media

MPYE agar medium is a bacterial medium that provides bacteria with a solid surface to grow on. This medium contains yeast extract, bactopeptone, minerals, and agar and its composition is given in Table A.1 indicated in Appendix A. After weighing their required amounts, they were added to 1 liter of distilled water and adjusted to pH 7.5 with 0.5 mM NaOH. The prepared medium was autoclaved (NÜVE OT 90L) for sterilization and then, when its temperature decreased to approximately 40 ^{o}C, it was poured into agar plates. This process was carried out in a laminar flow sterile cabinet (Bilser Biosafety Cabinet) to keep them sterile. After that, the solidified agar plates could be stored in the freezer at +4 ^{o}C.

The inoculation of bacteria, which were kept at -80 ^{o}C, was done on MPYE agar plates. To maintain the temperature constant at 30 ^{o}C, the incubator was used. Under the light, bacteria colonies were visible within 3-5 days, indicating that they were ready to grow in a liquid growth medium.

3.3.2 Growth Media

The growth medium was Biebl and Pfennig (BP) medium, which comprises 20 mM acetate and 10 mM glutamate as carbon and nitrogen sources, respectively (Biebl & Pfennig, 1981) (Table A.2).

To minimize measurement errors, stock solutions of required chemicals were prepared, and their composition is presented in Table A.3. To reach the required concentration of BP medium, the volume taken from stock solutions was calculated (Table A.4). The requisite amount was taken by an automatic pipette and completed to 1 liter with distilled water. Afterward, the pH of the solution was adjusted to 6.3-6.4 with 0.5 mM NaOH. The autoclave was used for sterilization at 121 ^{o}C for 20 minutes. After this step, the temperature of the medium was dropped to room temperature, and vitamins (thiamin, niacin, biotin), trace element solutions, and ferric citrate were added to it in a laminar flow sterile cabinet.

3.3.3 Hydrogen and PHB Production Media

In the hydrogen and PHB production medium, except for the carbon and nitrogen ratio, the same concentration of BP medium was used. The carbon and nitrogen supplies for this medium were 65 mM acetate and 2 mM glutamate, respectively. According to Özsoy Demiriz (2012), this C/N was found as the optimum ratio in which the highest total PHB amount was observed. The required volumes of the chemicals taken from their stock solutions are placed in Table A.5. The initial pH was adjusted to 6.3-6.4 as in the previous study (Özsoy Demiriz, 2012).

3.4 Improvement and Implementation of Gas Collection System

In the previous several studies, the water displacement method was used to collect the produced gas during the experiments (Oflaz & Koku, 2020; Özsoy Demiriz, 2012; Sagir et al., 2017). A gas collection unit and thin plastic pipes are two main components of this method. A 250 mL glass bottle serves as the gas collection unit, and it is connected to the reactor via a thin plastic pipe. The picture of water displacement unit is shown in Figure 3.1.

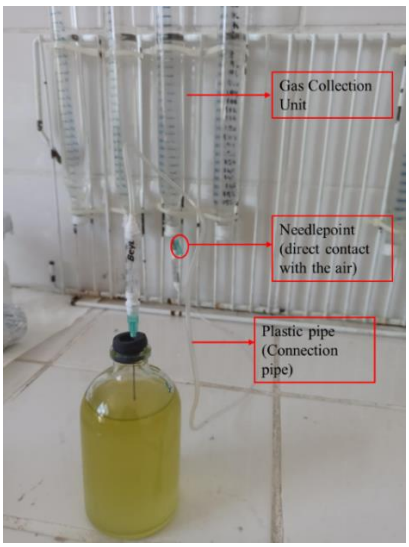

Figure 3.1 The picture of the water displacement unit

For this purpose, the needle tips on both ends of this are sealed using silicone. Further, a needlepoint that is in direct contact with the air connects to the gas collection unit. Thus, the produced gas by the reactor passes through a pipe, and then it is replaced with distilled water. In other words, as much distilled water as produced gas is discharged out from the needlepoint. Consequently, the produced gas volume was checked via this method every day.

Before starting up to experiment, the water displacement units were prepared carefully, and controls were done whether it was working properly or not. In order to check the units, a measured amount of gas was injected into the reactors and the gas collected in the water displacement unit was recorded. During the controls, no problem was detected in the water displacement units. However, even though this method is frequently used, two major obstacles made its usage difficult. First, there was a leak problem caused by the connection points of the gas collection unit, especially during the long-lasting experiments. In the water displacement system, bottlenecks were observed at the needle tips in contact with the air as shown in Figure 3.1. To avoid these problems, all parts of the gas collection system should be checked regularly, and even with backups if possible. As a result, using this method resulted in unreliable gas data and was time-consuming.

Considering these disadvantages of the water displacement method, another approach called the syringe method was used during the experiments in addition to the previous method to avoid its obstacles. To collect the generated gas, syringes with a volume capacity of 50 or 100 mL were used. It is important to mention that before starting the experiment, the syringes should be lubricated with glycerol for easier movement. In the small-scale experiments, 50 mL syringes were sufficient to collect gas data. However, for the larger scale 350 mL volume capacity reactors used in this study, 100 mL syringes (Latex-free siliconized, dicoNEX) were utilized and connected to the reactors all the time. Therefore, more reliable gas data had been obtained, as well as time was saved since it was quite easy to discharge the collected gas from them.

3.5 Sets Configurations and Reactors

In this study, six sets of experiments were conducted in total. The configurations of all sets are given in Table 3.1. For all sets, the temperature, initial pH, light intensity, initial carbon to nitrogen ratios were same. However, the sets differed in either the reactor volume preferred, the gas collection system or batch/fed-batch mode studied.

In Set1 and Set2, the main objective was to compare the H_2 and PHB accumulation in small-scale using *R.capsulatus* WT and YO3 strains. Set2 was a repeat of Set1 and both sets were operated in batch mode and the effective reactor volume was 50 mL. As gas collection system, syringes were used.

In Set3, the aim was to compare gas collection systems which were water displacement and syringe by using only *R.capsulatus* WT. The reactors were operated in batch mode and the effective reactor volume was 100 mL. Figure 3.2 depicts the bioreactors with 50 mL and 100 mL capacities.

The main aim of Sets 4-6 was to investigate and compare the H_2 production and PHB accumulation of two strains of *R.capsulatus* at a larger scale. To scale-up into larger reactor volume, glass and transparent reactors (Gordon's gin bottle, 350 mL) were selected. The effective volume was 350 mL. The picture of this bioreactor is shown in Figure 3.3. In photobioreactors, the light transmittance and even distribution of light are very important. Since the gin bottle was flat, it was sufficient to receive the light. The dimensions of reactor are shown in Figure 3.3. Additionally, this reactor was high temperature resistant for sterilization and economically cheap.

For these anaerobic bioreactors, 21 x 27 diameter and 30 mm height silicon stoppers were selected. The silicon material was flexible enough for stabbing the GC syringe, tight and elastic to prevent gas leakage.

Table 3.1 List of sets operated (for all sets; constant temperature: 30^{o}C, initial pH: 6.3-6.4, light intensity: 2500-2600 lux, initial acetate: 65 mM, initial glutamate: 2 mM)

Set No	Objective	Strains	Modes	Reactor Volume (mL)	Gas Collection Method[a]
1	H_2 and PHB accumulation in small scale	WT &YO3	Batch	50	S (50 mL capacity)
2	Repeat of Set1	WT &YO3	Batch	50	S (50 mL capacity)
3	Comparison of gas collection systems	WT	Batch	100	S (50 mL capacity) and WD
4	H_2 and PHB accumulation in larger scale	WT &YO3	Batch & Fed-Batch	350	WD
5	Repeat of Set4	WT &YO3	Batch & Fed-Batch	350	WD
6	Repeat of Set4	WT &YO3	Batch	350	S (100 mL capacity)

[a] S: syringe, WD: Water Displacement

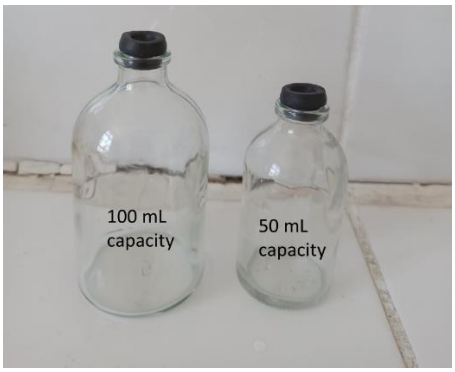

Figure 3.2 The picture of bioreactors with 50 mL and 100 mL capacities

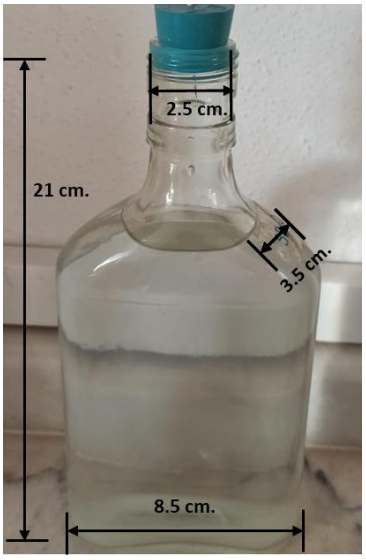

Figure 3.3 The picture of gin bottle with 350 mL capacity

In Set4 and Set5 (repeat of Set4), these bioreactors of 350 mL were operated in both batch and fed-batch modes. In the fed-batch reactors, the aim was to obtain the acetate concentration at 65 mM when the acetate reached to 30 mM, required amount of acetate solution were added and pH was adjusted 6.3. In these sets, water displacement method was used as a gas collection method.

Set6 was a repeat of Set4. However, Set6 differed in operational mode and gas collection method. The reactors were operated at batch mode and syringe were used

as the gas collection system. Compared to other sets, Set6 was the promising experiment due to the fact that the targeted values such as acetate and glutamate concentrations were most optimized.

3.6 Experimental Procedure and Set-up

The followings are the general experimental procedure that was applied similarly in all six sets.

Bacterial Growth

- The bacterial strains were initially kept at -80 ^{o}C in a deep freezer. *R.capsulatus* WT and YO3 strains, were inoculated into a solid agar plate to activate the bacteria. As the bacterial colonies became visible, a colony was chosen and inoculated into Eppendorf tubes which contained the 1.5 mL growth medium described in Section 3.3.2. After 2-3 days, 10% inoculation was done into a growth medium with 15 mL and 50 mL falcons, respectively. After 3-4 days, their dry cell weights were measured and around 0.1 g/L was sufficient to use in the experiment. The calibration curves for both strains, which were also used in the previous studies (Uyar, 2008; Öztürk, 2005), are given in Appendix C.

Bioreactor Set-up

- The reactors used in the sets are mentioned in Section 3.5. The reactor numbers and replicates of reactors in each set are given in Table 3.2.
- To prevent the contamination problem, all materials used in the experiment such as reactors, pipette tips, hydrogen and PHB production media were cleaned with bleach, tap water, and finally distilled water. Additionally, they were autoclaved at 120 ^{o}C, and 20 minutes.

Table 3.2 List of sampling for all sets

Set No	Number of reactors	Reactors [a]	Liquid Sampled reactor [b]	Total Liquid Sample amount (mL) [c]	Sample withdrawn replaced with	Analyses
1	8	4 WT - B 4 YO3 - B	1 out of 4 reactors for each strain	1.5	Argon	Daily gas and liquid PHB (only final day of set)
2	10	5 WT - B 5 YO3 - B	1 out of 5 reactors for each strain	2.0	Argon	Daily gas and liquid PHB (only final day of set)
3	3	1 WT - WD 2 WT - S	All reactors	2.5	Argon	Daily gas and liquid PHB (only final day of set)
4	6	2 WT- B & 1 WT-FB 2 YO3 - B & 1 YO3-FB	All reactors	20	Basal Medium	Daily gas, liquid, and PHB
5	6	2 WT- B & 1 WT-FB 2 YO3 - B & 1 YO3-F	All reactors	20	Basal Medium	Daily gas, liquid, and PHB
6	6	3 WT - B 3 YO3 - B	All reactors	20	Basal Medium	Daily gas, liquid, and PHB

[a] B: batch, FB: Fed-Batch, numbers in this column indicate the number of replicates conducted for each species

[b] Gas analysis was done for all rectors.

[c] 0.5 mL, 1 mL, 1.5 mL, and 15 mL amounts were required amount for pH analysis, cell dry weight analysis, HPLC analysis, and PHB analysis, respectively.

- 10% inoculation was done into a glass reactor containing H_2 and PHB medium, which was mentioned in Section 3.3.3. The schematic diagram of experimental set-up using water displacement method and picture of the experimental set-up with syringe are depicted in Figure 3.4 and Figure 3.5, respectively.
- Water displacement and syringe (50-100 mL capacity) were used in the sets as a gas collection unit. The methods used in each set are shown in Table 3.1.

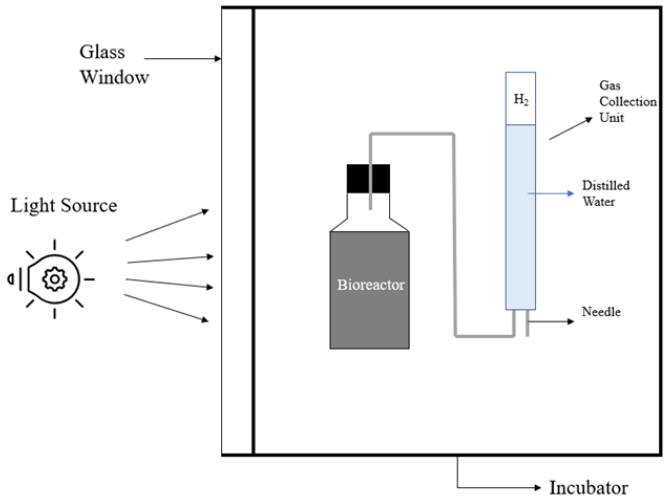

Figure 3.4 The schematic diagram of experimental set-up using water displacement method (Set 4 and 5)

Figure 3.5 The picture of the experimental set-up H_2 and PHB production using syringe method (Set6)

The reactors were operated under the constant operational conditions of 30 ^{o}C and 2500-2600 lux. Moreover, anaerobic conditions were prevailed in each reactor by flushing argon for 4-5 minutes for both strains of *R.capsulatus*.

Sampling for All Sets

The sampling differed in all sets. Reactor numbers, sample amounts, and sampled reactors varied with respect to the sets. The list of sampling for all sets are shown in Table 3.2. Moreover, the details of sampling were mentioned in Results and Discussion part of the relevant sections.

Throughout all sets, gas analysis was daily performed to investigate H_2 production and calculate H_2 productivity (Appendix H). In Set1-3, 1.5-2.5 mL sample was withdrawn from the reactors to the liquid analyses which were pH, OD, and HPLC. For PHB analysis, the liquid sample was withdrawn from all reactors only at the end of the experiment. Additionally, while the liquid sample was withdrawing, gas was injected into reactors as much as the amount of liquid withdrawn to prevent the negative pressure.

In Sets4-6, a 20 mL sample was withdrawn from for each reactor and a 20 mL basal medium, which had no carbon or nitrogen source, was added to prevent the negative pressure in the reactor. 15 mL of 20 mL sample was used for PHB analysis, while the remaining liquid sample was used for pH, OD, and HPLC analyses.

3.7 Analyses

Gas Analysis

The composition of produced biogas was analyzed by gas chromatography every day (GC-Agilent Technologies 6890N). The type of detector and column was a thermal conductivity detector (TCD) and Superco 1010 column, respectively. The temperatures of the oven, injector, and detector were set to 140 ^{o}C, 160 ^{o}C, and 170 ^{o}C, respectively and the flow of carrier gas which was argon was 26 mL/min.

The 100 μL gas sample was taken every day by using a micro syringe (Hamilton 22 GA 500 μL gas-tight syringe). Finally, to find the concentration of the biogas, gas calibration was done and these calibration curves, which are consisting of hydrogen, carbon dioxide, and nitrogen, and sample chromatogram are given in Appendix C-D. The gas calibration was done with the calibration gas, which contains 50% of hydrogen, 30% of carbon dioxide, 10% of nitrogen, and 10% of methane. The gas calibration was done only once throughout all sets.

Organic Acid Analysis

High-Performance Liquid Chromatography (HPLC, Shimadzu LC 20A-Prominence Series) with Alltech IOA-1000 column was used to analyze the concentration of organic acids. To measure organic acids, a UV detector was used. The concentration of the mobile phase was set to 0.85 mM H_2SO_4, using ultra-pure water. The mobile phase was filtered with a cellulose acetate membrane filter (0.45 μm, 47 mm) and sonicated for 1 hour. The flow rate of the mobile phase was set to 0.5 mL/min during the runs. Additionally, the column temperature was set at 66 ^{0}C and brought gradually to its final value. The run time for a sample was 50 minutes. The calibration curves for the organic acids were obtained using analytical grade chemicals. Details of the calibration procedure and the resulting curves are presented in Appendix E.

For organic acid analyses, liquid samples of 1.5 mL volume were withdrawn from reactors and centrifuged at 13,000 rpm for 10 minutes (Eppendorf MiniSpin 5452

Hamburg Microcentrifuge). By pouring supernatant into another Eppendorf tube, the received supernatant was filtered via sterile mixed cellulose ester (MCE) filters (0.22 μm, 25 mm, Millipore). The samples were injected using an autosampler (Shimadzu SIL-10AC) with an injection volume of 20 μL.

pH and Cell Concentration Analysis

The initial pH of reactor contents of all sets was set to 6.3-6.4. During the experimental runs, 2.5 mL of the 20 mL sample withdrawn daily from the reactors was used to analyze with a pH meter (Mettler Toledo MP220). The meter was calibrated regularly using pH buffer solutions at 4.01 and 7.00.

1 mL of the sample withdrawn was also used to measure the dry cell weight. For this purpose, a UV spectrophotometer (Shimadzu UV-1800) was used at 660 nm wavelength, and the calibration curves for both strains are given in Appendix B.

Light Intensity

Throughout the experimental period, the light intensity was checked regularly with a luxmeter (EXTECH HD450). The light intensity was set to 2500-2600 lux under illumination with 100W tungsten lamps.

3.8 PHB Analysis

To determine the PHB amount produced in the biomass, lyophilization, methanolysis, and GC methods were applied as followings (Braunegg, 1978; Özsoy Demiriz, 2012). First, calibration of PHB was done.

PHB Calibration

i. Pure PHB (Chemika, 81329) was weighed as 0.5, 1.0, 2.0, 5.0, and 10.0 mg.
ii. To dissolve the PHB, 2 mL chloroform was used. At this point, it was important to add weighed pure PHB into the chloroform (Isolab, 910.037).

iii. After dissolving them totally, 2 mL of 15% H_2SO_4 acidic methanol mixture was added to them.

iv. Samples, which were placed in total nitrogen test tubes resistant to high temperature and chloroform, a corrosive chemical, were incubated (WTW CR3200) at 100 ^{0}C for 4 hours. Before starting incubation, it was important to close the test tubes tightly and seal with a teflon band. Otherwise, the chloroform was prone to evaporate. At the end of the methanolysis, it was expected to convert PHB to methyl 3-hydroxybutyric acid. According to Braunegg et al. (1978), 4 hours is sufficient to convert the PHB into methyl esters of PHB (methyl 3-hydroxybutyric acid) completely.

v. After completing the incubation period, the samples were cooled down to room temperature, and 1 mL of distilled water was added into the vials. They were shaken for 10 minutes to mix well. Then, two phases were obtained, namely lower chloroform phase and upper water phase. The lower chloroform phase, which contains methyl 3-hydroxybutyric acid, was withdrawn with a 1 mL syringe, and it was filtered through a PTFE hydrophobic filter (0.22 μm and 25 mm).

vi. To measure the PHB concentration, GC (Agilent Technologies 6890N) analysis was used, and its specifications are given in Table 3.3. GC analysis was performed to detect the methyl 3-hydroxybutyric acid obtained by the acidic methanolysis. During the acidic methanolysis, the PHB accumulated in the biomass was converted into methyl esters of PHB (methyl 3-hydroxybutyric acid). Assuming that, all PHB was converted completely after 4 hours of methanolysis (Braunegg et al. (1978), the methyl 3-hydroxybutyric acid measured was related to the PHB amount accumulated. The calibration curve of PHB is given in Appendix F. The PHB calibration was done only once throughout all sets.

Table 3.3 The specifications of GC for PHB analysis

GC-PHB Analysis	
Column	HP-FFAP (30 m x 0.320 mm x 0.25 μm
Column Pressure	6.66 psi
Detector	Flame Ionization Detector (FID)
Carrier Gas	Argon
Gas Flow rate	1.0 mL/min.
Split Ratio	20:1
Detector Temperature	250 ^{o}C
Oven Temperature	70 -160 ^{o}C (Ramped 8 ^{o}C/min until max. temp.)
Back Inlet Temperature	230
Total Run Time	13.3 minutes
Injection Volume	1 μL

PHB Analysis for Bacterial Cell

To analyze the PHB accumulation daily, 15 mL of sample was withdrawn from the reactors every day and centrifuged at 13,000 rpm for 20 minutes (Thermo Scientific SL 16R). Thus, only bacterial pellet without any supernatant was obtained. Immediately after, the samples were placed into freeze dryer (Christ alpha 1-4 LD) for a day. Thus, under low temperature and vacuum, the water in the pellets could be removed. After, weighting the dried pellets, the steps ii-vi given above, in the PHB calibration part were applied. See Appendix G for sample chromatogram. In Figure 3.6, some steps are given.

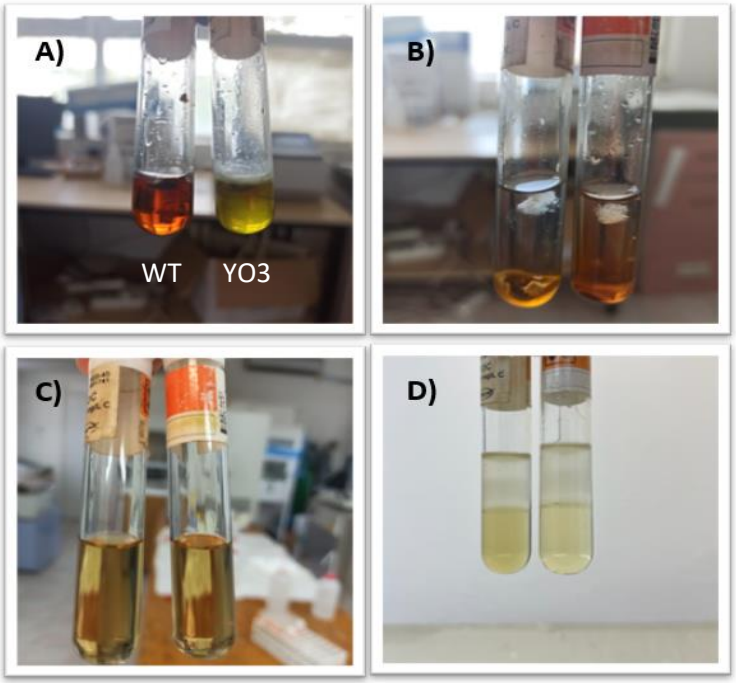

Figure 3.6 The photographs of PHB analysis steps for *R.capsulatus* WT and YO3 strains from left to right for each photos a) chloroform solution containing weighted bacterial pellet b) adding acidic methanol mixture to chloroform solution c) after incubation 100 ºC for 4 hours d) after adding 1 mL distilled water, phase separation

CHAPTER 4

RESULTS AND DISCUSSION

To investigate the correlation between produced H_2 and PHB accumulation by considering the differences in both WT and YO3 strains of *R.capsulatus*, a total of 6 sets were conducted. The list of sets was given in Table 3.1 above, where the objectives, modes of operation (batch or fed-batch), and primary parameters such as reactor volume and substrate concentration are summarized. Additionally, due to observed issues in the gas collection as will be discussed below, two different gas collector systems were utilized and these are also indicated in the table. The results and discussions for these sets are given in the following sections, in the same order as in Table 3.1.

Acetate is the essential metabolic intermediate since acetate can be consumed immediately for the TCA cycle or for storage as PHB synthesis. Thus, acetate is a proper carbon source compared to other organic acids for PHB production (Hustede et al., 1993; Uyar, et al., 2010). Additionally, the initial concentration, as well as carbon source, plays a major role in PHB production. According to Özsoy Demiriz (2012), the highest PHB amount (0.2 g/L) was obtained at 65 mM initial acetate concentration by using the *R.capsulatus* WT strain (Özsoy Demiriz, 2012). Thus, in this study, acetate was used as a carbon source with 65 mM initial concentration in all experiments based on the Özsoy Demiriz (2012)'s study.

PHB accumulation was affected by the initial pH. Khatipov et al. (1998) studied the effects of increasing pH from 6.8 to 7.5 on both H_2 and PHB production for *R.spaeroides*. In the case of using lactate as a carbon source, an increase in pH resulted in a decrease in H_2 production and an increase in PHB accumulation. When the acetate was utilized as a carbon source, both H_2 and PHB production increased with an increase in pH (Khatipov et al., 1998). However, in this study, the initial pH

was arranged to 6.3-6.4 based on the work of Özsoy Demiriz (2012)'s study, where the strain was *R.capsulatus* as in this study.

4.1 Comparison of H_2 Production and PHB Accumulation by *R.capsulatus* WT and YO3 strains in Small Scale (Set1 and Set2)

Set1 and repeated Set1 (Set2) were carried out with *R.capsulatus* WT and YO3 strains using 50 mL reactors. The details of the experimental conditions are given in Table 3.1. The main aim was to analyze produced H_2 and PHB accumulation. In addition to these analyses, pH, optical density (OD), and organic acid analyses were also monitored and recorded.

4.1.1 H_2 and PHB Production in Set1

For each strain, quadruplicate reactors were operated and H_2 analyses were done daily. 2 of 4 reactors for each strain were used to withdraw liquid samples and assumed that all reactors were operated parallel to each other.

Before presenting the results, it should be noted that although the targeted initial amount of acetate was 65 mM, it was analyzed as 40 mM. Thus the results were discussed considering 40 mM initial acetate. This error could have been caused by human error during the experimental set-up or might be calculation error.

The cumulative H_2 production of the YO3 strain was 1.89 times higher than that of WT as shown in Figure 4.1. Similarly, for the YO3 strain, the highest molar H_2 productivity was found as 0.534 mmol/(L.h) and this value was 1.65 times of the productivity of the WT strain.

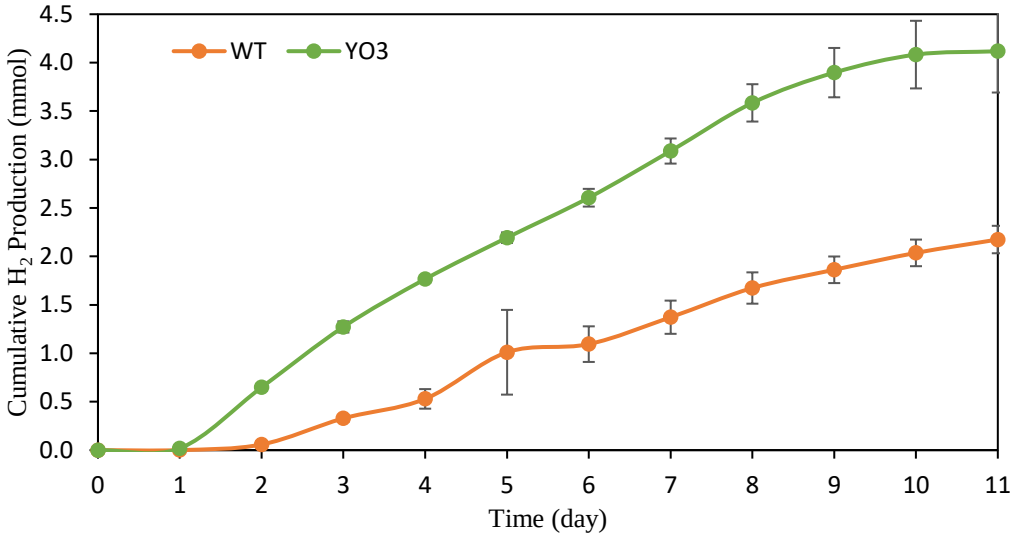

Figure 4.1 Cumulative H_2 production in Set1

It is accepted that the uptake hydrogenase enzyme works antagonistically to nitrogenase, which is a responsible enzyme for H_2 production (Koku et al., 2002). The YO3 strain of *R.capsulatus* lacks the uptake hydrogenase enzyme and so, it is theoretically expected to observe an enhanced H_2 production by YO3 strain under optimum conditions for H_2. In the literature, there was no study using acetate as carbon source by *R.capsulatus* YO3 strain to compare the results of this study. However, there are similar studies using different carbon sources for *R.capsulatus* YO3 strain. For instance, a study showed that the hup^- mutant type of *R.sphaeroides* produced 1.82 times higher H_2 than that of wild type of it with using 30 mM malate as a carbon source (Kim et al., 2006). In Set1, although the targeted amount of acetate in this thesis was selected as 65mM for enhancing the PHB production, the initial concentration in the reactors were approximately 40 mM. The 40 mM initial acetate concentration may have enhanced the H_2 production.

While the cumulative H_2 was found as 43 mmol $H_2/L_{reactor}$ for *R.capsulatus* WT, a study investigated by Özsoy Demiriz (2012), found 92 $H_2/L_{reactor}$ for WT at 50 mM initial acetate (Özsoy Demiriz, 2012). This lower H_2 production in Set1 could be

caused by two reasons. First, the 40 mM initial concentration of acetate in this study, which was less than the amount in Özsoy Demiriz (2012)'s study, could have caused less H_2 production. Secondly, air leakage, which was obtained in some reactors due to human error, could have caused less H_2 production. It should be noted that the liquid sample was withdrawn from reactors by a syringe. After withdrawing the samples, the syringe tip sometimes stuck in the septum and disconnected from the barrel (cylinder part), which caused entry of air into the headspace of the reactor. Consequently, GC results showed that N_2 % content of the reactors was more than 60%. As a result, it may have caused the WT to produce less H_2. The sample headspace gas compositions of a reactor with air leak is shown in Table I (Appendix).

Liquid samples for pH and OD analyses were withdrawn from only 1 of the 4 reactors for both strains. Assuming that all reactors were parallel among themselves, information about pH and OD were obtained. No air leakage was observed in these reactors. The pH change in time is shown in Figure 4.2. It shows a very similar curve to the pH change in observed Özsoy Demiriz (2012)'s study.

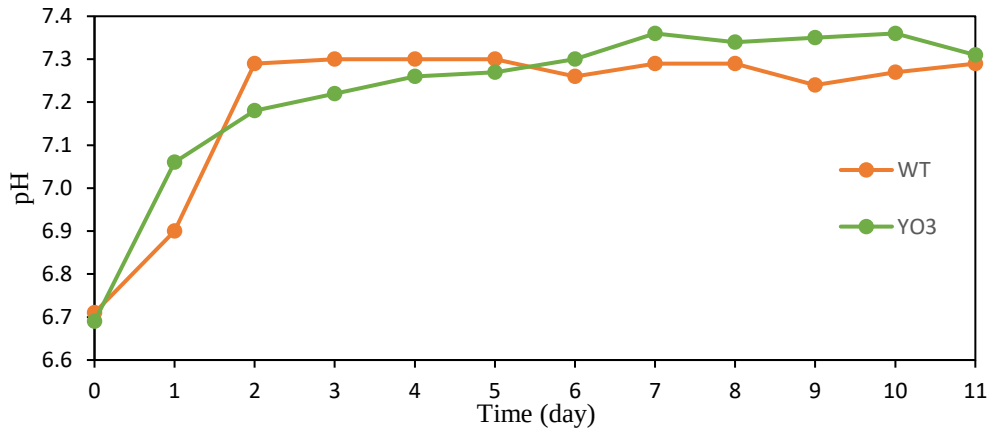

Figure 4.2 The pH variation during Set1

The bacterial growth process includes several phases which are lag, exponential, and stationary phases (Huang, 2013). The growth curves of WT and YO3 strains, which are shown in Figure 4.3, demonstrated two of these phases, namely exponential and stationary. The initial DCW for both strains was around 0.15 g/L, and an exponential phase was obtained until 4th day. After that, the stationary phase was obtained until the end of the experiment. The death phase was not observed since the experiment was ceased on the 11th day due to unexpected pandemic conditions and further lockdown. A lag phase was not observed.

Note that the decrease in DCW for both strains on the 3rd day was caused by human error. The reactor was not well mixed while the liquid sample was withdrawn that day. Therefore, this may have caused a non-uniformity in the sample.

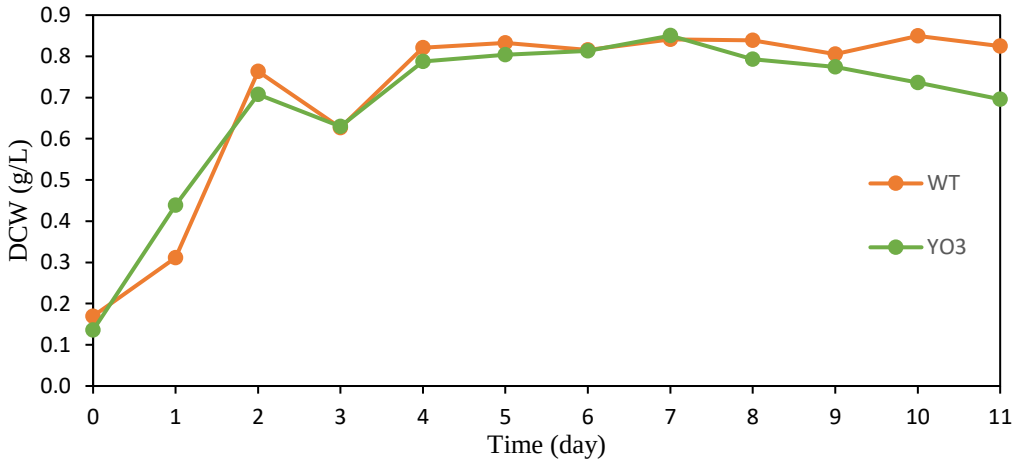

Figure 4.3 The growth curve of WT and YO3 strains in Set1

The results of organic acids are depicted in Table 4.1. The liquid samples for HPLC analysis were withdrawn only at the beginning and end of the experiment. Despite the targeted initial acetate concentration of 65 mM to enhance the PHB production, the initial acetate was found as 40.5 mM and 38 mM in the WT and YO3 reactors, respectively. The effect of the lower acetate concentration than targeted volume on H_2 production was already discussed above (in section with Figure 4.1).

Table 4.1 Concentration of organic acids in the photobioreactors (Day 0 is the start of experiment)

Day	Reactor	Acetic Acid (mM)	Lactic Acid (mM)	Formic Acid (mM)	Propionic Acid (mM)
0	WT	40.5	-	-	0.4
	YO3	37.9	-	-	1.5
11	WT	6.6	-	5.6	2.5
	YO3	4.1	1.3	5.1	1.7

While both lactic and formic acid concentrations were not obtained at the beginning of the Set1, formic acid was found at 5.6 mM and 5.1 mM for WT and YO3, respectively. The lactic acid concentration was found at 1.3 mM for only YO3. Finally, the propionic acid concentration to increased to 2.5 mM and 1.7 mM, respectively for WT and YO3.

Since the HPLC samples were withdrawn just for two days, which were the initial and final days of the experiment, the production and consumption of organic acids, during the experimental period were not observed.

PHB analysis was done at the end of the experiment since the total reactor volume was not high enough to withdraw PHB samples at interval periods. While the PHB% amount of the WT strain was found as 10.1±0.8%, the PHB amount was 11.7±3.7% for YO3 strain at the end of the experiment. In addition to the H_2 productivity of YO3 being around 2 times that of WT, PHB accumulation was also higher in the YO3 strain.

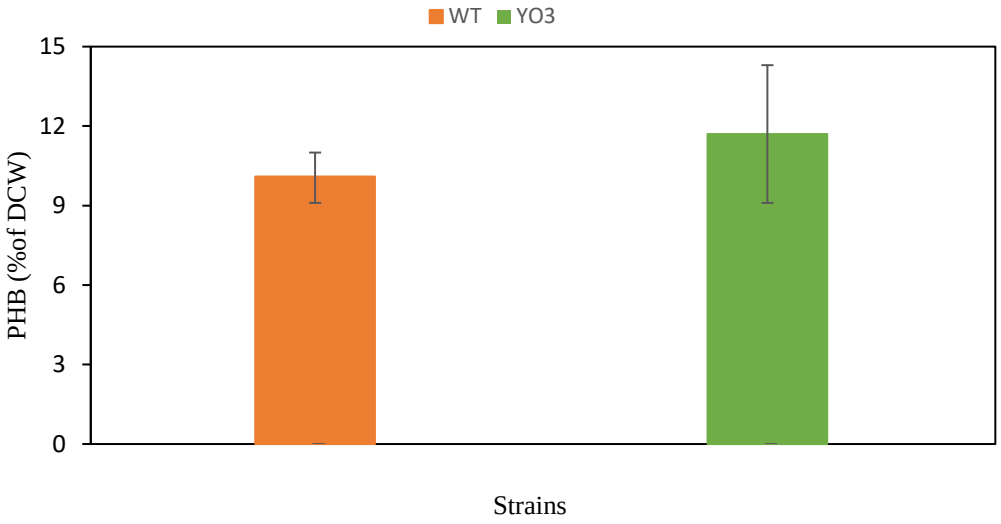

Figure 4.4 PHB accumulation at the final day of Set1

Due to the mandatory COVID-19 curfew during Set1, the reactor operation had to be terminated on the 11th day, despite continuing H_2 production. Thus, H_2 and PHB results were obtained before the total consumption of acetate. Due to these setbacks, Set2 was carried out as a repeat of Set1 to fix the problems mentioned above and to observe H_2 and PHB analyses by extending the operation time.

4.1.2 H_2 and PHB Production in Set2

In this experiment, which is a repetition of Set1, 10 reactors (five replicates for WT, five replicates for YO3 strain) were used and two of the reactors were allocated to withdraw liquid samples. Same as Set1, argon was injected into reactor to prevent the negative pressure while withdrawing liquid samples. The target initial concentration was 65 mM and it was analyzed as 58.1 ± 0.6 mM acetate.

Set2 lasted for 20 days and H_2 analysis was done every day. However, a gas leakage problem occurred at 4 reactors including 2 reactors from which liquid samples were withdrawn. In 2 of these 4 reactors, gas leakage was caused by human error. The

nitrogen amount of reactor headspace were found higher than 60% in the GC analysis. As mentioned in Set1, this error was due to the tip of the syringe getting stuck in the septum and disconnected from the barrel (cylinder part) while the liquid sample was withdrawn. The air was observed in the gas data taken from the GC (Biogas content of reactors is given in Tables I.17-I.26). In the other two reactors, air content was not detected according to GC results, but gas production stopped early after the 8th day. Due to this leakage problem in these reactor, they were not included in the H_2 calculations. The graph of cumulative H_2 production is shown in Figure 4.5. It was found that the cumulative H_2 results were in line with the Set1 results (Figure 4.1). Thus, the results are consistent within themselves.

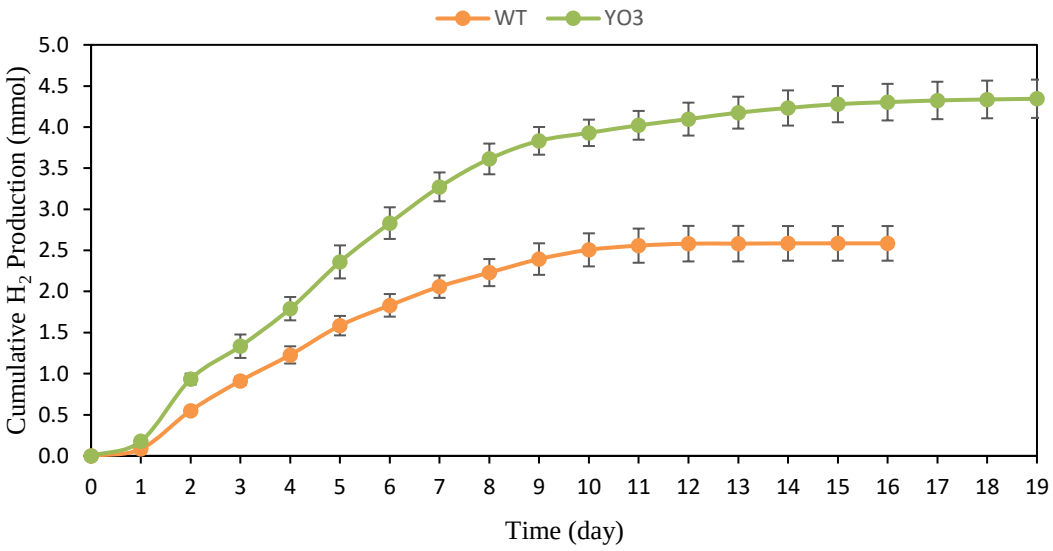

Figure 4.5 Cumulative H_2 production in Set2

The results of cumulative H_2 production for WT were found at 2.2 mmol and 2.6 mmol in Set1 and Set2, respectively on the 11th day and hydrogen production stopped thereafter in Set2. The reason of the cease of H_2 production could be the low amount of acetate in the reactor. According to HPLC analysis results, which are shown in Figure 4.6, there was no acetate on the 14th day. However, since the HPLC samples

were withdrawn within long intervals, the day acetate was consumed might be between 7th and 14th days.

On the other hand, for the YO3 strain, H_2 was produced steadily, albeit in smaller amounts after the 11th day. This could be associated with the availability of the carbon source during the entire set.

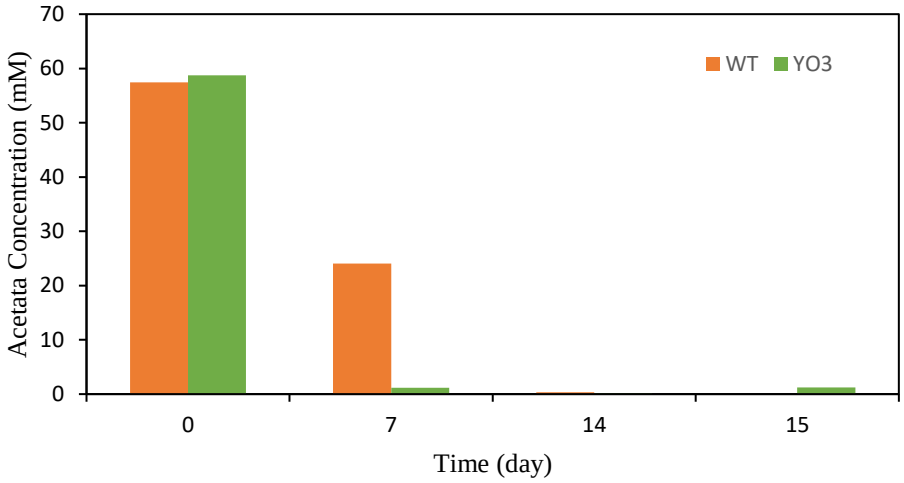

Figure 4.6 Acetic acid concentration at specified time points of Set2

The variation of pH and growth curves for the two strains are given in Figures 4.7 and 4.8, respectively. In Set1, pH was almost constant at 7.3 after the 2nd day for both strains (Figure 4.2). However, in Set2, the pH curve of WT was notably different from Set1 results. After the 2nd day, there was a continuously decreasing trend in pH.

For both Set1 and Set2, the reactors, in which the liquid samples were withdrawn were assumed to be operating in parallel to other reactors of their replicates. However, in Set2, the H_2 trends of reactors, from which liquid samples were withdrawn, were not similar to other reactors. Even if there was no air leakage in the reactors, which were used for taking liquid samples, H_2 production stopped in these

reactors. For this reason, it can be interpreted that the pH curve of WT may not reflect the exact pH change in its replicates reactors.

Compared to Set1, the growth curves of both strains in Set2 displayed consistently higher concentrations. While in Set1, the maximum dry cell weights (DCWs) for both strains were found 0.85 g/L, the maximum DCWs of WT and YO3 were 1.05 g/L and 0.92 g/L, respectively. In the growth curve of Set2, exponential, stationary, and death phases were obtained without lag phase. However, as mentioned in the previous paragraph, the H_2 production stopped in the reactors, which were used for taking liquid samples.

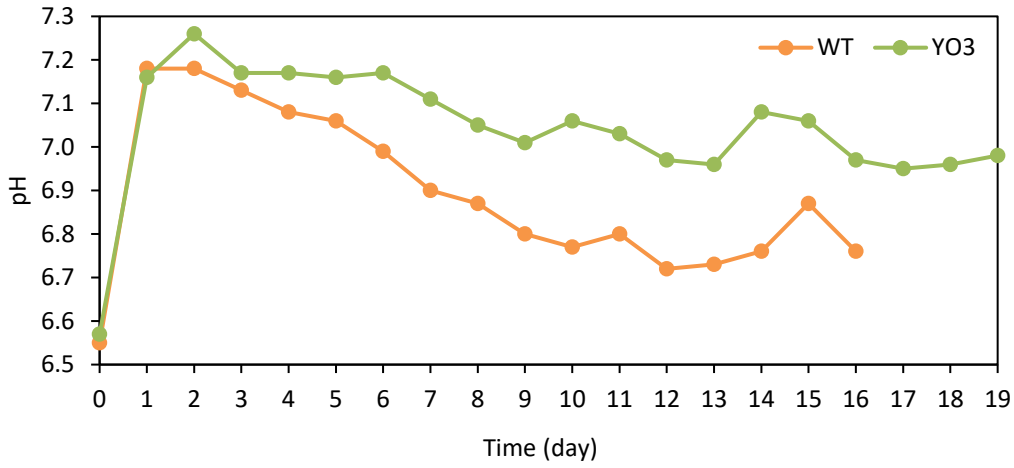

Figure 4.7 The pH variation during operating time in Set2

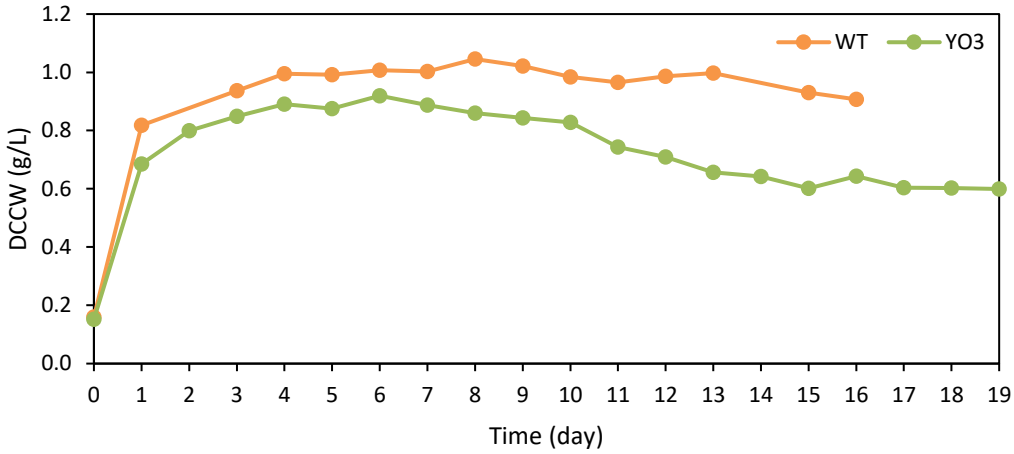

Figure 4.8 The growth curve of WT and YO3 strains in Set2

The PHB samples were withdrawn from all reactors at the end of the experiment. The 17th and 19th days were the final days for WT and YO3 strains, respectively. However, during PHB extraction methanolysis at 100 ^{o}C, most of the YO3 samples were lost and only one sample was measured. The PHB accumulation results for both strains are depicted in Figure 4.9. The PHB results were found at 5.3 ± 0.8% and 5.0% for WT and YO3, respectively.

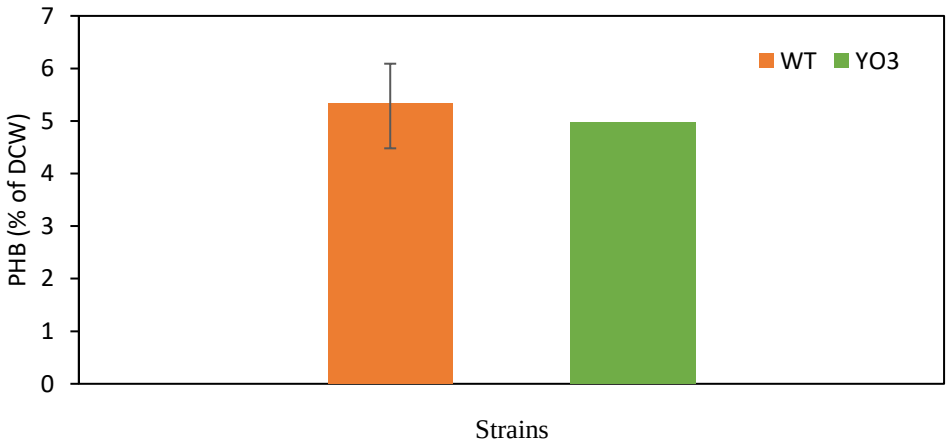

Figure 4.9 PHB accumulation analyzed at the final day of Set2

Compared to Set1 (Figure 4.4), PHB amounts were lower in Set2. It should be noted that Set2 lasted almost 9 days longer than Set1. It was interpreted that PHB, which was analyzed only at the final day of the Set2, decomposed due to the decrease or depletion of the carbon source in the environment.

Throughout Set1 and Set2, where the reactors of 50 mL volume were operated in batch mode using two strains of *R.capsulatus,* daily H_2 analyses were performed yet PHBs were analysed after the cease of the operation. Therefore, the relationship between H_2 and PHB could not be clearly understood from Set1 and Set2. On the other hand, it can be still suggested that the presence of the uptake hydrogenase enzyme has a significant impact on H_2 production. Besides the results of Set1 and Set2 helped provide insight and a deeper understanding and influenced the designs of the following experiments.

It was decided that all gas, liquid, and PHB analyses should be done daily. Thus, the relationships between them should be investigated by determining the H_2 and PHB amount daily. For this purpose, reactor design and investigation were done to scale up the reactor capacity. Besides, the comparison of gas collection methods, which are water displacement and syringe applications, were investigated before scaling up.

4.2 Comparison of Gas Collection Systems: Water Displacement and Syringe Methods

4.2.1 Results and Discussion of Set3

The comparison of H_2 production using water displacement and syringe methods by *R.capsulatus* WT was the main purpose of Set3 (before scaling the reactor's capacity up to 350 mL). When the reactor capacity scales up to 100 mL, the produced gas still will be increased. For this reason, the water displacement method, where higher volumes of gas can be captured and measured, is the most common method for

collecting the produced gas. However, there are bottlenecks of this method. The details of these bottlenecks are given in Section 3.4.

The cumulative H_2 production in mL is shown in Figure 4.10. Figure 4.10 volumes of showed that the syringe method was a more effective method with 1.4 times more hydrogen collected than the water displacement method.

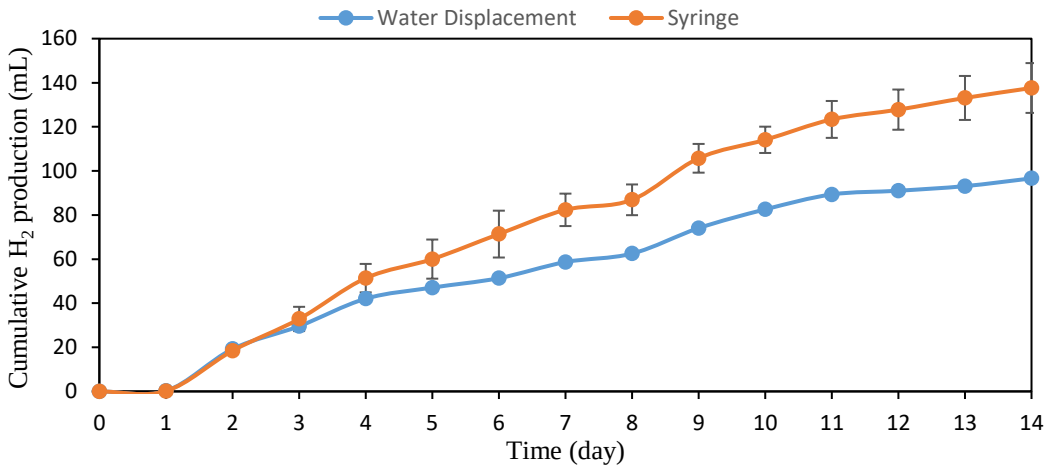

Figure 4.10 The cumulative H_2 production for two different gas collection methods in Set3

In addition to the a comparison of gas collection methods, liquid and PHB analyses were also done in Set3. The initial acetate in the reactors was 57.1 ± 3.7 mM. The 100 mL capacity of reactors was used in Set3, and while withdrawing liquid samples, argon was injected into reactors. The liquid samples were withdrawn from all reactors at certain time intervals and their raw data are shown in Table I.30. At the end of the experiment (16^{th} day), PHB accumulation was found as 11.8 ± 2.0% as the average volume of 4 replicate reactors. Compared to Set1 and Set2, Set3 was more similar to the study of Özsoy Demiriz et al. (2019) with respect to initial acetate concentration and reactor volume. Özsoy Demiriz found that PHB accumulated was 20% of DCW at 65 mM initial acetate with 120 mL capacity reactor after 18 days (at the end of the experiment) (Özsoy Demiriz et al., 2019). However, the PHB accumulation results in reactor content were lower than that of Özsoy Demiriz et al.

(2012)'s results which might be explained due to the scarcity of the available carbon source in reactor content. In this study, according to the HPLC results (Table I.29), the acetate was consumed totally by the 15th day. Since acetate was not analyzed between the 12th and 15th day, the exact day when acetate was consumed is cannot be determined. Therefore, the acetate might have been consumed early, and part of the PHB produced might have been consumed by the microorganism itself till end of the experiment (16th day).

4.3 Comparison of H_2 Production and PHB Accumulation (Set4 and Set5)

Since analyzing PHB only at the end of the previous experiments (Sets1,2, and 3) was not sufficient to understand the relationship between produced H_2 and PHB accumulation, three additional experiments were designed to analyze gas, liquid, and PHB daily. Since the PHB sample should be at least 15 mL to obtain enough biomass concentration for analysis, the capacity of 50 mL and 100 mL of the reactors were not sufficient enough for daily PHB analysis. For this purpose, different reactor designs on a large scale (capacity of 350 mL-750 mL) were investigated. Consequently, 350 mL capacity of glass, transparent, and resistant to high-temperature gin bottles were selected as bioreactors (Figure 3.3). These reactors were also proper economically compared to other reactor designs.

By scaling up to 350 mL of reactor volume, 20 mL of daily samples were withdrawn from all reactors and 15 mL of liquid was used for PHB analysis. The rest of them were used for the pH, OD, and HPLC analyses.

Results and Discussion of Set4 and Set5

Set4 was carried out as explained in Table 3.1 by WT and YO3 strains of *R.capsulatus* in both batch and fed-batch modes. In total, 6 reactors were operated and 4 of 6 reactors were operated in batch mode (2 replicate reactors for WT, 2 replicate reactors for YO3). The other 2 reactors were operating in fed-batch mode (1 reactor for WT, 1 reactor for YO3). All gas, liquid, and PHB analyses were done

daily. The average initial concentration of acetate was analyzed as 108.5 ± 18.7 mM. This amount of acetate was much more than the targeted (65mM value).

Although it was found in Set3 that the syringe was a more effective method for gas collection than water displacement method, the water displacement method was used for the collection of biogas in Set4 and Set5. The reason for it was that the capacity of the syringe was only 50 mL and the theoretical gas production was found to be higher than the 50 mL in the first few days. For this reason, water displacement was used as a gas collection method. However, air leakage was again obtained because of this method. According to the GC results, there was no air in all reactors. Thus, the leakage problem was caused only by the water displacement unit. The locations of these leaks are explained in detail in Section 3.4. Thus, the H_2 production data did not give reliable results, being probably lower than the actual amount of H_2 produced.

In Set 4, the air leakage was obtained in the water displacement devices operating for both batch and fed-batch reactors. Additionally, for fed-batch reactors, the main aim was to analyze the PHB production by achieving a constant acetate concentration of 65 mM after each daily feeding. For this purpose, when the acetate concentration was dropped to 30 mM, carbon and nitrogen feeding were done. However, the pH of feeding was not arranged to 6.4. Thus, the pH in the reactor decreased sharply. As a result, there was no H_2 production and PHB enhancement. The sharp decrease in pH affected the reactor negatively.

By considering all these problems which were high initial acetate concentration, leakage in the water displacement device, and a decrease in pH suddenly, the Set4 was repeated as the next experiment. In Set5, the average initial acetate concentration was found 69 ± 8.2 mM. Additionally, while feeding reactors, which were carried out in fed-batch mode, the pH of the feeding medium was set to 6.4 by 0.5 mM NaOH. It was observed that PHB accumulation averaged at 12% of DCW for both strains from the 4^{th} day to the 26^{th}. It showed that PHB accumulation was done under excess carbon and limited nitrogen source. On the other hand, the leakage problem

in water displacement equipment still continued and the collected gas data were not reliable to interpret.

In conclusion, since the collected gas data were not reliable in Set4 and Set5 because of leakage problems in water displacement equipment, the results of these sets were not presented in this thesis. In Appendix I, the raw data and figures of Set4 and the graph of H_2, pH, PHB, and acetate are given in Tables I.2 - I.5. The raw data and figures of Set5 are presented in Tables I.6-I.10.

4.4 Discussion of Relationship with H_2 and PHB Production via Different Two Strains of *R.capsulatus* (Set6)

4.4.1 Results and Discussion of Set6

According to the Set3 result, the syringe was a more effective method for gas collection method rather than water displacement. However, in Set4 and Set5, the water displacement method was used. The reason for this was the insufficient syringe volume. The syringe volume was 50 mL and theoretically, produced gas was expected to be more than 50 mL daily at the beginning of the experiment with increasing total reactor volume. Therefore, a 50 mL syringe for gas collection could not be used. Thus, 100 mL syringes (Latex-free siliconized, dicoNEX) were procured and used in Set6. The results of Set6 showed that there was no air leakage caused by syringes. In Tables I.10 and I.11, the gas composition graphs for two strains are presented, typically. Set6 was carried out by triple reactors for two strains of *R.capsulatus* in a batch mode. The H_2 productivities daily for both strains are shown in Figure 4.11.

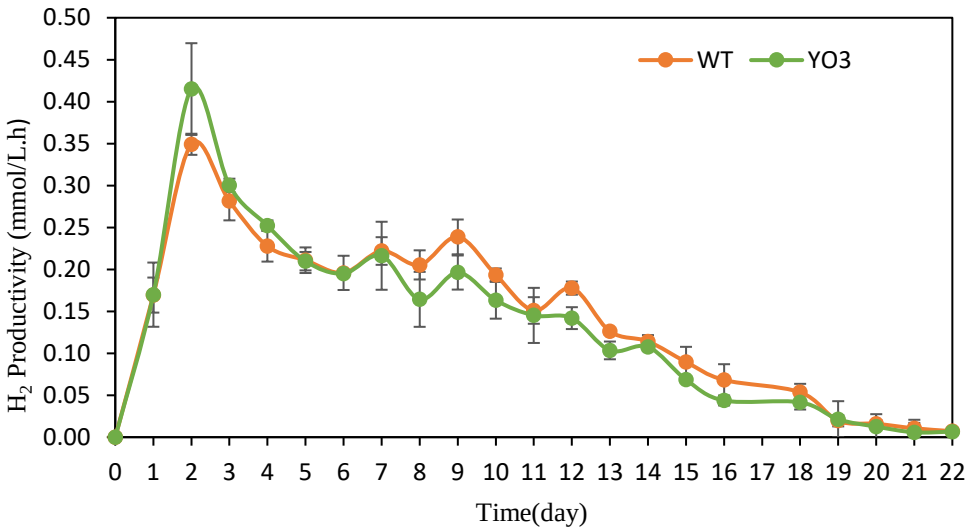

Figure 4.11 The graph of hydrogen productivities for both strains in Set6

The maximum H_2 productivity of YO3 was higher than that of WT on the 3rd day. However, the trend of H_2 productivities for both strains is quite similar. Furthermore, the gas results, which are shown in Table 4.2 as a comparison for both strains showed that the WT strain produced more H_2 cumulatively.

Table 4.2 Comparison of gas production results for both strains [a]

Gas Results	*R.capsulatus* WT	*R.capsulatus* YO3
Cumulative Gas Production (mL)	800 ± 27.8	725 ± 58.5
Cumulative H_2 Production (mL)	615 ± 17.4	573 ± 38.4
Cumulative H_2 /Reactor Volume(mmol $H_2/L_{reactor}$)	71 ± 2.7	66 ± 4.4
Max. H_2 Productivity (mmol/L.h)	0.35 ± 0.05	0.41 ± 0.01

[a] Average initial acetate concentration 60.3 ± 3.2 mM

In the literature, there was no study using acetate and analyzing comparing both H_2 and PHB production via *R.capsulatus* YO3. For this reason, it is quite limited to compare the results with similar studies in the literature with respect to H_2 and PHB production. The previous studies focused on increasing the H_2 since the YO3 mutant lacks the uptake hydrogenase enzyme, which catalyzes the produced H_2. For instance, Öztürk et al. (2006) reported that maximum H_2 production rate increased 1.4 and 1.3 times by *R.capsulatus* YO3 (hup^-) using 15 mM malate as a carbon source (Öztürk et al., 2006). Similarly, it was found that the maximum productivity of the YO3 strain was 1.2 times higher than that of WT. However, in this thesis study, the results were higher for the WT based on cumulative H_2 as shown in Table 4.2.

The high carbon to nitrogen ratio (C/N) and targeted 65 mM acetate, which were arranged to enhance the PHB production, may have caused the YO3 strain to produce less H_2. Androga et al. (2011) studied the effects of C/N on H_2 production on a large scale by using *R.capsulatus* YO3. 40 mM acetate and 4 mM glutamate (C/N=20) were found to be the optimum concentrations between 40-80 mM acetate and 2-4 mM glutamate to achieve the highest H_2 productivity (0.66 mmol/L.h) (Androga et al., 2011). In conclusion, compared to Androga's study, 65 mM acetate may be a high concentration to increase the H_2 production for YO3 strain, explaining that this WT strain produced more H_2.

The self-shading of cells frequently decreases light penetration and so, lowers H_2 production (Ma et al., 2012; Oh et al., 2011). For this study, it was found to be the opposite case for both strains. In this study, while taking samples daily, the 20 mL basal medium was injected into reactors. Thus, dilution was obtained by decreasing the bacterial concentration over the operating time. There has been a noticeable change in color from dark to light colors. Thus, it is suggested that the H_2 production was enhanced by preventing the self-shading of the cells. Similarly, according to the

study conducted by Oflaz (2019), it was found that the cumulative H_2 production increased after dilution.

Regarding the literature, there is no study comparing *R.capsulatus* WT and YO3 with respect to PHB production. However, considering *R.capsulatus* WT strain, Özsoy Demiriz (2012) reported, the cumulative H_2 productivity as 0.35 mmol/L.h at 65 mM initial acetate concentration by the 19th day. Similarly, in this study, it was found as 0.35 mmol/L.h in this thesis for WT strain, too. As a conclusion, the H_2 production results are comparable to that of Özsoy Demiriz (2012)'s results.

4.4.1.1 The pH results for R.capsulatus WT and YO3 in Set6

Figure 4.12 depicts the pH change over the operational time for both strains.

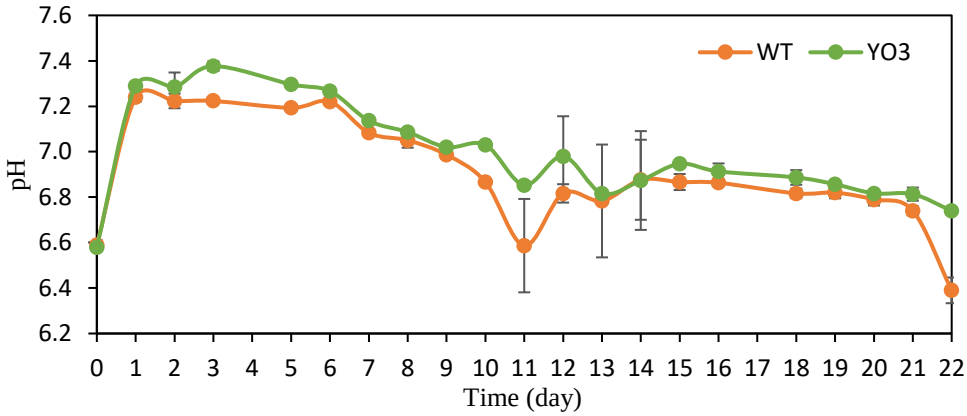

Figure 4.12 pH change over time in Set6

As a result of fermentation, organic acids are accumulated such as lactic, propionic, butyric, and formic acid. These fermentation products can cause the decrease in pH of the reactor medium. As shown in Figure 4.12, a decrease in pH might be caused by the accumulated organic acids in the reactors as also observed in this study (Section 4.4.1.4). On the other hand, during the experimental period, the liquid samples were withdrawn daily in 20 mL and replaced with a 20 mL basal medium

which had no carbon or nitrogen source as mentioned before. For this reason, the Set6 was carried out for long period (22 days), and adding basal medium led to the dilution of reactor content.

4.4.1.2 The Growth Curve Results for *R.capsulatus* WT and YO3 in Set6

The graph of growth curves for both strains is shown in Figure 2.13 and as seen in Figure 2.15, until the 3rd day, the bacterial growth phase was an exponential phase. After exponential phase, the stationary phase was not occurred clearly and the death phase has occurred instead of stationary phase. As mentioned in Section 4.4.1.1, the dilution effect might be affected the growth phases in the reactors.

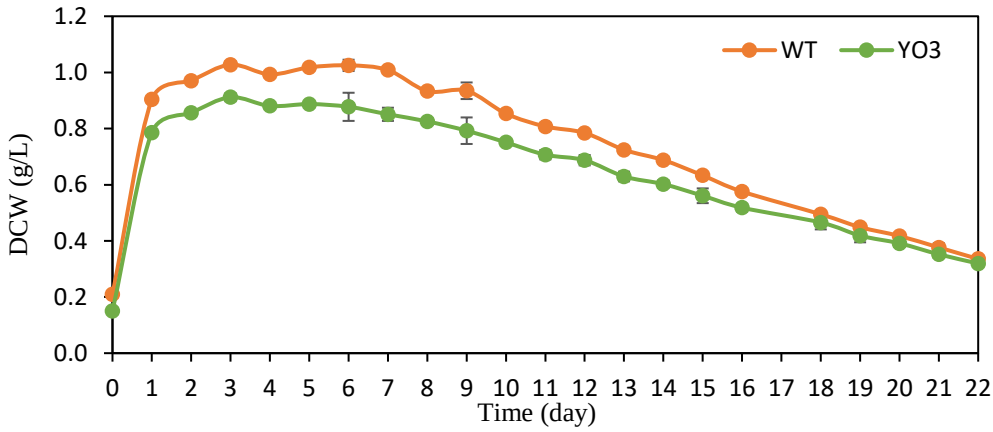

Figure 4.13 Growth curve for both strains in Set6

4.4.1.3 Comparison of PHB Production for two different strains of *R.capsulatus* in Set6

During the experiment, the biomass sample was daily withdrawn for PHB analysis from each reactor and for both strains daily. The sample volume was 15 mL, and this amount was sufficient to analyze them. In Figure 4.14, the results of PHB % of dry cell weight (DCW) are given as a bar graph and errors show the maximum and minimum values for them.

The maximum PHB amount of WT was obtained at 16.0% of DCW on the 3rd day, while it was obtained as 17.2% of DCW on the 6th day for YO3. After that, there was a decrease in the PHB amount until the 10th day for both strains. However, there was an increase in PHB accumulation for YO3 between days 10-11, 12-13, 15-16, and 19-20. For the WT strain, there was no fluctuation like the YO3 strain since increases in PHB amount were quite low. It can be speculated that the increase in PHB amount for YO3 might be caused by consumption of the organic acids produced by photofermentation such as iso butyric acid (see Section 4.4.1.4). As would be discussed in the following part, HPLC results showed that the concentration changes in isobutyric acid (Figure 4.21) were only for YO3. Reddy et al. (2020) found that butyric acid is a suitable carbon source for PHB production as well as acetate. Moreover, as mentioned in Section 2.5.1., although the acetate is directly converted into acetoacetyl-CoA to produce PHB, butyric acid is converted indirectly. Consequently, it can be interpreted that the YO3 strain of *R.capsulatus* accumulated more PHB than WT strain even if the operating conditions were the same and it can be related to the consumption of isobutyric acid, speculatively.

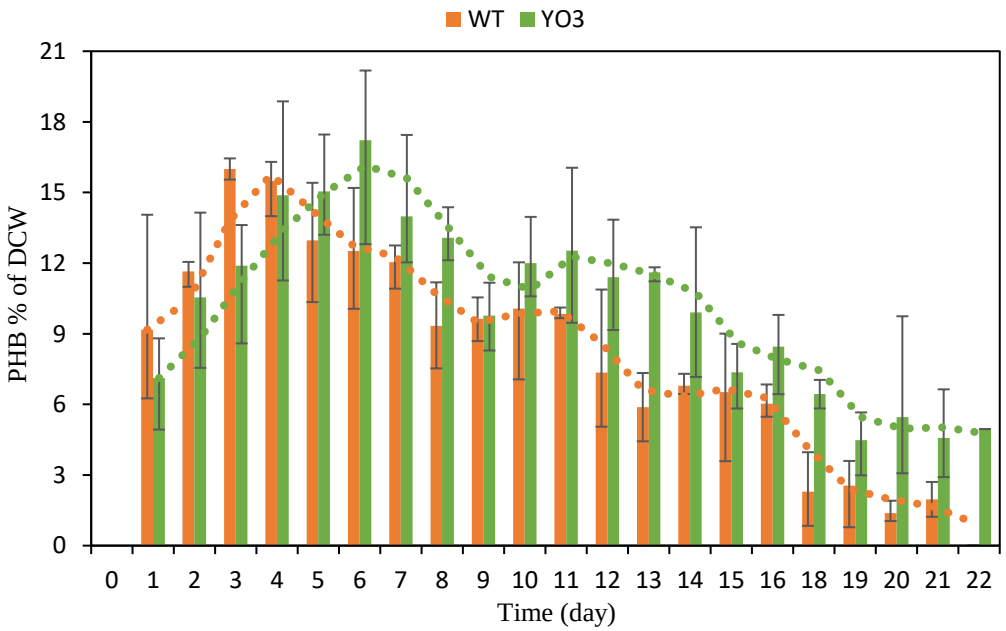

Figure 4.14 Comparison of PHB accumulation daily for both strains in Set6. Dotted lines indicate the average values

Doi et al. (1992) found that the rate of PHB degradation was roughly 10 times slower than the rate of its production. Similarly, the Set6 results showed that the PHB production rate was so high at the beginning of the experiment. However, the degradation of it occurred slowly compared to PHB synthesis.

It can be suggested that the effect of the presence of the uptake hydrogenase enzyme on the PHB production was not understandable directly from the results under the conditions studied. On the other hand, it can be interpreted speculatively. Theoretically, if a particular mutation suppresses hydrogenase synthesis, the mutant might be able to create more H_2 under optimum conditions for H_2 production. (Ooshima et al., 1998). However, according to the H_2 result in Set6, the WT strain of *R.capsulatus* produced more H_2 cumulatively than the YO3 strain, despite higher H_2 productivity being obtained for the YO3 strain (Table 4.2). As discussed before, production of lower H_2 by YO3 strain was caused by excess carbon source, which was selected considering the optimum conditions for PHB production, not H_2. Thus, it can be speculated that the metabolism of YO3 may be shifted to PHB accumulation instead of more H_2 production at that the average initial concentration of 61 mM. As a result, more PHB accumulation was obtained in the YO3 strain compared to WT.

This thesis includes the first investigation of both H_2 and PHB production for *R.capsulatus* YO3 using acetate as a carbon source. Consequently, this study cannot be comparable with the literature directly. On the other hand, for R.capsulatus WT strain, Özsoy Demiriz et al. (2019) studied the effect of initial acetate concentration. Therefore, the summary of results and its comparison with the study of Özsoy Demiriz et al. (2019) are shown in Table 4.3.

Özsoy Demiriz et al. (2019) found that the PHB accumulation was 20% of DCW on the 19th day (end of the experiment) for *R.capsulatus* WT. On the other hand, in that similar study, the maximum PHB amount for the WT strain was found 16% of DCW on the 3rd day. Compared to Özsoy Demiriz (2012)'s study, the method of PHB analysis was not similar even if the initial concentration of acetate was the same (Figure 4.14).

Table 4.3 The summary of the results of this study and that of Özsoy Demiriz et al. (2019)

	This study (Set6)		Özsoy Demiriz *et al.*, 2019
Strains	*R.capsulatus* WT	*R.capsulatus* YO3	*R.capsulatus* WT
Operation mode	Batch	Batch	Batch
Reactor Volume (mL)	350	350	120
Carbon & Nitrogen Source and Concentration (mM)	65 mM acetate and 2 mM glutamate	65 mM acetate and 2 mM glutamate	65 mM acetate and 2 mM glutamate
Operation Time (day)	22	22	18
Cumulative H_2 production (mmol $H_2/L_{reactor}$)	71	66	96
Maximum H_2 productivity (mmol/L.h)	0.35	0.41	0.35
Maximum bacterial concentration (g/L)	1.03	0.91	0.85
Maximum PHB Amount of DCW	16	17	20

4.4.1.4 Rates of H_2 production, PHB accumulation, and Acetate Consumption for Both Strains of *R.capsulatus* in Set6

To understand the relationship between H_2 production, PHB accumulation, and acetate utilization, Figures 4.15 and 4.16 was drawn for WT and YO3, respectively.

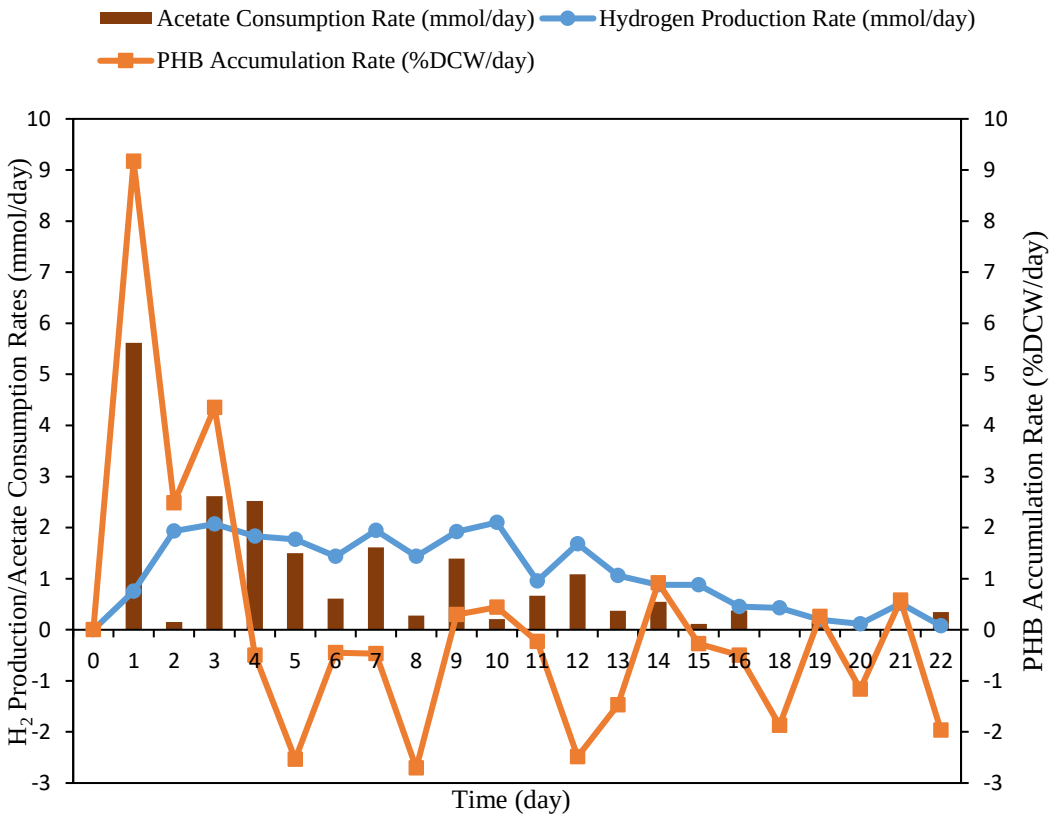

Figure 4.15 The graph of relationship between produced H_2 rate, PHB accumulation rate, and acetate consumption rate daily for *R.capsulatus* WT

On the first day of Set6, the acetate consumption rate, and H_2 and PHB production rates for WT were the maximum amounts. Until the 3rd day, H_2 and PHB rates continued to increase and reached their maximum values. After the 3rd day, both H_2 and PHB production rates fluctuated. The negative amount for PHB accumulation rate indicated that the PHB degradation occurred on these days. Throughout Set6, the accumulation rate of PHB increased, remained constant, and decreased. In the

case of comparison of H_2 and PHB production rates, it was found that until the 6th day, both production rates were directly proportional to each other. While H_2 and PHB production rates increased in the first 3 days, both rates, H_2 being slightly decreased in parallel between the 3rd and 6th days. Additionally, the acetate concentration decreased to about 20 mM from the initial concentration of 65 mM acetate by day 6 (Figure 4.17). With the decrease in the amount of carbon source in the medium of the reactor, both H_2 and PHB production rates decreased compared to the first 3 days of Set6. However, after the 6th day, the relationship between H_2 and PHB production rates was both inversely and directly proportional. It can be speculated that the reason for the increase in H_2 and PHB after the 6th day can be explained by the consumption of fermentation products such as lactic and formic acids.

Table 4.4 indicates the increase and decrease with respect to H_2 and PHB production/consumption rates and acetate consumption rate for a previous day. Table 4.4 is used for making it easier to follow their relationship with respect to the days.

H_2 and PHB production rate was directly related until the 6th day as shown in Table 4.4. While both rates increased together in the first 3 days, it was seen directly that both decreased in the next 3 days. In the table, the time intervals, which are marked with a star (*) represent the peaks of H_2 productivities for the WT strain. There were 4 peaks in total. In the 1st and 3rd peaks, which were on the days between 1-2 and 8-9, the relationship between H_2 and PHB was directly proportional. In the 2nd peak, while the H_2 production rate increased, the PHB production rate remained constant. Thus, it can not be concluded that there is direct correlation between them. However, an inverse relationship has been observed for H_2 and PHB production in the last peak (11th -12th days).

Table 4.4 The increase and decrease in rates of H_2 production, PHB accumulation and acetate consumption in time intervals for *R.capsulatus* WT [a]

Time Interval (day)	H_2 Production Rate (mmol/day)	PHB Accumulation (DCW%)	Acetate Consumption Rate (mmol/day)
0-1	+	+	+
1-2[b]	+	+	-
2-3	+	+	+
3-4	-	-	0
4-5	-	-	-
5-6	-	-	-
6-7[b]	+	0	+
7-8	-	-	-
8-9[b]	+	+	+
9-10	+	+	-
10-11	-	-	+
11-12[b]	+	-	+
12-13	-	-	-
13-14	-	+	+
14-15	0	-	-
15-16	-	-	+
16-18	0	-	-
18-19	-	+	-
19-20	-	-	-
20-21	+	+	-
21-22	-	-	+

[a] negative (-) sign represents the decrease in rates of H_2 and acetate, and PHB degradated, positive sign (+) represents the increase in H_2 and acetate rates, and PHB accumulated, and zero (0) sign represents no changes in rates for the previous day.

[b] Representing peaks of H_2 productivites for *R.capsulatus* WT in Set6

In conclusion, by considering the daily H_2 production, PHB accumulation, and acetate consumption rates for *R.capsulatus* WT, there was a complex correlation between them. For WT strain, when there was an available excess carbon source (20 mM-65mM), there was a directly proportional relationship between the H_2 and PHB production. However, when the acetate concentration decreased to low values of acetate (such as level of 20 mM in this study), a complex correlation has been found.

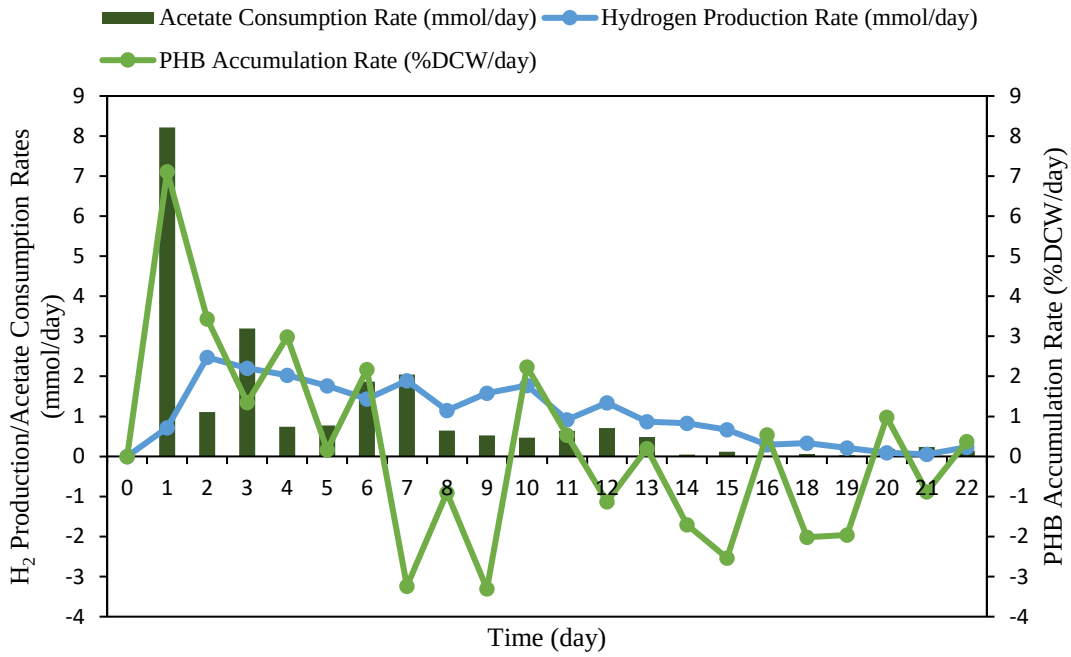

Figure 4.16 The graph of daily relationship between produced H_2 rate, PHB accumulation rate, and acetate consumption rate for *R.capsulatus* YO3

Like WT strain, the rates of H_2 production, PHB accumulation, and acetate consumption reached the maximum point on the 1st day of Set6 for *R.capsulatus* YO3. However, the H_2 production rate decreased and PHB accumulation rate increased between the 2nd and 7th days. After the 6th day, the relationship between H_2 and PHB was reversed most of the time. Table 4.5 indicates the H_2 production rates, PHB accumulation rates, and acetate consumption rates.

Table 4.5 The increase and decrease in rates of H_2 production, PHB accumulation and acetate consumption in time intervals for *R.capsulatus* YO3 [a]

Time Interval (day)	H_2 Production Rate (mmol/day)	PHB Accumulation Rate (DCW%)	Acetate Consumption Rate (mmol/day)
0-1	+	+	+
1-2	+	+	-
2-3	-	+	+
3-4	-	+	-
4-5	-	+	0
5-6	-	+	+
6-7	+	-	0
7-8	-	-	-
8-9	+	-	0
9-10	+	+	0
10-11	-	+	+
11-12	+	-	+
12-13	-	+	-
13-14	-	-	-
14-15	-	-	+
15-16	-	+	-
16-18	-	-	-
18-19	-	0	-
19-20	-	+	-
20-21	-	-	-
21-22	-	+	-

[a] negative (-) sign represents the decrease in rates of H_2 and acetate, and PHB degradated, positive sign (+) represents the increase in H_2 and acetate rates, and PHB accumulated, and zero (0) sign represents no changes in production or consumption rates for the previous day.

There is no study in the literature about enhancing the PHB production by *R. capsulatus* YO3. Several studies in the literature investigate the optimum conditions to improve the H_2 production by *R. capsulatus* YO3 strain.

Since *R. capsulatus* YO3 strain was deleted from the uptake hydrogenase enzyme, the studies found that YO3 produces more H_2 than WT strain under optimum H_2 production conditions. However, there was no study in the literature covering the conditions in this thesis. Considering the studies, which used other types of carbon sources such as 15 mM malate, 2 mM-10 mM molasses, and 5 mM sucrose, the initial amount of 65 mM acetate might be much higher than in other studies for enhancing the H_2 production. Thus, as shown in Table 4.5, after the 2^{nd} day, there was usually a decrease in H_2 production.

Between the 9^{th} and 10^{th} days, there was an increase in both H_2 and PHB production rates. It can be speculated that the reason for this increase could be caused by consumption of the isobutyric acid, as discussed before (Figure 4.21). From the HPLC results, isobutyric acid was obtained for only the YO3 strain. Consequently, it can be concluded that the correlation between H_2 and PHB production was reversed for *R.capsulatus* YO3. Finally, the results of acetate consumption rate do not indicate a clear correlation between the carbon source utilization and PHB production.

4.4.1.5 Production and Consumption of Organic Acids in Set6

R.capsulatus, which is a subclass of PNS bacteria, has the ability to both produce and consume organic acids such as lactic acid, formic acid, propionic acid, and butyric acid as a result of their metabolism. Hence, this process may cause complexity to their metabolic pathways such as H_2 production and PHB accumulation (Oflaz & Koku, 2020).

Figure 4.17 depicts the consumption of acetic acid with respect to time for WT and YO3 strains of *R.capsulatus*. The trend of consumption curves for both bacterial

strains was similar to each other. While the acetate was consumed completely for the *R.capsulatus* WT on the 22nd day, nearly 0.2 mM acetate remained in 65 mM acetate-containing media for the *R.capsulatus* YO3 on the 22nd day. The acetate consumption results do not show a direct relationship between the use of carbon sources and the H_2 production or synthesis of PHB (Section 4.4.1.4) . The small differences in the initial acetate concentration between the reactors, which are shown in Figure 4.17 with the error bar, may also affect both H_2 and PHB production.

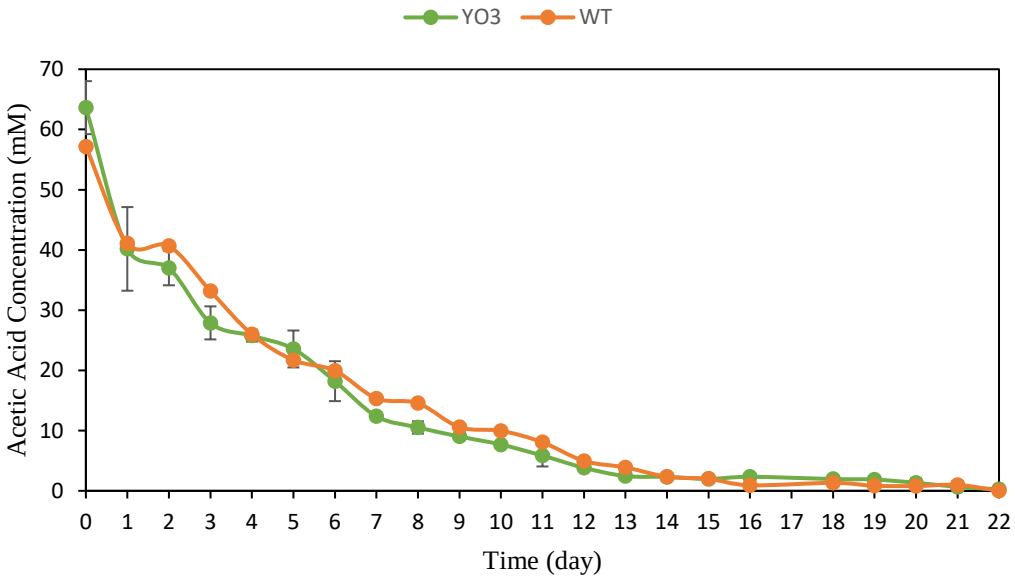

Figure 4.17 Acetic acid consumptions for both *R.capsulatus* WT and YO3 strains in Set6

As a result of the photofermentation processes of *R.capsulatus* WT and YO3, organic acids, which are lactic acid, formic acid, propionic acid, and isobutyric acid were produced and consumed as shown in Figures 4.18-4.21, respectively.

While the maximum lactic acid concentration was 1.6 mM for the WT strain, 2.0 mM was found as the maximum lactic acid for the YO3 strain. Moreover, it is seen from the graph of lactic acid concentration that the fluctuations were obtained for both strains. These fluctuations may have been due to both being produced and

consumed during the fermentation process. Since there was no study using acetate by *R.capsulatus* YO3, the comparison between produced organic acids and literature can not be done.

On the other hand, compared to the study of Özsoy Demiriz (2012), which was investigate the effect of initial acetate concentration on H_2 and PHB production by using *R.capsulatus* WT strain, the maximum lactic acid concentration of this study was 3.2 times higher than that of Özsoy Demiriz (2012)'s results. The reason for this difference could be caused by the taking liquid sample methods. A small volume of liquid samples was withdrawn every 2 days in Özsoy Demiriz (2012)'s study. On the other hand, in this study, the liquid sample was withdrawn for 20 mL daily so, this amount of sample could be caused a disturbance in the reactor.

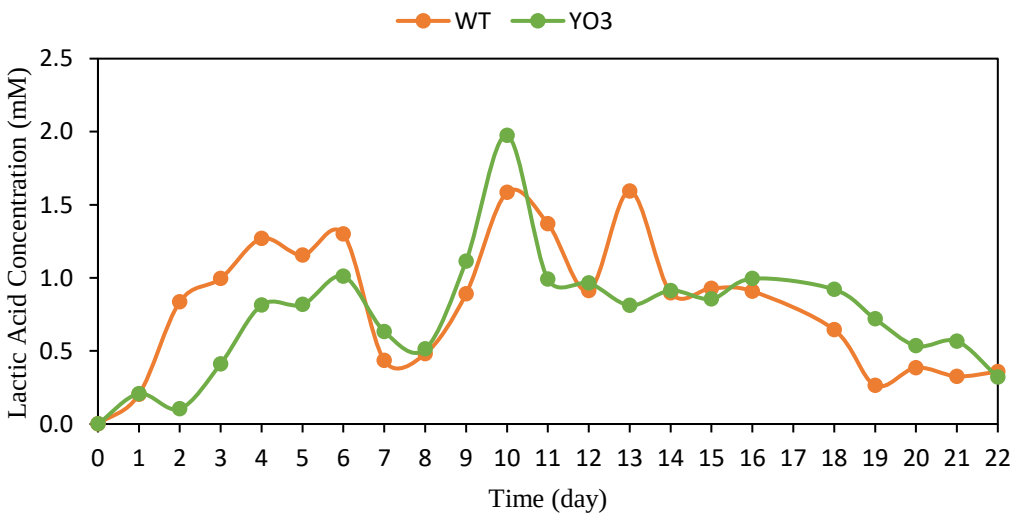

Figure 4.18 Lactic acid concentration for both *R.capsulatus* WT and YO3 strains in Set6

In Figure 4.19, the concentration changes of formic acid are shown for both strains. It is seen that the trends of formic acid concentrations for WT and YO3 strains were quite different. Moreover, there were fluctuations in the concentrations for formic acid like all produced organic acids in Set6.

The results of propionic concentration changes were quite different compared to Özsoy Demiriz's study for WT. It was found that no propionic acid was produced on the first day of the Özsoy's experiment, and 0.2 mM was found as the maximum concentration of propionic acid (Özsoy Demiriz, 2012). Additionally, although in a study that was quite different from the experimental conditions in this thesis, the propionic acid concentration was obtained between about 1 mM and 10 mM for 15 C/N using *R.capsulatus* YO3 (Oflaz, 2019).

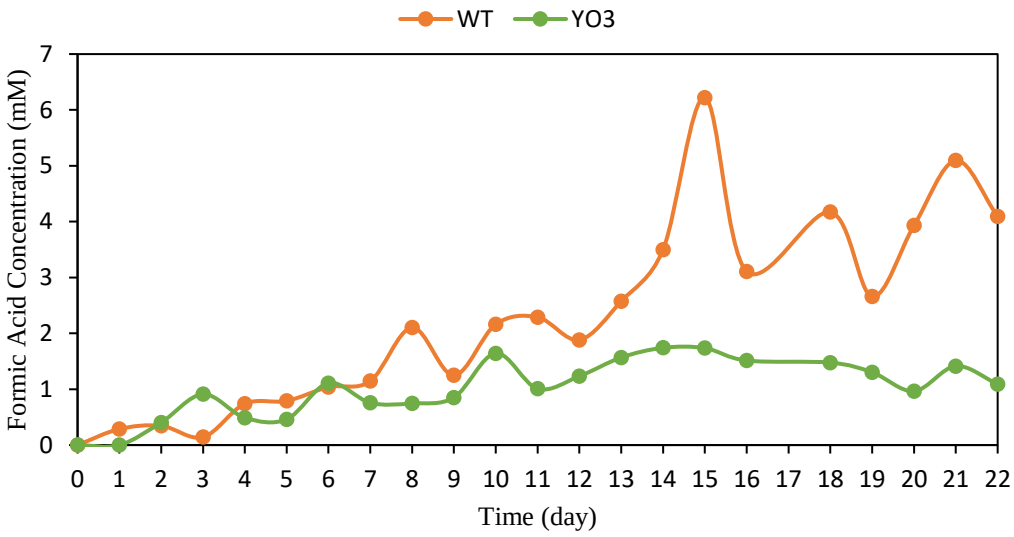

Figure 4.19 Formic acid concentration for both *R.capsulatus* WT and YO3 strain in Set6

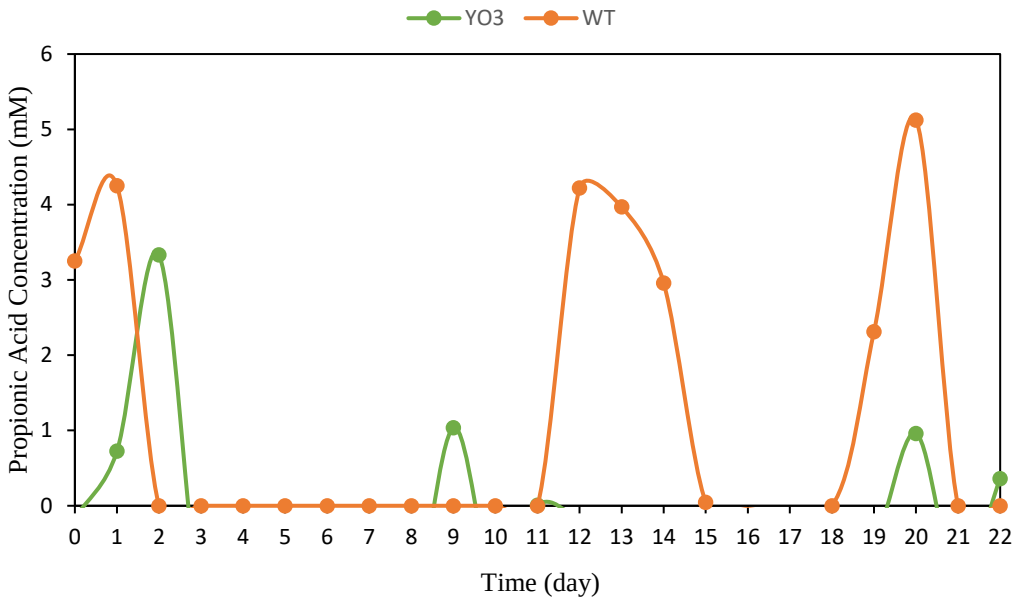

Figure 4.18 Propionic acid concentration for both *R.capsulatus* WT and YO3 strain in Set6

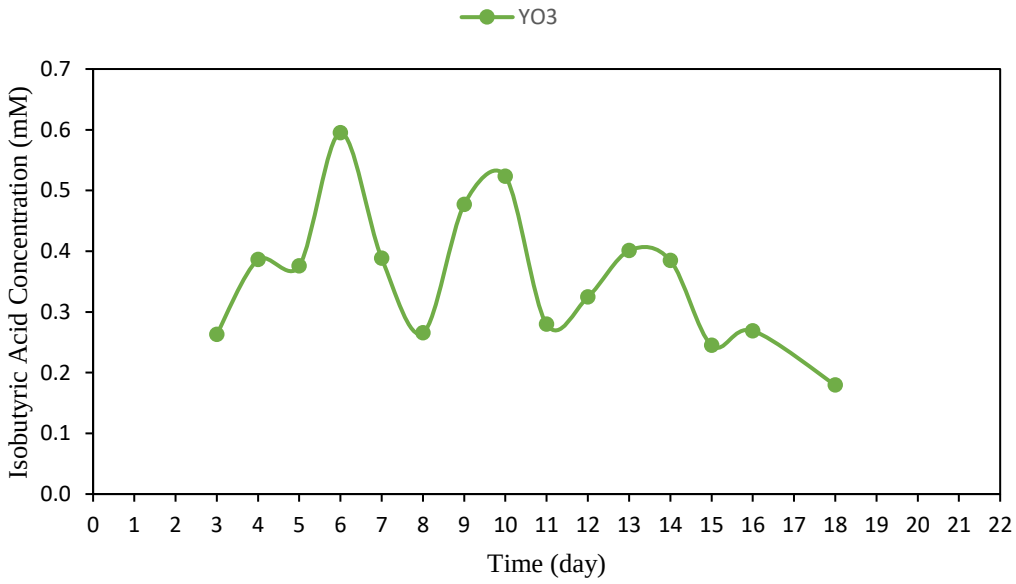

Figure 4.19 Isobutyric acid concentration for only *R.capsulatus* YO3 strain

The fluctuation on the isobutyric acid was obtained for only *R.capsulatus* YO3 with respect to the time in Figure 4.21. It can be speculated that PHB accumulation for this strain was higher than WT since the isobutyric acid was consumed. The fluctuations of isobutyric acid concentrations as shown in Figure 4.21 supported this speculation.

In conclusion, taking a high liquid sample volume for analyses in Set6 may have had a disturbance effect on the reactor and so, the results of produced and consumed organic acids fluctuated for this reason.

4.5 The Summary of Discussion for All Sets

In total, 6 Sets were operated, and the details of the experimental conditions are given in Table 3.1.

First, Set1 was operated in batch mode using 50 mL reactors to compare the H_2 production and PHB accumulation by *R.capsulatus* WT and YO3 strains, which was one of the aims of this thesis. During Set1, some problems were obtained. Air entered some reactors because of a human error made while taking the liquid samples. GC analyses also show high nitrogen percentages. Additionally, the targeted initial acetate concentration was 65 mM to enhance the PHB accumulation. However, the acetate concentration was at 25 mM lower than the targeted value. The results showed that the cumulative H_2 production of the YO3 strain was approximately 2 times higher than the WT strain. Lower initial acetate concentrations than 65 mM could have enhanced the H_2 instead of PHB production. On the other hand, the PHB analysis, which was done at the end of the experiment (11^{th} day) showed that the PHB accumulation was higher in the YO3 strain.

By considering the initial concentration less than targeted and the air leakage caused by human error, Set1 was repeated in Set2. In Set2, the initial acetate concentration was found at the targeted concentration (65 mM). However, the human error was repeated while withdrawing liquid samples and caused the errors in the gas data.

Additionally, similar to Set1, the PHB samples were withdrawn on the final day of the experiment since the reactor volume of 50 mL was not sufficient for the PHB samples daily. Sufficient biomass is needed for PHB analysis. Consequently, it was planned to increase the reactor volume to analyze the amount of PHB not only on the last day of the experiments but also on the previous days.

The main purpose of Set3 was to compare the efficiency of collecting produced gas by two different gas collection systems which were water displacement device and syringe with 50 mL capacity. Thus, before scaling up to the higher volume of reactors, Set3 was conducted. The results of high cumulative H_2 production showed that the gas collection was collected more efficiently with the syringe method.

Set4 and Set5 were designed to investigate the relationship between H_2 production and PHB accumulation daily in batch and fed-batch modes. Set5 was a repeat set of Set4. Among the reactor designs, gin bottles, which are glass, transparent, flat, and resistant to the high temperature for sterilizing with a 350 mL capacity were chosen as the reactors, both economically cheaper and sufficiently to receive the light. However, although the syringe method was a more efficient method for gas collection found in Set3, the water displacement method was applied for both Set4 and Set5. The reason for this was the theoretical gas to be produced being higher than 50 mL and the capacity of 50 mL syringes was not enough to collect that amount of gas. Yet, the air leakage in the water displacement was observed very often so, the gas data for both Sets 4 and 5 were not taken into account because of not reliable in interpreting the results for H_2 and PHB.

The goal for operating fed-batch reactors in Sets 4 and 5 was to keep it constant at 65 mM by adding acetate and glutamate when the acetate level dropped to 30 mM. However, there was a sharp pH drop since the pH of the feeding media was not adjusted to 6.5 in Set4. Thus, the sharp pH drop affected the fed-batch reactors negatively, and then, the PHB was degraded. On the other hand, the pH of the feeding media was arranged to 6.5 and the PHB results showed that PHB accumulation continued as long as there was a high amount of acetate in the medium in Set5.

In Set6, two reactor types were operated as triple for both strains of *R.capsulatus,* only in batch mode. The initial acetate concentration was 65 mM and the trends of H_2 productivities were so similar for both strains. While the H_2 produced by both strains was almost the same, the PHB amount was higher for the YO3 strain.

YO3 strain is a mutant with the uptake hydrogenase enzyme deleted and thus the produced H_2 volume was expected to be in higher amount compared to the WT strain under the optimum conditions for H_2 production. However, in Set6 there were stress conditions and so, this could have resulted in production of more PHB by YO3 strain. Additionally, the relationship between H_2 and PHB production was investigated for both strains with respect to their production rates. Until the acetate concentration in the medium was less than 20 mM, H_2 and PHB production was directly proportional for the *R.capsulatus* WT strain. For the *R.capsulatus* YO3 strain, it was found that H_2 and PHB production was usually inversely proportional.

CONCLUSIONS AND RECOMMENDATIONS

The main aim of this study was to compare the productions of hydrogen and PHB via two different bacterial strains, which were *R.capsulatus* WT and YO3 (hup^-). For this purpose, several experiments were operated to reveal the relationship between H_2 production and PHB accumulation. According to the results and discussions, conclusions are given in the following text:

- The initial acetate concentration affected the H_2 production for both strains. Although the targeted initial acetate concentration was 65 mM in all Sets, the initial acetate concentration was found at 40 mM in Set1. It was found that the cumulative H_2 production of the YO3 strain was 1.9 times higher than the WT strain. When the initial acetate concentration was 65 mM in Set6, the cumulative H_2 production for both strains was nearly equal to each other.
- Since the 50 mL reactor volume was not sufficient to take daily PHB samples, 350 mL capacity of glass, transparent, and resistant to high-temperature gin bottles were suitable for taking samples daily by *R.capsulatus*.
- Compared to the water displacement method, the use of syringes with 50 mL and 100 mL volumes was a more efficient method for collecting produced gas from bioreactors.
- The PHB results of reactors, which were operated at fed-batch modes, showed that the PHB accumulated as long as excess carbon was provided in the media of reactors.
- The DCW results for both strains in Set6 showed that taking a daily 20 mL liquid sample from the 350 mL reactor and then, adding basal medium without carbon and nitrogen source into the reactors to prevent negative pressure had a dilution effect on the system.
- The concentrations of produced organic acids fluctuated with respect to time. This fluctuations showed that organic acids, which are lactic acid, formic

acid, propionic acid, and isobutyric acid were both produced and consumed, in addition to the decrease in time due to the dilution effect.

- The maximum PHB accumulation of dry cell weight for both strains were obtained of different days of the operational period. The maximum PHB amount of WT was 16.4 ± 0.5% on the 3rd day, as it was found 20.2 ± 2.9 % for YO3 strain on the 6th day of Set6.
- The PHB accumulation rate was higher than the PHB degradation rate.
- The acetate consumption rate, H_2 production rate, and PHB accumulation rate reached the maximum on the first day of Set6 for both strains.
- For WT strain in Set6, there was a directly proportional relationship between H_2 and PHB production at higher than 20 mM acetate concentration. Low acetate amount (less than 20 mM in this study) caused a complex correlation between H_2 and PHB for *R.capsulatus* WT strain.
- For the YO3 strain in Set6, there was a generally reverse relationship between H_2 and PHB production.

Based on these conclusions, recommendations are done for future works. The detailed effect of acetate concentration on H_2 and PHB production capacity of both *R.capsulatus* WT and YO3 strains could be investigated. PHB sample volume could be optimized by operating at higher reactor volumes such as 1L-2L to prevent the dilution effect. Furthermore, the H_2 production and PHB accumulation could be investigated by using molasses, which contains 50% of sucrose for *R.capsulatus* YO3 (hup$^-$) in the fed-batch mode. As found in this thesis, the high amount of carbon source and limited nitrogen source maintained the PHB accumulation. Since molasses is a by-product of the sugar industry, using molasses as a carbon source may give the advantage to decrease the cost in the large scale.

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APPENDICES

A. Composition of the Media

Table A. 1 Composition of MPYE, solid medium

Compound	Concentration	Unit
Yeast extract	3.00	g/L
Bactopeptone	3.00	g/L
$MgCl_2$	0.32	g/L
$CaCl_2$	0.15	g/L
Agar	15.0	g/L

Table A. 2 Composition of Biebl and Pfennig

Compound	Concentration	Unit
KH_2PO_4	3.00	g/L
$MgSO_4.7H_2O$	0.50	g/L
$CaCl_2.2H_2O$	0.05	g/L
Na-Glutamate	1.85	g/L
Acetate	1.15	mL/L
Vitamin Solution (10X stock solution)	0.10	mL/L
Iron Citrate (50X stock solution)	0.50	mL/L
Trace Elements (10X stock solution)	0.10	mL/L

Table A. 3 Concentrations of Stock Solution

Compound	Concentration	Unit
KH_2PO_4	30.0	g/L
$MgSO_4.7H_2O$	50.0	g/L
$CaCl_2.2H_2O$	5.00	g/L
Na-Glutamate	37.2 (650 mM)	g/L
Acetate	1.15 (200 mM)	mL/L

Table A. 4 Composition of BP medium taken from stock solutions (20 mM acetate and 10 mM glutamate)

Compound	**Volume Taken from Stock Solution (mL)**	**Final Concentration**
KH_2PO_4	100.0	3.0 g/L
$MgSO_4.7H_2O$	10.0	0.5 g/L
$CaCl_2.2H_2O$	10.0	0.5 g/L
Na-Glutamate	50.0	20 mM
Acetate	30.8	10 mM

Table A. 5 Composition of hydrogen and PHB production medium taken from stock solutions (65 mM acetate and 2 mM glutamate)

Compound	**Volume Taken from Stock Solution (mL)**	**Final Concentration**
KH_2PO_4	100.0	3.0 g/L
$MgSO_4.7H_2O$	10.0	0.5 g/L
$CaCl_2.2H_2O$	10.0	0.5 g/L
Na-Glutamate	100.0	20 mM
Acetate	10.0	10 mM

After preparing the BP medium or hydrogen and PHB production medium from stocks, prepared medium is autoclaved at 121 ^oC for 20 minutes. After sterilization, 0.1 mL vitamin solution (from 10X stock solution), 0.5 mL iron citrate (from 50X stock solution), and 0.1 mL trace elements (10X stock solution) are added into the medium.

Table A. 6 Composition of trace element solution (10X)

Compound	**Amount**	**Unit**
$ZnCl_2$	700	mg/L
$MnCl_2.4H_2O$	1000	mg/L
H_3BO_3	600	mg/L
$CoCl_2.\ 6H_2O$	2000	mg/L
$CuCl_2.\ 2H_2O$	200	mg/L
$NiCl_2.\ 6H_2O$	200	mg/L
$Na_2MoO_4.2H_2O$	400	mg/L
HCl (25% v/v)	10	mL/L

Table A. 7 Composition of vitamin solution (10X)

Compound	**Amount**	**Unit**
Thiamin chloride hydrochloride	5.0	g/L
Niacin (Nicotinic acid)	5.0	g/L
D+ Biotin	0.15	g/L

5 g ferric citrate was dissolved in 100 mL distilled water for preparing ferric citrate solution (50X). Trace element and ferric citrate solution (50X) were sterilized by autoclaving and stored at +4ºC.

B. Calibration Curves of the Dry Cell Weight

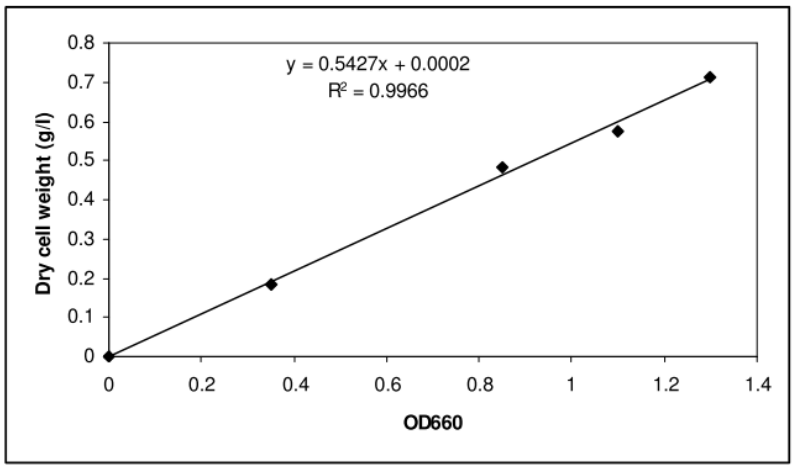

Figure B. 1 Calibration curve for *R.capsulatus* wild strain (DSM1710) (Uyar, 2008)

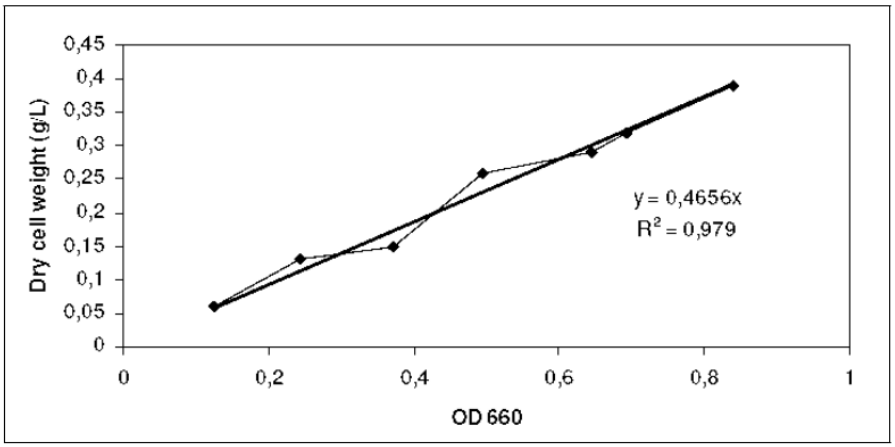

Figure B. 2 Calibration curve for *R.capsulatus* YO3 (hup$^-$) (Öztürk, 2005)

C. Gas Calibration Curves

Calibration gas, which contains 50% of hydrogen, 30 % of carbon dioxide, 10% of nitrogen, and 10% of methane was used.

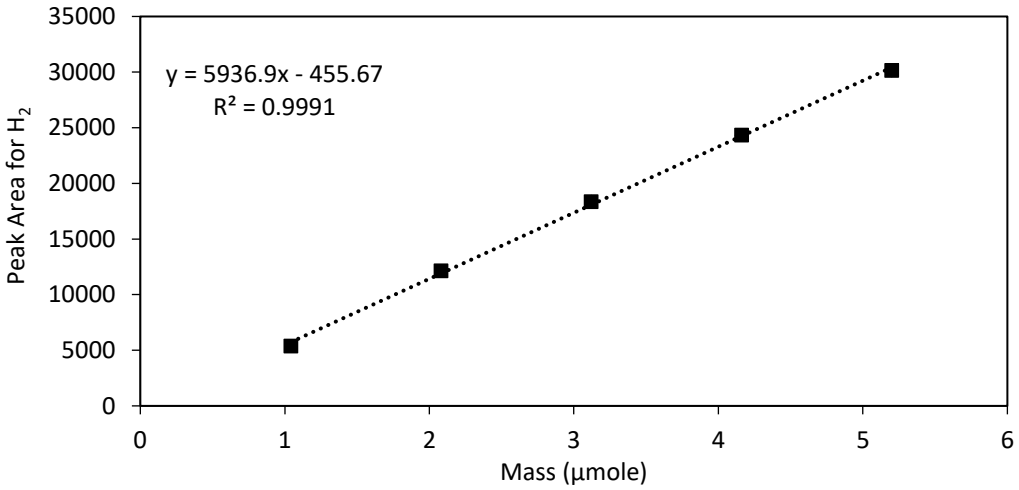

Figure C. 1 Calibration curve of hydrogen

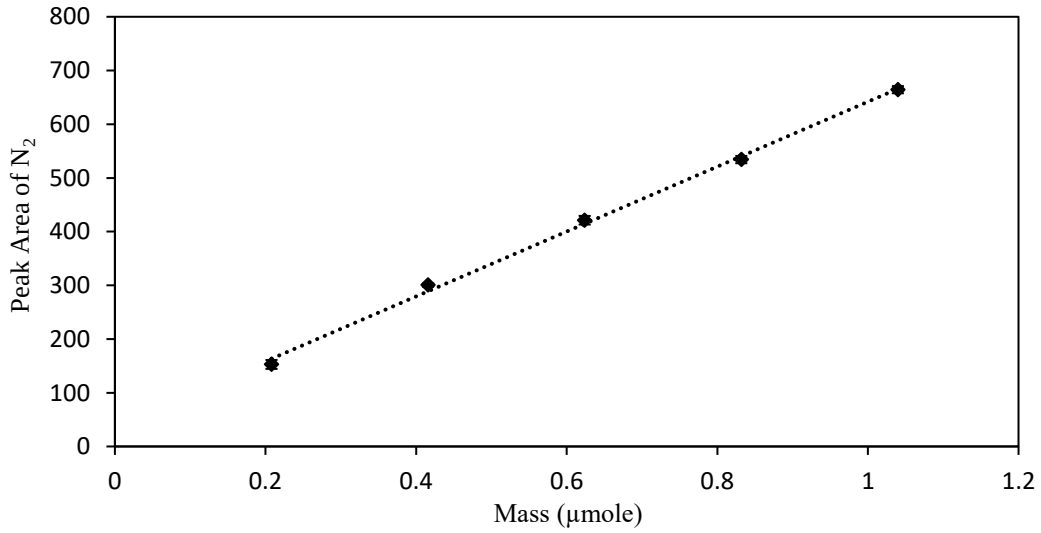

Figure C. 2 Calibration curve of nitrogen

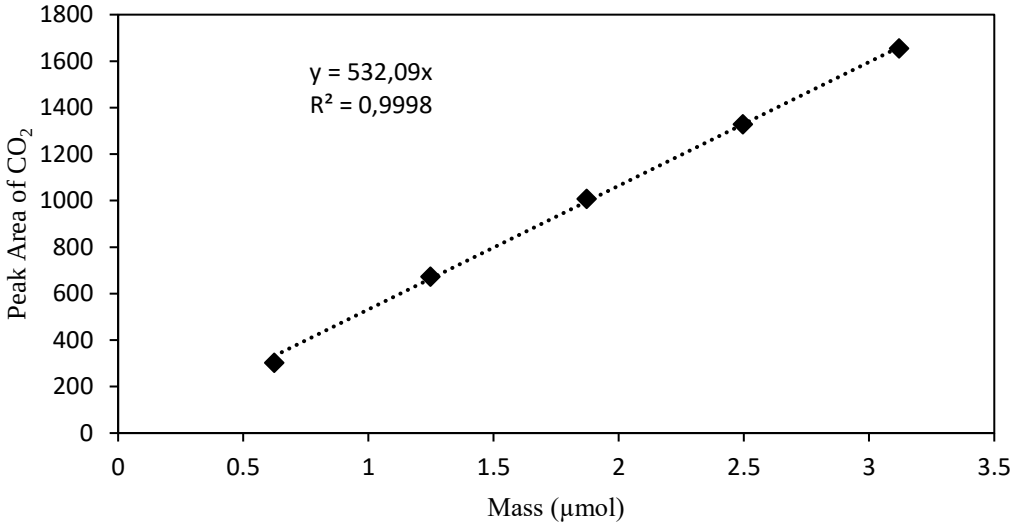

Figure C. 3 Calibration curve of carbon dioxide

D. Sample Gas Chromatogram for Gas Analysis

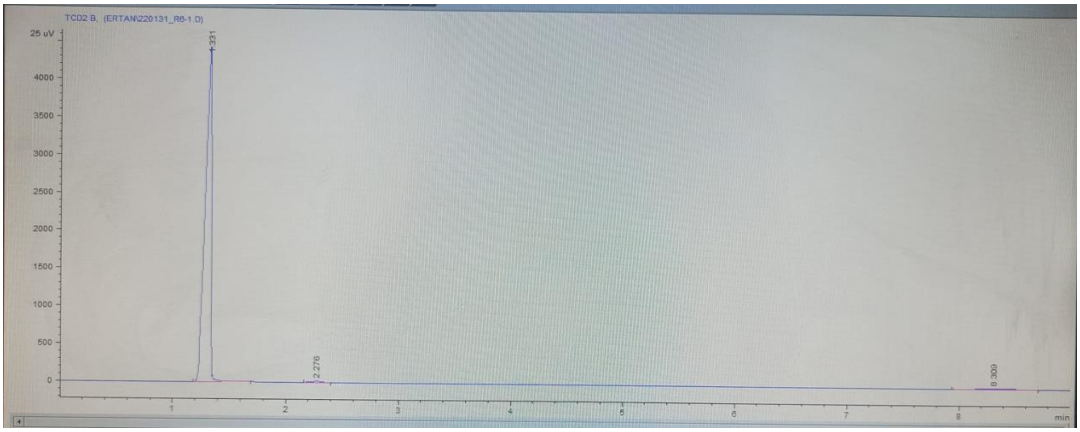

Figure D. 1 Sample chromatogram for gas analysis. Retention times: 1.33 min. for H_2, 2.27 min for N_2, 8.2 min. for CO_2

E. HPLC Calibration Curves of Organic Acids and Sample Chromatogram

Retention Times

To determine the retention time of organic acids, 10 mM of pure organic acids, which are shown in Table E.1 were injected into HPLC. Their retention times is given in Table E.1.

Table E. 1 Retention times of several organic acids in HPLC

Organic Acids	Retention Time (minutes)
Lactic Acid	20.1 - 21.4
Formic Acid	21.8 - 22.8
Acetic Acid	23.5 – 24.4
Propionic Acid	27.3 – 28.7
Isobutyric Acid	31.8 – 32.1
Butyric Acid	34.2 – 35.4

Volatile free acid mixture (VFAmix, 10 mM, CRM46975), which is consistent of heptanoic acid, lactic acid, formic acid, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, isocaproic acid, and hexanoic acid was used to make the calibration curves.

Different concentrations of VFAmix, which are 1.0 mM, 2.5 mM, 5.0 mM, 7.5 mM, and 10.0 mM were prepared by diluting with ultra-pure water. The calibration curves of organic acids are given in Figures E.1-E.5.

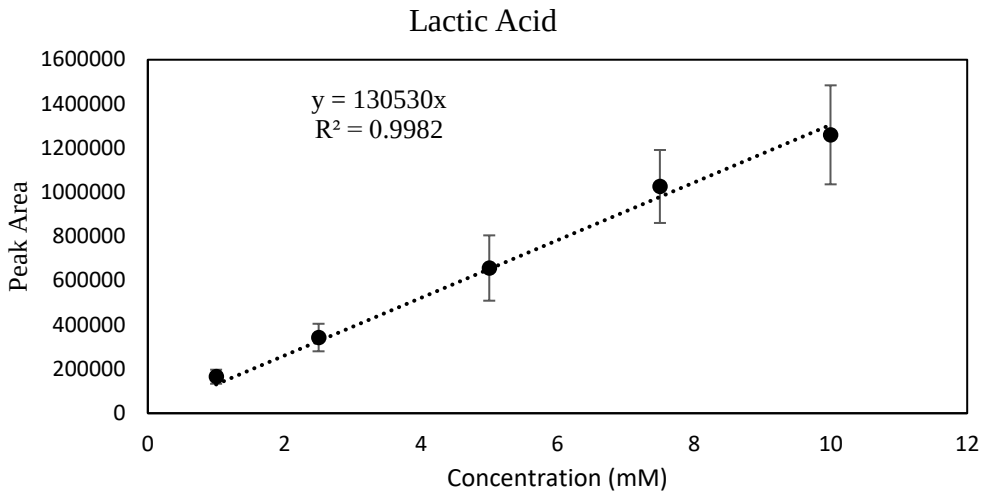

Figure E. 1 Calibration curve for lactic acid

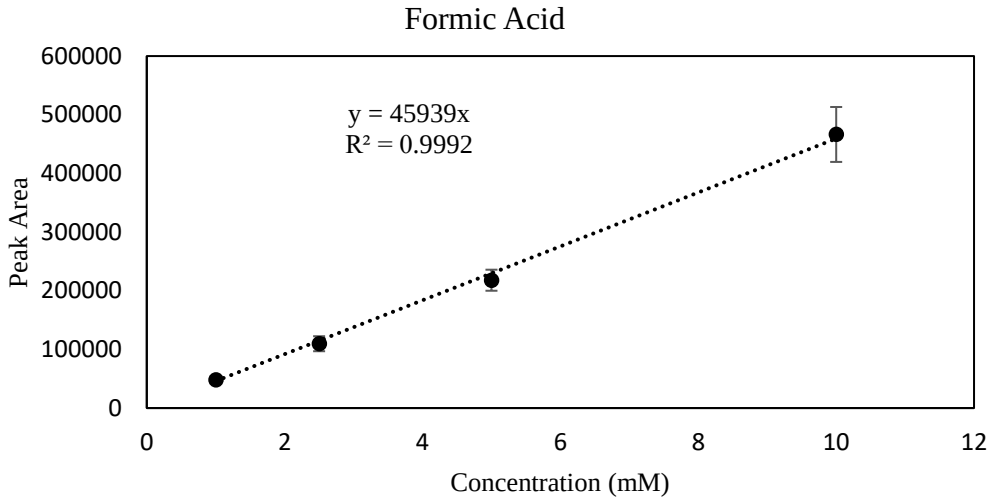

Figure E. 2 Calibration curve for formic acid

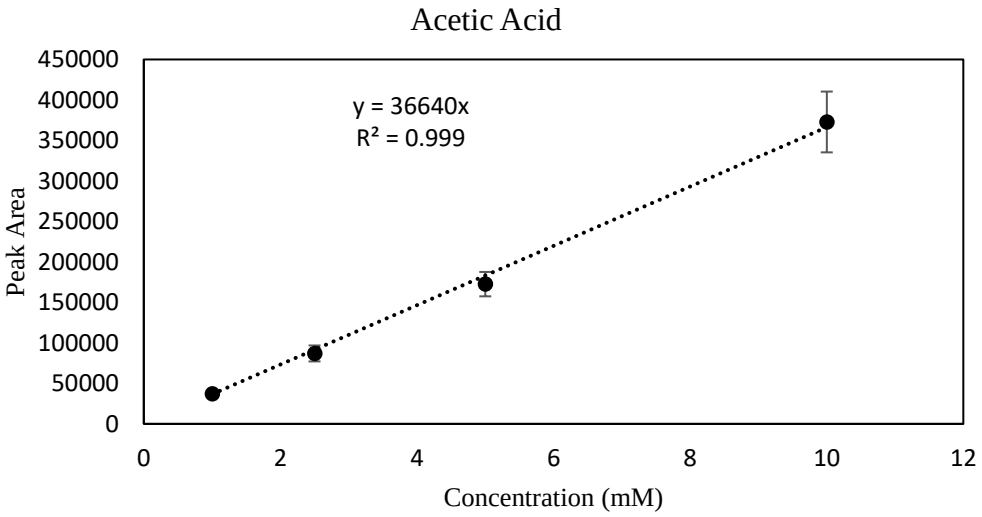

Figure E. 3 Calibration curve for acetic acid

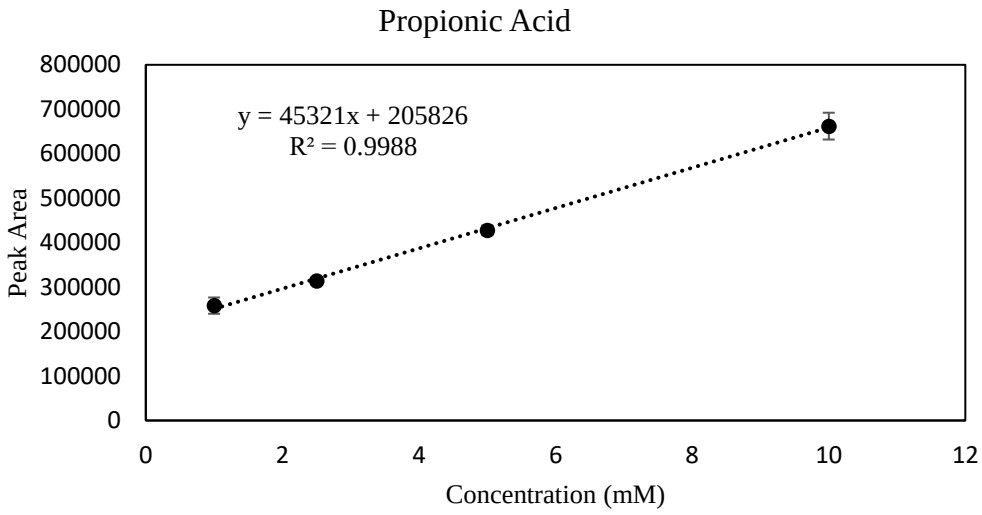

Figure E. 4 Calibration curve for propionic acid

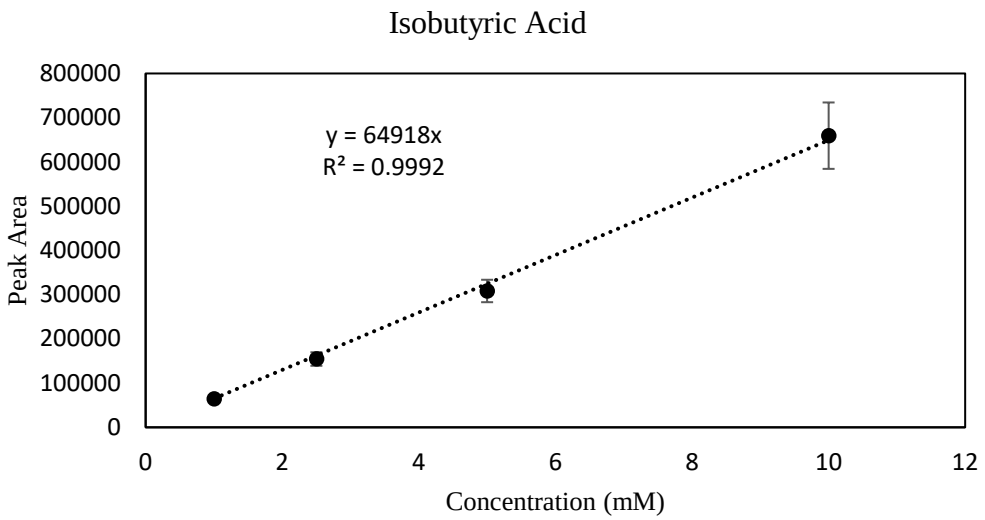

Figure E. 5 Calibration curve for isobutyric acid

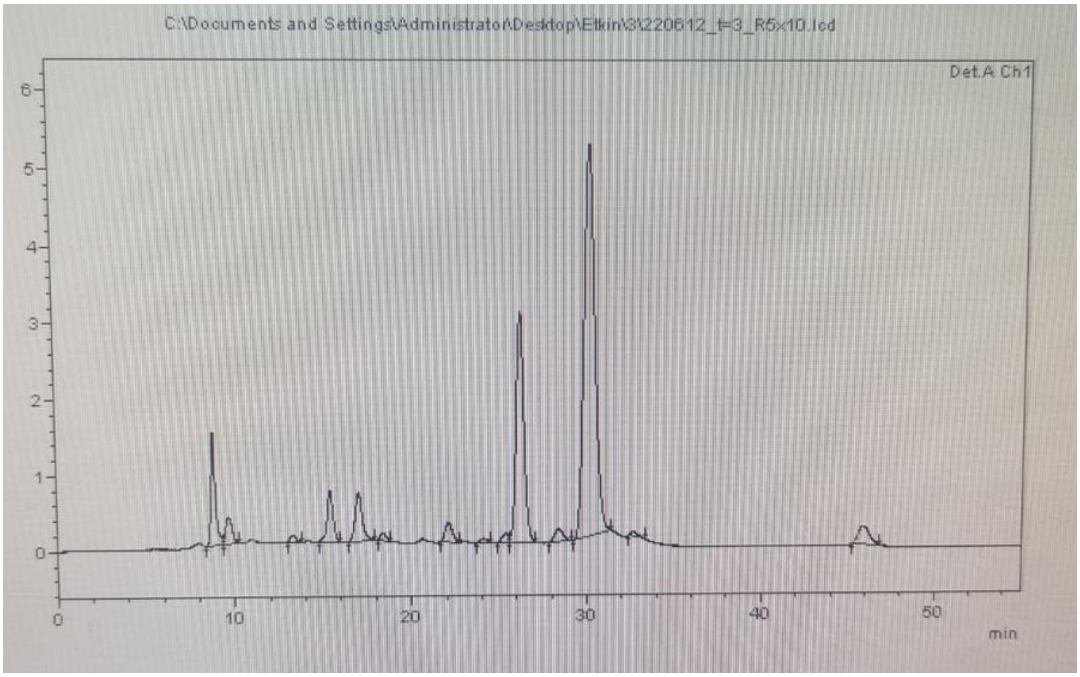

Figure E. 6 Sample HPLC chromogram for July 22, 2022

F. Calibration Curve of PHB

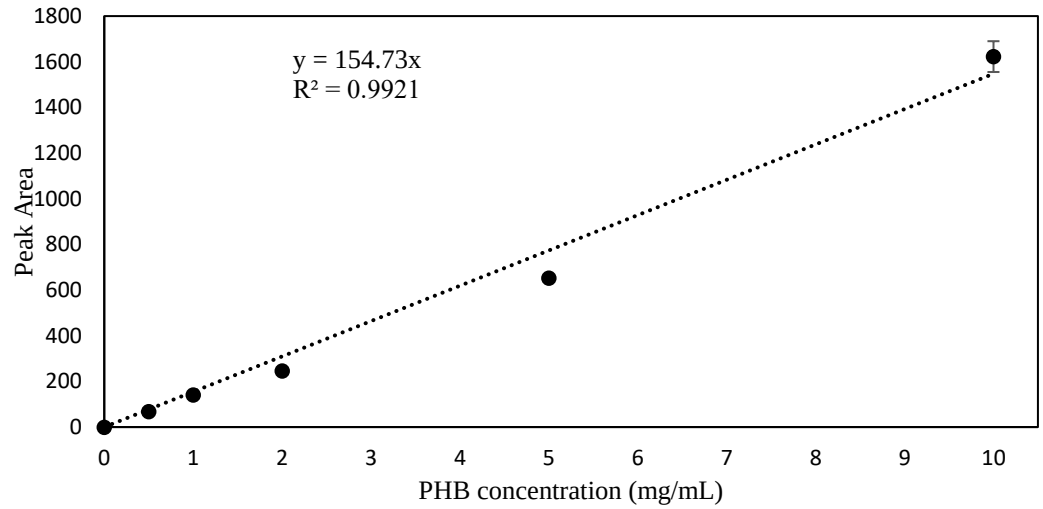

Figure F. 1 Calibration Curve of Standard PHB

G. Sample Gas Chromatogram for PHB Analysis

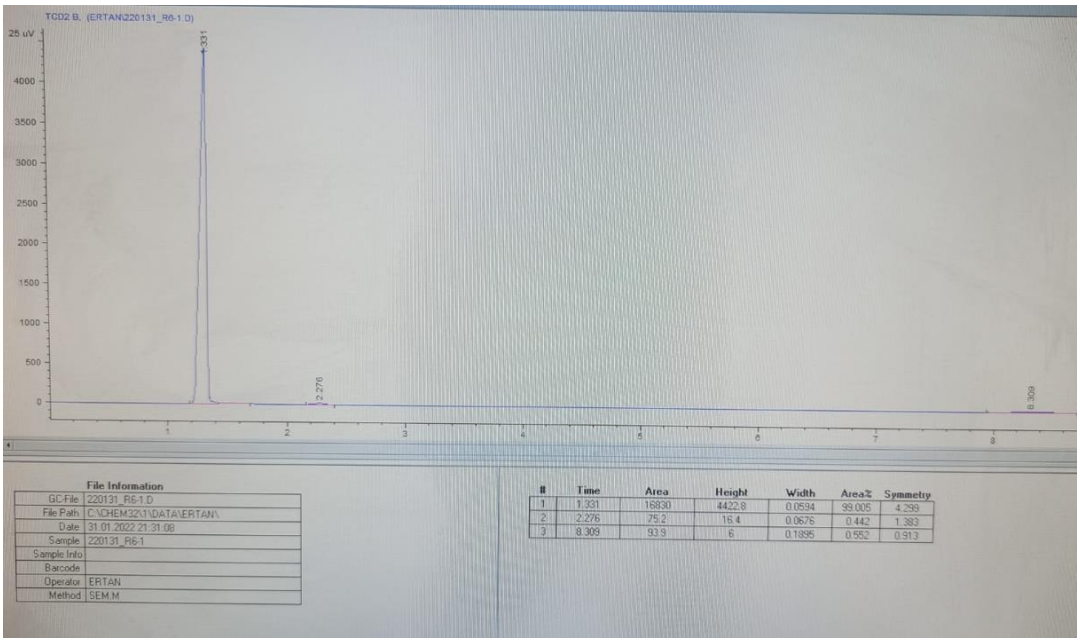

Figure G. 1 Sample Gas Chromatogram for PHB Analysis. The retention time is 8.8 minute for PHB.

H. Sample Calculation for Hydrogen Productivity

Sample calculation was performed for Set6, Reactor 2 on July 4, 2022. The produced biogas and hydrogen percentage were 83 mL and 91.8 %, respectively. The headspace pressure was 1 atm and the temperature of reactors was 301.15 K. -

$$V_{H_2} = (83\ mL).\left(\frac{91.8}{100}\right) = 76.2\ mL$$

$$n_{H_2} = \frac{(1\ atm).(76.2\ mL).(\frac{0.001L}{mL})}{\left(0.082\ \frac{atm.L}{mol.K}\right).(301.15\ K)} = 0.003\ mol$$

$$Productivity = \frac{H_2\ produced}{Reactor\ volume.Incubation\ ime}$$

$$Productivity = \frac{(0.003\ mol).(\frac{1000mmol}{mol})}{(350\ mL * 0.001\frac{L}{mL}).(22\ h)}$$

$$Productivity = 0.4\frac{mmol}{L.h}$$

I. Raw Data for All Sets

Raw Data for Set1

Table I. 1 Name of reactors in Set1. Note: All reactors were operated in batch

Reactor No	Bacterial Strain	Analyses
R1	*R.capsulatus* WT	GC, PHB
R2	*R.capsulatus* WT	GC, PHB
R3	*R.capsulatus* WT	GC, PHB
R4	*R.capsulatus* WT	GC, PHB, pH, OD, HPLC
R5	*R.capsulatus* YO3 (hup^-)	GC, PHB
R6	*R.capsulatus* YO3 (hup^-)	GC, PHB
R7	*R.capsulatus* YO3 (hup^-)	GC, PHB
R8	*R.capsulatus* YO3 (hup^-)	GC, PHB, pH, OD, HPLC

Table I. 2 Variation in organic acid concentration with time in Set1. Day 0 is the start of experiment

Date	Day	Reactor	Acetic Acid (mM)	Lactic Acid (mM)	Formic Acid (mM)	Propionic Acid (mM)
26.04.21	0	R4	40.5	-	-	0.4
		R8	37.9	-	-	1.5
07.05.21	11	R4	6.6	-	5.6	2.5
		R8	4.1	1.3	5.1	1.7

Table I. 3 Variation in pH, OD, and DCW with time in Set1

Reactors		R4			R8		
Date	Day	pH	OD 660	DCW (g/L)	pH	OD 660	DCW (g/L)
26.04.21	0	6.71	0.312	0.17	6.69	0.291	0.14
27.04.21	1	6.90	0.573	0.31	7.06	0.943	0.44
28.04.21	2	7.29	1.406	0.76	7.18	1.520	0.71
29.04.21	3	7.30	1.154	0.63	7.22	1.353	0.63
30.04.21	4	7.30	1.513	0.82	7.26	1.691	0.79
01.05.21	5	7.30	1.534	0.83	7.27	1.727	0.80
02.05.21	6	7.26	1.502	0.82	7.30	1.747	0.81
03.05.21	7	7.29	1.549	0.84	7.36	1.826	0.85
04.05.21	8	7.29	1.545	0.84	7.34	1.703	0.79
05.05.21	9	7.24	1.484	0.81	7.35	1.663	0.77
06.05.21	10	7.27	1.565	0.85	7.36	1.581	0.74
07.05.21	11	7.29	1.520	0.83	7.31	1.495	0.70

Table I. 4 The raw data in PHB analysis at the beginning and end of the Set1

Date	Day	Reactors	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB % of DCW
26.04.21	0	R0WT*	4.3	0	0
		R0YO3*	19.4	0	0
07.05.21	11	R1	27	2.5	9.1
		R2	9.6	1.1	11.0
		R3	12.3	1.3	10.5
		R4	17.7	1.8	9.9
		R5	36.3	-	-
		R6	37	5.3	14.3
		R7	29.5	-	-
		R8	16.5	1.5	9.1

*R0WT and R0YO3 which were incubated into growth medium (20mM acetate, 10mM glutamate)

Table I. 5 Daily variation of produced biogas and its content for R1 in Set1

Reactors		R1				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
26.04.21	0	0	0	0	0	100.0
27.04.21	1	1	1.9	11.4	2.1	84.6
28.04.21	2	4	46.2	11.8	4.4	37.6
29.04.21	3	13	58.9	4.6	4.1	32.5
30.04.21	4	0	9.8	74.6	6.4	9.3
01.05.21	5	12	73.5	18.7	9.3	0.0
02.05.21	6	16	80.3	9.0	12.1	0.0
03.05.21	7	11	66.3	5.1	11.4	17.1
04.05.21	8	11	76.4	7.5	14.8	1.2
05.05.21	9	6	70.8	5.0	14.8	9.4
06.05.21	10	6	74.3	6.8	17.3	1.6
07.05.21	11	4	79.0	5.9	18.3	0.0

Table I. 6 Daily variation of produced biogas and its content for R2 in Set1

Reactors		R2				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
26.04.21	0	0	0	0	0	100.0
27.04.21	1	1	1.9	7.5	1.9	88.6
28.04.21	2	5	35.3	7.2	3.8	53.6
29.04.21	3	12	57.0	3.0	5.2	34.8
30.04.21	4	10	72.9	6.1	7.3	13.6
01.05.21	5	11	64.2	3.7	7.8	24.4
02.05.21	6	11	77.8	4.4	10.9	6.8
03.05.21	7	9	70.5	3.4	11.5	14.6
04.05.21	8	10	67.7	4.2	12.3	15.8
05.05.21	9	6	75.0	4.5	15.1	5.4
06.05.21	10	6	65.8	4.4	13.7	16.0
07.05.21	11	5	75.5	9.4	16.8	-1.7

Table I. 7 Daily variation of produced biogas and its content for R3 in Set1

Reactors		R3				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
26.04.21	0	0	0	0	0	100.0
27.04.21	1	1	1.9	5.9	2.0	90.2
28.04.21	2	4	34.2	7.1	4.1	54.7
29.04.21	3	11	59.8	0.0	5.3	34.9
30.04.21	4	9	63.7	1.9	6.0	28.4
01.05.21	5	0	-	-	-	-
02.05.21	6	12	69.8	10.9	10.5	8.8
03.05.21	7	11	66.4	5.8	11.2	16.6
04.05.21	8	10	79.1	4.4	14.2	2.3
05.05.21	9	7	79.0	3.8	15.8	1.4
06.05.21	10	6	70.0	3.6	14.7	11.7
07.05.21	11	4	81.4	4.6	17.7	0.0

Table I. 8 Daily variation of produced biogas and its content for R4 in Set1

Reactors		R4				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
26.04.21	0	0	0	0	0	100.0
27.04.21	1	0	1.9	10.4	1.7	86.0
28.04.21	2	4	28.4	9.1	3.6	58.9
29.04.21	3	11	57.6	12.0	5.8	24.6
30.04.21	4	8	67.0	10.1	7.8	15.1
01.05.21	5	12	39.6	3.5	8.2	48.7
02.05.21	6	14	62.2	3.7	11.6	22.6
03.05.21	7	11	66.8	3.8	13.1	16.2
04.05.21	8	11	69.4	2.9	14.6	13.1
05.05.21	9	7	69.1	3.1	15.3	12.4
06.05.21	10	8	59.2	2.7	13.8	24.3
07.05.21	11	6	71.8	3.8	17.9	6.5

Table I. 9 Daily variation of produced biogas and its content for R5 in Set1

Reactors		R5				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
26.04.21	0	0	0	0	0	100.0
27.04.21	1	2	18.1	6.7	2.7	72.5
28.04.21	2	24	71.9	1.0	8.1	19.0
29.04.21	3	23	68.5	1.7	9.8	20.0
30.04.21	4	16	69.5	2.1	10.3	18.0
01.05.21	5	13	76.2	2.2	11.6	10.0
02.05.21	6	13	81.4	2.4	13.2	3.1
03.05.21	7	14	79.6	2.0	15.1	3.3
04.05.21	8	15	70.6	1.8	14.6	13.1
05.05.21	9	11	72.9	2.0	16.0	9.0
06.05.21	10	0	7.6	75.9	11.3	5.1
07.05.21	11	4	33.9	50.9	12.0	0.0

Table I. 10 Daily variation of produced biogas and its content for R6 in Set1

Reactors		R6				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
26.04.21	0	0	0	0	0	100.0
27.04.21	1	2	16.1	6.4	2.9	74.6
28.04.21	2	24	62.8	1.1	7.3	28.8
29.04.21	3	21	68.0	2.4	9.8	19.8
30.04.21	4	16	82.6	3.0	13.0	1.5
01.05.21	5	16	83.4	2.8	13.6	0.3
02.05.21	6	14	80.8	3.2	15.2	0.8
03.05.21	7	17	66.8	1.6	14.0	17.7
04.05.21	8	18	76.8	2.6	17.2	3.4
05.05.21	9	13	70.1	2.5	17.1	10.3
06.05.21	10	13	74.4	3.9	19.6	2.1
07.05.21	11	7	76.3	9.6	21.1	-7.0

Table I. 11 Daily variation of produced biogas and its content for R7 in Set1

Reactors		R7				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
26.04.21	0	0	0	0	0	100.0
27.04.21	1	2	14.3	6.0	2.6	77.1
28.04.21	2	25	61.8	1.0	7.2	29.9
29.04.21	3	23	72.6	1.6	10.8	15.0
30.04.21	4	17	75.3	2.6	12.2	9.9
01.05.21	5	16	69.5	2.1	11.9	16.5
02.05.21	6	14	74.4	2.7	14.1	8.8
03.05.21	7	18	79.3	2.6	17.0	1.1
04.05.21	8	18	76.9	2.8	18.5	1.8
05.05.21	9	13	73.3	1.2	19.2	6.3
06.05.21	10	0	5.4	62.9	9.2	22.5
07.05.21	11	7	41.2	45.3	14.8	0.0

Table I. 12 Daily variation of produced biogas and its content for R8 in Set1

Reactors		R8				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
26.04.21	0	0	0	0	0	100.0
27.04.21	1	4	21.2	4.9	3.3	70.7
28.04.21	2	25	62.1	7.5	8.1	22.4
29.04.21	3	25	64.2	5.4	10.5	19.9
30.04.21	4	19	63.4	2.1	11.8	22.8
01.05.21	5	14	66.1	2.0	13.2	18.7
02.05.21	6	14	62.5	3.5	14.5	19.5
03.05.21	7	19	58.8	3.4	15.1	22.8
04.05.21	8	19	60.6	2.9	17.3	19.3
05.05.21	9	9	64.5	5.1	21.2	9.2
06.05.21	10	5	57.8	11.2	23.0	8.0
07.05.21	11	2	56.9	11.2	24.1	7.8

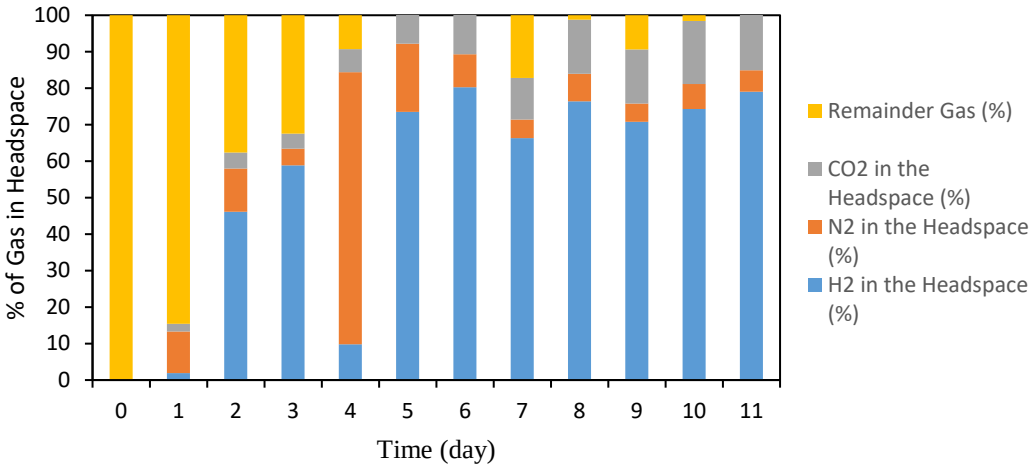

Figure I. 1 Sample graph of biogas concent for R1 in Set1

Raw Data for Set2

Table I. 13 Name of reactors in Set2. Note: All reactors were operated in batch

Reactor No	Bacterial Strain	Analyses
R1	*R.capsulatus* WT	GC, PHB
R2	*R.capsulatus* WT	GC, PHB, pH, OD, HPLC
R3	*R.capsulatus* WT	GC, PHB
R4	*R.capsulatus* WT	GC, PHB
R5	*R.capsulatus* WT	GC, PHB
R6	*R.capsulatus* YO3 (hup^-)	GC, PHB
R7	*R.capsulatus* YO3 (hup^-)	GC, PHB, pH, OD, HPLC
R8	*R.capsulatus* YO3 (hup^-)	GC, PHB
R9	*R.capsulatus* YO3 (hup^-)	GC, PHB
R10	*R.capsulatus* YO3 (hup^-)	GC, PHB

Table I. 14 Variation in organic acid concentration with time in Set2. Day 0 is the start of experiment

Date	Day	Reactor	Organic Acids (mM)			
			Acetic acid	Lactic Acid	Formic acid	Propionic acid
16.08.21	0	R2	57.5	-	-	2.5
		R7	58.8	-	-	2.1
23.08.21	7	R2	24.1	1.4	1.4	1.1
		R7	1.2	1.8	18.0	1.8
30.08.21	14	R2	0.4	1.2	13.0	2.8
		R7	0.2		11.9	2.3
31.08.21	15	R1	-	0.1	50.1	2.0
		R2	-	-	20.5	1.4
		R3	-	0.2	56.0	1.0
		R4	-	0.2	68.1	0.6
		R5	-	0.2	49.6	2.0
		R7	1.3	3.5	30.4	1.0
01.09.21	16	R6	2.2	3.0	15.4	1.8
02.09.21	17	R1	0.1	89.6	-	0.3
		R3	-	0.1	50.9	1.5
		R4	0.1	60.3	-	0.8
		R5	-	0.2	79.0	-

Table I. 15 Variation in pH, OD, and DCW with time in Set2

Reactors		R1			R6		
Date	Day	pH	OD 660	DCW (g/L)	pH	OD 660	DCW (g/L)
16.08.21	0	6.55	0.294	0.16	6.57	0.326	0.15
17.08.21	1	7.18	1.508	0.82	7.16	1.472	0.69
18.08.21	2	7.18	0.000	0.00	7.26	1.717	0.80
19.08.21	3	7.13	1.726	0.94	7.17	1.823	0.85
20.08.21	4	7.08	1.833	0.99	7.17	1.914	0.89
21.08.21	5	7.06	1.827	0.99	7.16	1.881	0.88
22.08.21	6	6.99	1.856	1.01	7.17	1.975	0.92
23.08.21	7	6.90	1.847	1.00	7.11	1.905	0.89
24.08.21	8	6.87	1.926	1.05	7.05	1.847	0.86
25.08.21	9	6.80	1.881	1.02	7.01	1.811	0.84
26.08.21	10	6.77	1.813	0.98	7.06	1.779	0.83
27.08.21	11	6.80	1.779	0.97	7.03	1.596	0.74
28.08.21	12	6.72	1.816	0.99	6.97	1.524	0.71
29.08.21	13	6.73	1.837	1.00	6.96	1.411	0.66
30.08.21	14	6.76	1.171	0.00	7.08	1.380	0.64
31.08.21	15	6.87	1.713	0.93	7.06	1.293	0.60
01.09.21	16	6.76	1.671	0.91	6.97	1.381	0.64
02.09.21	17	-	-	-	6.95	1.298	0.60
03.09.21	18	-	-	-	6.96	1.294	0.60
04.09.21	19	-	-	-	6.98	1.287	0.60

Table I. 16 The raw data in PHB analysis at the end of the Set2

Date	Day	Reactors	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB% of DCW	Avg. PHB% of DCW
02.09.21	17	R3	12.8	0.788	6.2	6.1
			11.6	0.673	5.3	
			4.6	0.304	2.4	
		R4	12.5	0.538	4.3	4.5
			13.5	0.596	4.8	
			5.2	0.263	2.1	
		R5	11.4	0.552	4.8	5.4
			10.4	0.629	5.5	
04.09.21	19	R10	9.4	0.59	6.2	5.0
			9.0	0.41	4.4	
			6.9	0.26	2.8	

Note that the PHB samples of R1, R2, R6, R7, R8, and R9 were lost during methanolysis at 100 ^{o}C.

Table I. 17 Daily variation of produced biogas and its content for R1 in Set2

Reactors		R1				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
16.08.21	0	0	0.0	0.0	0.0	100.0
17.08.21	1	6	32.7	7.6	3.4	56.3
18.08.21	2	10	57.2	6.1	5.0	31.7
19.08.21	3	13	73.2	9.3	9.0	8.5
20.08.21	4	12	71.3	5.7	11.2	11.8
21.08.21	5	12	71.3	10.4	13.8	4.5
22.08.21	6	9	63.6	8.8	14.6	13.0
23.08.21	7	8	53.3	8.3	14.2	24.2
24.08.21	8	5	62.6	12.2	19.0	6.3
25.08.21	9	4	45.6	9.4	16.0	29.1
26.08.21	10	1	50.4	11.7	20.5	17.4
27.08.21	11	0	48.6	13.6	21.0	16.8
28.08.21	12	0	42.5	13.3	21.1	23.1
29.08.21	13	0	12.0	3.9	6.5	77.6
30.08.21	14	0	16.5	6.2	13.6	63.8
31.08.21	15	0	13.3	14.0	17.5	55.1
01.09.21	16	0	6.7	15.2	15.9	62.3

Table I. 18 Daily variation of produced biogas and its content for R2 in Set2

Reactors		R2				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
16.08.21	0	0	0.0	0.0	0.0	100.0
17.08.21	1	4	30.8	7.3	4.1	57.8
18.08.21	2	16	64.4	5.2	7.8	22.7
19.08.21	3	12	74.4	5.0	8.7	11.9
20.08.21	4	10	66.3	6.1	9.5	18.1
21.08.21	5	14	78.4	4.9	12.2	4.5
22.08.21	6	11	62.2	3.4	11.1	23.3
23.08.21	7	10	67.8	3.3	13.4	15.5
24.08.21	8	0	59.4	3.4	13.3	23.9
25.08.21	9	0	4.0	78.3	9.3	8.4
26.08.21	10	0	2.8	54.2	8.7	34.3
27.08.21	11	0	2.6	41.5	8.2	47.8
28.08.21	12	0	2.4	53.5	10.5	33.6
29.08.21	13	0	3.3	53.3	9.5	33.9
30.08.21	14	0	2.9	51.2	8.3	37.5
31.08.21	15	0	2.1	62.9	8.1	26.9
01.09.21	16	0	7.5	54.0	6.9	31.6

Table I. 19 Daily variation of produced biogas and its content for R3 in Set2

Reactors		R3				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
16.08.21	0	0	0.0	0.0	0.0	100.0
17.08.21	1	6	35.5	4.6	3.7	56.2
18.08.21	2	18	65.5	3.9	6.2	24.3
19.08.21	3	13	65.2	3.4	7.6	23.8
20.08.21	4	10	69.9	4.4	10.0	15.7
21.08.21	5	12	76.7	5.2	12.1	6.0
22.08.21	6	8	71.7	5.4	13.3	9.6
23.08.21	7	8	66.6	5.3	13.9	14.2
24.08.21	8	6	72.0	6.7	17.1	4.1
30.08.21	14	0	48.6	13.4	23.8	14.1
31.08.21	15	0	44.6	15.6	25.2	14.6
01.09.21	16	0	31.0	20.0	24.3	24.7

Table I. 20 Daily variation of produced biogas and its content for R4 in Set2

Reactors		R4				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
16.08.21	0	0	0.0	0.0	0.0	100.0
17.08.21	1	6	33.1	3.6	4.1	59.3
18.08.21	2	16	67.3	5.0	6.2	21.6
19.08.21	3	13	68.3	4.3	8.1	19.3
20.08.21	4	10	73.8	5.2	11.0	10.0
21.08.21	5	12	72.9	6.1	13.2	7.7
22.08.21	6	8	74.1	7.3	16.2	2.4
23.08.21	7	8	74.5	7.8	18.6	0.0
24.08.21	8	6	60.0	6.6	16.9	16.5
25.08.21	9	5	64.6	9.1	20.6	5.7
26.08.21	10	3	57.6	9.6	20.3	12.5
27.08.21	11	1	50.3	12.4	20.5	16.9
28.08.21	12	0	39.9	14.1	20.9	25.1
29.08.21	13	0	34.3	19.1	25.6	21.1
30.08.21	14	2	13.0	37.0	26.7	23.3
31.08.21	15	0	3.1	46.5	28.0	22.3
01.09.21	16	0	2.2	41.7	24.8	31.3

Table I. 21 Daily variation of produced biogas and its content for R5 in Set2

Reactors		R5				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
16.08.21	0	0	0.0	0.0	0.0	100.0
17.08.21	1	6	35.3	4.7	3.4	56.6
18.08.21	2	17	70.8	3.5	6.0	19.7
19.08.21	3	13	75.5	4.1	8.2	12.2
20.08.21	4	12	78.1	4.5	11.0	6.4
21.08.21	5	14	68.0	4.4	11.4	16.1
22.08.21	6	9	78.2	3.3	15.6	2.8
23.08.21	7	9	61.8	5.0	14.3	18.8
24.08.21	8	7	72.3	6.3	18.2	3.2
25.08.21	9	7	68.4	6.8	19.2	5.6
26.08.21	10	5	61.0	9.4	18.5	11.0
27.08.21	11	2	65.6	9.3	21.8	3.3
28.08.21	12	1	58.6	12.0	25.0	4.4
29.08.21	13	0	35.1	10.0	18.7	36.2
30.08.21	14	0	28.7	15.4	20.2	35.7
31.08.21	15	0	20.9	33.2	26.3	19.7
01.09.21	16	0	2.5	35.1	23.8	38.7

Table I. 22 Daily variation of produced biogas and its content for R6 in Set2

Reactors		R6				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
16.08.21	0	0	0.0	0.0	0.0	100.0
17.08.21	1	10	48.1	4.0	4.3	43.6
18.08.21	2	12	72.4	3.1	6.8	17.7
19.08.21	3	16	80.4	4.0	10.2	5.5
20.08.21	4	16	63.3	2.9	9.8	24.0
21.08.21	5	19	63.9	3.6	11.9	20.6
22.08.21	6	15	70.7	4.3	15.3	9.7
23.08.21	7	12	56.4	6.9	14.3	22.4
24.08.21	8	7	54.8	4.9	16.4	23.9
25.08.21	9	6	59.2	6.9	20.4	13.4
26.08.21	10	4	58.9	7.8	22.4	10.9
27.08.21	11	4	50.5	8.2	21.5	19.8
28.08.21	12	3	44.0	8.1	20.7	27.1
29.08.21	13	3	36.6	8.6	18.9	35.9
30.08.21	14	1	38.6	10.7	21.6	29.1
31.08.21	15	0	41.4	14.9	26.0	17.7
01.09.21	16	0	35.0	15.4	23.8	25.8
02.09.21	17	0	31.1	19.9	24.1	24.8
03.09.21	18	0	26.6	19.6	23.7	30.1
04.09.21	19	0	21.3	17.8	21.4	39.5

Table I. 23 Daily variation of produced biogas and its content for R7 in Set2

Reactors		R7				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
16.08.21	0	0	0.0	0.0	0.0	100.0
17.08.21	1	10	52.9	5.1	4.9	37.1
18.08.21	2	24	70.2	3.6	8.5	17.8
19.08.21	3	15	76.8	3.6	10.7	8.9
20.08.21	4	16	78.0	3.8	13.1	5.2
21.08.21	5	20	74.9	3.6	14.2	7.3
22.08.21	6	16	60.7	3.3	13.8	22.3
23.08.21	7	0	11.9	52.6	11.9	23.6
24.08.21	8	14	54.8	19.0	19.7	6.5
25.08.21	9	13	57.8	7.7	21.0	13.4
26.08.21	10	6	60.5	8.9	23.5	7.1
27.08.21	11	1	59.5	11.5	26.0	3.0
28.08.21	12	1	54.7	13.3	26.6	5.4
29.08.21	13	0	43.4	14.1	23.7	18.7
30.08.21	14	0	40.2	19.5	26.3	14.1
31.08.21	15	0	33.4	22.4	27.1	17.0
01.09.21	16	0	26.2	23.9	26.6	23.2
02.09.21	17	0	18.7	22.9	22.1	36.3
03.09.21	18	8	2.5	4.9	46.9	45.8
04.09.21	19	0	2.7	8.5	46.8	42.0

Table I. 24 Daily variation of produced biogas and its content for R8 in Set2

Reactors		R8				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
16.08.21	0	0	0.0	0.0	0.0	100.0
17.08.21	1	10	48.6	3.6	4.3	43.4
18.08.21	2	26	75.2	3.0	8.6	13.2
19.08.21	3	16	80.3	3.5	11.2	5.1
20.08.21	4	17	66.1	2.6	11.1	20.3
21.08.21	5	19	77.2	3.5	14.7	4.5
22.08.21	6	17	67.5	3.3	15.4	13.7
23.08.21	7	14	60.2	6.0	15.3	18.5
24.08.21	8	11	71.0	4.5	19.9	4.7
25.08.21	9	9	59.2	4.4	18.8	17.6
26.08.21	10	3	58.6	5.1	20.4	15.9
27.08.21	11	5	51.9	8.0	20.5	19.6
28.08.21	12	1	61.5	8.8	27.0	2.7
29.08.21	13	3	54.1	11.7	25.9	8.3
30.08.21	14	2	52.8	10.4	26.3	10.4
31.08.21	15	2	54.6	13.6	29.8	2.1
01.09.21	16	1	53.5	14.6	30.0	1.9
02.09.21	17	1	50.6	18.3	32.8	0.0
03.09.21	18	1	48.0	19.0	31.0	2.0
04.09.21	19	0	47.3	20.9	31.4	0.4

Table I. 25 Daily variation of produced biogas and its content for R9 in Set2

Reactors		R9				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
16.08.21	0	0				100.0
17.08.21	1	8	52.5	3.9	4.4	39.3
18.08.21	2	24	72.4	3.1	8.3	16.3
19.08.21	3	14	68.9	2.4	8.7	20.1
20.08.21	4	14	77.0	3.2	11.7	8.1
21.08.21	5	18	66.4	2.9	11.6	19.1
22.08.21	6	16	75.9	3.4	15.7	5.0
23.08.21	7	14	81.5	3.6	19.1	-4.2
24.08.21	8	11	75.7	4.2	19.6	0.6
25.08.21	9	8	74.1	3.3	21.0	1.6
26.08.21	10	4	72.3	2.9	21.9	2.9
27.08.21	11	3	58.2	8.5	21.1	12.3
28.08.21	12	3	64.4	7.5	25.2	2.9
29.08.21	13	4	62.6	8.0	26.7	2.6
30.08.21	14	2	58.4	8.1	25.4	8.1
31.08.21	15	2	62.8	10.2	29.1	0.0
01.09.21	16	1	59.7	10.6	28.3	1.5
02.09.21	17	1	59.0	12.6	29.2	0.0
03.09.21	18	1	61.6	11.1	30.7	0.0
04.09.21	19	1	59.7	13.4	30.7	0.0

Table I. 26 Daily variation of produced biogas and its content for R10 in Set2

Reactors		R10				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
16.08.21	0	0	0.0	0.0	0.0	100.0
17.08.21	1	8	55.3	5.3	4.9	34.5
18.08.21	2	28	70.8	2.9	8.4	17.9
19.08.21	3	16	46.9	2.5	6.6	44.0
20.08.21	4	16	78.5	3.4	13.1	5.0
21.08.21	5	20	77.8	3.6	14.9	3.7
22.08.21	6	17	71.0	3.6	16.2	9.2
23.08.21	7	18	73.4	2.8	17.7	6.2
24.08.21	8	13	72.7	0.9	20.1	6.3
25.08.21	9	8	62.9	2.6	19.5	14.9
26.08.21	10	4	63.9	5.1	21.2	9.8
27.08.21	11	4	70.0	4.0	23.5	2.5
28.08.21	12	5	68.0	3.6	24.2	4.2
29.08.21	13	3	64.9	5.5	24.8	4.8
30.08.21	14	3	66.9	5.8	24.9	2.5
31.08.21	15	2	69.0	4.4	26.8	0.0
01.09.21	16	1	67.2	5.2	26.0	1.5
02.09.21	17	1	68.2	8.7	26.2	0.0
03.09.21	18	1	69.5	5.4	27.6	0.0
04.09.21	19	1	67.3	5.6	27.2	0.0

Raw Data for Set3

Table I. 27 Name of reactors in Set3. Note: All reactors were operated in batch

Reactor No	Bacterial Strain	Gas Collection
R1	*R.capsulatus* WT	Water Displacement
R2	*R.capsulatus* WT	Water Displacement
R3	*R.capsulatus* WT	Syringe
R4	*R.capsulatus* WT	Syringe

Table I. 28 The raw data in PHB analysis at the end of the Set3

Date	Day	Reactors	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB% of DCW
17.11.2021	16	R1	14.8	2.0	13.4
			18.6	2.1	11.3
			15.6	2.5	15.8
		R2	15.4	1.5	9.6
			16	1.6	10.3
			15.5	1.4	8.9
		R3	15	1.8	11.9
			20.5	2.6	12.7
			10.9	1.3	11.8
		R4	17.1	1.9	11.3
			23.3	3.4	14.7
			15.1	1.6	10.4

Table I. 29 Variation in organic acid concentration with time in Set3. Day 0 is the start of experiment

Date	Day	Reactors	Concentration of Organic Acids (mM)			
			Lactic Acid	Formic Acid	Acetic Acid	Propionic Acid
01.11.21	0	R1	-	-	59.78	1.49
		R2	-	-	57.19	1.58
		R3	-	-	59.56	1.72
		R4	-	-	51.68	2.10
04.11.21	3	R1	0.10	1.6	32.40	2.11
		R2	0.13	1.8	41.11	1.39
		R3	0.10	1.7	33.79	1.87
		R4	0.12	1.6	32.72	1.94
10.11.21	9	R1	-	3.9	4.53	3.27
		R2	-	4.2	3.79	3.69
		R3	0.04	9.2	11.60	2.36
		R4	0.04	15.1	12.61	1.47

Table I.29 Continued

12.11.21	11	R1	-	9.7	3.46	3.13
		R2	0.03	20.1	6.17	1.64
		R3	0.02	8.9	4.92	3.18
		R4	0.05	25.9	10.25	0.99
13.11.21	12	R1	0.03	27.8	5.29	0.98
		R2	0.05	32.1	5.88	0.62
		R3	0.20	29.6	11.11	0.62
		R4	0.04	36.5	6.12	0.51
15.11.21	15	R1	-	42.6	-	1.05
		R2	-	44.9	-	0.53
		R3	-	45.2	3.01	2.20
		R4	-	47.2	0.05	1.03
16.11.21	16	R1	-	50.5	-	0.66
		R2	-	44.5	-	0.77
		R3	0.03	46.2	-	0.74
		R4	0.03	51.8	-	0.89

Table I. 30 Variation in pH, OD, and DCW with time in Set3

Date	Day	Reactors	pH	OD 660	DCW (g/L)
01.11.21	0	R1	6.65	0.414	0.22
		R2	6.64	0.410	0.22
		R3	6.63	0.400	0.22
		R4	6.65	0.419	0.23
02.11.21	1	R3	6.98	1.190	0.65
03.11.21	2	R3	7.33	1.704	0.92
04.11.21	3	R3	7.30	1.816	0.99
05.11.21	4	R3	7.23	1.901	1.03
06.11.21	5	R3	7.18	1.907	1.04
07.11.21	6	R3	7.24	1.879	1.02
08.11.21	7	R3	7.20	1.923	1.04
09.11.21	8	R3	7.24	2.006	1.09
10.11.21	9	R3	7.23	1.954	1.06
11.11.21	10	R3	7.20	1.976	1.07

Table I.30 Continued

12.11.21	11	R3	7.08	1.985	1.08
13.11.21	12	R3	6.91	1.924	1.04
14.11.21	13	R3	7.01	1.956	1.06
15.11.21	14	R3	6.68	1.907	1.04
16.11.21	15	R3	6.98	1.977	1.07
17.11.21	16	R1	6.92	1.846	1.00
		R2	7.02	1.970	1.07
		R3	7.05	1.952	1.06
		R4	6.98	1.936	1.05

Table I. 31 Daily variation of produced biogas and its content for R1 in Set3

Reactors		R1				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
01.11.21	0	0	0.0	0.0	0.0	100.0
02.11.21	1	3	6.5	16.7	2.9	74.0
03.11.21	2	27	70.5	4.8	4.0	20.7
04.11.21	3	14	74.0	8.8	5.7	11.6
05.11.21	4	17	73.3	11.9	7.7	7.0
06.11.21	5	7	71.9	15.1	9.1	3.9
07.11.21	6	6	71.3	14.3	11.1	3.2
08.11.21	7	11	66.4	14.5	12.2	7.0
09.11.21	8	6	62.2	16.6	12.2	9.0
10.11.21	9	17	67.9	11.8	15.3	5.0
11.11.21	10	13	65.4	11.1	15.7	7.8
12.11.21	11	11	61.6	13.8	17.0	7.6

Table I.31 Continued

13.11.21	12	3	56.4	11.1	18.3	14.3
14.11.21	13	4	52.4	15.0	18.0	14.6
15.11.21	14	7	50.0	15.6	20.8	13.6
16.11.21	15	2	39.4	22.9	20.1	17.6
17.11.21	16	1	15.0	11.5	8.5	65.1

Table I. 32 Daily variation of produced biogas and its content for R2 in Set3

Reactors		R2				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
01.11.21	0	0	0.0	0.0	0.0	100.0
02.11.21	1	1	3.1	19.6	3.0	74.3
03.11.21	2	0	59.7	8.7	3.8	27.8
04.11.21	3	0	70.3	6.5	5.6	17.5
05.11.21	4	0	71.1	10.9	9.0	9.0
06.11.21	5	0	66.9	18.0	10.9	4.1
07.11.21	6	0	61.5	17.6	12.6	8.3
08.11.21	7	0	54.7	15.9	13.3	16.1
09.11.21	8	0	54.3	24.0	15.0	6.8
10.11.21	9	0	69.9	7.0	15.4	7.8
11.11.21	10	0	68.7	7.1	17.0	7.3
12.11.21	11	0	64.7	8.8	16.7	9.8
13.11.21	12	0	60.7	8.6	16.1	14.5
14.11.21	13	0	46.3	21.6	17.6	14.5
15.11.21	14	0	44.4	23.0	21.7	10.9
16.11.21	15	0	32.8	32.2	20.0	15.0
17.11.21	16	0	11.0	51.5	20.2	17.2

Table I. 33 Daily variation of produced biogas and its content for R3 in Set3

Reactors		R3				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
01.11.21	0	0	0.0	0.0	0.0	100.0
02.11.21	1	1	4.5	12.2	2.9	80.4
03.11.21	2	29	67.1	2.4	4.7	25.9
04.11.21	3	22	78.4	3.9	6.5	11.2
05.11.21	4	24	79.9	4.8	8.6	6.7
06.11.21	5	13	82.8	5.1	10.0	2.1
07.11.21	6	17	76.3	5.9	11.5	6.3
08.11.21	7	15	59.9	4.1	10.2	25.8
09.11.21	8	6	75.5	9.0	13.6	1.8
10.11.21	9	24	77.2	5.8	16.3	0.8
11.11.21	10	12	66.6	6.8	15.2	11.4
12.11.21	11	14	81.1	8.4	20.0	0.0
13.11.21	12	8	62.1	8.5	16.7	12.8
14.11.21	13	10	59.1	8.1	16.7	16.2
15.11.21	14	9	60.5	8.1	19.1	12.2
16.11.21	15	9	60.3	7.9	19.1	12.7
17.11.21	16	2	39.4	18.7	19.8	22.1

Table I. 34 Daily variation of produced biogas and its content for R4 in Set3

Reactors		R4				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2 %	Remainder Gas (%)
01.11.21	0	0	0.0	0.0	0.0	100.0
02.11.21	1	4	5.9	14.0	3.1	77.0
03.11.21	2	27	64.6	6.2	4.0	25.1
04.11.21	3	16	74.9	5.9	6.0	13.2
05.11.21	4	23	79.2	7.1	9.4	4.2
06.11.21	5	10	72.9	10.4	10.6	6.1
07.11.21	6	14	74.9	9.7	13.6	1.9
08.11.21	7	18	73.9	7.0	14.6	4.4
09.11.21	8	7	72.1	6.3	14.8	6.8
10.11.21	9	25	78.2	5.8	18.8	0.0
11.11.21	10	13	70.2	7.1	18.0	4.8
12.11.21	11	12	62.9	7.9	17.3	11.9
13.11.21	12	6	65.1	8.6	19.1	7.2
14.11.21	13	8	58.6	10.0	19.0	12.4
15.11.21	14	6	59.8	10.5	22.2	7.5
16.11.21	15	2	54.2	12.6	21.7	11.5
17.11.21	16	0	40.9	18.4	23.1	17.6

Raw Data for Set4

Table I. 35 Name of reactors in Set4

Reactor No	Bacterial Strain	Operation Mode
R1	*R.capsulatus* WT	Batch
R2	*R.capsulatus* WT	Batch
R3	*R.capsulatus* YO3 (hup^-)	Batch
R4	*R.capsulatus* YO3 (hup^-)	Batch
R5	*R.capsulatus* WT	Fed-Batch
R6	*R.capsulatus* YO3 (hup^-)	Fed-Batch

Table I. 36 Variation in pH, OD, and DCW with time for R1 in Set4

Reactors		R1		
Date	Day	pH	OD 660	DCW (g/L)
23.01.22	0	6.48	0.236	0.13
24.01.22	1	6.79	0.995	0.54
25.01.22	2	7.04	1.552	0.84
26.01.22	3	7.07	1.644	0.89
27.01.22	4	6.99	1.709	0.93
28.01.22	5	6.68	1.506	0.82
29.01.22	6	6.95	1.785	0.97
30.01.22	7	6.97	1.672	0.91
31.01.22	8	7.06	1.579	0.86
01.02.22	9	6.92	1.471	0.80
02.02.22	10	7.00	1.419	0.77
03.02.22	11	7.04	1.534	0.83
04.02.22	12	6.94	1.310	0.71
05.02.22	13	7.00	1.237	0.67

Table I. 36 Continued

06.02.22	14	7.00	1.121	0.61
07.02.22	15	6.85	0.991	0.54
08.02.22	16	-	0.865	0.47
09.02.22	17	6.42	0.606	0.33
10.02.22	18	6.57	0.482	0.26
12.02.22	20	-	-	-
13.02.22	21	-	-	-
14.02.22	22	6.84	0.836	0.45
15.02.22	23	-	-	-
16.02.22	24	6.88	0.481	0.26

Table I. 37 Variation in pH, OD, and DCW with time for R2 in Set4

Reactors		R2		
Date	Day	pH	OD 660	DCW (g/L)
23.01.22	0	6.48	0.267	0.15
24.01.22	1	6.88	1.219	0.66
25.01.22	2	7.12	1.863	1.01
26.01.22	3	7.03	1.617	0.88
27.01.22	4	7.13	1.818	0.99
28.01.22	5	6.68	1.622	0.88
29.01.22	6	7.02	1.813	0.98
30.01.22	7	6.96	1.671	0.91
31.01.22	8	7.04	1.631	0.89
01.02.22	9	7.00	1.535	0.83
02.02.22	10	6.96	1.507	0.82
03.02.22	11	7.04	1.496	0.81
04.02.22	12	6.92	1.406	0.76

Table I. 37 Continued

05.02.22	13	6.94	1.280	0.69
06.02.22	14	6.96	1.160	0.63
07.02.22	15	6.86	1.008	0.55
08.02.22	16	-	0.821	0.45
09.02.22	17	6.48	0.624	0.34
10.02.22	18	6.57	0.547	0.30
12.02.22	20	6.74	0.617	0.34

Table I. 38 Variation in pH, OD, and DCW with time for R3 in Set4

Reactors		R3		
Date	Day	pH	OD 660	DCW (g/L)
23.01.22	0	6.00	0.225	0.10
24.01.22	1	7.19	1.508	0.70
25.01.22	2	7.13	1.786	0.83
26.01.22	3	7.19	1.766	0.82
27.01.22	4	7.26	1.915	0.89
28.01.22	5	7.23	1.943	0.90
29.01.22	6	6.83	1.800	0.84
30.01.22	7	7.19	1.872	0.87
31.01.22	8	7.20	1.717	0.80
01.02.22	9	7.16	1.730	0.81
02.02.22	10	7.15	1.793	0.83
03.02.22	11	7.13	1.642	0.76
04.02.22	12	7.07	1.543	0.72
05.02.22	13	7.12	1.402	0.65
06.02.22	14	7.06	1.353	0.63

Table I. 38 Continued

07.02.22	15	6.92	1.322	0.62
08.02.22	16	-	1.155	0.54
09.02.22	17	6.56	0.932	0.43
10.02.22	18	6.60	0.853	0.40
14.02.22	22	6.89	0.786	0.37
16.02.22	24	6.85	0.789	0.37

Table I. 39 Variation in pH, OD, and DCW with time for R4 in Set4

Reactors		R4		
Date	Day	pH	OD 660	DCW (g/L)
23.01.22	0	6.59	0.432	0.20
24.01.22	1	7.19	1.517	0.71
25.01.22	2	7.18	1.795	0.84
26.01.22	3	7.27	1.847	0.86
27.01.22	4	7.35	1.929	0.90
28.01.22	5	7.31	1.940	0.90
29.01.22	6	7.23	1.919	0.89
30.01.22	7	7.22	1.895	0.88
31.01.22	8	7.22	1.805	0.84
01.02.22	9	7.21	1.735	0.81
02.02.22	10	7.12	1.719	0.80
03.02.22	11	7.12	1.620	0.75
04.02.22	12	7.06	1.575	0.73
05.02.22	13	7.10	1.448	0.67
06.02.22	14	7.04	1.430	0.67
07.02.22	15	6.95	1.413	0.66
08.02.22	16	-	1.195	0.56

Table I.39 Continued

09.02.22	17	6.55	1.026	0.48
10.02.22	18	6.90	0.951	0.44
14.02.22	22	6.86	0.833	0.39
16.02.22	24	6.5	0.547	0.25

Table I. 40 Variation in pH, OD, and DCW with time for R5 in Set4

Reactors		R5		
Date	Day	pH	OD 660	DCW (g/L)
23.01.22	0	6.49	0.269	0.15
24.01.22	1	7.43	2.091	1.13
25.01.22	2	6.39	2.059	1.12
26.01.22	3	6.41	1.941	1.05
27.01.22	4	6.29	1.949	1.06
28.01.22	5	6.43	1.587	0.86
29.01.22	6	6.44	1.420	0.77
30.01.22	7	6.33	1.276	0.69
31.01.22	8	-	1.328	0.72
01.02.22	9	6.42	1.222	0.66
02.02.22	10	6.47	1.049	0.57
03.02.22	11	6.43	1.014	0.55
04.02.22	12	6.39	0.82	0.45
05.02.22	13	4.80	0.815	0.44
06.02.22	14	4.79	0.933	0.51
07.02.22	15	4.77	0.78	0.42
08.02.22	16	4.79	0.847	0.46
09.02.22	17	4.74	0.761	0.41

Table I. 41 Variation in pH, OD, and DCW with time for R6 in Set4

Reactors		R6		
Date	Day	pH	OD 660	DCW (g/L)
23.01.22	0	6.54	0.276	0.13
24.01.22	1	7.54	2.042	0.95
25.01.22	2	6.72	2.004	0.93
26.01.22	3	6.82	1.883	0.88
27.01.22	4	6.81	1.773	0.83
28.01.22	5	6.82	1.755	0.82
29.01.22	6	6.82	1.600	0.74
30.01.22	7	6.73	1.574	0.73
31.01.22	8	-	1.463	0.68
01.02.22	9	6.75	1.620	0.75
02.02.22	10	6.81	1.393	0.65
03.02.22	11	6.91	1.398	0.65
04.02.22	12	6.87	1.212	0.56
05.02.22	13	4.81	1.312	0.61
06.02.22	14	4.78	1.293	0.60
07.02.22	15	4.82	1.173	0.55
08.02.22	16	4.85	1.228	0.57
09.02.22	17	4.83	1.179	0.55

Table I. 42 Daily variation of PHB for R1 in Set4

Reactors		R1		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB % of DCW
23.01.22	0	-	-	-
24.01.22	1	6.7	0.3	5.1
25.01.22	2	10.6	0.9	8.8
26.01.22	3	10.0	0.9	9.2
27.01.22	4	8.4	0.7	8.5
28.01.22	5	2.8	0.6	20.4
29.01.22	6	5.8	1.2	20.0
30.01.22	7	8.5	1.0	11.6
31.01.22	8	7.8	0.9	11.1
01.02.22	9	7.7	-	-
02.02.22	10	13.6	0.9	6.6
03.02.22	11	8.0	1.4	17.2
04.02.22	12	7.3	0.7	10.0
05.02.22	13	4.5	0.5	10.7
06.02.22	14	5.6	0.4	6.9
07.02.22	15	3.0	0.1	3.6
08.02.22	16	2.7	0.2	7.9
09.02.22	17	4.2	0.1	2.7
10.02.22	18	3.9	0.0	0.7

Table I. 43 Daily variation of PHB for R2 in Set4

Reactors		R2		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB% of DCW
23.01.22	0			
24.01.22	1	6.6	0.4	6.0
25.01.22	2	10.5	0.9	8.2
26.01.22	3	10.3	0.8	7.9
27.01.22	4	10.0	1.2	11.5
28.01.22	5	9.1	0.7	7.5
29.01.22	6	10.3	1.4	13.9
30.01.22	7	8.1	1.1	13.4
31.01.22	8	6.7	0.4	6.6
01.02.22	9	8.0	0.9	10.7
02.02.22	10	13.3	0.7	5.1
03.02.22	11	7.7	0.9	11.3
04.02.22	12	6.8	0.8	11.7
05.02.22	13	4.9	0.6	12.9
06.02.22	14	6.2	0.5	8.7
07.02.22	15	3.7	0.2	5.0
08.02.22	16	3.9	0.3	6.6
09.02.22	17	2.9	0.0	0.9
10.02.22	18	3.6	0.0	0.5

Table I. 44 Daily variation of PHB for R3 in Set4

Reactors		R3		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB% of DCW
23.01.22	0	-	-	-
24.01.22	1	9.4	0.4	4.3
25.01.22	2	11.4	0.7	5.8
26.01.22	3	10.8	0.9	8.1
27.01.22	4	13.4	1.5	11.5
28.01.22	5	10.1	1.0	9.6
29.01.22	6	9.7	1.4	14.0
30.01.22	7	7.5	0.9	12.1
31.01.22	8	10.0	1.2	12.5
01.02.22	9	8.3	1.0	12.1
02.02.22	10	7.0	1.4	20.6
03.02.22	11	9.8	1.7	17.5
04.02.22	12	6.0	-	-
05.02.22	13	8.3	1.0	12.2
06.02.22	14	7.0	0.7	9.5
07.02.22	15	5.9	0.7	12.0
08.02.22	16	6.1	0.5	8.8
09.02.22	17	5.6	0.5	8.4
10.02.22	18	4.3	-	-

Table I. 45 Daily variation of PHB for R4 in Set4

Reactors		R4		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB% of DCW
23.01.22	0	-	-	-
24.01.22	1	10.0	0.4	3.8
25.01.22	2	12.3	0.8	6.3
26.01.22	3	11.0	1.3	11.7
27.01.22	4	11.9	1.5	12.3
28.01.22	5	9.7	2.1	22.0
29.01.22	6	11.7	1.5	13.1
30.01.22	7	13.0	1.6	12.4
31.01.22	8	9.7	1.2	12.3
01.02.22	9	11.7	1.8	15.1
02.02.22	10	7.5	1.2	16.0
03.02.22	11	11.3	1.5	12.9
04.02.22	12	9.6	1.3	14.0
05.02.22	13	7.0	0.7	10.5
06.02.22	14	7.7	0.9	11.1
07.02.22	15	6.9	0.7	10.7
08.02.22	16	3.3	0.2	5.0
09.02.22	17	5.9	0.4	7.5
10.02.22	18	4.4	0.3	7.2

Table I. 46 Daily variation of PHB for R5 in Set4

Reactors		R5		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB% of DCW
01.02.22	9	12.1	2.0	16.2
02.02.22	10	9.3	2.5	27.2
03.02.22	11	13.4	2.4	17.7
04.02.22	12	12.6	1.6	12.7
05.02.22	13	4.1	0.5	12.3
06.02.22	14	7.5	0.6	7.6
07.02.22	15	7.2	0.4	5.2
08.02.22	16	4.9	0.3	6.4
09.02.22	17	4.9	0.3	6.6
10.02.22	18	6.7	0.4	6.6

Table I. 47 Daily variation of PHB for R6 in Set4

Reactors		R6		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB% of DCW
01.02.22	9	10.6	2.0	18.8
02.02.22	10	11.2	2.2	19.5
03.02.22	11	13.1	2.3	17.5
04.02.22	12	12.1	1.3	11.0
05.02.22	13	10.1	1.2	12.2
06.02.22	14	9.3	1.3	13.8
07.02.22	15	7.3	0.9	12.6
08.02.22	16	4.3	0.6	14.6
09.02.22	17	7.5	0.9	12.4

Table I. 48 Daily variation of produced biogas and its content for R1 in Set4

Reactors		R1				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
23.01.22	0	0	0.0	0.0	0.0	100.0
24.01.22	1	4	2.5	6.7	0.0	90.8
25.01.22	2	50	74.4	3.7	3.2	18.7
26.01.22	3	88	61.6	2.1	3.9	32.5
27.01.22	4	118	73.0	1.4	7.2	18.3
28.01.22	5	120	64.6	1.7	9.1	24.6
29.01.22	6	64	82.7	2.0	12.1	3.2
30.01.22	7	66	79.0	1.8	12.5	6.8
31.01.22	8	84	83.0	3.5	16.0	0.0
01.02.22	9	65	78.2	2.3	15.4	4.1
02.02.22	10	56	79.3	2.2	17.1	1.5
03.02.22	11	74	75.2	2.0	18.0	4.8
04.02.22	12	76	74.6	1.6	18.9	4.9
05.02.22	13	86	67.2	2.3	16.1	14.5
06.02.22	14	72	71.5	3.3	18.0	7.2
07.02.22	15	62	67.2	2.8	17.8	12.2
09.02.22	17	42	69.8	3.1	19.4	7.7
10.02.22	18	32	67.4	3.1	19.5	10.0
12.02.22	20	2	54.0	13.2	20.8	12.0
14.02.22	22	8	44.2	19.8	18.4	17.6
16.02.22	24	0	-	-	-	-

Table I. 49 Daily variation of produced biogas and its content for R2 in Set4

Reactors		R2				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
23.01.22	0	0	0.0	0.0	0.0	100.0
24.01.22	1	3	7.4	32.9	2.0	57.6
25.01.22	2	0	58.6	4.5	2.8	34.1
26.01.22	3	0	56.9	3.3	4.0	35.8
27.01.22	4	0	23.4	6.7	2.7	67.2
28.01.22	5	0	79.9	3.8	11.9	4.4
29.01.22	6	28	66.5	2.8	9.1	21.6
30.01.22	7	0	71.4	3.9	13.0	11.8
31.01.22	8	0	74.1	4.7	14.9	6.3
01.02.22	9	0	77.4	4.0	16.9	1.7
02.02.22	10	0	74.1	3.4	17.3	5.2
03.02.22	11	0	73.5	3.0	18.9	4.5
04.02.22	12	0	71.4	2.5	19.7	6.3
05.02.22	13	0	67.0	1.4	0.0	31.5
06.02.22	14	0	66.4	3.4	19.9	10.3
07.02.22	15	0	47.3	8.4	20.0	24.3
09.02.22	17	0	54.6	9.5	22.8	13.2
10.02.22	18	0	40.4	19.9	23.0	16.7
12.02.22	20	0	0.3	59.4	20.7	19.6

Table I. 50 Daily variation of produced biogas and its content for R3 in Set4

Reactors		R3				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
23.01.22	0	0	0.0	0.0	0.0	100.0
24.01.22	1	31	67.0	3.6	2.4	27.0
25.01.22	2	118	74.2	3.6	3.9	18.2
26.01.22	3	94	37.0	3.0	3.2	56.8
27.01.22	4	92	80.8	1.9	6.3	10.9
28.01.22	5	96	81.2	2.3	7.8	8.7
29.01.22	6	60	62.3	3.6	6.5	27.5
30.01.22	7	46	85.0	2.8	10.1	2.2
31.01.22	8	63	43.6	1.8	6.9	47.7
01.02.22	9	11	106.3	3.1	12.5	-22.0
02.02.22	10	42	80.7	3.2	12.8	3.3
03.02.22	11	38	84.3	2.6	14.4	-1.3
04.02.22	12	48	78.2	2.8	15.4	3.7
05.02.22	13	58	68.8	2.9	14.7	13.6
06.02.22	14	44	72.2	2.4	16.4	9.1
07.02.22	15	36	66.7	5.3	15.0	12.9
09.02.22	17	8	66.4	5.9	16.9	10.8
10.02.22	18	8	70.4	7.1	17.9	4.6
12.02.22	20	8	55.7	13.2	16.7	14.4
14.02.22	22	8	56.0	14.6	17.0	12.3
16.02.22	24	0	-	-	-	-

Table I. 51 Daily variation of produced biogas and its content for R4 in Set4

Reactors		R4				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
23.01.22	0	0	0.0	0.0	0.0	100.0
24.01.22	1	41	43.5	10.9	2.0	43.5
25.01.22	2	0	76.4	2.8	2.4	18.4
26.01.22	3	36	29.6	2.0	1.7	66.7
27.01.22	4	2	95.0	2.2	6.5	0.0
28.01.22	5	0	66.5	1.9	2.2	29.5
29.01.22	6	0	58.3	7.3	6.0	28.4
30.01.22	7	0	81.2	4.4	9.8	4.6
31.01.22	8	16	78.2	6.7	11.7	3.4
01.02.22	9	12	76.5	4.5	12.8	6.3
02.02.22	10	0	76.3	4.9	14.1	4.7
03.02.22	11	0	75.7	5.0	15.8	3.6
04.02.22	12	0	76.3	3.6	16.8	3.4
05.02.22	13	0	67.2	4.2	16.0	12.6
06.02.22	14	12	66.3	5.1	16.8	11.8
07.02.22	15	10	62.1	6.7	17.9	13.2
09.02.22	17	0	53.7	14.4	19.9	12.1
10.02.22	18	4	47.9	19.8	19.5	12.7
11.02.22	19	1	-	-	-	-
12.02.22	20	3	34.1	28.8	19.9	17.2
14.02.22	22	4	38.9	26.3	19.8	14.9
16.02.22	24	0	-	-	-	-

Table I. 52 Daily variation of produced biogas and its content for R5 in Set4

Reactors		R5				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
23.01.22	0	0	0.0	0.0	0.0	100.0
24.01.22	1	4	11.3	9.9	0.8	78.0
25.01.22	2	62	78.8	2.5	1.9	16.8
26.01.22	3	92	62.3	2.7	3.4	31.6
27.01.22	4	95	93.8	2.1	6.1	-1.9
28.01.22	5	68	63.1	8.5	4.8	23.7
29.01.22	6	38	83.2	8.3	6.4	2.1
30.01.22	7	36	73.9	12.3	6.6	7.2
31.01.22	8	32	64.1	7.9	6.4	21.7
01.02.22	9	22	67.9	16.6	8.5	7.1
02.02.22	10	20	33.8	6.7	55.7	3.8
03.02.22	11	8	28.2	15.7	51.2	4.8
04.02.22	12	8	24.2	21.9	46.7	7.2
05.02.22	13	4	18.3	27.0	39.6	15.2
06.02.22	14	4	13.1	34.7	35.7	16.5
07.02.22	15	0	8.8	40.8	34.5	16.0
08.02.22	16	2	-	-	-	-
09.02.22	17	0	3.8	48.6	27.3	20.3
10.02.22	18	0	3.7	51.8	26.1	18.4
11.02.22	19	3	-	-	-	-
12.02.22	20	5	14.4	43.7	23.9	18.1
14.02.22	22	0	-	-	-	-

Table I. 52 Continued

15.02.22	23	-	-	-	-	-
16.02.22	24	0	-	-	-	-
17.02.22	25	-	-	-	-	-
18.02.22	26	0	71.2	9.0	33.5	-13.8
19.02.22	27	12	25.5	15.2	39.3	20.1
20.02.22	28	-	-	-	-	-
21.02.22	29	-	-	-	-	-
22.02.22	30	8	4.8	20.2	30.5	44.4
23.02.22	31	-	-	-	-	-
24.02.22	32	0	1.1	19.8	31.2	48.0

Table I. 53 Daily variation of produced biogas and its content for R6 in Set4

Reactors		R6				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
23.01.22	0	0	0.0	0.0	0.0	100.0
24.01.22	1	4	6.9	13.1	1.3	78.7
25.01.22	2	74	78.6	3.0	2.7	15.7
26.01.22	3	102	68.1	1.9	3.1	26.9
27.01.22	4	62	90.0	2.2	3.5	4.3
28.01.22	5	82	88.4	2.6	2.9	6.1
29.01.22	6	60	89.4	2.7	3.5	4.4
30.01.22	7	62	87.5	2.5	4.0	6.0
31.01.22	8	49	71.5	2.8	4.4	21.3
01.02.22	9	23	87.7	3.8	6.3	2.3
02.02.22	10	20	56.8	9.0	32.4	1.9

Table I. 53 Continued

02.02.22	10	20	56.8	9.0	32.4	1.9
03.02.22	11	20	62.3	3.9	30.5	3.3
04.02.22	12	14	62.7	3.1	27.8	6.4
05.02.22	13	8	58.5	4.5	24.9	12.1
06.02.22	14	6	59.0	5.9	23.5	11.6
07.02.22	15	8	48.0	17.0	21.5	13.5
08.02.22	16	12	-	-	-	-
09.02.22	17	10	61.1	9.1	20.1	9.7
10.02.22	18	4	62.0	11.2	19.1	7.7
11.02.22	19	8	-	-	-	-
12.02.22	20	8	68.5	7.3	18.0	6.3
13.02.22	21	-	-	-	-	-
14.02.22	22	16	64.3	7.6	17.9	10.3
15.02.22	23	-	-	-	-	-
16.02.22	24	0	67.7	8.8	17.0	6.5
17.02.22	25	-	-	-	-	-
18.02.22	26	10	28.3	6.8	55.4	9.5
19.02.22	27	2	18.2	11.7	50.3	19.7
20.02.22	28	-	-	-	-	-
21.02.22	29	-	-	-	-	-
22.02.22	30	16	4.8	16.6	36.1	42.5
23.02.22	31	0	-	-	-	-
24.02.22	32	0	3.6	8.0	43.9	44.5

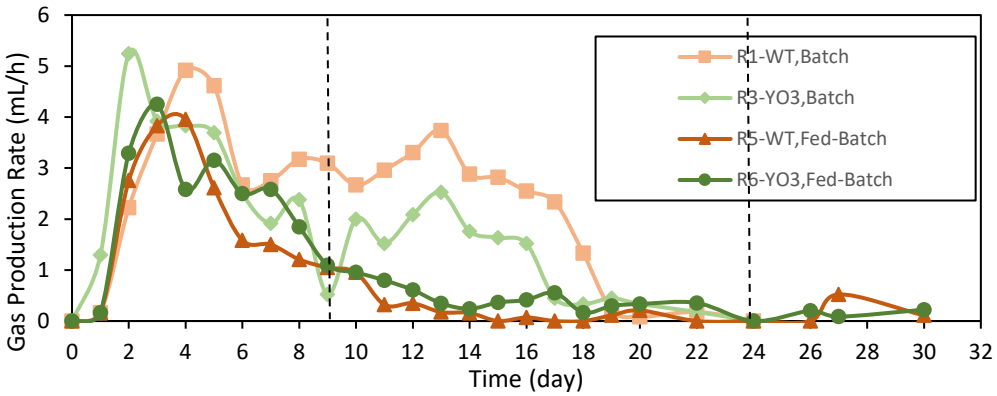

Figure I. 2 The graph of biogas rate in Set4

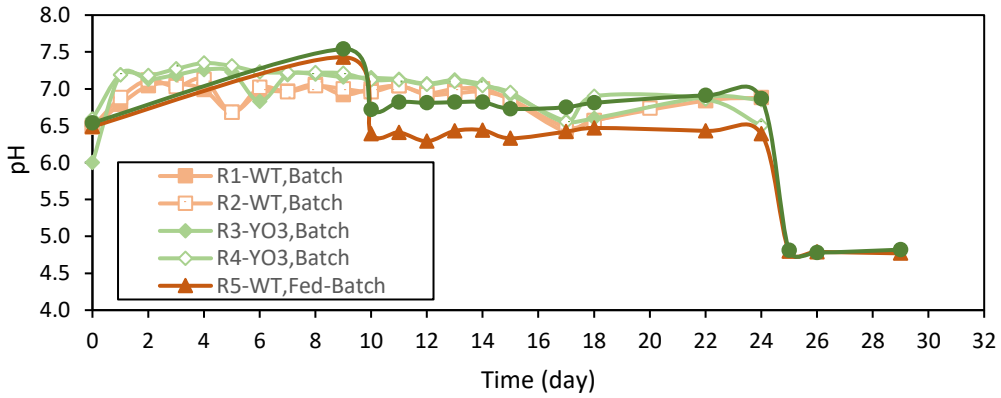

Figure I. 3 pH changes over time in Set4

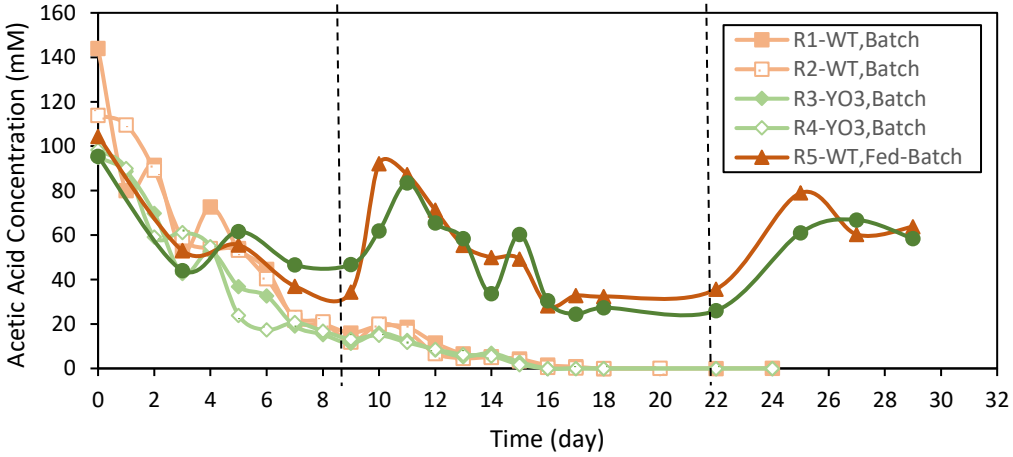

Figure I. 4 Variation of acetic acid with time in Set4

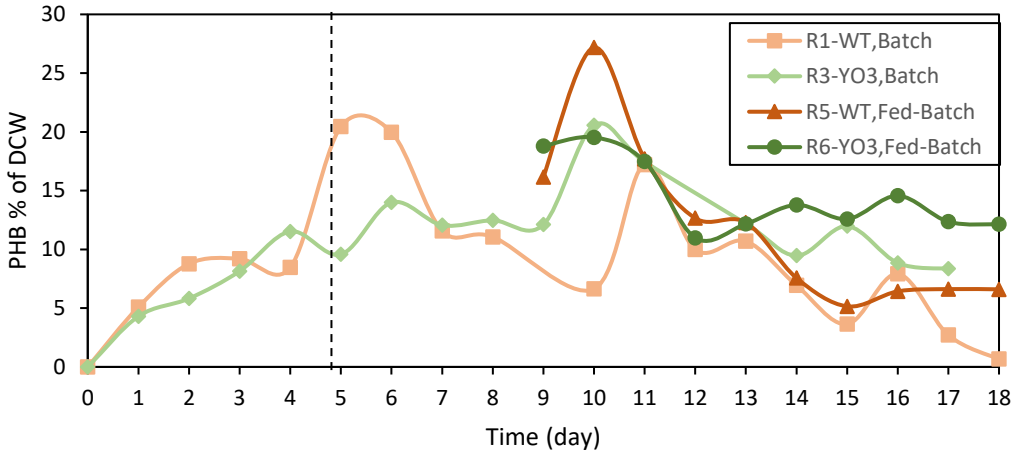

Figure I. 5 The PHB% of DCW for reactors in Set4

Raw Data for Set5

Table I. 54 Name of reactors in Set5

Reactor No	Bacterial Strain	Operation Mode
R1	*R.capsulatus* WT	Batch
R2	*R.capsulatus* WT	Batch
R3	*R.capsulatus* YO3 (hup^-)	Batch
R4	*R.capsulatus* YO3 (hup^-)	Batch
R5	*R.capsulatus* WT	Fed-Batch
R6	*R.capsulatus* YO3 (hup^-)	Fed-Batch

Table I. 55 Variation in pH, OD, and DCW with time for R1 in Set5

Reactors		R1		
Date	Day	pH	OD 660	DCW (g/L)
03.04.22	0	6.62	0.383	0.21
05.04.22	2	7.28	1.783	0.97

Table I.55 Continued

06.04.22	3	7.28	1.762	0.96
07.04.22	4	7.23	1.761	0.96
08.04.22	5	7.06	1.786	0.97
09.04.22	6	7.03	1.667	0.90
10.04.22	7	7.16	1.582	0.86
11.04.22	8	7.00	1.529	0.83
12.04.22	9	6.91	1.571	0.85
13.04.22	10	7.04	1.420	0.77
14.04.22	11	6.99	1.528	0.83
15.04.22	12	6.98	1.526	0.83
16.04.22	13	6.93	1.175	0.64
17.04.22	14	6.85	1.102	0.60
18.04.22	15	6.84	0.849	0.46
19.04.22	16	6.86	0.746	0.41
20.04.22	17	6.78	0.930	0.50

Table I. 56 Variation in pH, OD, and DCW with time for R2 in Set5

Reactors		R2		
Date	Day	pH	OD 660	DCW (g/L)
03.04.22	0	6.62	0.383	0.21
05.04.22	2	7.25	1.723	0.94
06.04.22	3	7.29	1.811	0.98
07.04.22	4	7.29	1.869	1.01
08.04.22	5	7.15	1.910	1.04
09.04.22	6	7.09	1.831	0.99
10.04.22	7	7.17	1.760	0.96
11.04.22	8	7.04	1.702	0.92

Table I. 56 Continued

12.04.22	9	6.96	1.675	0.91
13.04.22	10	7.11	1.599	0.87
14.04.22	11	6.96	1.578	0.86
15.04.22	12	6.98	1.624	0.88
16.04.22	13	6.96	1.315	0.71
17.04.22	14	6.88	1.313	0.71
18.04.22	15	6.84	1.223	0.66
19.04.22	16	6.89	0.997	0.54
20.04.22	17	6.82	1.121	0.61
21.04.22	18	6.85	0.859	0.47
22.04.22	19	6.84	0.749	0.41
23.04.22	20	6.75	0.743	0.40

Table I. 57 Variation in pH, OD, and DCW with time for R3 in Set5

Reactors		R3		
Date	Day	pH	OD 660	DCW (g/L)
03.04.22	0	6.56	0.475	0.22
05.04.22	2	7.28	1.773	0.83
06.04.22	3	7.45	1.924	0.90
07.04.22	4	7.55	1.965	0.91
08.04.22	5	7.41	1.972	0.92
09.04.22	6	7.30	2.040	0.95
10.04.22	7	7.40	1.865	0.87
11.04.22	8	7.20	1.838	0.86
12.04.22	9	7.16	1.811	0.84
13.04.22	10	7.17	1.669	0.78
14.04.22	11	7.03	1.654	0.77

Table I. 57 Continued

15.04.22	12	7.04	1.561	0.73
16.04.22	13	7.04	1.465	0.68
17.04.22	14	7.00	1.405	0.65
18.04.22	15	6.96	1.329	0.62
19.04.22	16	7.00	1.180	0.55
20.04.22	17	6.91	1.134	0.53
21.04.22	18	6.89	1.051	0.49
22.04.22	19	6.89	1.159	0.54
23.04.22	20	6.88	0.850	0.40
24.04.22	21	6.85	0.719	0.33
25.04.22	22	6.85	0.705	0.33
26.04.22	23	6.83	0.707	0.33

Table I. 58 Variation in pH, OD, and DCW with time for R4 in Set5

Reactors		R4		
Date	Day	pH	OD 660	DCW (g/L)
03.04.22	0	6.56	0.475	0.22
04.04.22	1	-	-	-
05.04.22	2	7.31	1.761	0.82
06.04.22	3	7.48	1.938	0.90
07.04.22	4	7.50	1.966	0.92
08.04.22	5	7.36	1.997	0.93
09.04.22	6	7.36	1.934	0.90
10.04.22	7	7.37	1.874	0.87
11.04.22	8	7.19	1.842	0.86
12.04.22	9	7.13	1.787	0.83
13.04.22	10	7.18	1.669	0.78

Table I. 58 Continued

14.04.22	11	7.02	1.677	0.78
15.04.22	12	7.04	1.553	0.72
16.04.22	13	7.06	1.400	0.65
17.04.22	14	6.97	1.331	0.62
18.04.22	15	6.92	1.238	0.58
19.04.22	16	6.93	1.035	0.48
20.04.22	17	6.88	1.062	0.49

Table I. 59 Variation in pH, OD, and DCW with time for R5 in Set5

Reactors		R5		
Date	Day	pH	OD 660	DCW (g/L)
03.04.22	0	6.62	0.383	0.21
06.04.22	3	7.34	1.900	1.03
07.04.22	4	7.47	1.932	1.05
08.04.22	5	7.06	1.965	1.07
09.04.22	6	6.96	1.922	1.04
10.04.22	7	7.10	1.988	1.08
11.04.22	8	7.23	1.946	1.06
12.04.22	9	7.04	1.986	1.08
13.04.22	10	7.45	1.918	1.04
14.04.22	11	7.12	2.001	1.09
15.04.22	12	7.09	2.014	1.09
16.04.22	13	7.30	1.915	1.04
17.04.22	14	7.08	1.974	1.07
18.04.22	15	7.10	1.920	1.04
19.04.22	16	7.17	1.804	0.98

Table I. 59 Continued

20.04.22	17	7.07	1.827	0.99
21.04.22	18	7.08	1.786	0.97
22.04.22	19	7.04	1.834	1.00
23.04.22	20	7.14	1.713	0.93
24.04.22	21	7.26	1.695	0.92
25.04.22	22	7.08	1.690	0.92
26.04.22	23	7.17	1.593	0.86
27.04.22	24	7.05	1.601	0.87
28.04.22	25	7.06	1.663	0.90
29.04.22	26	7.07	1.552	0.84

Table I. 60 Variation in pH, OD, and DCW with time for R6 in Set5

Reactors		R6		
Date	Day	pH	OD 660	DCW (g/L)
03.04.22	0	6.56	0.475	0.22
06.04.22	3	7.50	1.930	0.90
07.04.22	4	7.57	1.996	0.93
08.04.22	5	7.12	1.998	0.93
09.04.22	6	7.06	2.018	0.94
10.04.22	7	7.21	1.924	0.90
11.04.22	8	7.27	1.962	0.91
12.04.22	9	7.12	1.917	0.89
13.04.22	10	7.55	1.844	0.86
14.04.22	11	7.13	1.867	0.87
15.04.22	12	7.09	1.819	0.85
16.04.22	13	7.44	1.705	0.79
17.04.22	14	7.09	1.731	0.81

Table I. 60 Continued

18.04.22	15	7.14	1.633	0.76
19.04.22	16	7.21	1.602	0.75
20.04.22	17	7.15	1.574	0.73
21.04.22	18	7.09	1.567	0.73
22.04.22	19	7.30	1.578	0.73
23.04.22	20	7.15	1.507	0.70
24.04.22	21	7.31	1.484	0.69
25.04.22	22	7.18	1.744	0.81
26.04.22	23	7.16	1.445	0.67
27.04.22	24	7.12	1.522	0.71
28.04.22	25	7.08	1.614	0.75
29.04.22	26	7.06	1.473	0.69

Table I. 61 Daily variation of PHB for R1 in Set5

Reactors		R1		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB % of DCW
03.04.22	0	3.3	0.0	0.0
05.04.22	2	11.8	1.3	11.2
06.04.22	3	10.3	1.3	13.0
07.04.22	4	11.7	0.8	6.8
08.04.22	5	12.5	1.3	10.3
09.04.22	6	11.1	1.0	9.1
10.04.22	7	7.7	0.8	10.1
11.04.22	8	7.1	0.5	7.2
12.04.22	9	5.7	0.6	10.4
13.04.22	10	7.0	0.5	6.9

Table I. 61 Continued

14.04.22	11	7.6	0.5	6.6
15.04.22	12	7.1	0.6	7.8
16.04.22	13	7.2	0.5	6.3
18.04.22	15	4.1	0.0	0.0
19.04.22	16	4.7	0.0	0.0
20.04.22	17	14.0	0.0	0.0

Table I. 62 Daily variation of PHB for R2 in Set5

Reactors		R2		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB % of DCW
03.04.22	0	3.3	0.0	0.0
05.04.22	2	12.1	1.3	10.4
06.04.22	3	12.8	1.5	12.1
07.04.22	4	13.2	1.3	9.7
08.04.22	5	14.9	1.5	10.0
09.04.22	6	13.4	1.6	11.6
11.04.22	8	10.0	0.8	7.9
12.04.22	9	10.6	1.3	12.0
13.04.22	10	8.5	0.6	7.1
14.04.22	11	5.2	0.5	10.2
15.04.22	12	6.6	0.4	5.7
16.04.22	13	9.0	0.8	8.4
17.04.22	14	6.9	0.6	9.0
18.04.22	15	5.4	0.4	7.1
19.04.22	16	5.6	0.4	6.3

Table I. 62 Continued

20.04.22	17	3.6	0.0	0.0
21.04.22	18	5.0	0.0	0.0
22.04.22	19	3.8	0	0
23.04.22	20	12.7	0	0

Table I. 63 Daily variation of PHB for R3 in Set5

Reactors		R3		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB % of DCW
03.04.22	0	2.1	0.0	0.0
05.04.22	2	7.6	0.7	8.8
06.04.22	3	10.9	1.1	10.2
07.04.22	4	14.2	1.8	12.9
08.04.22	5	14.1	2.1	15.1
09.04.22	6	14.1	1.9	13.5
10.04.22	7	10.8	1.3	12.2
11.04.22	8	11.8	1.5	12.5
12.04.22	9	9.3	1.2	12.9
13.04.22	10	8.2	0.8	9.9
14.04.22	11	6.3	0.3	4.8
15.04.22	12	5.1	0.7	13.8
16.04.22	13	9.6	1.2	12.7
18.04.22	15	7.5	0.6	8.0
20.04.22	17	6.2	0.4	6.3
21.04.22	18	6.0	0.5	8.2
22.04.22	19	4.7	0.3	6.0

Table I. 63 Continued

23.04.22	20	4.3	0.2	5.7
24.04.22	21	3.8	0.1	2.7
25.04.22	22	4.1	0.2	4.7
26.04.22	23	11.9	0.6	4.7

Table I. 64 Daily variation of PHB for R4 in Set5

Reactors		R4		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB % of DCW
03.04.22	0	2.1	0.0	0.0
05.04.22	2	17.5	1.6	9.1
06.04.22	3	13.2	1.1	8.4
07.04.22	4	14.9	1.3	9.0
09.04.22	6	14.3	2.0	13.7
11.04.22	8	10.0	1.1	11.5
12.04.22	9	10.4	1.3	13.0
13.04.22	10	9.8	1.3	13.0
14.04.22	11	5.2	0.5	9.6
15.04.22	12	9.3	1.5	16.0
16.04.22	13	9.7	1.0	10.0
17.04.22	14	8.1	0.7	8.9
18.04.22	15	7.5	0.6	7.9
19.04.22	16	6.5	0.4	5.5
20.04.22	17	18.1	0.8	4.6

Table I. 65 Daily variation of PHB for R5 in Set5

Reactors		R5		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB% of DCW
03.04.22	0	3.3	0.0	0.0
06.04.22	3	16.4	2.1	12.6
07.04.22	4	13.7	1.7	12.5
17.04.22	14	8.7	1.1	12.8
29.04.22	26	25	3.0	11.9

Table I. 66 Daily variation of PHB for R6 in Set5

Reactors		R6		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB% of DCW
03.04.22	0	2.1	0.0	0.0
06.04.22	3	11.6	0.9	8.2
07.04.22	4	13.6	1.5	11.2
17.04.22	14	12.2	1.7	14.3
29.04.22	26	23.0	2.9	12.4

Table I. 67 Daily variation of produced biogas and its content for R1 in Set5

Reactors		R1				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
03.04.22	0	0	0.0	0.0	0.0	100.0

Table I. 67 Continued

05.04.22	2	6	63.0	18.7	3.3	14.9
06.04.22	3	22	102.0	4.8	6.6	-13.3
07.04.22	4	36	70.6	3.9	8.4	17.1
08.04.22	5	0	86.0	5.0	12.8	0.0
09.04.22	6	16	68.7	13.9	14.1	3.4
10.04.22	7	4	78.0	2.5	16.8	2.7
11.04.22	8	2	78.5	3.1	18.7	0.0
12.04.22	9	8	78.2	2.6	20.6	0.0
13.04.22	10	13	70.4	4.8	21.6	3.3
14.04.22	11	7	74.2	2.9	22.0	0.9
15.04.22	12	2	74.9	3.3	23.0	0.0
16.04.22	13	4	70.4	5.5	25.2	0.0
17.04.22	14	2	64.9	6.4	24.7	4.0
18.04.22	15	0	56.3	16.7	27.1	0.0
19.04.22	16	2	13.8	54.9	26.6	4.7
20.04.22	17	0	1.0	66.4	23.1	9.5

Table I. 68 Daily variation of produced biogas and its content for R2 in Set5

Reactors		R2				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
03.04.22	0	0	0.0	0.0	0.0	100.0
05.04.22	2	40	74.6	8.8	3.4	13.2
06.04.22	3	84	92.2	1.2	5.6	1.0
07.04.22	4	106	90.3	2.2	7.4	0.1
09.04.22	6	56	88.2	4.6	8.8	-1.6

Table I. 68 Continued

10.04.22	7	40	69.0	2.2	9.7	19.0
11.04.22	8	8	90.6	2.4	12.5	-5.5
12.04.22	9	0	92.3	1.2	14.1	-7.6
13.04.22	10	65	73.2	8.1	14.7	4.0
14.04.22	11	58	81.0	4.1	18.1	-3.2
15.04.22	12	10	87.0	1.6	18.6	-7.2
16.04.22	13	24	82.5	1.6	19.4	-3.5
17.04.22	14	6	77.2	2.3	19.7	0.9
18.04.22	15	2	76.0	1.7	19.7	2.6
20.04.22	17	24	81.2	1.5	21.8	-4.6
21.04.22	18	34	77.4	3.1	22.5	-3.1
22.04.22	19	10	76.6	2.0	22.2	-0.8
23.04.22	20	2	73.5	3.3	22.7	0.5
24.04.22	21	2	61.9	14.7	22.5	0.8
25.04.22	22	0	28.8	43.5	18.9	8.8

Table I. 69 Daily variation of produced biogas and its content for R3 in Set5

Reactors		R3				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
03.04.22	0	0	0.0	0.0	0.0	100.0
05.04.22	2	80	78.7	6.6	3.3	11.4
06.04.22	3	94	104.6	1.1	4.8	-10.5
07.04.22	4	84	97.4	1.7	5.0	-4.1

Table I. 60 Continued

08.04.22	5	38	89.6	3.7	5.7	1.0
09.04.22	6	36	88.3	3.8	7.1	0.8
10.04.22	7	54	87.2	2.4	8.8	1.6
11.04.22	8	38	94.0	1.4	10.5	-5.9
12.04.22	9	44	88.3	2.1	12.1	-2.4
13.04.22	10	42	90.0	3.6	14.4	-8.0
14.04.22	11	28	85.0	4.6	14.9	-4.6
15.04.22	12	36	86.7	1.9	16.6	-5.2
16.04.22	13	58	79.0	2.5	17.3	1.3
17.04.22	14	24	78.8	2.9	17.8	0.5
18.04.22	15	24	80.4	2.0	18.8	-1.1
19.04.22	16	36	89.5	3.0	20.7	-13.2
20.04.22	17	2	93.3	3.4	20.8	-17.6
21.04.22	18	20	74.4	2.8	20.4	2.4
22.04.22	19	12	73.7	3.5	20.7	2.1
23.04.22	20	8	70.8	5.8	18.4	5.0
24.04.22	21	6	53.8	16.1	17.2	12.8
25.04.22	22	2	58.8	15.8	19.4	6.0
26.04.22	23	0	58.5	19.5	19.0	2.9

Table I. 70 Daily variation of produced biogas and its content for R4 in Set5

Reactors		R4				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
03.04.22	0	0	0.0	0.0	0.0	100.0
05.04.22	2	0	80.3	5.3	3.7	10.7

Table I. 70 Continued

06.04.22	3	0	83.4	6.9	4.8	4.9
07.04.22	4	4	89.2	4.3	5.8	0.8
08.04.22	5	4	84.6	8.2	7.3	0.0
09.04.22	6	36	65.3	12.6	8.0	14.1
10.04.22	7	44	86.0	2.8	10.5	0.7
11.04.22	8	0	76.5	4.7	11.1	7.7
12.04.22	9	38	67.4	7.5	12.2	12.9
13.04.22	10	43	81.2	3.9	15.9	0.0
14.04.22	11	29	82.2	4.1	17.0	-3.3
15.04.22	12	0	80.3	2.9	18.5	-1.8
16.04.22	13	40	76.0	3.8	20.6	0.0
17.04.22	14	4	61.2	12.6	20.6	5.6
18.04.22	15	0	62.9	15.3	23.4	-1.6
19.04.22	16	2	51.4	22.4	25.2	1.1
20.04.22	17	0	41.6	29.2	24.4	4.9

Table I. 71 Daily variation of produced biogas and its content for R5 in Set5

Reactors		R5				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
03.04.22	0	0	0.0	0.0	0.0	100.0
05.04.22	2	84	78.5	4.8	3.7	12.9
06.04.22	3	98	108.5	1.2	5.7	-15.3
07.04.22	4	86	89.9	3.3	6.1	0.7
08.04.22	5	66	78.5	3.0	16.1	2.5
09.04.22	6	48	93.5	3.2	18.0	-14.7

Table I. 71 Continued

10.04.22	7	63	79.0	2.3	17.5	1.2
11.04.22	8	57	79.4	2.1	17.7	0.8
12.04.22	9	52	79.6	1.3	17.3	1.8
13.04.22	10	53	68.0	9.0	17.1	5.8
14.04.22	11	35	76.6	3.6	17.9	1.9
15.04.22	12	38	76.7	3.3	18.0	2.0
16.04.22	13	52	81.0	3.1	19.0	-3.2
17.04.22	14	26	74.6	2.2	17.8	5.4
18.04.22	15	8	86.7	4.0	19.5	-10.2
19.04.22	16	34	76.2	3.1	19.6	1.1
20.04.22	17	14	7.6	2.2	19.7	70.6
21.04.22	18	16	76.5	3.0	20.0	0.5
22.04.22	19	14	78.4	3.7	20.9	-3.0
23.04.22	20	14	74.9	2.8	18.2	4.2
24.04.22	21	8	66.8	4.0	16.2	13.0
25.04.22	22	8	74.2	2.8	20.3	2.7
26.04.22	23	12	75.6	3.7	20.5	0.2
27.04.22	24	2	76.1	4.1	20.9	-1.1
28.04.22	25	2	77.7	3.9	20.6	-2.1
29.04.22	26	0	80.6	4.4	20.5	-5.4

Table I. 72 Daily variation of produced biogas and its content for R6 in Set5

Reactors		R6				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
05.04.22	2	70	84.9	4.2	3.8	7.1
06.04.22	3	88	106.3	3.4	4.1	-13.8
07.04.22	4	96	80.6	5.9	4.3	9.2
08.04.22	5	18	81.1	4.3	12.7	2.0
10.04.22	7	20	84.1	3.0	13.9	-1.0
11.04.22	8	20	72.9	7.4	13.1	6.6
12.04.22	9	18	62.6	10.9	13.4	13.1
13.04.22	10	23	69.7	5.1	14.4	10.8
14.04.22	11	11	73.5	3.0	14.4	9.1
15.04.22	12	12	79.7	3.1	16.0	1.2
16.04.22	13	18	79.3	4.4	16.7	-0.3
17.04.22	14	8	76.7	3.7	16.5	3.1
18.04.22	15	6	72.9	7.3	14.9	4.8
19.04.22	16	16	78.2	4.7	16.1	0.9
20.04.22	17	6	77.9	4.6	16.2	1.3
21.04.22	18	8	76.4	5.3	16.4	2.0
22.04.22	19	8	77.2	4.9	14.6	3.3
23.04.22	20	10	75.3	4.6	14.1	6.0
24.04.22	21	8	74.3	6.2	14.0	5.5
25.04.22	22	2	74.9	5.0	15.9	4.3
26.04.22	23	12	75.3	5.8	15.6	3.3
27.04.22	24	2	78.0	5.3	16.2	0.5
28.04.22	25	4	78.5	5.6	16.0	-0.1
29.04.22	26	11	61.9	4.1	12.5	21.5

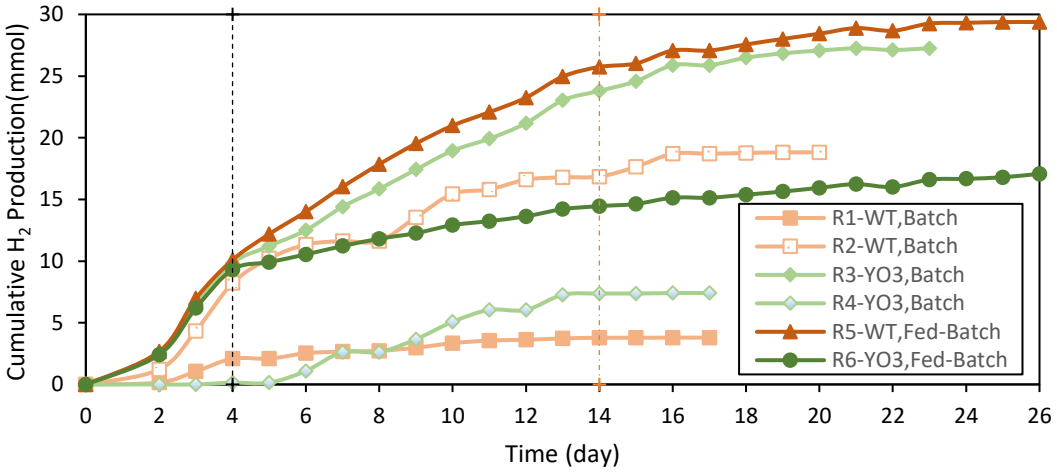

Figure I. 6 The cumulative H_2 production in Set5

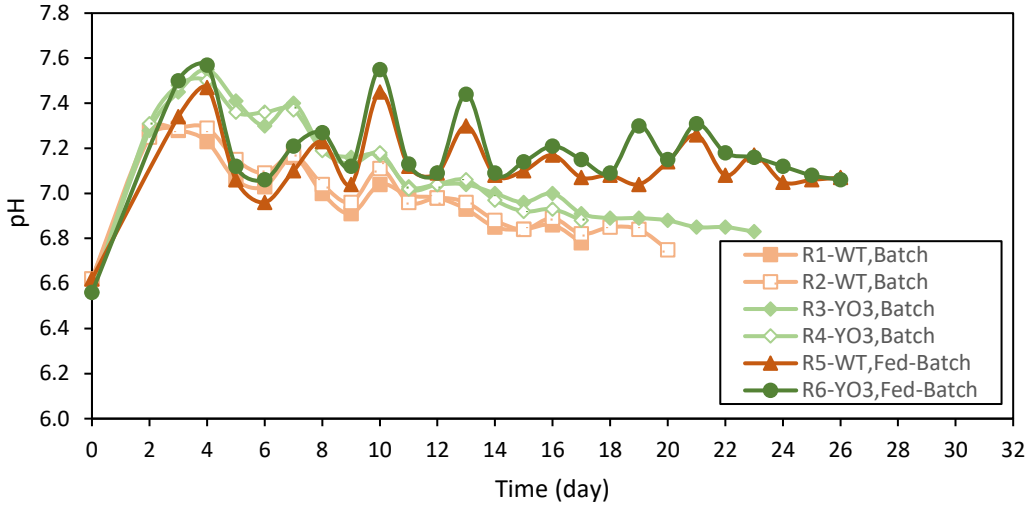

Figure I. 7 pH changes over time in Set5

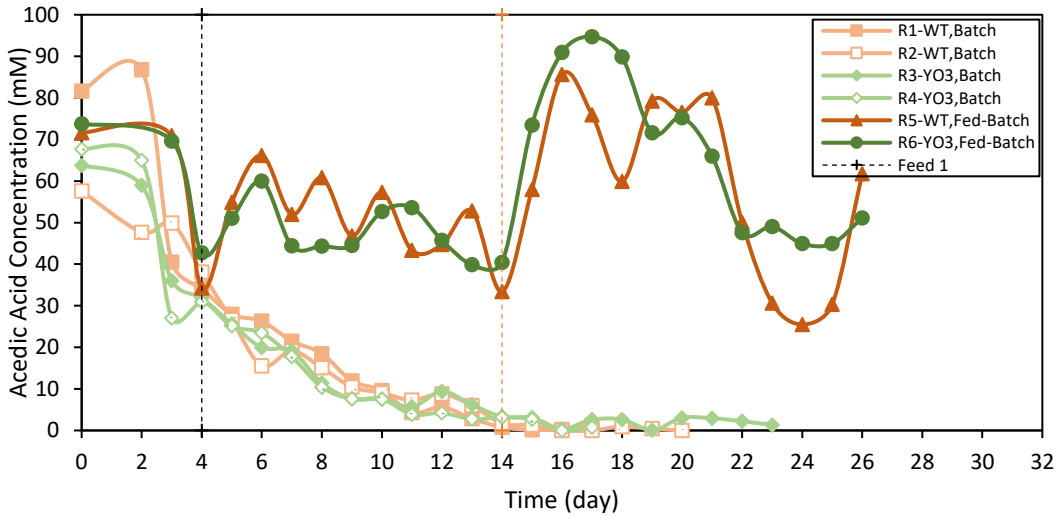

Figure I. 8 Variation of acetic acid with time in Set5

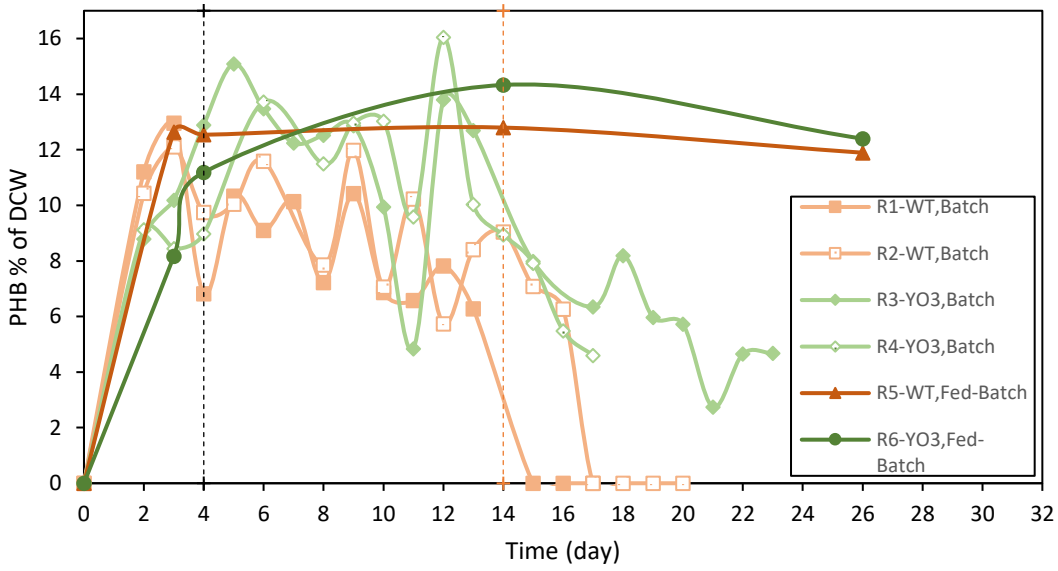

Figure I. 9 PHB% of DCW for reactors in Set5

Raw Data for Set6

Table I. 73 Name of reactors in Set6. Note: All reactors were operated in batch

Reactor No	Bacterial Strain
R1	*R.capsulatus* WT
R2	*R.capsulatus* WT
R3	*R.capsulatus* YO3 (hup^-)
R4	*R.capsulatus* YO3 (hup^-)
R5	*R.capsulatus* WT
R6	*R.capsulatus* YO3 (hup^-)

Table I. 74 Variation in pH, OD, and DCW with time for R1 in Set6

Reactors		R1		
Date	Day	pH	OD 660	DCW (g/L)
02.06.22	0	6.61	0.374	0.20
03.06.22	1	7.24	1.664	0.90
04.06.22	2	7.21	1.789	0.97
05.06.22	3	7.22	1.901	1.03
06.06.22	4	-	1.835	1.00
07.06.22	5	7.20	1.879	1.02
08.06.22	6	-	1.995	1.08
09.06.22	7	7.07	1.887	1.02
10.06.22	8	7.07	1.735	0.94
11.06.22	9	7.00	1.688	0.92
12.06.22	10	6.63	1.570	0.85
13.06.22	11	6.58	1.490	0.81
14.06.22	12	6.53	1.412	0.77
15.06.22	13	6.58	1.316	0.71
16.06.22	14	6.88	1.265	0.69

Table I. 74 Continued

17.06.22	15	6.86	1.142	0.62
18.06.22	16	6.84	1.042	0.57
20.06.22	18	6.84	0.879	0.477
21.06.22	19	6.85	0.785	0.426
22.06.22	20	6.81	0.767	0.416

Table I. 75 Variation in pH, OD, and DCW with time for R2 in Set6

Reactors		R2		
Date	Day	pH	OD 660	DCW (g/L)
02.06.22	0	6.57	0.386	0.21
03.06.22	1	7.26	1.674	0.91
04.06.22	2	7.26	1.795	0.97
05.06.22	3	7.23	1.864	1.01
06.06.22	4	-	1.835	1.00
07.06.22	5	7.20	1.866	1.01
08.06.22	6	-	1.842	1.00
09.06.22	7	7.12	1.808	0.98
10.06.22	8	7.04	1.726	0.94
11.06.22	9	6.98	1.821	0.99
12.06.22	10	7.00	1.576	0.86
13.06.22	11	6.63	1.517	0.82
14.06.22	12	6.96	1.480	0.80
15.06.22	13	6.89	1.371	0.74
16.06.22	14	6.91	1.293	0.70
17.06.22	15	6.86	1.222	0.66
18.06.22	16	6.87	1.090	0.59
20.06.22	18	6.82	0.963	0.52

Table I. 75 Continued

21.06.22	19	6.80	0.872	0.47
22.06.22	20	6.79	0.792	0.43
23.06.22	21	6.78	0.710	0.386
24.06.22	22	6.39	0.618	0.336

Table I. 76 Variation in pH, OD, and DCW with time for R3 in Set6

Reactors		R3		
Date	Day	pH	OD 660	DCW (g/L)
02.06.22	0	6.59	0.398	0.22
03.06.22	1	7.22	1.655	0.90
04.06.22	2	7.20	1.780	0.97
05.06.22	3	7.22	1.914	1.04
06.06.22	4	-	1.819	0.99
07.06.22	5	7.18	1.881	1.02
08.06.22	6	7.22	1.829	0.99
09.06.22	7	7.06	1.878	1.02
10.06.22	8	7.04	1.698	0.92
11.06.22	9	6.98	1.657	0.90
12.06.22	10	6.97	1.570	0.85
13.06.22	11	6.55	1.456	0.79
14.06.22	12	6.96	1.444	0.78
15.06.22	13	6.88	1.316	0.71
16.06.22	14	6.84	1.243	0.67
17.06.22	15	6.88	1.135	0.62
18.06.22	16	6.88	1.049	0.57
20.06.22	18	6.79	0.892	0.48
21.06.22	19	6.81	0.824	0.45

Table I. 76 Continued

22.06.22	20	6.77	0.747	0.41
23.06.22	21	6.70	0.677	0.37

Table I. 77 Variation in pH, OD, and DCW with time for R4 in Set6

Reactors		R4		
Date	Day	pH	OD 660	DCW (g/L)
02.06.22	0	6.60	0.327	0.15
03.06.22	1	7.30	1.702	0.79
04.06.22	2	7.33	1.835	0.85
05.06.22	3	7.40	1.930	0.90
07.06.22	5	7.31	1.885	0.88
08.06.22	6	7.27	1.854	0.86
09.06.22	7	7.13	1.810	0.84
10.06.22	8	7.09	1.782	0.83
11.06.22	9	7.03	1.775	0.83
12.06.22	10	7.01	1.609	0.75
13.06.22	11	6.96	1.510	0.70
14.06.22	12	6.98	1.462	0.68
15.06.22	13	6.98	1.337	0.62
16.06.22	14	6.88	1.285	0.60
17.06.22	15	6.91	1.192	0.55
18.06.22	16	6.89	1.135	0.53
20.06.22	18	6.89	0.989	0.460
21.06.22	19	6.85	0.886	0.413
22.06.22	20	6.85	0.827	0.385
23.06.22	21	6.80	0.721	0.336
24.06.22	22	6.72	0.670	0.312

Table I. 78 Variation in pH, OD, and DCW with time for R5 in Set6

Reactors		R5		
Date	Day	pH	OD 660	DCW (g/L)
02.06.22	0	6.57	0.317	0.15
03.06.22	1	7.30	1.679	0.78
04.06.22	2	-	1.836	0.85
05.06.22	3	7.37	1.974	0.92
06.06.22	4	-	1.884	0.88
07.06.22	5	7.28	1.917	0.89
08.06.22	6	7.27	1.866	0.87
09.06.22	7	7.15	1.842	0.86
10.06.22	8	7.09	1.782	0.83
11.06.22	9	7.01	1.670	0.78
12.06.22	10	7.04	1.608	0.75
13.06.22	11	6.65	1.494	0.70
14.06.22	12	-	1.480	0.69
15.06.22	13	6.57	1.349	0.63
16.06.22	14	6.86	1.322	0.62
17.06.22	15	6.98	1.213	0.56
18.06.22	16	6.90	1.101	0.51
20.06.22	18	6.87	1.019	0.47
21.06.22	19	6.85	0.911	0.42
22.06.22	20	6.80	0.862	0.40
23.06.22	21	6.82	0.763	0.36
24.06.22	22	6.76	0.702	0.33

Table I. 79 Variation in pH, OD, and DCW with time for R6 in Set6

Reactors		R6		
Date	Day	pH	OD 660	DCW (g/L)
02.06.22	0	6.57	0.326	0.15
03.06.22	1	7.27	1.682	0.78
04.06.22	2	7.24	1.843	0.86
05.06.22	3	7.36	1.969	0.92
06.06.22	4	-	1.900	0.88
07.06.22	5	7.30	1.915	0.89
08.06.22	6	7.26	1.934	0.90
09.06.22	7	7.13	1.830	0.85
10.06.22	8	7.08	1.756	0.82
11.06.22	9	-	1.661	0.77
12.06.22	10	7.04	1.621	0.75
13.06.22	11	6.95	1.550	0.72
14.06.22	12	-	1.487	0.69
15.06.22	13	6.90	1.369	0.64
16.06.22	14	6.88	1.273	0.59
17.06.22	15	6.95	1.208	0.56
18.06.22	16	6.95	1.103	0.51
20.06.22	18	6.90	0.991	0.46
21.06.22	19	6.87	0.900	0.42
22.06.22	20	6.80	0.832	0.39
23.06.22	21	6.82	0.785	0.37

Table I. 80 Daily variation of PHB for R1 in Set6

Reactors		R1		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB% of DCW
03.06.22	1	5.0	0.7	14.1
04.06.22	2	13.3	1.6	12.1
05.06.22	3	12.2	2.0	16.4
06.06.22	4	12.7	2.1	16.3
07.06.22	5	13.2	2.0	15.4
08.06.22	6	9.4	1.4	15.2
09.06.22	7	13.8	1.8	12.8
10.06.22	8	11.1	1.0	9.3
11.06.22	9	10.8	1.0	9.7
12.06.22	10	9.5	1.1	11.1
13.06.22	11	7.7	0.8	9.7
14.06.22	12	7.6	0.8	10.9
15.06.22	13	7.0	0.4	5.9
16.06.22	14	6.8	0.5	7.3
17.06.22	15	5.8	0.2	3.6
18.06.22	16	5.9	0.4	6.8
20.06.22	18	5.0	0.1	2.1
21.06.22	19	4.0	0.0	0.8
22.06.22	20	14.3	0.1	1.0

Table I. 81 Daily variation of PHB for R2 in Set6

Reactors		R2		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB% of DCW
03.06.22	1	11.9	0.9	7.2
04.06.22	2	11.7	1.4	11.9
06.06.22	4	13.2	2.1	16.2
07.06.22	5	12.1	1.3	10.3
08.06.22	6	11.7	1.4	12.3
09.06.22	7	12.7	1.6	12.5
10.06.22	8	10.5	1.2	11.2
11.06.22	9	10.3	1.1	10.5
12.06.22	10	9.5	0.7	7.1
13.06.22	11	6.7	0.6	9.7
14.06.22	12	8.1	0.5	6.1
15.06.22	13	8.4	0.6	7.3
16.06.22	14	8.0	0.5	6.4
17.06.22	15	7.4	0.5	7.0
18.06.22	16	6.2	0.4	5.8
20.06.22	18	6.3	0.1	0.8
21.06.22	19	3.2	0.1	3.3
22.06.22	20	4.7	0.1	1.9
23.06.22	21	4.8	0.1	1.2

Table I. 82 Daily variation of PHB for R3 in Set6

Reactors		R3		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB% of DCW
03.06.22	1	9.9	0.6	6.3
04.06.22	2	11.9	1.3	11.0
05.06.22	3	11.0	1.7	15.5
06.06.22	4	14.4	2.0	14.0
07.06.22	5	11.9	1.6	13.1
08.06.22	6	11.3	1.1	10.1
09.06.22	7	8.9	1.0	10.9
10.06.22	8	9.6	0.7	7.5
11.06.22	9	5.7	0.5	8.7
12.06.22	10	9.2	1.1	12.0
13.06.22	11	8.8	0.9	10.1
14.06.22	12	6.8	0.3	5.1
15.06.22	13	7.7	0.3	4.4
16.06.22	14	5.6	0.4	6.6
17.06.22	15	6.8	0.6	9.0
18.06.22	16	6.0	0.3	5.5
20.06.22	18	4.5	0.2	4.0
21.06.22	19	3.7	0.1	3.6
22.06.22	20	4.6	0.1	1.2
23.06.22	21	12.7	0.3	2.7

Table I. 83 Daily variation of PHB for R4 in Set6

Reactors		R4		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB% of DCW
03.06.22	1	9.2	0.7	7.6
04.06.22	2	12.8	1.3	9.9
05.06.22	3	13.6	1.2	8.6
06.06.22	4	13.0	2.5	18.9
07.06.22	5	13.4	2.3	17.5
08.06.22	6	12.8	2.6	20.2
09.06.22	7	12.6	1.5	12.0
10.06.22	8	10.0	1.4	14.4
11.06.22	9	10.2	1.0	9.8
12.06.22	10	10.9	1.5	14.0
13.06.22	11	8.9	1.1	12.1
14.06.22	12	6.5	0.9	13.8
15.06.22	13	7.5	0.9	11.8
16.06.22	14	5.3	0.5	9.0
17.06.22	15	6.9	0.4	5.8
18.06.22	16	6.2	0.4	6.4
20.06.22	18	6.5	0.4	5.8
21.06.22	19	5.3	0.3	5.7
22.06.22	20	4.2	0.1	3.6
23.06.22	21	3.0	0.1	4.2

Table I. 84 Daily variation of PHB for R5 in Set6

Reactors		R5		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB% of DCW
03.06.22	1	9.1	0.8	8.8
04.06.22	2	15.6	1.2	7.6
05.06.22	3	14.0	1.9	13.6
06.06.22	4	14.0	1.6	11.3
07.06.22	5	14.9	2.0	13.2
08.06.22	6	12.2	1.6	12.8
09.06.22	7	12.4	1.5	12.5
10.06.22	8	12.1	1.5	12.7
11.06.22	9	11.1	0.9	8.3
12.06.22	10	10.8	1.1	10.6
13.06.22	11	4.2	0.4	9.5
14.06.22	12	6.9	0.8	11.2
15.06.22	13	8.1	1.0	11.8
16.06.22	14	8.0	0.6	7.2
17.06.22	15	7.0	0.6	8.6
18.06.22	16	6.1	0.6	9.8
20.06.22	18	6.6	0.5	7.0
21.06.22	19	5.4	0.2	3.0
22.06.22	20	3.5	0.3	9.7
23.06.22	21	4.5	0.1	2.9
24.06.22	22	14.2	0.7	5.0

Table I. 85 Daily variation of PHB for R6 in Set6

Reactors		R6		
Date	Day	Weight of bacterial pellet (mg)	PHB in the biomass (mg)	PHB% of DCW
03.06.22	1	9.6	0.5	4.9
04.06.22	2	12.4	1.8	14.1
05.06.22	3	13.8	1.9	13.5
06.06.22	4	10.6	1.5	14.5
07.06.22	5	11.7	1.7	14.5
08.06.22	6	12.9	2.4	18.7
09.06.22	7	11.6	2.0	17.4
10.06.22	8	12.2	1.5	12.1
11.06.22	9	12.3	1.4	11.2
12.06.22	10	10.1	1.2	11.5
13.06.22	11	9.7	1.6	16.1
14.06.22	12	6.9	0.6	9.2
15.06.22	13	7.0	0.8	11.2
16.06.22	14	6.5	0.9	13.5
17.06.22	15	6.5	0.5	7.7
18.06.22	16	6.4	0.6	9.1
21.06.22	19	6.0	0.3	4.8
22.06.22	20	4.8	0.1	3.1
23.06.22	21	15.0	1.0	6.6

Table I. 86 Variation in organic acid concentration with time for R1 in Set6

Reactors		R1			
Date	Day	Lactic Acid (mM)	Formic Acid (mM)	Acetic Acid (mM)	Propionic Acid (mM)
02.06.22	0	0.0	0.0	55.0	0.0
03.06.22	1	0.2	0.4	39.6	10.0
04.06.22	2	0.7	0.0	42.9	0.0
05.06.22	3	0.9	0.0	33.8	0.0
06.06.22	4	1.3	0.7	29.2	0.0
07.06.22	5	0.9	0.7	16.4	0.0
08.06.22	6	1.1	1.4	20.0	0.0
09.06.22	7	0.2	1.8	15.5	0.0
10.06.22	8	1.0	2.3	15.2	0.0
11.06.22	9	0.8	2.8	9.8	0.0
12.06.22	10	1.9	4.9	10.1	8.6
13.06.22	11	2.1	6.0	8.2	7.0
14.06.22	12	1.0	5.2	4.4	0.0
15.06.22	13	2.1	1.5	2.6	0.0
16.06.22	14	1.0	1.7	1.7	0.0
17.06.22	15	0.9	7.1	1.9	0.0
18.06.22	16	0.5	4.5	1.0	0.0
20.06.22	18	0.7	7.7	1.3	0.0
21.06.22	19	0.1	1.4	0.2	5.4
22.06.22	20	0.1	1.8	0.1	5.1

Table I. 87 Variation in organic acid concentration with time for R2 in Set6

Reactors		R2			
Date	Day	Lactic Acid (mM)	Formic Acid (mM)	Acetic Acid (mM)	Propionic Acid (mM)
02.06.22	0	0.0	0.0	53.4	3.2
03.06.22	1	0.3	0.5	46.6	2.0
04.06.22	2	1.0	0.5	43.8	0.0
05.06.22	3	0.9	0.0	30.8	0.0
06.06.22	4	1.1	0.6	21.4	0.0
07.06.22	5	1.2	0.5	20.9	0.0
08.06.22	6	1.1	0.6	17.9	0.0
09.06.22	7	0.9	0.5	13.9	0.0
10.06.22	8	0.2	1.9	14.1	0.0
11.06.22	9	0.8	0.5	9.7	0.0
12.06.22	10	1.2	0.7	10.8	12.9
13.06.22	11	1.1	0.7	9.1	11.1
14.06.22	12	1.0	0.3	6.1	7.7
15.06.22	13	1.0	4.7	4.9	7.0
16.06.22	14	0.8	4.1	2.8	3.0
17.06.22	15	1.0	6.2	2.5	3.1
18.06.22	16	1.7	1.2	1.2	0.0
20.06.22	18	0.7	0.1	1.5	0.0
21.06.22	19	0.1	1.7	1.2	0.0
22.06.22	20	0.6	5.3	1.3	0.0
23.06.22	21	0.2	5.2	1.1	0.0
24.06.22	22	0.4	4.1	0.0	0.0

Table I. 88 Variation in organic acid concentration with time for R3 in Set6

Reactors		R3			
Date	Day	Lactic Acid (mM)	Formic Acid (mM)	Acetic Acid (mM)	Propionic Acid (mM)
02.06.22	0	0.0	0.0	63.0	0.0
03.06.22	1	0.2	0.0	37.0	0.7
04.06.22	2	0.7	0.5	35.3	3.6
05.06.22	3	1.2	0.4	34.9	0.0
06.06.22	4	1.4	0.9	27.4	0.0
07.06.22	5	1.4	1.1	27.8	0.0
08.06.22	6	1.6	1.1	21.9	0.0
09.06.22	7	0.2	1.1	16.6	0.0
10.06.22	8	0.2	2.2	14.3	0.0
11.06.22	9	1.1	0.5	12.3	7.4
12.06.22	10	1.7	0.9	9.0	4.8
13.06.22	11	0.8	0.2	6.8	3.0
14.06.22	12	0.8	0.1	4.4	0.7
15.06.22	13	1.7	1.5	4.2	1.0
16.06.22	14	0.9	4.6	2.5	0.0
17.06.22	15	0.9	5.3	1.6	0.0
18.06.22	16	0.5	3.6	0.5	0.5
20.06.22	18	0.6	4.7	1.1	0.0
21.06.22	19	0.5	4.9	1.1	0.0
22.06.22	20	0.5	4.7	1.0	0.0
23.06.22	21	0.5	4.9	0.9	0.0

Table I. 89 Variation in organic acid concentration with time for R4 in Set6

Reactors		R4				
Date	Day	Lactic Acid (mM)	Formic Acid (mM)	Acetic Acid (mM)	Propionic Acid (mM)	Isobutyric Acid (mM)
02.06.22	0	0.0	0.0	68.6	0.0	0.0
03.06.22	1	0.2	0.0	32.2	1.9	0.0
04.06.22	2	0.0	1.2	34.3	1.6	0.0
05.06.22	3	0.0	1.8	27.1	1.4	0.2
06.06.22	4	0.8	0.3	26.5	0.0	0.3
07.06.22	5	0.9	0.4	25.2	0.0	0.3
08.06.22	6	1.4	1.8	22.0	0.0	1.1
09.06.22	7	0.2	1.2	12.8	0.0	0.0
10.06.22	8	0.1	1.1	11.7	0.0	0.0
11.06.22	9	0.9	0.7	9.4	1.3	0.5
12.06.22	10	1.7	1.2	7.5	0.0	0.6
13.06.22	11	1.2	1.1	7.5	0.0	0.3
14.06.22	12	0.9	1.0	3.9	0.3	0.4
15.06.22	13	0.4	1.8	2.9	0.6	0.4
16.06.22	14	0.9	1.1	2.0	1.0	0.3
17.06.22	15	0.5	2.0	1.3	0.0	0.5
18.06.22	16	1.0	1.5	2.3	0.0	0.2
20.06.22	18	0.9	1.5	2.2	0.0	0.2
21.06.22	19	0.8	1.5	2.4	0.0	0.0
22.06.22	20	0.8	1.5	2.2	0.0	0.0
23.06.22	21	0.7	1.2	1.7	0.0	0.0
24.06.22	22	0.3	1.1	0.2	0.4	0.0

Table I. 90 Variation in organic acid concentration with time for R5 in Set6

Reactors		R5				
Date	Day	Lactic Acid (mM)	Formic Acid (mM)	Acetic Acid (mM)	Propionic Acid (mM)	Isobutyric Acid (mM)
02.06.22	0	0.0	0.0	62.2	0.0	0.0
03.06.22	1	0.2	0.0	43.2	0.8	0.0
04.06.22	2	0.2	0.0	36.7	6.3	0.0
05.06.22	3	0.7	0.5	30.9	0.0	0.3
06.06.22	4	0.7	0.7	26.2	0.0	0.5
07.06.22	5	0.7	0.5	25.5	0.0	0.4
08.06.22	6	0.8	0.5	16.6	0.0	0.3
09.06.22	7	0.7	0.5	12.0	0.5	0.0
10.06.22	8	0.7	0.5	9.9	0.7	0.0
11.06.22	9	1.2	0.8	8.6	0.8	0.4
12.06.22	10	2.2	2.0	7.8	0.0	0.5
13.06.22	11	0.9	1.0	6.1	0.4	0.4
14.06.22	12	0.9	1.2	4.0	0.2	0.3
15.06.22	13	0.9	1.3	2.3	0.0	0.4
16.06.22	14	0.7	2.3	2.4	0.0	0.4
17.06.22	15	1.0	1.6	2.3	0.0	0.0
18.06.22	16	1.0	1.7	2.4	0.0	0.0
20.06.22	18	0.8	1.4	1.9	0.0	0.0
21.06.22	19	0.6	1.1	1.5	0.0	0.0
22.06.22	20	0.4	0.6	0.7	2.2	0.0
23.06.22	21	0.5	1.4	0.0	0.0	0.0

Table I. 91 Variation in organic acid concentration with time for R6 in Set6

Reactors		R6				
Date	Day	Lactic Acid (mM)	Formic Acid (mM)	Acetic Acid (mM)	Propionic Acid (mM)	Isobutyric Acid (mM)
02.06.22	0	0.0	0.0	60.1	0.3	0.0
03.06.22	1	0.2	0.0	45.1	0.0	0.0
04.06.22	2	0.2	0.0	40.0	2.1	0.0
05.06.22	3	0.6	0.5	25.6	1.2	0.3
06.06.22	4	0.9	0.5	24.6	0.0	0.3
07.06.22	5	0.8	0.5	20.0	0.0	0.3
08.06.22	6	0.8	1.0	16.0	0.0	0.3
09.06.22	7	1.0	0.6	12.2	0.0	0.8
10.06.22	8	0.7	0.6	9.9	0.0	0.5
11.06.22	9	1.2	1.0	9.1	1.1	0.5
13.06.22	11	0.9	0.9	3.9	0.4	0.2
14.06.22	12	1.1	1.5	3.5	0.0	0.3
15.06.22	13	1.1	1.6	2.2	0.0	0.4
16.06.22	14	1.2	1.8	2.5	0.0	0.5
17.06.22	15	1.1	1.7	2.3	0.0	0.2
18.06.22	16	1.0	1.4	0	0.0	0.3
20.06.22	18	1.0	1.6	1.7	0.0	0.1
21.06.22	19	0.8	1.4	1.8	0.0	0.0
22.06.22	20	0.4	0.8	1.1	0.6	0.0
23.06.22	21	0.6	1.7	0.2	0.0	0.0

Table I. 92 Daily variation of produced biogas and its content for R1 in Set6

Reactors		R1				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
02.06.22	0	0	0.0	0.0	0.0	100.0
03.06.22	1	48	80.6	3.4	3.3	12.7
04.06.22	2	70	79.3	2.9	3.5	14.4
05.06.22	3	58	90.4	3.0	4.8	1.8
06.06.22	4	48	92.5	2.6	5.9	-1.1
07.06.22	5	44	93.7	4.4	7.0	-5.1
08.06.22	6	39	89.3	2.9	8.1	-0.3
09.06.22	7	65	89.1	2.3	10.6	-2.0
10.06.22	8	50	83.7	2.9	11.9	1.6
11.06.22	9	62	82.8	2.7	13.7	0.8
12.06.22	10	74	77.4	2.4	14.9	5.3
13.06.22	11	36	80.9	3.0	16.7	-0.6
14.06.22	12	56	79.7	2.3	17.8	0.3
15.06.22	13	34	80.2	3.0	18.6	-1.8
16.06.22	14	28	78.1	3.8	19.0	-0.9
17.06.22	15	28	79.1	4.3	19.2	-2.6
18.06.22	16	16	75.4	4.8	18.6	1.3
20.06.22	18	28	63.1	13.9	19.3	3.7
21.06.22	19	0	57.7	14.3	18.3	9.8
22.06.22	20	0	39.7	18.4	18.3	23.6

Table I. 93 Daily variation of produced biogas and its content for R2 in Set6

Reactors		R2				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
02.06.22	0	0	0.0	0.0	0.0	100.0
03.06.22	1	53	83.2	2.9	3.3	10.6
04.06.22	2	83	91.8	2.1	4.1	2.1
05.06.22	3	67	74.0	1.9	3.8	20.2
06.06.22	4	55	81.4	1.9	4.9	11.8
07.06.22	5	48	93.0	2.5	6.9	-2.4
08.06.22	6	39	92.0	2.6	7.8	-2.4
09.06.22	7	58	71.3	1.8	7.6	19.3
10.06.22	8	38	89.0	2.1	11.1	-2.2
11.06.22	9	56	85.5	2.1	12.7	-0.3
12.06.22	10	64	82.5	2.1	14.4	1.0
13.06.22	11	24	79.6	2.8	15.3	2.4
14.06.22	12	50	77.1	2.5	16.0	4.4
15.06.22	13	34	81.3	2.7	17.5	-1.4
16.06.22	14	28	80.7	3.4	17.9	-2.0
17.06.22	15	28	78.2	4.5	17.3	0.0
18.06.22	16	14	81.4	4.1	18.1	-3.6
20.06.22	18	32	76.7	3.9	18.0	1.4
21.06.22	19	14	76.7	4.7	18.0	0.6
22.06.22	20	7	76.4	5.4	17.0	1.2
23.06.22	21	6	76.2	6.7	16.9	0.3
24.06.22	22	2	71.3	8.5	16.5	3.7

Table I. 94 Daily variation of produced biogas and its content for R3 in Set6

Reactors		R3				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
02.06.22	0	0	0.0	0.0	0.0	100.0
03.06.22	1	40	68.8	3.3	2.7	25.3
04.06.22	2	73	92.7	2.3	4.1	1.0
05.06.22	3	68	75.4	2.0	4.2	18.4
06.06.22	4	56	83.4	2.2	5.5	8.9
07.06.22	5	51	89.3	2.3	7.0	1.3
08.06.22	6	40	90.1	2.7	8.3	-1.0
09.06.22	7	52	86.0	2.3	9.9	1.8
10.06.22	8	36	85.9	2.7	11.2	0.2
11.06.22	9	52	83.2	2.3	12.9	1.7
12.06.22	10	56	81.4	2.3	14.5	1.7
13.06.22	11	27	82.7	3.6	15.8	-2.0
14.06.22	12	50	82.6	3.1	17.2	-3.0
15.06.22	13	30	78.9	3.1	16.9	1.2
16.06.22	14	27	76.6	3.7	17.2	2.5
17.06.22	15	28	75.4	5.7	16.9	2.0
18.06.22	16	13	78.2	5.2	17.8	-1.2
20.06.22	18	30	72.4	5.2	17.3	5.1
21.06.22	19	6	66.6	11.1	16.9	5.4
22.06.22	20	5	67.2	10.1	15.9	6.8
23.06.22	21	0	62.0	12.4	15.0	10.6

Table I. 95 Daily variation of produced biogas and its content for R4 in Set6

Reactors		R4				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
02.06.22	0	0	0.0	0.0	0.0	100.0
03.06.22	1	48	81.9	3.3	3.0	11.8
04.06.22	2	84	90.8	3.3	3.1	2.8
05.06.22	3	54	92.8	2.3	3.8	1.1
06.06.22	4	52	92.5	2.2	4.8	0.5
07.06.22	5	46	87.4	2.5	5.7	4.4
08.06.22	6	36	87.1	3.6	6.9	2.4
09.06.22	7	50	87.3	2.2	8.7	1.9
10.06.22	8	32	88.1	2.8	10.3	-1.2
11.06.22	9	40	85.8	2.7	11.6	-0.1
12.06.22	10	50	84.2	3.1	13.3	-0.6
13.06.22	11	26	82.3	3.9	14.2	-0.4
14.06.22	12	40	80.4	2.9	15.0	1.7
15.06.22	13	26	79.9	3.4	15.4	1.4
16.06.22	14	26	79.3	4.1	16.4	0.2
17.06.22	15	16	77.8	6.0	15.7	0.5
18.06.22	16	8	78.2	5.7	16.5	-0.4
20.06.22	18	24	76.0	5.7	17.5	0.8
21.06.22	19	6	72.5	7.8	17.4	2.3
22.06.22	20	4	67.0	11.3	15.1	6.6
23.06.22	21	2	68.1	13.4	15.5	2.9
24.06.22	22	2	64.9	13.8	15.3	6.0

Table I. 96 Daily variation of produced biogas and its content for R5 in Set6

Reactors		R5				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
02.06.22	0	0	0.0	0.0	0.0	100.0
03.06.22	1	48	82.0	3.5	2.9	11.7
04.06.22	2	96	84.1	2.1	3.5	10.2
05.06.22	3	64	91.2	2.4	4.1	2.3
06.06.22	4	62	87.7	2.5	4.8	4.9
07.06.22	5	52	89.4	2.9	6.4	1.3
08.06.22	6	44	87.8	3.3	7.6	1.3
09.06.22	7	58	87.3	2.0	9.4	1.3
10.06.22	8	36	87.6	3.1	10.9	-1.5
11.06.22	9	52	80.8	2.6	11.9	4.7
12.06.22	10	54	79.9	2.5	13.2	4.4
13.06.22	11	26	80.6	3.4	14.1	1.9
14.06.22	12	44	80.1	4.0	15.1	0.7
15.06.22	13	26	82.5	4.0	16.2	-2.7
16.06.22	14	28	78.0	4.4	16.2	1.4
17.06.22	15	28	75.5	7.1	16.3	1.0
18.06.22	16	14	75.9	5.6	16.5	2.0
20.06.22	18	26	74.8	5.8	17.2	2.2
21.06.22	19	10	73.0	8.3	17.5	1.2
22.06.22	20	4	69.8	11.5	15.4	3.3
23.06.22	21	4	68.7	11.6	15.1	4.6
24.06.22	22	2	66.1	13.9	15.3	4.7

Table I. 97 Daily variation of produced biogas and its content for R6 in Set6

Reactors		R6				
Date	Day	Daily Produced Biogas (mL)	Biogas Content			
			H_2%	N_2%	CO_2%	Remainder Gas (%)
02.06.22	0	0	0.0	0.0	0.0	100.0
03.06.22	1	40	78.8	3.9	2.8	14.5
04.06.22	2	86	92.8	2.1	4.2	0.8
05.06.22	3	61	90.4	2.1	4.4	3.1
06.06.22	4	58	82.8	1.9	4.7	10.6
07.06.22	5	48	91.6	3.4	6.7	-1.7
08.06.22	6	40	90.8	3.6	8.1	-2.5
09.06.22	7	50	92.0	2.5	10.1	-4.6
10.06.22	8	28	91.2	2.9	11.4	-5.6
11.06.22	9	46	88.7	3.0	13.1	-4.8
12.06.22	10	56	82.2	2.5	14.1	1.2
13.06.22	11	30	84.8	3.5	15.4	-3.7
14.06.22	12	38	84.2	3.6	16.3	-4.1
15.06.22	13	26	85.4	4.2	17.3	-6.9
16.06.22	14	24	79.1	4.9	16.9	-0.9
17.06.22	15	20	81.1	5.8	17.4	-4.3
18.06.22	16	6	77.5	5.8	17.3	-0.6
20.06.22	18	16	75.0	7.0	17.5	0.5
21.06.22	19	6	72.8	9.1	17.3	0.8
22.06.22	20	2	68.2	11.7	14.8	5.3
23.06.22	21	0	57.4	9.8	15.7	17.1

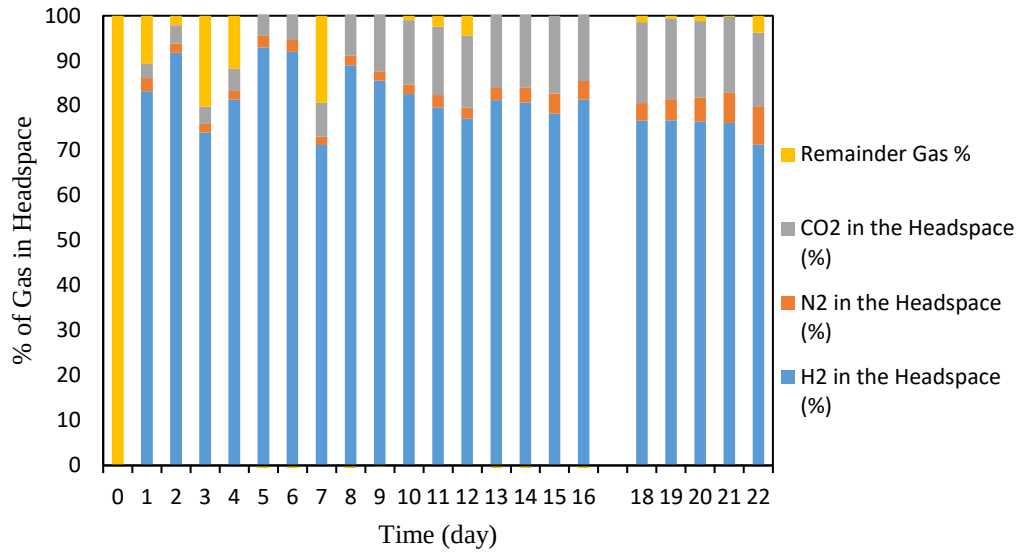

Figure I. 10 Sample graph of biogas content for R2 (WT strain) in Set6

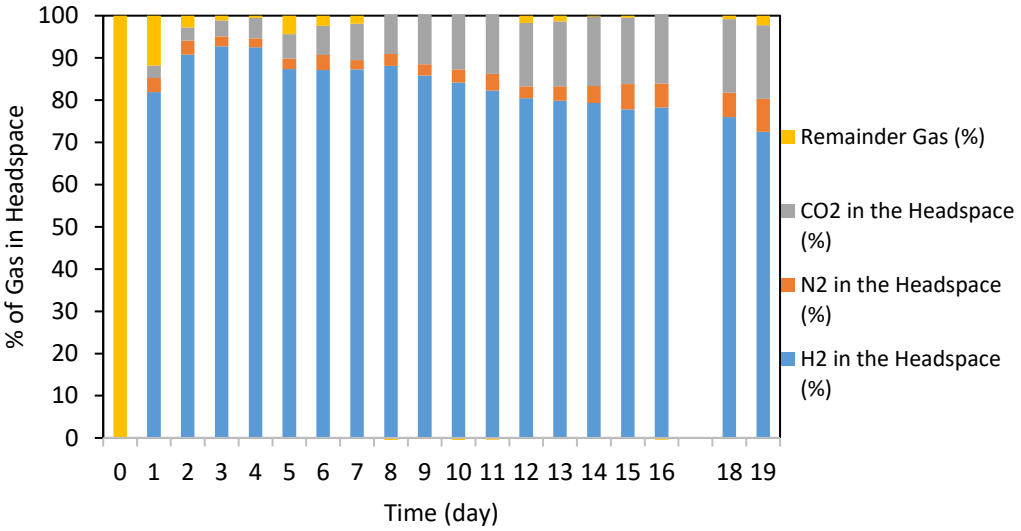

Figure I. 11 Sample graph of biogas content for R4 (YO3 strain) in Set6