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Modelling and simulation of syngas fermentation for the production of biofuels precursors

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Summary:

Production of acetic acid and ethanol by syngas fermentation process could be an alternative way of biofuel production, and is therefore of interest to study.

In accordance to literature review, gas fermentation process can be implemented by the Wood-Ljungdahl pathway, where as a guarantor of his passage might be acetogenic bacteria. The purpose of this thesis is formation of a mathematical model of biological process and scrutiny of parameters that effect on production rate, such as the CO/H_2 ratio, partial pressure, temperature and pH.

Development of a mathematical model was based on Haldane model of microbial growth and thermodynamic calculations, for five potential products - ethanol, acetic acid, methanol, lactate and methane and transformation process from acetic acid to ethanol.

Simulation was conducted to prove correctness of formed mathematical model and to show how can initial conditions effect on biofuel production rate. For this, were performed three sub-models (Model 1, 2 and 3) and Model 4, that include all sub-models as one process. Where Model 1 describes acetic acid production with carbon monoxide as electron donor; Model 2 - with hydrogen as electron donor; and Model 3 - transformation of acetic acid to ethanol.

The simulation of Model 4 shows that the consumption of CO, CO_2, H_2 will take around 175 days under given conditions. Also, based on Model 4, was proved the influence of:

- initial biomass value on lag phase (Test 1);
- partial pressure on production rate (Test 2);
- maximum specific substrate utilization rate on reaction time (Test 3).

Studied process has a potential for further future research.

Preface

In deciding the choice of my master's thesis topic, I spent just a moment. But the preparation of the thesis has required the application of all learning that I have gained throughout my life. I knew that achieving the desired results would take hours of painstaking work and perseverance. I also understood that my restricted knowledge and experience would complicate the implementation of tasks. However, working under the leadership of Carlos Dinamarca and Rune Bakke reduced the impact of these constraints. Accordingly, I wish to express my gratitude to my supervisors for their patience and assistance.

Despite the fact that this work was written as a university requirement for the successful completion of my master's degree, I must also express my appreciation to the management of University College of Southeast Norway (USN). Their work has given me the opportunity to study and advance in the science that I truly love.

It is very important to express thanks to my family members and to my best friends Zheka, Vlad, Andrey and Stefan. Your support and motivation gave me confidence in solving difficulties throughout my life, and especially these two years.

It is expected that the reader of this thesis has a basic knowledge about microbiology and chemical thermodynamics.

Porsgrunn, 15.05.2017

Rostyslav Kravchuk

List of abbreviations

Abbreviation:	Description:
Acetyl-CoA	Acetyl coenzyme A.
CODH	Carbon monoxide dehydrogenase.
CSTR	Continuous stirred tank reactor.
NADH	Nicotinamide adenine dinucleotide.
NADHP	Nicotinamide adenine dinucleotide phosphate.
UAc	Undissociated acetic acid.
ADM1	Anaerobic Digestion Model №1.
Aquasim	Computer Program for the Identification and Simulation of Aquatic Systems.

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1 Introduction

Non-renewable sources such as oil, coal and natural gas have been and still remain as the main sources of raw materials for the fuel production and organic synthesis products (BBC.CO.UK, 2014). However, the use of these sources has limits and at present humanity is approaching them. The diversification of the raw material base and the development of alternative sources of raw materials become a new necessity. Which in turn will contribute to solving the problems of the ecological state of the planet. It was the latter that determined the active development of many environmental projects, the main of them being the Montreal (UNEP, 1987) and Kyoto (UNFCCC, 1997) Protocols, the Paris Agreement (UNFCCC, 2016).

Throughout the 20th century, various methods for obtaining biofuels from diverse sources, both organic and inorganic, were studied (Marchetti et al., 2007). One of such sources can be synthesis gas, which is, mainly, a mixture of hydrogen, carbon monoxide and carbon dioxide. All of them are important components in fermentation process (Bengelsdorf et al., 2013). It can be argued that the biofuel production through biological methods will be one of the priorities for meeting the priority needs of mankind in the future.

One of the alternatives to obtain the biofuel can be the process of combining the syngas and the metabolic Wood-Ljungdahl pathway. The passage of this combination requires the use of Acetogenic bacteria. These bacteria, through the reduction of carbon dioxide to carbon monoxide and hydrogen oxidation, allow the passage of the metabolic recovery pathway of acetyl-coenzyme A (acetyl CoA). Further, by the conversion of acetyl CoA is possible to obtain diverse products such as ethanol, butanol, pyruvate and acetate.

The passage of this pathway is influenced by a sufficient number of parameters, such as the type of bacteria, reactor design, the ratio between the syngas components, partial pressure, temperature redox potential and pH (Kundiyana et al., 2011). In this regard, the assessment of the significance of each of the factors and the rationale for the optimal approaches that ensure the maximum amount of biofuels are very relevant. One of the most important links in solving this problem is modeling of the influence of individual factors and simulating the whole process. These two points are the aim of this master's project.

1.1 Outline of the master’s thesis

This master thesis report consists of seven chapters. First, introduction that briefly describe the prerequisites and the main issues for writing the master thesis, in order to inform the reader about the information that the author thinks is necessary for a better understanding of the thesis. This chapter is followed by a detailed literature review. Which contain a full explanation of Wood-Ljungdahl pathway with main parameters affecting on biofuel production. Then chapter 3, where a model is developed for the mathematical description of the processes described in the foregoing chapter. In the fifth chapter, a creation and presentation of results from AquaSim software simulation based on mathematical syngas fermentation model. Finally, discussion and conclusion of the results obtained in the fourth and fifth chapters.

1.2 Thesis objective(s)

The tasks given for this master thesis are presented below.

1. Literature review of syngas fermentation process for the biofuel precursors. It should contain the relevant microbial catabolic pathway; review of fermentation bacteria which are suitable, and list of parameters that can affect on production rate.
2. Creation and development of a mathematical model to describe syngas fermentation process. The key issues are: stoichiometric equations; growth rate; gas-liquid phase mass balance; mass transfer phenomena; kinetic expressions.
3. Based on the AquaSim software, creating a visual representation of the calculation of model to confirm that the process of synthesis gas fermentation for biofuel is appropriate.

2 Literature review

This review will focus on describing syngas fermentation process. It will include a description of the most important factors and parameters, which can have effect on fermentation process. As an overview of bacteria, involve in syngas fermentation and biochemistry pathways.

2.1 Syngas

Syngas is a mixture of gases, the main components are carbon monoxide (CO), hydrogen (H_2) and carbon dioxide (CO_2) (Bengelsdorf et al., 2013). Syngas also include other species in much smaller concentrations: water (H_2O); methane (CH_4); ethene (C_2H_4); ethane (C_2H_6); ethyne (C_2H_2); benzene (C_6H_6); naphthalene ($C_{10}H_8$); ammonia (NH_3); nitrogen oxides (NO_X); sulphur dioxide (SO_2); and hydrogen sulphide (H_2S) (Xu et al., 2011, Daniell et al., 2012).
The first known production way of obtaining synthesis gas was gasification of coal. This method was implemented in England in the 30's. Subsequently, this method was replaced by methods based on the use of oil and natural gas. However, due to a significant reduction in world oil resources, the significance of the coal gasification process again began to increase.
Today, there are three main methods for obtaining synthesis (Karakhanov, 1997):
1. Gasification of coal. This process is based on the interaction of coal with water vapor (Mckendry, 2001) and occurs according to the next equation:

$$C + H_2O \rightarrow H_2 + CO \tag{2.1}$$

This reaction is endothermic, and equilibrium at a temperature of 900-1000 ˚C is shifted to the right. Various technological processes have been developed that use a steam-oxygen blast, thanks to which, along with the above reaction, an exothermic combustion reaction of coal takes place in parallel, which ensures the necessary heat balance. It's equation:

$$C + \frac{1}{2}O_2 \rightarrow CO \tag{2.2}$$

2. Conversion of methane (Mckendry, 2001). This reaction of water vapor and methane interaction is carried out at elevated temperature (800-900 ˚C) and pressure in the presence of nickel catalysts (Ni-Al2O3). The formula for this process is:

$$CH_4 + H_2O \rightarrow CO + 3H_2 \tag{2.3}$$

Also, as feedstock in this process, any raw material containing a hydrocarbon can be used instead of methane.

3. Partial oxidation of hydrocarbons. This process, occurring at temperatures above 1300 ˚C, is the thermal oxidation of hydrocarbons.

$$C_nH_{2n} + \left(2 + \frac{1}{2}n\right)O_2 \rightarrow nCO + (n+1)H_2 \tag{2.4}$$

2.2 Fermentation of synthesis gas by microbial catalyst

Anaerobic microorganisms such as acetogens and methanogens could serve as catalysts for the conversion of inorganic substrates such as CO, CO_2 and H_2 into fuel (Younesi et al., 2006). Acetogens are found to be capable of metabolizing single-carbon compounds to produce ethanol and other high molecular weight products via acetogenic fermentation (Grethlein et al.,

1991). Syngas can be metabolized to ethanol and butanol by several microbial catalysts (Grethlein et al., 1991). The stoichiometry for some of the products from syngas is written below.

Ethanol:

$$6CO + 3H_2O \rightarrow CH_3CH_2OH + 4CO_2 \quad (2.5)$$

$$2CO_2 + 6H_2 \rightarrow CH_3CH_2OH + 3H_2O \quad (2.6)$$

Acetic acid:

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2 \quad (2.7)$$

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \quad (2.8)$$

Methanol:

$$3CO + 2H_2O \rightarrow CH_3OH + 2CO_2 \quad (2.9)$$

$$CO_2 + 3H_2 \rightarrow CH_3OH + H_2O \quad (2.10)$$

Methane:

$$4CO + 2H_2O \rightarrow CH_4 + 3CO_2 \quad (2.11)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (2.12)$$

2.2.1 Synthesis gas fermentation bacteria

For the production of biofuels, synthesis gas can use different types of microorganisms. Such as phototrophic, acetogenous, and methanogenic (Kaster et al., 2011) bacteria. The most studied microorganisms able to synthesize biofuel are predominantly acetogenous bacteria.

Acetogenic bacteria (anaerobes) are organisms capable of growing on organic carbon or chemolithotropically (Vos et al., 2009). Than by using the acetyl-CoA pathway, fermentation that bacteria with acetic acid as a product. To date, there are more than 100 acetogenic species, representing 22 genera. Among them, there are two main and most studied genera *Acetobacterium* and *Clostridium* (Drake et al., 2008).

2.2.2 Biological water-gas shift reaction

$$CO + H_2O \rightarrow CO_2 + 2H^+ + 2e^- \quad (2.13)$$

$$0.5H_2 \rightarrow H^+ + e^- \quad (2.14)$$

Equation (2.13) describes the anaerobic process of CO oxidization catalyzed by Ni-CODH enzyme. Then, with the help of the CO-tolerant hydrogenase, protons are reduced to molecular hydrogen (2.14). Using ATP synthase, ATP synthesis is carried out, as a consequence, the protons are transferred through the cytoplasmic membrane.

$$CO + H_2O \rightarrow CO_2 + H_2 \quad (2.15)$$

The biological water-gas shift reaction (2.15) is caused by the interaction of Ni-CODH and CO-tolerant hydrogenase. The obtained CO is partially transformed into the biomass and the

components of the cell, and the remaining carbon dioxide and hydrogen are released from the system (Henstra et al., 2007).

Table 2.1. Hydrogenogenic bacteria

Mesophilic bacteria (hydrogenogenic bacteria)	Substrate	Temperature (°C)	pH	Product	Reference
Citrobacter sp. Y19	CO	30-40	5.0-8.0	H_2	(Jung et al., 1999b)
Rhodopseudomonas palustris	CO	30	7.0	H_2	(Jung et al., 1999a)
Rhodospirillum rubrum	CO	30	6.8	H_2	(Kerby et al., 1995)
Rubrivivax gelatinosus	CO	35	7.5	H_2	(Maness and Weaver, 2002)

2.2.3 Wood-Ljungdahl pathway

The acetyl coenzyme-A (Acetyl-CoA) pathway is formed as an intermediate product of the metabolic pathway during acetogenesis. This pathway, unlike other ways of fixing COs, is not cyclical (Thauer, 2007). According to the (Hu et al., 1982), one possible way for the reductive of acetyl-CoA can be proposed - the Wood-Ljungdahl pathway.

Wood-Ljungdahl pathway was represented by Dr. Harold G. Wood and Dr. Lars G. Ljungdahl. They described the enzymology and biochemistry of CO/CO_2 fixation by anaerobic acetogenic bacteria (Ljungdahl, 1986, Pezacka and Wood, 1984, Hu et al., 1982). This pathway is based on a series of biochemical reactions used by some bacteria and archea (anaerobic chemolithoautotrophs).

This way can be shown in a cyclic form (Figure 2.1), however, unlike the reverse cycle of Krebs and the Calvin cycle, the reductive pathway of acetyl-CoA is not cyclic.

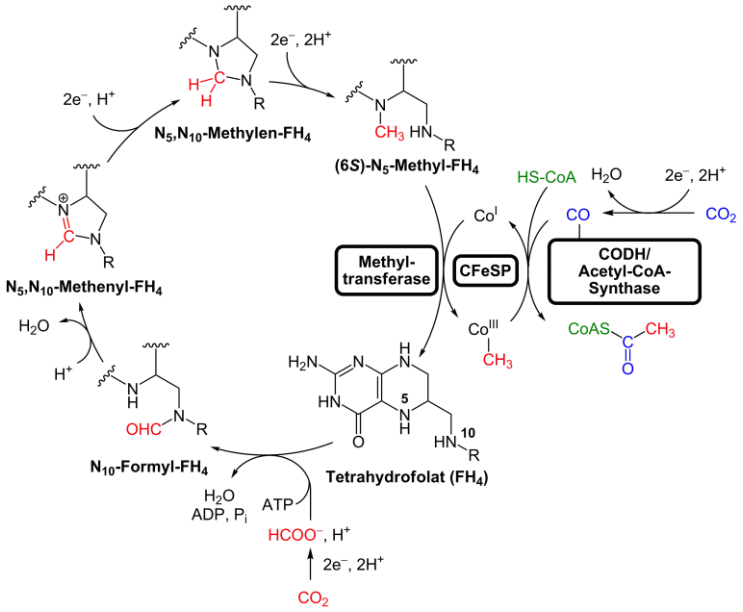

Figure 2.1. Wood-Ljungdahl pathway in a cyclic form (Ragsdale and Pierce, 2008)

CO, CO_2 and H_2 are used as energy and carbon source for Wood-Ljungdahl pathway. This pathway has two separate branches: the carbonyl branch and methyl branch. In the carbonyl branch, CO is used for the synthesis of acetyl-CoA. Carbon dioxide is reduced to carbon monoxide by the enzyme CO-dehydrogenase (CODH).

The methyl branch is a more complex and time-consuming process, which also requires carbon dioxide (CO_2 can be extracellular or obtained by oxidizing CO). This process contains several stages in which the CO_2 molecule is reduced to CO by successive enzymatic reactions with the methyl group of acetyl-CoA. The final product of acetyl-CoA can be metabolic products (acetic acid, ethanol) and biomass (Ragsdale and Pierce, 2008).

2.2.3.1 The Methyl branch

To reduce CO_2 to CO in the methyl branch, it is necessary to combine 6 electrons (H^+) and H_4folate to obtain CH_3-H_4folate (Figure 2.2) (Abubackar et al., 2011).

The first reaction in the branch is the two-electron reduction of CO_2 through NADH (nicotinamide adenine dinucleotide) to form formate (HCOOH), which is catalyzed by formate dehydrogenase.

$$CO_2 + 2H^+ + 2e^- \rightarrow HCOOH \qquad (2.26)$$

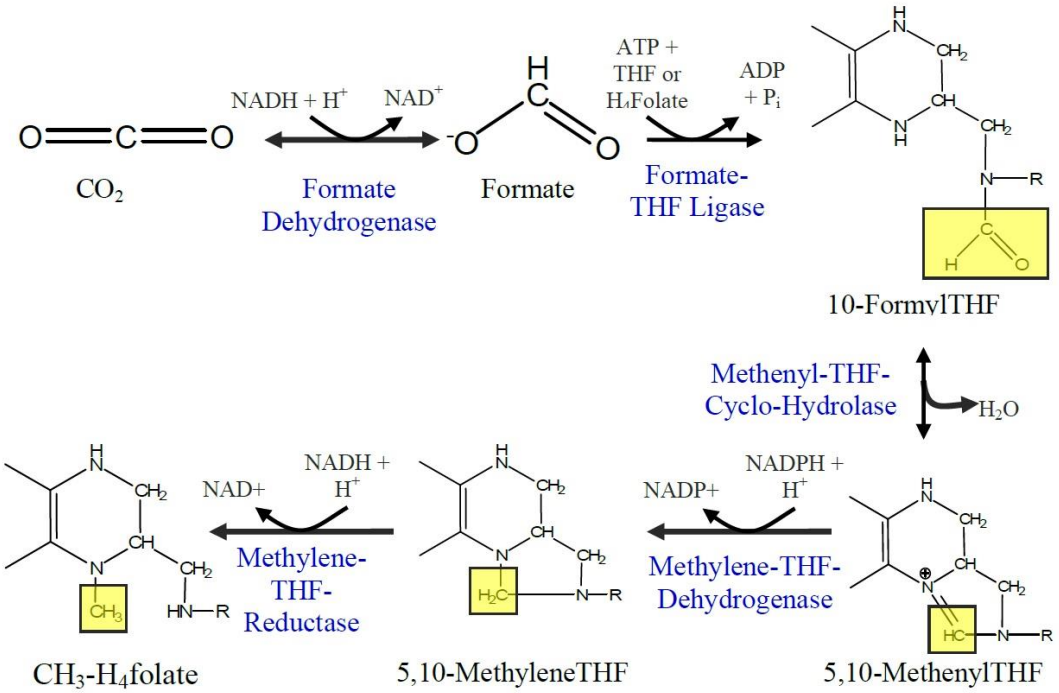

Figure 2.2. Methyl branch (Drake and Daniel, 2004)

Then, formyl-tetrahydrofolate synthetase forms a combination of formate with tetrahydrofolate (H_4folate). As a result formyl-H_4folate arises due to one molecule of adenosine triphosphate (ATP), which is converted into adenosine diphosphate (ADP).

$$HCOOH + ATP + H_4folate \rightarrow 10 - \text{formyl} - H_4folate + ADP + Pi \qquad (2.37)$$

After this, 5,10-methenyl-H_4folate is obtained by dehydration of 10-formyl-H_4folate

$$10 - \text{formyl} - H_4folate + H^+ \rightarrow 5{,}10 - methenyl - H_4folate + H_2O \qquad (2.48)$$

In the next stage is dehydrogenase. With the help of NADH or NADPH as the reducing agent, reduces the methenyl group to methylene 5,10-methylene-H_4folate.

$$5{,}10 - methenyl - H_4folate + NAD(P)H \rightarrow 5{,}10 - methylene - H_4folate + NAD(P)^+ \qquad (2.59)$$

Finally, 5.10 methylene-H_4folate is reduced to 5-methyl-H_4folate. This reduction is catalyzed by an oxygen-sensitive enzyme, (5,10-methylene-H_4folate -reductase).

$$5{,}10 - methylene - H_4folate + 2H^+ + 2e^- \rightarrow 5 - methyl - H_4folate \qquad (2.20)$$

The last stage of the methyl branch is the transfer of 5-methyl-H_4folate to the reduced cobalt in the corrinoid protein or enzyme (Co-FeSP), with the formation of an organometallic and inactive intermediate methyl-Co(III).

$$5 - methyl - H_4folate + E - [Co] \rightarrow FH4 + E - [Co] - CH_3 \qquad (2.21)$$

(Ragsdale, 2008, Clark and Ljungdahl, 1984, Diekert and Wohlfarth, 1994, Diekert and Wohlfarth, 1991).

2.2.3.2 The Carbonyl Branch

In the carbonyl branch, as in the methyl branch, two electrons reduce CO_2 to CO (Abubackar et al., 2011). But, in this case, by using the enzyme CODH.

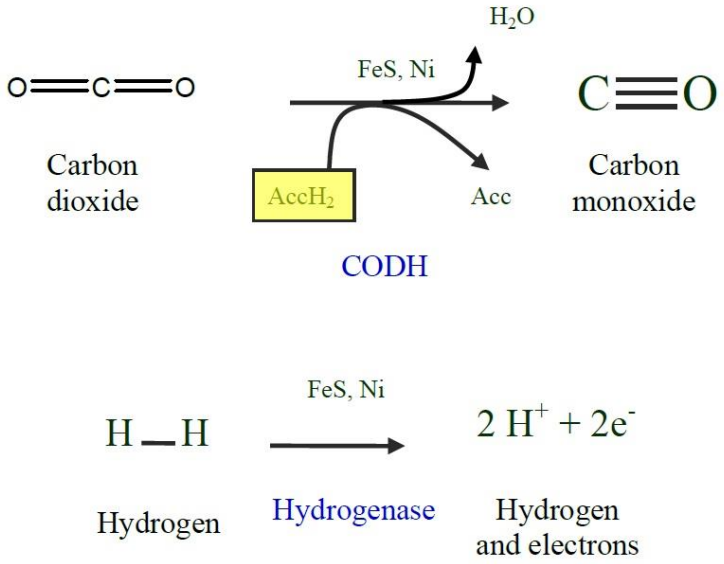

Figure 2.3. The carbonyl branch (Drake and Daniel, 2004)

Exist monofunctional and bifunctional CODH enzyme. In the case of the carbonyl branch, bifunctional CODH participates. It is a linkage of monofunctional CODH and acetyl-CoA-synthase (ACS). This bond provides the recovery of CO. Then organometallic methylcobamide from the methyl branch and carbon monoxide from the carbonyl branch are catalyst the synthesis of acetyl-CoA (Menon and Ragsdale, 1996). Moreover, this will happen if carbon monoxide is not possible to get from the environment.

2.2.3.3 Formation of acetyl-CoA

Figure 2.4 represented the final stage of the formation of acetyl-CoA. On the first step, with help of the methyltransferase enzyme (MeTr), involves the transformation of the H_4folate to organometallic methylcobamide (CH3-Co(III)) (Seravalli et al., 1999).

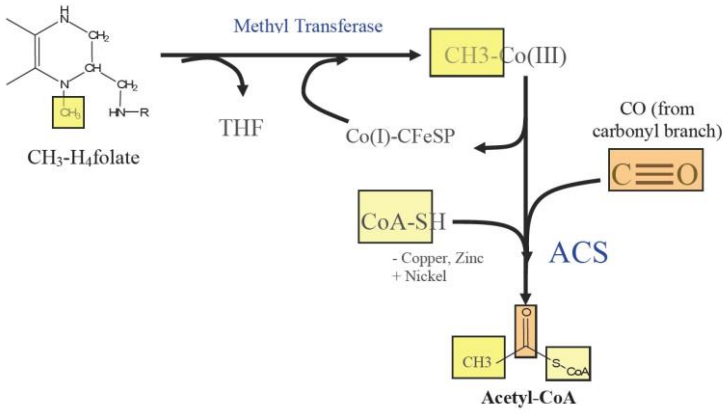

Figure 2.4. Formation of acetyl-CoA (Drake and Daniel, 2004)

The next step is to combine CO from the carbonyl branch and CH_3-Co (III) to form the acetyl-CODH moiety. Then to form acetyl-CoA, the ACS catalyzes the condensation of the acetyl moiety with free coenzyme-A.

2.2.3.4 Products of Wood-Ljungdahl pathway

After the acetyl-CoA formation, it can be used in a few ways. In case of thesis as a generation of ATP by the production of acetic acid. According to the objective of that master's thesis, the biofuel production (ethanol) and the generation of acetate (acetogenesis) are considered. In more detail, the transformation of acetyl-CoA into products and possible bacteria for this process is represented in the Figure 2.5.

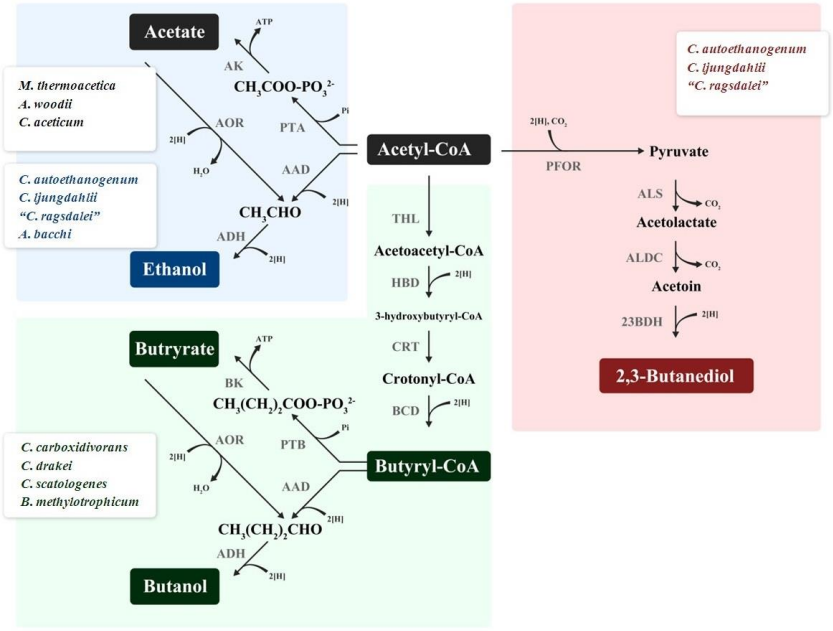

Figure 2.5. Transformation of acetyl-CoA into products (Daniell et al., 2012)

The simplest way of obtaining the ethanol is to transform acetyl-CoA into acetaldehyde, and then into ethanol. In the acidogenic phase, phosphotransacetylase catalyzes the conversion of acetyl-CoA to acetylphosphate.

$$Acetyl - CoA + P_i \rightarrow Acetylphosphate + P_i \tag{2.22}$$

Then, is the conversion of acetylphosphate to acetate with the release of ATP by phosphorylation of ADP.

$$\begin{aligned} Acetylphosphate + ADP \rightarrow Acetate + ATP 6CO + 3H_2O \\ \rightarrow CH_3CH_2OH + 4CO_2 \end{aligned} \tag{2.23}$$

In the slow growth conditions, solvents are formed (Rao and Mutharasan 1989). In this phase, acetyl-CoA is transformed into acetaldehyde with help of acetaldehyde dehydrogenase.

$$Acetyl - CoA + NADH + H^+ \rightarrow Acetaldehyne + NAD^+ + CoA - SH \tag{2.24}$$

The final stage is the conversion of acetaldehyde to ethanol under the influence of alcohol dehydrogenase during the oxidation of NADH.

$$Acetaldehyne + NADH + H^+ \rightarrow Ethanol + NAD^+ \tag{2.25}$$

2.2.3.5 Energy of Acetyl-CoA pathway

To support metabolic processes, the cells use chemical energy, which is transported by using a universal energy carrier - ATP.

There are two types of growth heterotrophic and autotrophic. During the first, ATP is generated by the phosphorylated substrate level (SLP). However, in autotrophic conditions, to stimulate the synthesis of ATP, phosphorylation is based on the chemosmotic ion gradient. In this case, the energy conservation in acetogenes combines an exergonic reaction for transporting ions through the membrane. As a result, the driving force for the synthesis of ATP is the deposition of an ionic gradient through the membrane (Muller, 2003).

In Wood-Ljungdahl pathway, one mole of ATP is used to activate formate, which must be restored to form H_4folate, which recovers to acetyl-CoA. Since the formation of acetyl-CoA has a negative energy balance, ATP is used to achieve equality by formation of SLP in the reaction of acetate kinase in the formation of acetate. (Zeikus et al., 1985) described that the generation of ATP in acetogenes occurs via electron transport phosphorylation associated with the dehydrogenation of CODH and the hydrogenating H_4folate -bound reactions.

2.2.4 Process parameters

The process of biofuel production (ethanol) is also accompanied by the production of by-products, such as acetate. Due to the fact that bacteria can get more energy to produce one mole of acetate than for biofuels (see Gibbs energy), the bioprocess needs to be controlled towards solventogenesis. Thus, a set of measures to optimize the conditions of fermentation in the bioreactor can be considered. It is necessary to take into account not only physical parameters, but also to understand the microbiological component of the process. In this section will be consider a number of basic physical parameters, the management of which can improve the quality and quantity of biofuels produced.

2.2.4.1 pH

This parameter has a direct effect on the growth of bacteria, the metabolic process, on the initial reaction parameters, and on the final products of the reaction (Pereira et al., 2009). In the case of biofuel production, the optimal pH will be between 5.5 and 6.5 (Table 2.2). However, in acetogenous bacteria, such as the genus Clostridium, the process is divided into two phases, acidogenesis and solventogenesis. During the first phase, according to the Wood-Ljungdahl pathway, there is a rapid growth of cells and the production of organic acids. As a result of the accumulation of organic acids, the pH drops, thereby causing phase transitions (Abubackar et al., 2012). Solventogenesis is accompanied by slow cell growth and biofuel production (Cotter et al., 2009a).

A possible shift in pH can be possible by stimulating the production of biofuels, resulting in phase separation. For example, (Richter et al., 2013), researching the ethanol production using *Clostridium ljungdahl*. They created a two-stage system to prove the effect of pH shift on ethanol production. In the first stage, a CSTR type bioreactor with a pH of 5.5 was used, and a bubbler column reactor with a pH of 4.5-4.8 was used in the second stage. As a result, a high level of growth was retained in the first reactor. In the second, artificially caused solventogenesis with cell recirculation (10 g/l). Based on the results of the study, a large increase in ethanol production was demonstrated.

Table 2.2. An overview of the optimum temperature and pH of most important acetogens

Microorganism	T_{opt} ()	pH_{opt}	Reference
Acetobacterium woodii	30	7.0-7.2	(Genthner and Bryant, 1987)

Acetogenum kivui	66	6.4	(Leigh et al., 1981)
Alkalibaculum bacchi	37	8.0-8.5	(Allen et al., 2010)
Clostridium aceticum	30	8.5	(Sim et al., 2008)
Clostridium autoethanogenum	37	5.8-6.0	(Kopke et al., 2011)
Clostridium carboxidivorans	38	6.2.	(Liou et al., 2005)
Clostridium ljungdahlii	37	6	(Daniell et al., 2012)
Clostridium ragsdalei	37	5.5-6.0	(Lewis et al., 2010)
Moorella thermoaceticum	55-60	6.8	(Drake and Daniel, 2004)
Moorella thermoautotrophicum	58	6.1	(Savage et al., 1987)

2.2.4.2 Temperature

Usually a temperature increase of 10 degrees doubles the rate constants of chemical reactions. In the case of biofuel production by fermentation of synthesis gas, the optimal growth temperature of microorganisms is 30-40 degrees (mesophylls) and 50-80 degrees (thermophiles) (Table 2.2). However, the temperature affects not only the rate of cell growth, but affects the solubility of gas synthesis. According to Henry's law, the solubility of CO and H_2 increases with decreasing temperature, thereby improving the mass transfer of gas into liquid, the influence of temperature on the production of ethanol has been proved. (Kundiyana et al., 2011). For example, (Ramio-Pujol et al., 2015) investigated *Clostridium Carbonidivorans P7* and *Clostridium Ragsdalei* was confirmed that lowering the temperature by a few degrees from the optimal leads to an increase in the solubility of gases and an increase in the biofuel production rate.

2.2.4.3 Syngas composition

The synthesis gas composition has a few factors of influence on the biofuels production. First of all, it is the influence of the relationship between CO/H_2. Secondly, it is the effect of impurities in the synthesis gas.

The relationship between CO/H_2 in the Wood-Ljungdahl pathway plays an important role. According to section 1, the carbonyl branch is possible without the process of reducing CO2 into CO, due to the direct use of CO taken from outside. But, in the methyl branch, the presence of H_2 and CO_2 is necessary (see Section 1). In this case, the energy required for the pathway is obtained from hydrogen and the CODH enzyme. Also, was proven that increase the ethanol production in the *Clostridium ljungdahlii* by increasing hydrogen concentration (Gaddy et al., 2007).

The second point is the effect of other chemical species in syngas, such as sulfur, NH_3, NO, etc. According to the studies, most of these impurities slow down the cell growth and change the phase transition parameters described in section 1, etc. To reduce the impact of this factor, there are many options for purifying gas synthesis before use (Xu et al., 2011, Ahmeda et al., 2006).

2.2.4.4 Partial pressure

The gases that form the synthes gas (CO_2, CO and H_2) have a low solubility. This phenomenon complicates the process of obtaining biofuel. According to numerous studies, a direct effect on the growth of cells and the inhibition of the enzyme hydrogenase by changing the partial pressure was justified (Pereira et al., 2009). In case of cell growth, this effect can be explained from a microbiological point of view. For example, change of P(CO) and/or P(CO)/P(CO_2) effect on cell growth and product formation during synthes gas fermentation by *Clostridium Carbonidivorans P7*. The experiment show that ricing partial pressure of carbon monoxide will rise production rate of ethanol (Hurst and Lewis, 2010).

As for the inhibition of the hydrogenase enzyme, firstly, free CO is used to exhaustion, and only after that the consumption of CO_2 and H_2 begins. Then, by increasing the partial pressure of carbon monoxide, the reaction rate will be higher. But at the same time, increasing the partial pressure of carbon dioxide will slow down the reaction rate. However, the total elimination of CO_2 from the reaction will not improve the production of biofuels. The results of a study by (Heiskanen et al., 2007), showed that the use of CO_2 and CO in the methyl branch raises the production of acetate (C (CO)> C (CO_2)).

(Skidmore et al., 2013, Pereira et al., 2009, Abubackar et al., 2012, Abubackar et al., 2011)

2.2.4.5 Mass transfer limitations

There are two factors that have effect on the mass transfer of gas into the liquid (Abubackar et al., 2011). First is the solubility of gases. As the solubility of the main components of syngas is low the mass transfer - slow. This will reduce the availability of the enriched substrate for effective growth of microorganisms in the medium.

Second is a kinetic-growth. While a low metabolic activity of the cells, the rate of substrate consumption will be lower than the rate of its supply to microorganisms.

To minimize the impact of these two factors, different types of bioreactors can be used. For this purpose, the volumetric gas-liquid mass transfer coefficient ($k_L a$) can be used as a criterion for comparing the efficiency of mass transfer between different types of bioreactors. This coefficient is calculated by the following equations:

$$\frac{dS}{dt} = (k_L a) * (S^* - S) \tag{2.26}$$

$$\ln\left(\frac{S^* - S_0}{S^* - S}\right) = (k_L a) * t \tag{2.27}$$

Where S^* – concentration of saturated dissolved gas in the liquid phase;

S_0 – concentration of dissolved gas in the liquid phase at the zero point in time;

S – concentration of the gas in a liquid phase at time;

t – time.

In Table 2.3 comparisons of the types of bioreactors by different $k_L a$ (Munasinghe and Khanal, 2010).

Table 2.3. Volumetric mass transfer coefficient ($k_L a$)

Reactor design	Bacteria	kLa (h^{-1})	Reference
CSTR	*n/a*	38	
CSTR	*SRB*mixed culture*	31 for CO, 75 for H_2	
CSTR	*C. ljungdahlii*	35 for CO	
CSTR	*R. rubrum*	28.1 for CO	(Cowger et al., 1992)
CSTR	*R. rubrum*	101 for CO	(Cowger et al., 1992)
Stirred tank	*SRB*mixed culture*	104 for CO	(Ungerman and Heindel, 2007)
Packed bubble column	*R. rubrum*	2.1	(Cowger et al., 1992)
Trickle bed	*R. rubrum*	55.5	(Cowger et al., 1992)
Trickle bed	*SRB*mixed culture*	121 for CO, 335 for H_2	
Trickle bed	*C. ljungdahlii*	137 for CO	
Stirred tank	*R. rubrum*	71.8	(Younesi et al., 2008)

2.2.4.6 Redox potential

The last parameter in this review, whose effect is proved, is the redox level or redox potential.

The redox potential is a measure of the proximity of electrons in solution in comparison with hydrogen. Solutions with a positive redox potential are able to oxidize hydrogen, while solutions with a negative redox potential may lead to hydrogen reduction. The redox potential makes possible to understand what will happen with the electrons in the solution when a new component is added into this solution. Usually it is measured in volts (V), millivolts (mV) or Eh (1 Eh = 1 mV).

The process of obtaining biofuel requires a large donation of electrons. Therefore, the process of synthesis gas fermentation towards biofuel production can be influenced parameters as: initial composition of CO, H_2 and CO_2; dynamic changes in the composition of CO, H_2 and

CO_2 during fermentation. The optimal range of redox potential for biofuel production differs with types of bacteria. To date, many articles have been published that discuss the effect of the parameters described above on the production of alcohols. For example, according to some publications, the following conclusions can be drawn:

- More negative potential contributes to the production of alcohols rather than acids, since alcohols are more reduced (Kim and Bajapai, 1988);

- The solvent production process can be caused by electron donors. For example, methyl viologen, addition of which promoted the formation of solvents (Rao and Ward, 1987);

- Hydrogen sulphide (Jee and Nishio, 1987), sodium sulfide (Rao and Ward, 1987), methyl viologen (Rao and Ward, 1987), ascorbic acid and others can be used to control the potential.

3 Modelling and simulation

The development of syngas fermentation model will be represented in this chapter. It consists of two subsections: formation of a mathematical model with a description of methods and verification of this model through the software AquaSIM. An illustration of the conceptual model is presented in Figure 3.1, below.

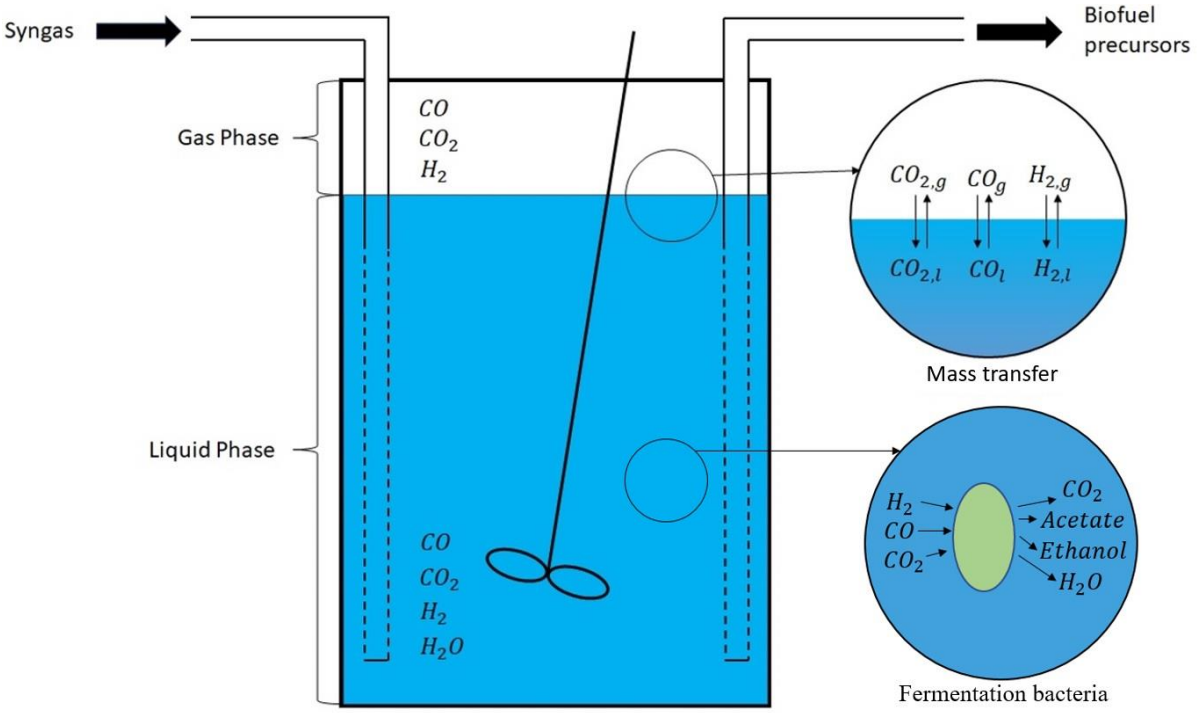

Figure 3.1. The conceptual model of syngas fermentation reactor

Syngas feds into the reactor where fermentation bacteria produce biofuel. Liquid and gas phase are taken into account as well as the mass transfer between them. Calculation of liquid and gas mass balances, mass transfer limitation approximates the model to the natural course of the process in nature.

Below is presented step-by-step mathematical model creation to describe of the process.

3.1 Thermodynamics of the bioprocess

Thermodynamics calculations are based on (Rittmann and McCarthy, 2001).

To simplify it the following assumptions are made:

- The energy capture efficiency is 60%;
- NH_4^+ is used as nitrogen source;
- CO_2 is used as carbon source.

A thermodynamic reaction is based on half reactions, an oxidation reaction and a reducing reaction, and this is referred to as catabolism, where the material that are oxidized are called electron donors and those being reduced are electron acceptors.

The determination if this process is feasible will be decided by the Gibbs free energy. The Gibbs free energy determines if reactions are exothermic or endothermic. Exothermic means

that the reaction will release energy and endothermic means the reaction needs energy. Equitation 1 describes how the Gibbs free energy is calculated.

$$\Delta G^0 = \sum_i N_{i,P}\,\Delta G^0_{i,P} - N_{i,R}\Delta G^0_{i,R} \tag{3.1}$$

Where ΔG^0 – Gibbs free energy;

$N_{i,P}$ – Stochiometric coefficient of reactants;

$N_{i,R}$ – Stochiometric coefficient products;

$\Delta G^0_{i,P}$ – Gibbs free energy for reactants;

$\Delta G^0_{i,R}$ – Gibbs free energy for products.

3.1.1 Electron acceptor

Syngas fermentation process use carbon dioxide as electron acceptor. The CO_2 will be reduced based on the electron charge of the electron donor. In the Table 3.1 shown five possible electron donor half-reactions. According to the values of Gibbs free energy, the Methane production is the most attractive than production of ethanol or acetic acid.

Table 3.1. Half-reactions for electron acceptor

	Half-reactions	$\Delta G°\ [\frac{\text{kJ}}{e^-\ eq}]$
No.1 Ethanol	$\frac{1}{6}CO_2 + H^+ + e^- \rightarrow \frac{1}{12}CH_3CH_2OH + \frac{1}{4}H_2O$	31.18
No.2 Acetic acid	$\frac{1}{4}CO_2 + H^+ + e^- \rightarrow \frac{1}{8}CH_3COOH + \frac{1}{4}H_2O$	30.1655
No.3 Methanol	$\frac{1}{6}CO_2 + H^+ + e^- \rightarrow \frac{1}{6}CH_3OH + \frac{1}{6}H_2O$	36.84
No.4 Lactate	$\frac{1}{4}CO_2 + \frac{1}{12}HCO_3^- + H^+ + e^- \rightarrow \frac{1}{12}CH_3CHOHCOO^- + \frac{1}{3}H_2O$	32.29
No.5 Methane	$\frac{1}{8}CO_2 + H^+ + e^- \rightarrow \frac{1}{8}CH_4 + \frac{1}{4}H_2O$	23.53

3.1.2 Electron donor

Electron donor is usually the substrate. Which is utilize for energy production and cell synthesis, thus bacteria growth. When all the substrates are consumed, some of the bacteria will decay and become new substrate for the remaining bacteria.

As for the electron donor, there are two possible options (Table 3.1): carbon monoxide and hydrogen. They will oxidize and will donate electrons.

Table 3.2. Half-reactions for electron donor

Reactions for electron donor	**Half-reactions**	$\Delta G°\ [\frac{\text{kJ}}{e^- eq}]$
No.6	$\frac{1}{2}CO + \frac{1}{2}H_2O \rightarrow \frac{1}{2}CO_2 + H^+ + e^-$	-49.886
No. 7	$0.5H_2 \rightarrow H^+ + e^-$	-39.87

3.1.3 Bacterial cell synthesis

In addition to electron donor and acceptor half-reactions, it is important to involve carbon and nitrogen source for cell synthesis (biomass). As carbon source is possible to use HCO_3^-/CO_2 and for nitrogen - NH_4^+, NO_3^-.

Following Table 3.3 shows the half reaction for cell synthesis, where the carbon source (HCO_3^-/CO_2) and nitrogen source (NH_4^+, NO_3^-) are utilized for growth.

Table 3.3. Half-reaction for bacterial cell synthesis

Reaction for bacterial cell synthesis	**Half-reaction**
No. 8	$0.59HCO_3^- + 0.12NH_4^+ + 1.47H^+ + e^- \rightarrow 0.59CH_{1,8}O_{0,5}N_{0,2} + 1.47H_2O$
No. 9	$0.24CO_2 + 0.05NH_4^+ + 0.95H^+ + e^- \rightarrow 0.24CH_{1,8}O_{0,5}N_{0,2} + 0.36H_2O$
No. 10	$0.17CO_2 + 0.03NO_3^- + 1.03H^+ + e^- \rightarrow 0.17\ CH_{1,8}O_{0,5}N_{0,2} + +0.36H_2O$

3.1.4 Total metabolic process

The metabolism is the total chemical process of the cell. Metabolism is the utilizing of the substrate for bacteria growth and energy production (Rittmann and McCarthy, 2001). The calculation of this process is via equation 3.

$$R = f_e R_a + f_s R_{CS} - R_d \quad (3.2)$$

$$f_e + f_s = 1 \quad (3.3)$$

Where f_s – cell synthesis;
f_e – energy production;
R_a – half reaction for electron acceptor;
R_{cs} – half reaction of cell synthesis;
R_d – half reaction of electron donor.

Cell synthesis and the energy production is calculated by equation 3 and 4.

$$\Delta G_s = \frac{\Delta G_p}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon} \quad (3.4)$$

Where ΔG_S – energy required to synthesis from a given carbon source;
ΔG_p – energy required to convert the carbon source to pyruvate;
ΔG_{pc} – energy required to convert pyruvate carbon to cellular carbon;
ε – energy capture efficiency;
n – the energy transfer efficiency for conversion of carbon to pyruvate.

Because of two possible electron donor reaction are necessary to calculate ΔG_S for CO as electron donor and for H_2 as electron donor. Below in Table 3.4 and Table 3.5 are represented calculations for ΔG_p and ΔG_{pc} .

Available energy for cell growth are taking from exergonic reactions. These reactions are catalyzed by enzymes in the cell. Energy losses for heat production reduce the amount of captured energy by the bacteria. According to the (Tchobanoglous et al., 2014), the energy capture efficiency is 60% as the most common value.

Table 3.4. CO as electron donor

ε	Assume 0.6
$\Delta G_p\ [\frac{\text{kJ}}{e^-\ eq}]$	84.976
$\Delta G_{pc}\ [\frac{\text{kJ}}{e^-\ eq}]$	14.15
n	+1

Table 3.5. H_2 as electron donor

ε	Assume 0.6
$\Delta G_p\ [\frac{\text{kJ}}{e^-\ eq}]$	74.96
$\Delta G_{pc}\ [\frac{\text{kJ}}{e^-\ eq}]$	12.48
n	+1

f_s and f_e is possible to get from equation

$$\frac{f_e}{f_s} = \frac{-\Delta G_s}{\varepsilon(\Delta G_R)} \quad (3.5)$$

Table 3.6 shows the biological stoichiometric, based on equation above. All steps are represented in 49Appendix A. The AquaSim simulation build on R2_CO and R2_H2 stoichiometric reactions.

Table 3.6. Biological stoichiometric

Reaction	Half-reaction combinations	Stoichiometric biological reactions (R)
R1_CO	No. 1 & 6	$33.3CO + 0.2NH_4^+ + 16.2H_2O \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 5.2CH_3CH_2OH + 21.93CO_2 + 0.173H^+$
R1_H2	No. 1 & 7	$20.4CO_2 + 60.24H_2 + 0.2NH_4^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 9.68CH_3CH_2OH + 0.22H^+ + 30.55H_2O$
R2_CO	No. 2 & 6	$31.64CO + 0.21NH_4^+ + 15.24H_2O \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 7.33CH_3COOH + 2.09H^+ + 15.87CO_2$
R2_H2	No. 2 & 7	$27.13CO_2 + 54.35H_2 + 0.21NH_4^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 13.06CH_3COOH + 27.63H_2O + 0.21H^+$
R3_CO	No. 3 & 6	$40.32CO + 26.08H_2O + 0.21NH_4^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 12.74CH_3OH + 26.58CO_2 + 0.21H^+$
R3_H2	No. 3 & 7	$57.75CO_2 + 172.41H_2 + 0.21NH_4^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 56.76CH_3OH + 58.27H_2O + 0.21H^+$
R4_CO	No. 4 & 6	$35.71CO + 11.81H_2O + 0.21NH_4^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 5.43CH_3CHOHCOO^- + 17.93CO_2 + 0.21H^+$
R4_H2	No. 4 & 7	$48.02CO_2 + 78.12H_2 + 0.2NH_4^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 12.67CH_3CHOHCOO^- + 55.19H_2O + 0.22H^+$
R5_CO	No. 5 & 6	$23.81CO + 11.47H_2O + 0.2NH_4^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 5.43CH_4 + 17.38CO_2 + 0.21H^+$

R5_H2	No. 5 & 7	$16.6CO_2 + 33.3H_2 + 0.2NH_4^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 7.8CH_4 + 17.13H_2O + 0.21H^+$

3.1.5 Biomass growth

The yield is related to biomass growth, the yield indicates how much the biomass growth will be, and can be determined by equation 3.6.

$$Y = \frac{\text{biomass produced}}{\text{substrate used}} \tag{3.6}$$

Table 3.7 is show the calculated values of biomass yield growth.

Table 3.7. Yield

Name	**Overall reactions**	**Yield, g VVS/g COD**
Ethanol_CO	$6CO + 3H_2O \rightarrow CH_3CH_2OH + 4CO_2$	0.0468
Ethanol_ H_2	$2CO_2 + 6H_2 \rightarrow CH_3CH_2OH + 3H_2O$	0.0254
Acetic acid_ CO	$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2$	0.0484
Acetic acid_ H_2	$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$	0.0282
Methanol_ CO	$3CO + 2H_2O \rightarrow CH_3OH + 2CO_2$	0.0398
Methanol_ H_2	$CO_2 + 3H_2 \rightarrow CH_3OH + H_2O$	0.0091
Lactate_ CO	$6CO + HCO_3^- + 2H_2O \rightarrow CH_3CHOHCOO^- + 3CO_2$	0.0441
Lactate_ H_2	$3CO_2 + HCO_3^- + 6H_2 \rightarrow CH_3CHOHCOO^- + 4H_2O$	0.0197
Methane_ CO	$4CO + 2H_2O \rightarrow CH_4 + 3CO_2$	0.0642
Methane_ H_2	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	0.0463

Unfortunately, yield calculations are far from real value. Up to date is still hard to calculate the real value of energy capture efficiency for each product during the reaction. Figure 3.2 represented change of yield by using ε value from 10% till 60%. To recalculate yield units used data from Appendix A.

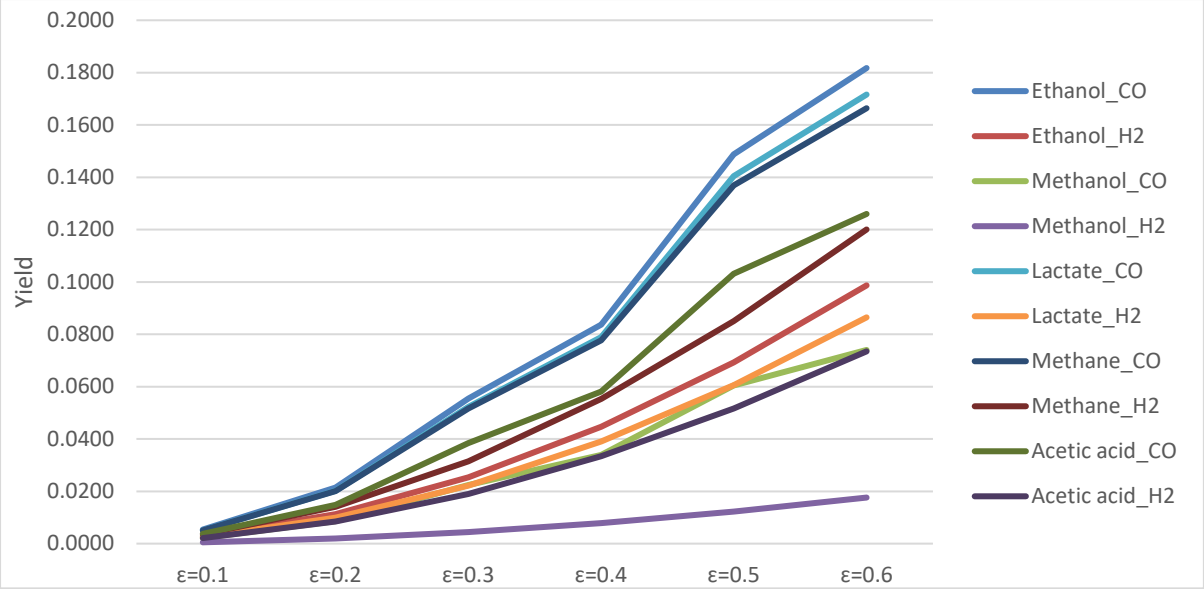

Figure 3.2. Change of yield value based on energy capture efficiency

3.2 Growth rate and kinetic expressions

The next step in the creation of syngas fermentation model is to determine the rate of the bacteria growth. To date, the most used method for calculating the rate is by the Monod equation (Liu, 2006).

$$\mu = X\mu_{max}\frac{S}{(K_S + S)} \tag{3.7}$$

Where μ – specific growth rate;
X – concentration of biomass;
μ_{max}– maximum specific grow rate;
S – growth limiting substrate concentration;
K_S – half saturation constant.

The Monod model, 1949, is one of the oldest model of microbial growth kinetics (Monod, 1949). It based on concentration of single controlled substance. However, presence of some inhibitory substance reduces the accuracy of Monod model. Due to the fact that syngas fermentation process is a complex biological process with inhibitory substances, it is necessary to approximate the model to natural conditions by using more complex models. According to (Arellano-Plaza et al., 2007) it is possible to use a wide range of different substrate inhibition models, such as Haldane model (Wang and Loh, 1999, Haldane, 1930) and Moser model (Moser, 1958).

3.2.1 Specific growth rate (R_d - carbon monoxide)

The stoichiometric reaction R2_CO describes the process of growth of biomass and the production of acetic acid by use carbon monoxide as electron donor. The Haldane model is suitable to explain the process with inhibition at high levels of carbon monoxide (Mohammadi et al., 2014).

$$r_1 = X * \mu_1 = X * \mu_1^{max} * \frac{S_{CO}}{K_{CO} + S_{CO} + \frac{S_{CO}^2}{K_{I,CO}}} * \frac{K_{I,UAc}}{K_{I,UAc} + S_{UAc}} \quad (3.8)$$

$$S_{UA} = S_{Ac} - \frac{10^{(pH-pKa)} * S_{Ac}}{10^{(pH-pKa)} + 1} \quad (3.9)$$

Where $K_{I,i}$ – inhibition constant that shows the representation of the affinity for the substrates;

pKa – logarithmic acid dissociation constant.

Growths rate will be inhibited then the S_{CO} go beyond the substrate inhibition constant (Younesi et al., 2005).

Aslo, the reserch made by (Wangt and Wang, 1984) showed that main inhibitor was the undissociated acetic acid (UAc) instead of the ionized acetate ion. So, to describe the inhibition of growth of undissociated acetic acid it is possible to involve the inhibition constant. The concentration of UAc is high, and could be calculated by the Equation 3.9.

3.2.2 Specific growth rate (R_d – hydrogen)

To describe the growth process with two substrates, combined kinetic models can be used according to (Arellano-Plaza et al., 2007). To describe reaction R2_H2, the combination of the kinetics of Boulton and Moser (Boulton, 1980, Moser, 1958) was chosen. Equation 3.10 also include inhibitory effect of CO, at which growth was not manifested. To explain limitation effect of both substrates possible to use the same way as in Section 3.2.1. In also consist of two Haldane model and inhibition by UAc. The specific growth rate is described by Equation 3.10:

$$r_2 = X * \mu_2 = X * \mu_2^{max} * \frac{S_{CO_2}}{K_{CO_2} + S_{CO_2} + \frac{S_{CO_2}^2}{K_{I,CO_2}}} * \frac{S_{H_2}}{K_{H_2} + S_{H_2} + \frac{S_{H_2}^2}{K_{I,H_2}}} * \frac{K_{I,UAc}}{K_{I,UAc} + S_{UAc}} \quad (3.10)$$

It is worth remembering that presence of carbon monoxide can reduce the specific growth rate, r_2, due to features of the Wood-Ljungdahl pathway. Inhibition of carbon monoxide is conditionally shows in the equation, as a product of process of CO_2 reduction to CO.

3.2.3 Transformation of acetic acid to ethanol

To build transformation model possible to use water-gas shift reaction (see Section 2.2.2). Then, the stoichiometric reaction can be built by summation of the next equations:

$$CH_3COOH + H^+ + e^- \rightarrow CH_3CH_2OH + H_2O \quad (3.11)$$

$$\frac{1}{2}CO + \frac{1}{2}H_2O \rightarrow \frac{1}{2}CO_2 + H^+ + e^- \quad (3.12)$$

Where electron donor is carbon monoxide.

As a result, the next expression will be setted:

$$13.8CH_3COOH + 29.76CO + 14.4H_2O + 2.08NH_4^+ + 0.23H^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 13.8CH_3CH_2OH + 28.75CO_2 \quad (3.13)$$

Using the method described in Section 3.2.1, is possible to form specific growth rate on acetic acid to get ethanol.

$$r_3 = X * \mu_3 = X * \mu_3^{max} * \frac{S_{CO}}{K_{CO} + S_{CO} + \frac{S_{CO}^2}{K_{I,CO}}} * \frac{S_{Ac}}{K_{Ac} + S_{Ac}} * \frac{K_{UAc}}{K_{UAc} + S_{UAc}} \quad (3.14)$$

3.2.4 Stoichiometric expressions

The stoichiometry equations can be transform to the stoichiometric coefficient. The results of transformation are shown in Table 3.8 (Vandecasteele, 2016). It contains the production of biomass, acetic acid and transformed ethanol. The consumption of nitrogen source and water are assumed to be neglected. The horizontal row shows the list of components. The vertical row shows the processes, mentioned above. A positive stoichiometric coefficient shows the production of that component while a negative sign indicates consumption. The yield coefficient (Y) presented in Table 3.7 and Figure 3.2. All of them are based on stoichiometric equations.

Table 3.8. Stoichiometric coefficient of biomass growths

	CO	CO_2	H_2	$Biomass$	$Acetic\ acid$	$Ethanol$
Specific growth rate (R_d - carbon monoxide)	$\frac{-1}{Y_1}$	$\frac{0.5}{Y_1} - 0.0175$	–	1	$\frac{0.25}{Y_1} - 0.5$	–
Specific growth rate (R_d - hydrogen)	–	$-\frac{0.5}{Y_2} + 0.0175$	$\frac{-1}{Y_2}$	1	$\frac{0.25}{Y_2} - 0.5$	–
Transformation Acetic acid to Ethanol	$\frac{-2}{Y_3}$	$\frac{2}{Y_3}$	–	–	$\frac{-1}{Y_3}$	1

3.3 Gas phase mass balances

To simplify the gas phase (headspace) model, the following assumptions are made:

- Perfectly mixed headspace;
- No reactions occurs in the headspace;
- Volatile components neglected;

- Dilution by water evaporation neglected;
- The headspace volume is constant;
- The temperature is constant.

The mass balance of headspace consists of carbon dioxide, carbon monoxide and hydrogen. It could be calculated by equation 3.15:

$$\frac{d(V_H * S_{gas,i})}{dt} = k_L a_i * (S_i^* - S_i) * V_L \quad (3.15)$$

Total headspace pressure possible to calculate by calculation of total number of moles (n_{gas}) inside the headspace. Also, important to mention that the pressure in headspace must be determined on each time step, because of mass transfer between the phases.

$$n_{gas} = V_H * \sum_i S_{gas,i} \quad (3.16)$$

Having the total number of moles is possible to find the total pressure ($p_{headspace}$) using ideal gas law (Clapeyron, 1834):

$$p_{headspace} = \frac{n_{gas} * R * T}{V_H} \quad (3.17)$$

Where R – the ideal, or universal, gas constant;

T – temperature.

Eventually, by using Raoult's law (Raoult, 1887), the partial pressure (p_i) can be calculated:

$$p_i = \frac{p_{headspace} * V_H * S_{gas,i}}{n_{gas}} \quad (3.18)$$

3.4 Liquid phase mass balances

To simplify the liquid phase model, the following assumptions are made:

- Perfectly mixed reactor;
- Dilution by water neglected;
- The liquid volume is constant;
- The temperature is constant.

The mass balance of liquid phase must be found for each component individually (CO, CO_2, H_2, Biomass, Acetic Acid and Ethanol). Moreover, calculation of compounds which take part in gas phase mass balance should consists of: mass transfer between phases and biological conversion. To make calculation possible to use equation 3.20 and 3.21:

$$\frac{d(V_L * S_i)}{dt} = k_L a_i * (S_i^* - S_i) * V_L + r_{c,i} * V_L \quad (3.20)$$

$$r_{c,i} = \sum_i r_i * v_i \quad (3.21)$$

Where $r_{c,i}$ –conversion rate for each component. It can be calculated by using compounds from the Table 3.8.

$$\begin{cases} \frac{d(S_{CO})}{dt} = k_L a_{CO} * (S^*_{CO} - S_{CO}) + r_{c,CO} & (3.22) \\ \frac{d(S_{CO_2})}{dt} = k_L a_{CO_2} * \left(S^*_{CO_2} - S_{CO_2}\right) + r_{c,CO_2} & (3.23) \\ \frac{d(S_{H_2})}{dt} = k_L a_{H_2} * \left(S^*_{H_2} - S_{H_2}\right) * + r_{c,H_2} & (3.24) \end{cases}$$

$$\begin{cases} r_{c,CO} = \left(-\frac{\mu_{X1}}{Y_{X1}} - 2\frac{\mu_{E1}}{Y_{E1}}\right) * X & (3.25) \\ r_{c,CO_2} = \left(\mu_{X1}\left(\frac{0.5}{Y_{X1}} - 0.0175\right) - \mu_{X2}\left(\frac{0.5}{Y_{X2}} + 0.0175\right) + 2\frac{\mu_{E1}}{Y_{E1}}\right) * X & (3.26) \\ r_{c,H_2} = -\frac{\mu_{X2}}{Y_{X2}} * X & (3.27) \end{cases}$$

Because of assumption that biomass ethanol and acetate cannot change the phase, these components in liquid mass balances consist only of the conversion rate.

$$\begin{cases} \frac{d(S_X)}{dt} = (\mu_{X1} + \mu_{X2}) * X = r_{c,X} & (3.28) \\ \frac{d(S_{Ac})}{dt} = \left(\mu_{X1}\left(\frac{0.25}{Y_{X1}} - 0.5\right) + \mu_{X2}\left(\frac{0.25}{Y_{X2}} - 0.5\right) - \frac{\mu_{E1}}{Y_{E1}}\right) * X = r_{c,Ac} & (3.29) \\ \frac{d(S_E)}{dt} = \mu_{E1} * X = r_{c,E} & (3.30) \end{cases}$$

3.5 Mass transfer between headspace and liquid phase

Due to the fact that biological process contains the gasses, is important to involve mass transfer between phases into the model.

$K_L a$ is the gas-liquid transfer coefficient, it represents the rate of transfer in both directions (See Section 2.2.4.5). The coefficient is based on experimental data and in this model, $K_L a$ is assumed the to be 0.1 based on ADM1 (D.J.Batstone et al., 2002).

According to (Sander, 2015), it is important to find the right distribution of compounds between the phases. This can be achieved using the Henry's law of diffusion (Henry, 1803).

Henry's law is represented in Equation 3.31:

$$K_{H,i} = \frac{S^*_i}{p_i} \quad (3.31)$$

Where $K_{H,i}$ – Henry's law constant
The Van't Hoff equation (Hoff, 1884) is used to recalculate the Henry's law coefficient due to temperature change:

$$K^{new}_{H,i} = K_{H,i} * e^{\frac{\Delta H^0_{w,i}}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)} \quad (3.32)$$

Where $K_{H,i}^{new}$ – Henry's law constant with new temperature;
$K_{H,i}$ – Henry's law for 298 K*;*
$\Delta H_{w,i}^{0}$ – enthalpy of reaction from liquid to gas phase;
R – universal gas law constant;
T_1 – temperature 298.15k K;
T_2 – temperature 310.15 K.

3.6 Simulation

The software "Computer Program for the Identification and Simulation of Aquatic Systems" (Aquasim) is a tool for modeling of biological processes. In this report Aquasim is used to simulate syngas fermentation. The simulation is based on growth rate, and developed partly on Anaerobic Digestion Model No. 1 (AMD1) to develop a simplified biological process (D.J.Batstone et al., 2002).

To simplify the model simulation, the following assumptions are made:

- The model is based on *Clostridium ljungdahlii* bacteria (Younesi et al., 2005);
- Reactor design is batch reactor;
- Reactor volume is 1 L;
- The headspace volume is 0.05 L;
- Reactor and headspace are perfectly mixed;
- The temperature is 310.15 K;
- The pH is 6.7;
- The time step is 1 minute;
- kLa is equal to 200 (D.J.Batstone et al., 2002).

3.6.1 Simulation development

Under the literary review and model development, the simulation of syngas fermentation process could be divided to three sub-models (Model 1, 2 and 3) and one complex model (Model 4), that include all sub-models mentioned above as one process. A separate simulation for each sub-model is an important step of detecting possible errors and verifying the calculations correctness. All the parameters for the simulation are available in Appendix B Aquasim Parameters.

Models 1, 2 and 3 are based on stoichiometric reaction R2_CO, R2_H2 and (3.13) respectively. Their initial conditions are basically the same. Among them can be distinguished:

- External pressure (P_{atm}=1 atm);
- Biomass value ($X_{biomass}$=0.0001 mol);
- Maximum specific substrate utilization rate (k=6);

Initial amount of carbon monoxide, carbon dioxide, hydrogen and nitrogen source are corresponding to the values in stoichiometric reaction in moles.

Since the Model 4 is a general model of syngas fermentation process, the initial values of reagents are selected in accordance with Wood-Ljungdahl pathway.

4 Results of simulation of syngas fermentation

4.1 Model 1

Figure 4.1 and Figure 4.2 show simulation of the Model 1, where carbon monoxide is electron donor. In this model were calculated production of acetic acid, carbon dioxide and biomass. The lag phase continues for ~5 days from the beginning of the simulating. After ~29 days the reaction reaches the stoichiometric balance according to equation R2_CO.

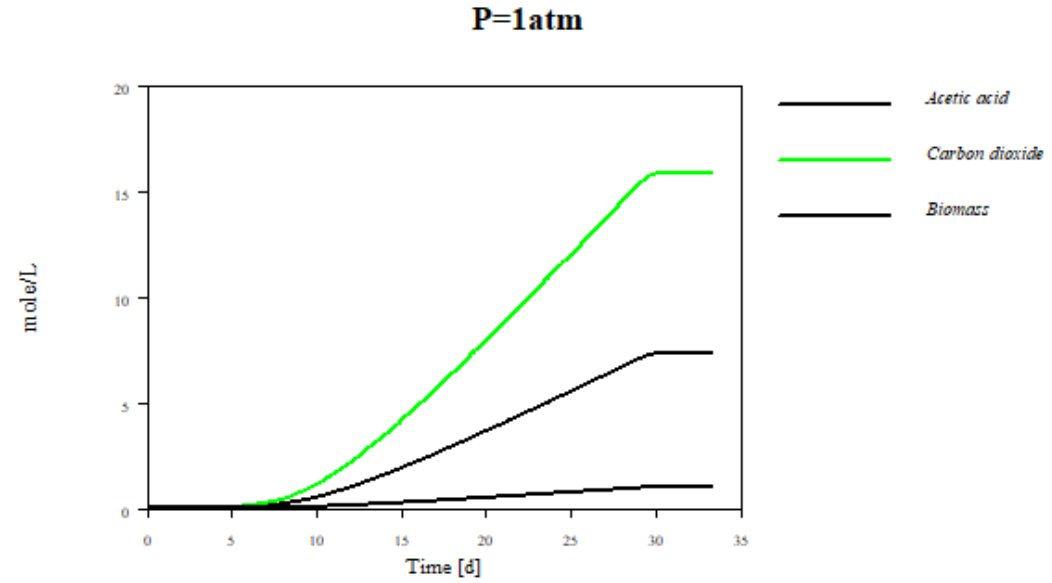

Figure 4.1. Plot of the simulated reactor (model 1)

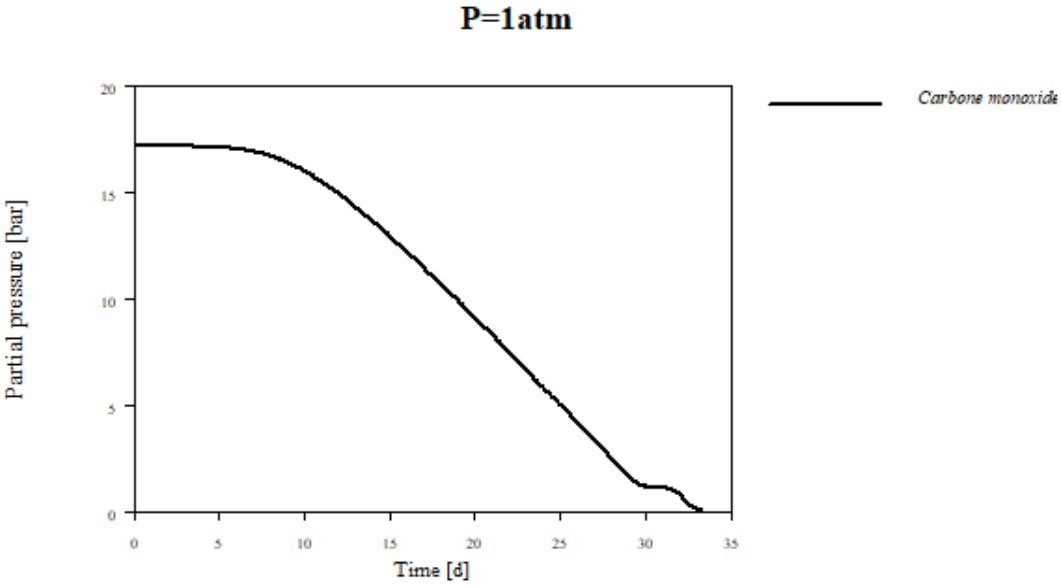

Figure 4.2. Plot of the partial pressure change (model 1)

4.2 Model 2

Figure 4.3 and Figure 4.4 plots of simulation of the Model 2, where hydrogen is electron donor. In this model were calculated production of acetic acid and biomass. The lag phase continues for ~40 days from the beginning of the simulating. After ~300 days the reaction reaches the stoichiometric balance according to equation R2_H2.

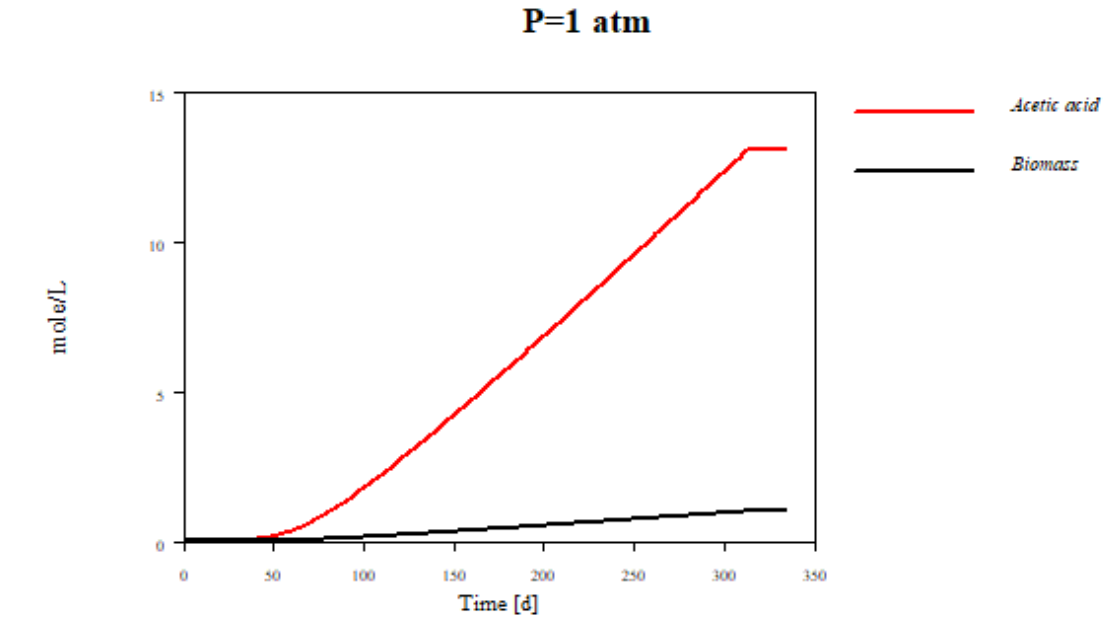

Figure 4.3. Plot of the simulated reactor (model 2)

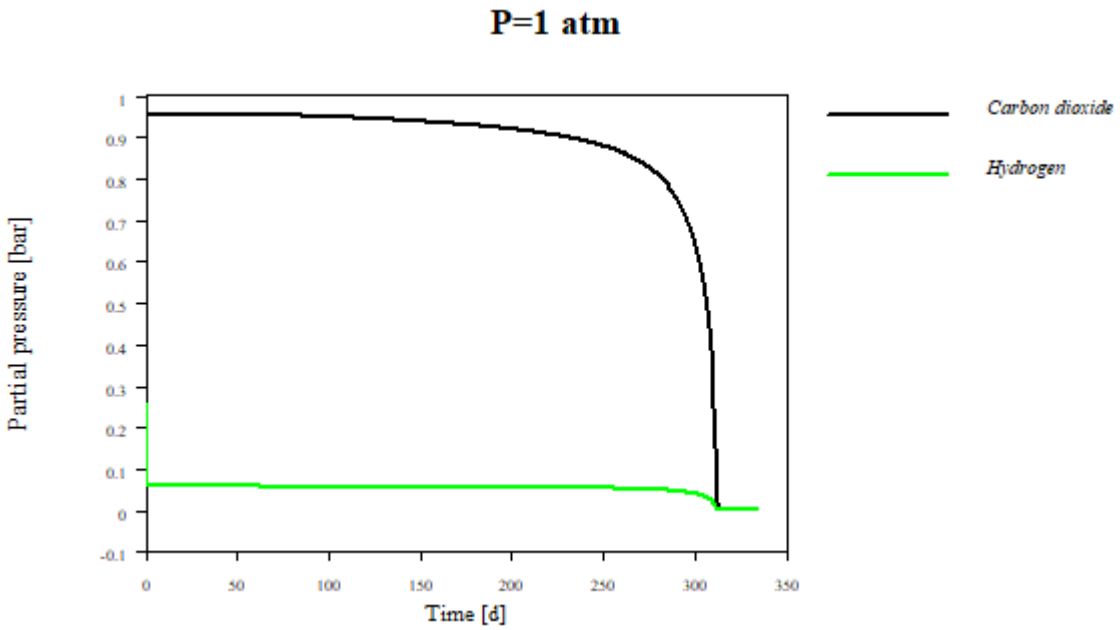

Figure 4.4. Plot of the partial pressure change (model 2)

4.3 Model 3 (Acetic acid to Ethanol)

Simulation of the Model 3 represent at Figure 4.5 and Figure 4.6. In this model were calculated production of ethanol, carbon dioxide and biomass. The lag phase continues for ~1000 days (~2.7 years) from the beginning of the simulation. After ~6100 days (~16.7 years) the reaction reaches the stoichiometric balance according to equation (3.13).

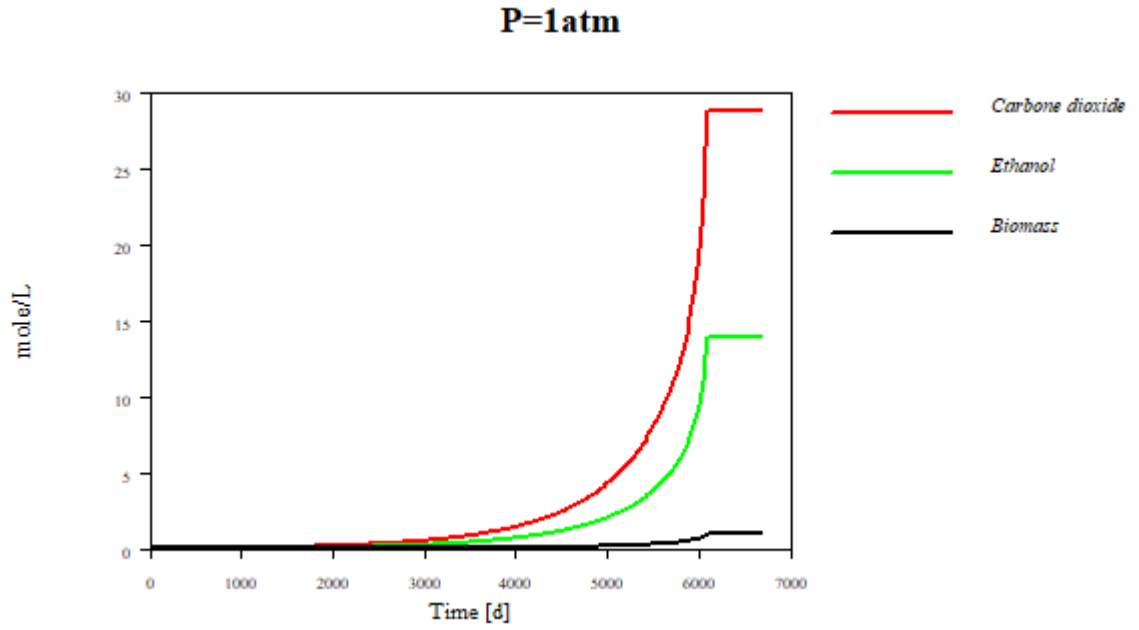

Figure 4.5. Plot of the simulated reactor (model 3)

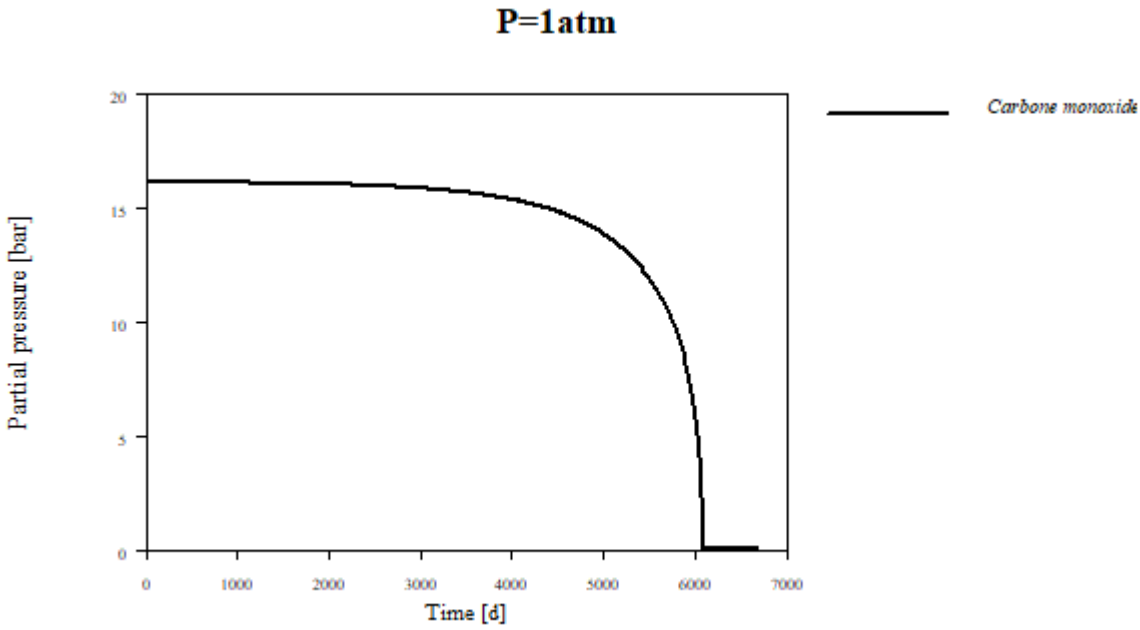

Figure 4.6. Plot of the partial pressure change (model 3)

4.4 Model 4

The simulation of the Model 4, with the same initial conditions, as in models 1,2 and 3, shown in the Figure 4.7 and Figure 4.8. In this model were calculated production of acetic acid, ethanol and biomass. The lag phase continues around 4 days. After ~170 days the carbon monoxide and hydrogen will be consumed.

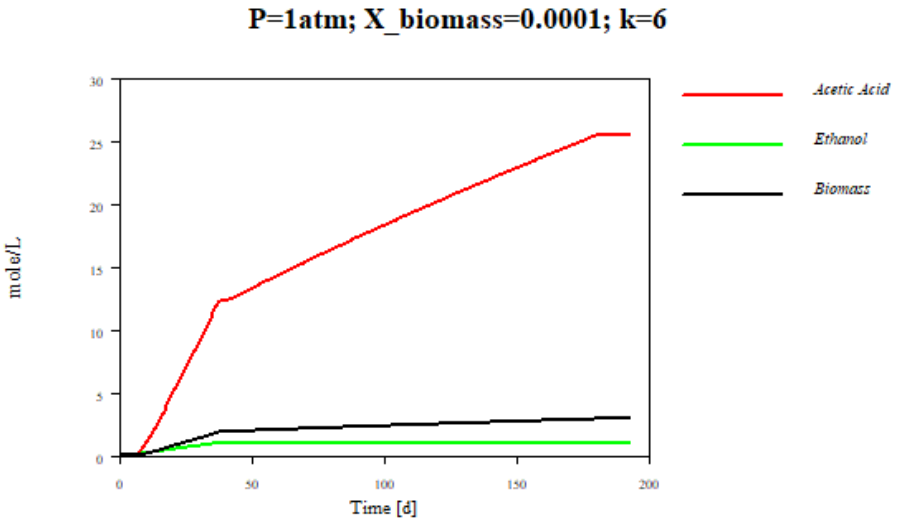

Figure 4.7. Plot of the simulated reactor (model 4)

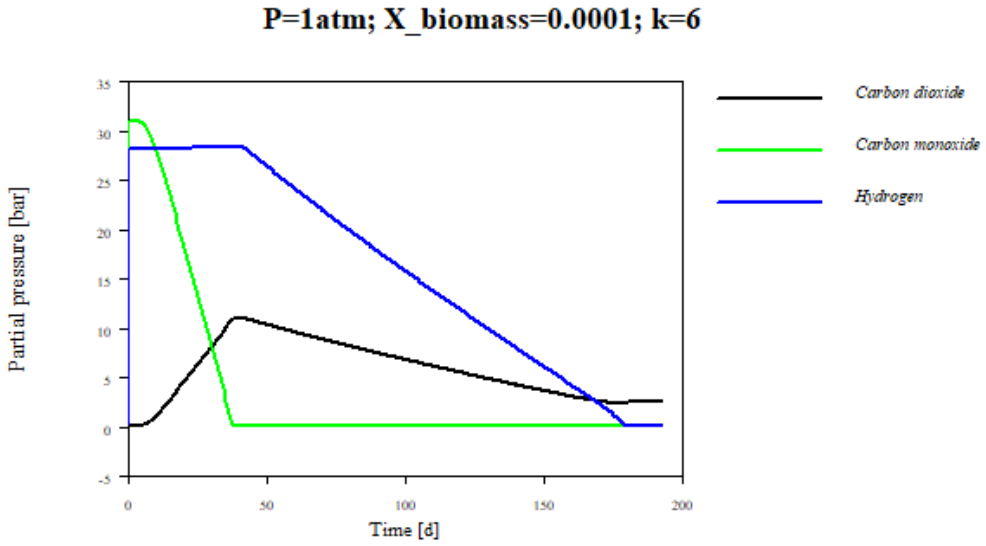

Figure 4.8. Plot of the partial pressure change (model 4)

Because of the Model 4 include all models mentioned above as one process, it going to use as a basic model to make tests. All tests are based on a change of the values that may affect the passage of the reaction. These values are:

- Initial value of biomass ($X_{biomass}$);
- Atmospheric pressure (P_{atm});
- Maximum specific substrate utilization rate (k).

4.4.1 Test 1 ($X_{biomass}$)

This test has been done to find the effect of change of initial biomass value on the reaction rate. Initial biomass value varies from 0.00001 to 0.001 mol.

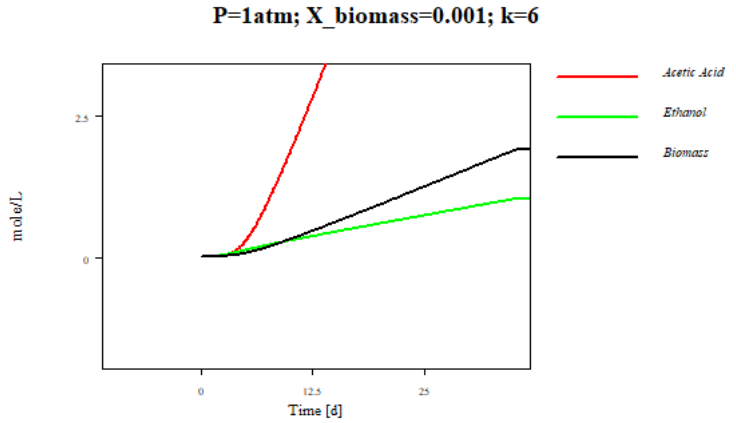

Figure 4.9. Test 1 ($X_{biomass}$=0.001)

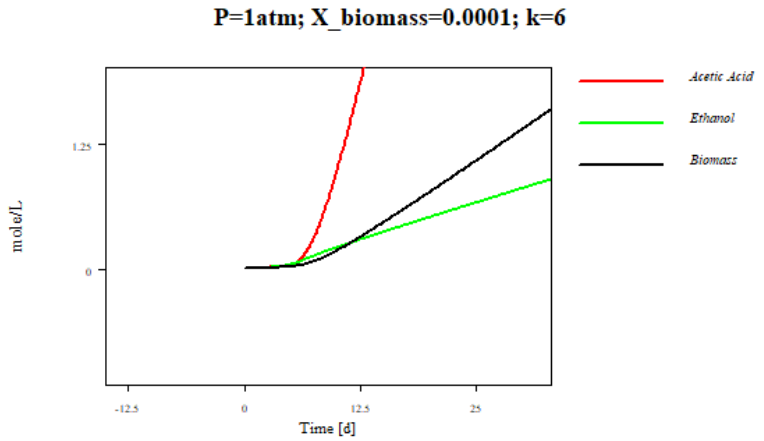

Figure 4.10. Test 1 ($X_{biomass}$=0.0001)

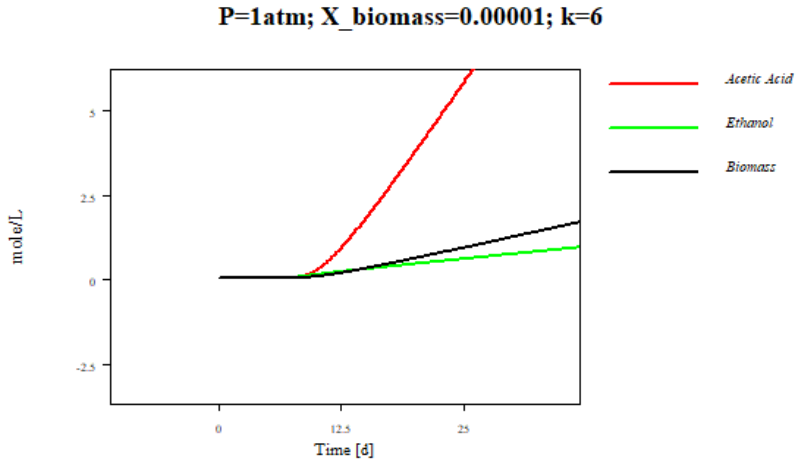

Figure 4.11. Test 1 ($X_{biomass}$=0.00001)

4.4.2 Test 2 (P_{atm})

According to literature review, the solubility of gases can be increased or decreased by change of external pressure. Representation of this effect is shown below.

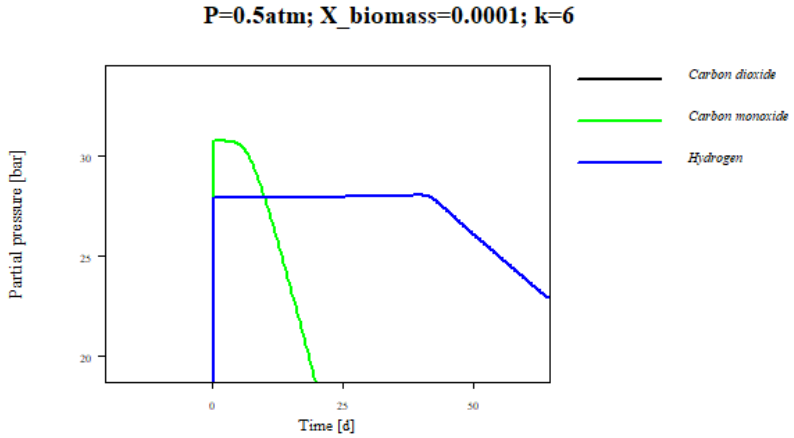

Figure 4.12. Test 2 (P_{atm}=0.5atm)

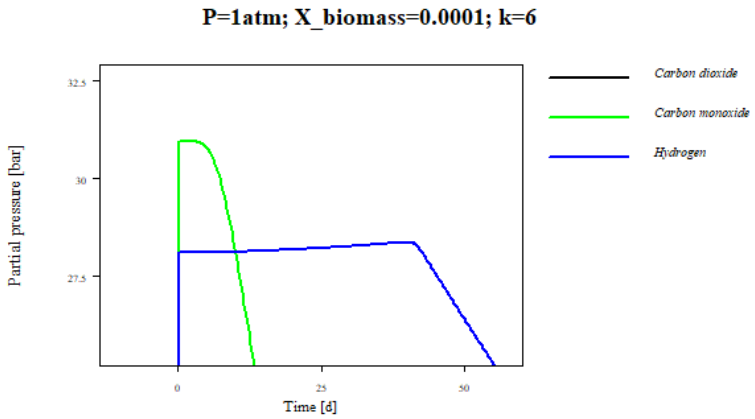

Figure 4.13. Test 2 (P_{atm}=1atm)

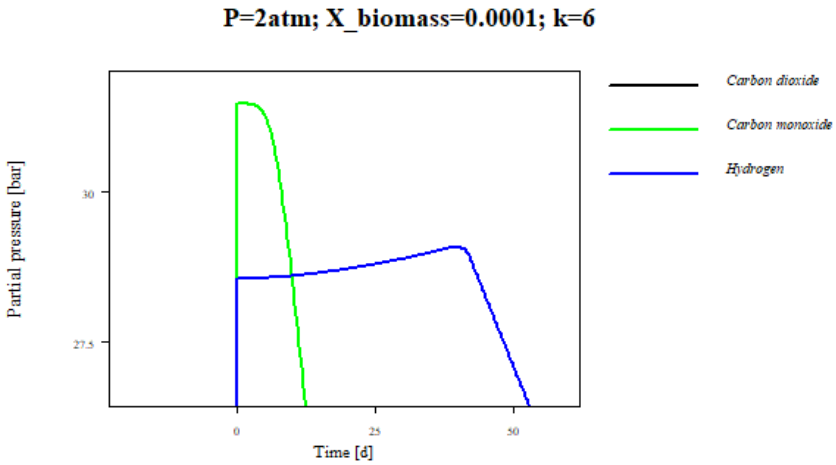

Figure 4.14. Test 2 (P_{atm}=2atm)

4.4.3 Test 3 (k)

Based on (Tchobanoglous et al., 2014) maximum specific substrate utilization rate can be in range from 2 to 10. Thus, Thus, it can influence on the reaction rate.

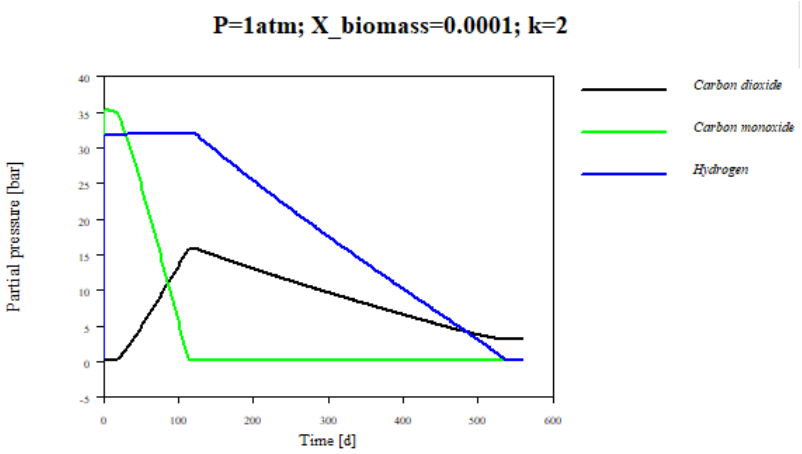

Figure 4.15. Test 3 (k=2)

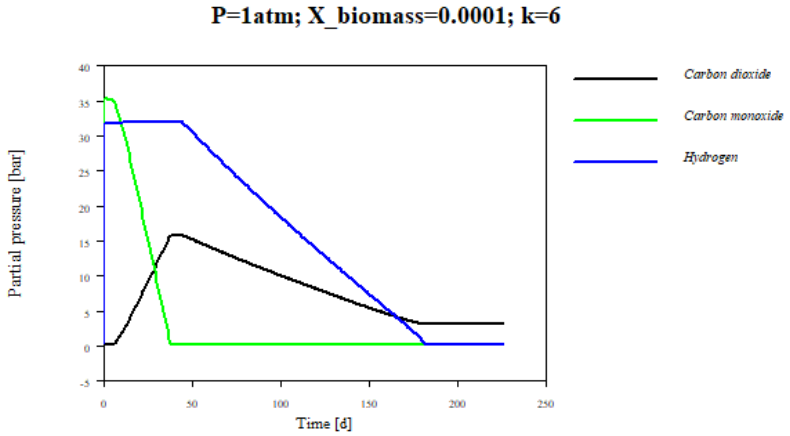

Figure 4.16. Test 3 (k=6)

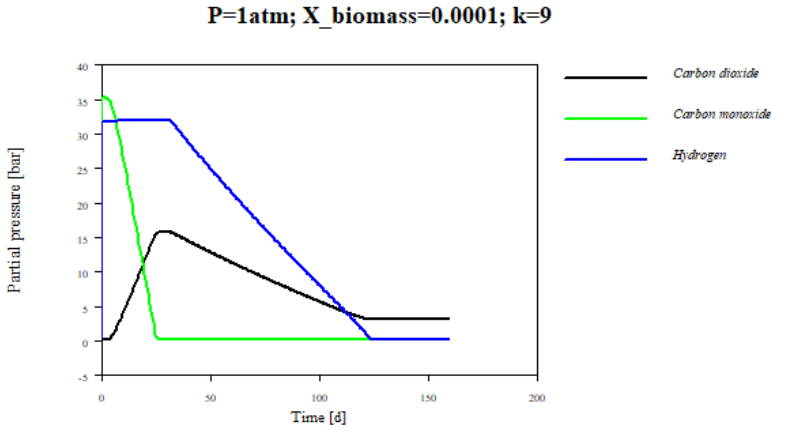

Figure 4.17. Test 3 (k=9)

Discussions

Under the literary review, the metabolic Wood-Ljungdahl pathway was studied. It gave a better understanding of the reactions, that are form the syngas fermentation process with production of acetic acid and ethanol.

According to the calculations of the Gibbs free energy, were determined five potential products (ethanol, acetic acid, methanol, lactate and methane) and transformation process from acetic acid to ethanol. All of them are exothermic. From the energy point of view, the methane production (R5_CO and R5_H2) are more probable than ethanol production (R1_CO and R1_H2).

In Rittmann and McCarthy, (2001), the energy capture efficiency is assumed to be 60% and its effect on the reaction is poorly described. But based on the biomass yield growth diagram for different products (Figure 3.2), can be seen that a change of the efficiency value from 10% to 60% gave an increase in the yield (R3_H2) by 3.5 times, and for yield (R3_H2) by 33.4 times under identical initial conditions.

The calculated biomass yield indicates that most of the energy is utilized for bacterial catabolism (f_e), rather than bacteria growth (f_s) (Appendix A Calculations).

The simulation was performed in the software Aquasim. The simulation only concerns the biological process of reaction R2_CO, R2_H2 and (3.13) due to lack of time

According to the results obtained, if each sub-model (Model 1or 2 or 3) passed independently from each other, such reactions could take up to 16 years with the same initial conditions.

Carbon monoxide was consumed within 40 days and after that began the consumption of carbon dioxide and hydrogen. It can be explained by Wood-Ljungdahl pathway, where to build the carbonyl branch can be used only carbon monoxide. Also, in accordance with that fact might be related that the total amount of ethanol obtained is several times less than that of acetic acid.

The initial amount of biomass in the simulation was an assumption, but was proved that this value affect the duration of the lag phase (Test 1). A higher amount of biomass in the substrate can accelerate the onset of the reaction. Unfortunately, the literature review does not contain references or studies of experiments that correlate with this result.

Compared to the experiment from the literature and Test 2, could be seen that influence of external pressure in the simulation is correlated with experiment. In the Figure 4.12, Figure 4.13 and Figure 4.14 can be seen that partial pressure of hydrogen was higher with external pressure P=2atm. At the same, with the P=0,5atm, the partial pressure was lower than at P=1atm.

According to the (Tchobanoglous et al., 2014), the typical value of maximum specific substrate utilization rate is 5. Unfortunately, confirmation and description of this assumption was not found. That is why the different k value was simulated. With the k=2, simulation time was ~530 days, with k=6 it takes ~175 days and with k=9, ~125 days.

Conclusion

Syngas fermentation process with biofuel production is a feasible process and is possible to simulate.

Simulation show that complete reagents consumption occurred after 175 days, in applied initial conditions.

Improving accuracy of modeling biological process can be achieved by selection of energy capture efficiency for each stoichiometric reaction separately.

Escalation of external pressure promotes an increase in the partial pressure of CO, CO_2, H_2 in the substrate, so production rate of acetic acid and ethanol also increases.

Production of acetic acid with carbon monoxide as electron donor is faster than with hydrogen as electron donor.

Due to the fact that the production of ethanol is several times less than acetic acid, during simulation R2_CO, R2_H2 and (3.13), adding the reactions R1_CO and R1_H2 is important to get process closer to real biological process.

Continuance of lag phase depends on initial biomass value.

Disadvantage of the created model is inability to specify physical parameters of reactor (height, width, depth). This leads to the impossibility of calculating kLa, thereby reducing the accuracy of calculations of the mass transfer between phases.

Experimental data is needed to confirm the correctness of this model and to calibrate it.

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Appendix A Calculations

Ethanol_CO		$\Delta G°\ [\frac{\text{kJ}}{e^-\ eq}]$
Reaction No. 1	$\frac{1}{6}CO_2 + H^+ + e^- \rightarrow \frac{1}{12}CH_3CH_2OH + \frac{1}{4}H_2O$	31.18
Reaction No. 7	$\frac{1}{2}CO + \frac{1}{2}H_2O \rightarrow \frac{1}{2}CO_2 + H^+ + e^-$	-49.886
Overall	$6CO + 3H_2O \rightarrow CH_3CH_2OH + 4CO_2$	-18.706

$$\Delta G_s = \frac{\Delta G_p}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon} = \frac{84{,}976}{0{,}6^1} + \frac{14{,}15}{0{,}6} = 165.21\ [\frac{\text{kJ}}{e^-\ mol}]$$

$$\frac{f_e}{f_s} = \frac{-\Delta G_s}{\varepsilon(\Delta G_R)} = \frac{-165.21}{0.6\times(-18.706)} = 14.7199$$

$$f_e + f_s = 1{,}0$$

$$14.7199 f_s + f_s = 1{,}0 \rightarrow f_s = 0{,}0636\ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$f_e = 1 - f_s = 0{,}9364\ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$For\ biomass\ (CH_{1,8}O_{0,5}N_{0,2}),\ 1\ g\ cells = 1{,}36\ g\ COD$$

$$Y_{S^0} = \frac{0{,}0636}{1{,}36} = 0{,}0468\ \text{, g VVS/g COD}$$

$$R = f_e R_a + f_s R_{CS} - R_d$$

$$0.156CO_2 + 0.9364H^+ + 0.9364e^- \rightarrow 0.078CH_3CH_2OH + 0.234H_2O$$

$$0.015CO_2 + 0.003NH_4^+ + 0.061H^+ + 0.0636e^- \rightarrow 0.015CH_{1,8}O_{0,5}N_{0,2} + 0.023H_2O$$

$$\frac{1}{2}CO + \frac{1}{2}H_2O \rightarrow \frac{1}{2}CO_2 + H^+ + e^-$$

$$R = 33.3CO + 0.2NH_4^+ + 16.2H_2O \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 5.2CH_3CH_2OH + 21.93CO_2 + 0.173H^+$$

Ethanol_H2		$\Delta G°\ [\frac{\text{kJ}}{e^-\ eq}]$
Reaction No. 1	$\frac{1}{6}CO_2 + H^+ + e^- \rightarrow \frac{1}{12}CH_3CH_2OH + \frac{1}{4}H_2O$	31.18
Reaction No. 8	$0.5H_2 \rightarrow H^+ + e^-$	-39.87

Overall	$2CO_2 + 6H_2 \rightarrow CH_3CH_2OH + 3H_2O$	-8.69

$$\Delta G_s = \frac{\Delta G_p}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon} = \frac{74.96}{0{,}6^1} + \frac{12.48}{0{,}6} = 145.73\ [\frac{\text{kJ}}{e^-\ eq}]$$

$$\frac{f_e}{f_s} = \frac{-\Delta G_s}{\varepsilon(\Delta G_R)} = \frac{-145.73}{0.6\times(-8.69)} = 27.9497$$

$$f_e + f_s = 1{,}0$$

$$27.9497 f_s + f_s = 1{,}0 \rightarrow f_s = 0{,}0345\ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$f_e = 1 - f_s = 0{,}9655\ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$For\ biomass\ (CH_{1,8}O_{0,5}N_{0,2}), 1\ g\ cells = 1{,}36\ g\ COD$$

$$Y_{S^0} = \frac{0{,}0345}{1{,}36} = 0{,}0254\ \text{, g VVS/g COD}$$

$$0.161CO_2 + 0.9655H^+ + 0.9655e^- \rightarrow 0.0804CH_3CH_2OH + 0.2414$$

$$0.0083CO_2 + 0.0017NH_4^+ + 0.0327H^+ + 0.0345e^- \rightarrow 0.0083CH_{1,8}O_{0,5}N_{0,2} + 0.0124H_2O$$

$$0.5H_2 \rightarrow H^+ + e^-$$

$$R = 20.4CO_2 + 60.24H_2 + 0.2NH_4^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 9.68CH_3CH_2OH + 0.22H^+ + 30.55H_2O$$

Acetic acid_CO		$\Delta G°\ [\frac{\text{kJ}}{e^-\ eq}]$
Reaction No. 2	$\frac{1}{4}CO_2 + H^+ + e^- \rightarrow \frac{1}{8}CH_3COOH + \frac{1}{4}H_2O$	30.1655
Reaction No. 7	$\frac{1}{2}CO + \frac{1}{2}H_2O \rightarrow \frac{1}{2}CO_2 + H^+ + e^-$	-49.886
Overall	$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2$	-19.4205

$$\frac{f_e}{f_s} = \frac{-\Delta G_s}{\varepsilon(\Delta G_R)} = \frac{-165.21}{0.6\times(-19.4205)} = 14.1783$$

$$f_e + f_s = 1{,}0$$

$$14.1783 f_s + f_s = 1{,}0 \rightarrow f_s = 0{,}0659\ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$f_e = 1 - f_s = 0{,}9341\ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$For\ biomass\ (CH_{1,8}O_{0,5}N_{0,2}), 1\ g\ cells = 1{,}36\ g\ COD$$

$$Y_{S^0} = \frac{0{,}0659}{1{,}36} = 0{,}0484 \text{ , g VVS/g COD}$$

$$0.2335CO_2 + 0.9341H^+ + 0.9341e^- \rightarrow 0.1167CH_3COOH + 0.2335H_2O$$

$$0.0158CO_2 + 0.0033NH_4^+ + 0.0626H^+ + 0.0659e^- \rightarrow 0.0158CH_{1,8}O_{0,5}N_{0,2} + 0.0237H_2O$$

$$\frac{1}{2}CO + \frac{1}{2}H_2O \rightarrow \frac{1}{2}CO_2 + H^+ + e^-$$

$$R = 31.64CO + 0.21NH_4^+ + 15.24H_2O \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 7.33CH_3COOH + 2.09H^+ + 15.87CO_2$$

Acetic acid_H2		$\Delta G° \left[\frac{\text{kJ}}{e^- \, eq}\right]$
Reaction No. 2	$\frac{1}{4}CO_2 + H^+ + e^- \rightarrow \frac{1}{8}CH_3COOH + \frac{1}{4}H_2O$	30.1655
Reaction No. 8	$0.5H_2 \rightarrow H^+ + e^-$	-39.87
Overall	$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$	-9.7045

$$\frac{f_e}{f_s} = \frac{-\Delta G_s}{\varepsilon(\Delta G_R)} = \frac{-145.73}{0.6\times(-9.7045)} = 25.0279$$

$$f_e + f_s = 1{,}0$$

$$25.0279f_s + f_s = 1{,}0 \rightarrow f_s = 0{,}0384 \ \frac{g \ cell \ COD}{g \ COD \ used}$$

$$f_e = 1 - f_s = 0{,}9616 \ \frac{g \ cell \ COD}{g \ COD \ used}$$

$$For \ biomass \left(CH_{1,8}O_{0,5}N_{0,2}\right), 1 \ g \ cells = 1{,}36 \ g \ COD$$

$$Y_{S^0} = \frac{0{,}0384}{1{,}36} = 0{,}0282 \text{ , g VVS/g COD}$$

$$0.2404CO_2 + 0.9616H^+ + 0.9616e^- \rightarrow 0.1202CH_3COOH + 0.2404H_2O$$

$$0.0092CO_2 + 0.0019NH_4^+ + 0.0365H^+ + 0.0384e^- \rightarrow 0.0092CH_{1,8}O_{0,5}N_{0,2} + 0.0138H_2O$$

$$0.5H_2 \rightarrow H^+ + e^-$$

$$R = 27.13CO_2 + 54.35H_2 + 0.21NH_4^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 13.06CH_3COOH + 27.63H_2O + 0.21H^+$$

Methanol_CO		$\Delta G° \left[\frac{\text{kJ}}{e^- \, eq}\right]$
Reaction No. 4	$\frac{1}{6}CO_2 + H^+ + e^- \rightarrow \frac{1}{6}CH_3OH + \frac{1}{6}H_2O$	36.84

Reaction No. 7	$\frac{1}{2}CO + \frac{1}{2}H_2O \rightarrow \frac{1}{2}CO_2 + H^+ + e^-$	-49.886
Overall	$3CO + 2H_2O \rightarrow CH_3OH + 2CO_2$	-15.046

$$\frac{f_e}{f_s} = \frac{-\Delta G_s}{\varepsilon(\Delta G_R)} = \frac{-165.21}{0.6\times(-15.045)} = 18.3018$$

$$f_e + f_s = 1{,}0$$

$$18.3018 f_s + f_s = 1{,}0 \rightarrow f_s = 0{,}0518 \ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$f_e = 1 - f_s = 0{,}9482 \ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$For\ biomass\ (CH_{1,8}O_{0,5}N_{0,2}),\ 1\ g\ cells = 1{,}36\ g\ COD$$

$$Y_{S^0} = \frac{0{,}0518}{1{,}36} = 0{,}0398 \text{ , g VVS/g COD}$$

$$0.158CO_2 + 0.9482H^+ + 0.9482e^- \rightarrow 0.158CH_3OH + 0.158H_2O$$

$$0.0124CO_2 + 0.0026NH_4^+ + 0.0492H^+ + e^- \rightarrow 0.0124CH_{1,8}O_{0,5}N_{0,2} + 0.0186H_2O$$

$$\frac{1}{2}CO + \frac{1}{2}H_2O \rightarrow \frac{1}{2}CO_2 + H^+ + e^-$$

$$R = 40.32CO + 26.08H_2O + 0.21NH_4^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 12.74CH_3OH + 26.58CO_2 + 0.21H^+$$

<u>Methanol_H2</u>		$\Delta G°\ [\frac{\text{kJ}}{e^-\ eq}]$
Reaction No. 4	$\frac{1}{6}CO_2 + H^+ + e^- \rightarrow \frac{1}{6}CH_3OH + \frac{1}{6}H_2O$	36.84
Reaction No. 8	$0.5H_2 \rightarrow H^+ + e^-$	-39.87
Overall	$CO_2 + 3H_2 \rightarrow CH_3OH + H_2O$	-3.03

$$\frac{f_e}{f_s} = \frac{-\Delta G_s}{\varepsilon(\Delta G_R)} = \frac{-145.73}{0.6\times(-3.03)} = 80.1595$$

$$f_e + f_s = 1{,}0$$

$$80.1595 f_s + f_s = 1{,}0 \rightarrow f_s = 0{,}0123 \ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$f_e = 1 - f_s = 0.9877 \ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$For\ biomass\ (CH_{1,8}O_{0,5}N_{0,2}),\ 1\ g\ cells = 1{,}36\ g\ COD$$

$$Y_{S^0} = \frac{0{,}0123}{1{,}36} = 0{,}0091 \text{ , g VVS/g COD}$$

$$0.1646CO_2 + 0.9877H^+ + 0.9877e^- \rightarrow 0.1646CH_3OH + 0.1646H_2O$$

$$0.0029CO_2 + 0.0006NH_4^+ + 0.0117H^+ + 0.0123e^- \rightarrow 0.0029CH_{1,8}O_{0,5}N_{0,2} + 0.0044H_2O$$

$$0.5H_2 \rightarrow H^+ + e^-$$

$$R = 57.75CO_2 + 172.41H_2 + 0.21NH_4^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 56.76CH_3OH + 58.27H_2O + 0.21H^+$$

Lactate_CO		$\Delta G°\ [\frac{\text{kJ}}{e^-\ eq}]$
Reaction No. 5	$\frac{1}{4}CO_2 + \frac{1}{12}HCO_3^- + H^+ + e^- \rightarrow \frac{1}{12}CH_3CHOHCOO^- + \frac{1}{3}H_2O$	32.29
Reaction No. 7	$\frac{1}{2}CO + \frac{1}{2}H_2O \rightarrow \frac{1}{2}CO_2 + H^+ + e^-$	-49.886
Overall	$6CO + HCO_3^- + 2H_2O \rightarrow CH_3CHOHCOO^- + 3CO_2$	-17.596

$$\frac{f_e}{f_s} = \frac{-\Delta G_s}{\varepsilon(\Delta G_R)} = \frac{-165.21}{0.6\times(-17.596)} = 15.6484$$

$$f_e + f_s = 1{,}0$$

$$15.6484f_s + f_s = 1{,}0 \rightarrow f_s = 0{,}06\ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$f_e = 1 - f_s = 0{,}94\ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$For\ biomass\ (CH_{1,8}O_{0,5}N_{0,2}),\ 1\ g\ cells = 1{,}36\ g\ COD$$

$$Y_{S^0} = \frac{0{,}06}{1{,}36} = 0{,}0441 \text{ , g VVS/g COD}$$

$$0.235CO_2 + 0.078HCO_3^- + 0.94H^+ + 0.94e^- \rightarrow 0.078CH_3CHOHCOO^- + 0.313H_2O$$

$$0.014CO_2 + 0.003NH_4^+ + 0.057H^+ + 0.06e^- \rightarrow 0.014CH_{1,8}O_{0,5}N_{0,2} + 0.0216H_2O$$

$$\frac{1}{2}CO + \frac{1}{2}H_2O \rightarrow \frac{1}{2}CO_2 + H^+ + e^-$$

$$R = 35.71CO + 11.81H_2O + 0.21NH_4^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 5.43CH_3CHOHCOO^- + 17.93CO_2 + 0.21H^+$$

Lactate_H2		$\Delta G°\ [\frac{\text{kJ}}{e^- eq}]$
Reaction No. 5	$\frac{1}{4}CO_2 + \frac{1}{12}HCO_3^- + H^+ + e^- \rightarrow \frac{1}{12}CH_3CHOHCOO^- + \frac{1}{3}H_2O$	32.29
Reaction No. 8	$0.5H_2 \rightarrow H^+ + e^-$	-39.87
Overall	$3CO_2 + HCO_3^- + 6H_2 \rightarrow CH_3CHOHCOO^- + 4H_2O$	-7.58

$$\frac{f_e}{f_s} = \frac{-\Delta G_s}{\varepsilon(\Delta G_R)} = \frac{-145.73}{0.6\times(-7.58)} = 36.3258$$

$$f_e + f_s = 1{,}0$$

$$36.3258 f_s + f_s = 1{,}0 \rightarrow f_s = 0{,}0268\ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$f_e = 1 - f_s = 0{,}9732\ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$For\ biomass\ (CH_{1,8}O_{0,5}N_{0,2}),\ 1\ g\ cells = 1{,}36\ g\ COD$$

$$Y_{S^0} = \frac{0{,}0268}{1{,}36} = 0{,}0197\ \text{, g VVS/g COD}$$

$$0.2433CO_2 + 0.0811HCO_3^- + 0.9732H^+ + 0.9732e^- \rightarrow 0.0811CH_3CHOHCOO^- + 0.3244H_2O$$

$$0.0064CO_2 + 0.0013NH_4^+ + 0.0254H^+ + 0.0268e^- \rightarrow 0.0064CH_{1,8}O_{0,5}N_{0,2} + 0.0096H_2O$$

$$0.5H_2 \rightarrow H^+ + e^-$$

$$R = 48.02CO_2 + 78.12H_2 + 0.2NH_4^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 12.67CH_3CHOHCOO^- + 55.19H_2O + 0.22H^+$$

Methane_CO		$\Delta G°\ [\frac{\text{kJ}}{e^- eq}]$
Reaction No. 6	$\frac{1}{8}CO_2 + H^+ + e^- \rightarrow \frac{1}{8}CH_4 + \frac{1}{4}H_2O$	23.53
Reaction No. 7	$\frac{1}{2}CO + \frac{1}{2}H_2O \rightarrow \frac{1}{2}CO_2 + H^+ + e^-$	-49.886
Overall	$4CO + 2H_2O \rightarrow CH_4 + 3CO_2$	-26.356

$$\frac{f_e}{f_s} = \frac{-\Delta G_s}{\varepsilon(\Delta G_R)} = \frac{-165.21}{0.6\times(-26.356)} = 10.4473$$

$$f_e + f_s = 1{,}0$$

$$10.4473 f_s + f_s = 1{,}0 \rightarrow f_s = 0{,}0873 \ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$f_e = 1 - f_s = 0{,}9127 \ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$For\ biomass\ (CH_{1,8}O_{0,5}N_{0,2}),\ 1\ g\ cells = 1{,}36\ g\ COD$$

$$Y_{S^0} = \frac{0{,}0659}{1{,}36} = 0{,}0642 \text{ , g VVS/g COD}$$

$$0.114CO_2 + 0.9127H^+ + 0.9127e^- \rightarrow 0.114CH_4 + 0.2282H_2O$$

$$0.021CO_2 + 0.0043NH_4^+ + 0.0829H^+ + 0.0873e^- \rightarrow 0.021CH_{1,8}O_{0,5}N_{0,2} + 0.031H_2O$$

$$\frac{1}{2}CO + \frac{1}{2}H_2O \rightarrow \frac{1}{2}CO_2 + H^+ + e^-$$

$$R = 23.81CO + 11.47H_2O + 0.2NH_4^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 5.43CH_4 + 17.38CO_2 + 0.21H^+$$

Methane_H2		$\Delta G°\ [\frac{\text{kJ}}{e^-\ eq}]$
Reaction No. 6	$\frac{1}{8}CO_2 + H^+ + e^- \rightarrow \frac{1}{8}CH_4 + \frac{1}{4}H_2O$	23.53
Reaction No. 8	$0.5H_2 \rightarrow H^+ + e^-$	-39.87
Overall	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	-16.34

$$\frac{f_e}{f_s} = \frac{-\Delta G_s}{\varepsilon(\Delta G_R)} = \frac{-145.73}{0.6\times(-16.34)} = 14.8643$$

$$f_e + f_s = 1{,}0$$

$$14.8643 f_s + f_s = 1{,}0 \rightarrow f_s = 0{,}063 \ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$f_e = 1 - f_s = 0{,}937 \ \frac{g\ cell\ COD}{g\ COD\ used}$$

$$For\ biomass\ (CH_{1,8}O_{0,5}N_{0,2}),\ 1\ g\ cells = 1{,}36\ g\ COD$$

$$Y_{S^0} = \frac{0{,}0384}{1{,}36} = 0{,}0463 \text{ , g VVS/g COD}$$

$$0.117CO_2 + 0.937H^+ + 0.937e^- \rightarrow 0.117CH_4 + 0.234H_2O$$

$$0.015CO_2 + 0.0031NH_4^+ + 0.0598H^+ + 0.063e^- \rightarrow 0.015CH_{1,8}O_{0,5}N_{0,2} + 0.023H_2O$$

$$0.5H_2 \rightarrow H^+ + e^-$$

$$R = 16.6CO_2 + 33.3H_2 + 0.2NH_4^+ \rightarrow CH_{1,8}O_{0,5}N_{0,2} + 7.8CH_4 + 17.13H_2O + 0.21H^+$$

	M	COD	e mol	1/e mol	new coef
Biomass	24.600	1.366	4.200	0.238	
CO2	44.000	0.000	0.000		
H2	2.000	8.000	2.000	0.500	
Ethanol	46.000	2.087	12.000	0.083	3.903
Acetic Acid	60.052	1.070	8.032	0.125	2.612
Methanol	32.000	1.500	6.000	0.167	1.951
Lactate	90.000	1.067	12.000	0.083	3.903
Methane	16.000	4.000	8.000	0.125	2.602

	ε=0.1					
	ΔGp	A	fs	fa	Y	Y_new
Ethanol_CO	991.260	529.916	0.002	0.998	0.001	0.005
Ethanol_H2	874.400	1006.214	0.001	0.999	0.001	0.003
Acetic Acid_CO	991.260	510.419	0.002	0.998	0.001	0.004
Acetic Acid_H2	874.400	901.025	0.001	0.999	0.001	0.002
Methanol_CO	991.260	658.820	0.002	0.998	0.001	0.002
Methanol_H2	874.400	2885.809	0.000	1.000	0.000	0.000
Lactate_CO	991.260	563.344	0.002	0.998	0.001	0.005
Lactate_H2	874.400	1153.562	0.001	0.999	0.001	0.002
Methane_CO	991.260	376.104	0.003	0.997	0.002	0.005
Methane_H2	874.400	535.129	0.002	0.998	0.001	0.004

	ε=0.2					
	ΔGp	A	fs	fa	Y	Y_new
Ethanol_CO	495.630	132.479	0.007	0.993	0.005	0.021
Ethanol_H2	437.200	251.554	0.004	0.996	0.003	0.011
Acetic Acid_CO	495.630	127.605	0.008	0.992	0.006	0.015
Acetic Acid_H2	437.200	225.256	0.004	0.996	0.003	0.008
Methanol_CO	495.630	164.705	0.006	0.994	0.004	0.009
Methanol_H2	437.200	721.452	0.001	0.999	0.001	0.002
Lactate_CO	495.630	140.836	0.007	0.993	0.005	0.020
Lactate_H2	437.200	288.391	0.003	0.997	0.003	0.010
Methane_CO	495.630	94.026	0.011	0.989	0.008	0.020

Methane_H2	437.200	133.782	0.007	0.993	0.005	0.014

	ε=0.3					
	ΔGp	A	fs	fa	Y	Y_new
Ethanol_CO	283.710	50.556	0.019	0.981	0.014	0.055
Ethanol_H2	291.467	111.802	0.009	0.991	0.006	0.025
Acetic Acid_CO	283.710	48.696	0.020	0.980	0.015	0.038
Acetic Acid_H2	291.467	100.114	0.010	0.990	0.007	0.019
Methanol_CO	283.710	62.854	0.016	0.984	0.011	0.022
Methanol_H2	291.467	320.645	0.003	0.997	0.002	0.004
Lactate_CO	283.710	53.745	0.018	0.982	0.013	0.052
Lactate_H2	291.467	128.174	0.008	0.992	0.006	0.022
Methane_CO	283.710	35.882	0.027	0.973	0.020	0.052
Methane_H2	291.467	59.459	0.017	0.983	0.012	0.032

	ε=0.4					
	ΔGp	A	fs	fa	Y	Y_new
Ethanol_CO	247.815	33.120	0.029	0.971	0.021	0.084
Ethanol_H2	218.600	62.888	0.016	0.984	0.011	0.045
Acetic Acid_CO	247.815	31.901	0.030	0.970	0.022	0.058
Acetic Acid_H2	218.600	56.314	0.017	0.983	0.013	0.033
Methanol_CO	247.815	41.176	0.024	0.976	0.017	0.034
Methanol_H2	218.600	180.363	0.006	0.994	0.004	0.008
Lactate_CO	247.815	35.209	0.028	0.972	0.020	0.079
Lactate_H2	218.600	72.098	0.014	0.986	0.010	0.039
Methane_CO	247.815	23.507	0.041	0.959	0.030	0.078
Methane_H2	218.600	33.446	0.029	0.971	0.021	0.055

	ε=0.5					
	ΔGp	A	fs	fa	Y	Y_new
Ethanol_CO	170.229	18.201	0.052	0.948	0.038	0.149
Ethanol_H2	174.880	40.249	0.024	0.976	0.018	0.069
Acetic Acid_CO	170.229	17.531	0.054	0.946	0.040	0.103

Acetic Acid_H2	174.880	36.041	0.027	0.973	0.020	0.052
Methanol_CO	170.229	22.628	0.042	0.958	0.031	0.060
Methanol_H2	174.880	115.432	0.009	0.991	0.006	0.012
Lactate_CO	170.229	19.349	0.049	0.951	0.036	0.140
Lactate_H2	174.880	46.142	0.021	0.979	0.016	0.061
Methane_CO	170.229	12.918	0.072	0.928	0.053	0.137
Methane_H2	174.880	21.405	0.045	0.955	0.033	0.085

	ε=0.6					
	ΔGp	A	fs	fa	Y	Y_new
Ethanol_CO	165.210	14.720	0.064	0.936	0.047	0.182
Ethanol_H2	145.733	27.950	0.035	0.965	0.025	0.099
Acetic Acid_CO	165.210	14.178	0.066	0.934	0.048	0.126
Acetic Acid_H2	145.733	25.028	0.038	0.962	0.028	0.073
Methanol_CO	165.210	18.301	0.052	0.948	0.038	0.074
Methanol_H2	145.733	80.161	0.012	0.988	0.009	0.018
Lactate_CO	165.210	15.648	0.060	0.940	0.044	0.172
Lactate_H2	145.733	32.043	0.030	0.970	0.022	0.086
Methane_CO	165.210	10.447	0.087	0.913	0.064	0.166
Methane_H2	145.733	14.865	0.063	0.937	0.046	0.120

Appendix B Aquasim Parameters

Parameters	Description	Units	Value
$\Delta H^0_{K_a,CO_2}$	enthalpy of reaction CO_2 -> HCO_3	J/mole	7646
$\Delta H^0_{K_a,NH_3}$	enthalpy of reaction NH_4 -> NH_3	J/mole	51965
$\Delta H^0_{K_w,CO_2}$	enthalpy of reaction of CO_2 (g) -> CO_2 (aq)	J/mole	-19410
$\Delta H^0_{K_w,CO}$	enthalpy of reaction of CO (g) -> CO (aq)	J/mole	-11000
$\Delta H^0_{K_w,H_2}$	enthalpy of reaction of H_2 (g) -> H_2 (aq)	J/mole	-4180
$\Delta H^0_{K_w,H_2O}$	enthalpy of reaction of H_2O (g) -> H_2O (liq)	J/mole	-44000
$\Delta H^0_{K_w,NH_3}$	enthalpy of reaction of NH_4 (g) -> NH_4 (aq)	J/mole	-87320
k	Max specific substrate utilization rate		6
K_{a,CO_2}	CO_2 acidity constant with temperature correction	mole	-
K_{a,NH_3}	NH_3 acidity constant with temperature correction	mole	-
K_{H,CO_2}	Henry's law coefficient for CO_2 with temperature correction	M(liq)*M(gas)$^{-1}$	-
$K_{H,CO}$	Henry's law coefficient for CO with temperature correction	M(liq)*M(gas)$^{-1}$	-
K_{H,H_2}	Henry's law coefficient for H_2 with temperature correction	M(liq)*M(gas)$^{-1}$	-
K_{H,NH_3}	Henry's law coefficient for NH_3 with temperature correction	M(liq)*M(gas)$^{-1}$	-
$K_{I,UAc}$	Inhibition constant of undissociated acetic acid	M. L^{-1}	0.0062
$K_L a_{CO_2}$	Gas-liquid transfer coefficient of CO_2		200
$K_L a_{CO}$	Gas-liquid transfer coefficient of CO		200

$K_L a_{H_2}$	Gas-liquid transfer coefficient of H_2		2000
K_{H_2}	Saturation constant value for H_2	M. L^{-1}	0.00022
K_{CO_2}	Saturation constant value for CO_2	M. L^{-1}	0.00022
K_{CO}	Saturation constant value for CO	M. L^{-1}	0.00069
K_{UAc}	Saturation constant value for UAc	M. L^{-1}	0.0005
$\mu_{max}1$	Maximum specific growth rate	d^{-1}	-
$\mu_{max}2$	Maximum specific growth rate	d^{-1}	-
$\mu_{max}3$	Maximum specific growth rate	d^{-1}	-
pH	pH		6.7
pK_{a,CO_2}	$-\log_{10}[K_{a,CO_2}]$ at 298K		6.35
$pK_{a,Ac}$	$-\log_{10}[K_{a,Ac}]$ at 298K		4.75
pK_{a,NH_3}	$-\log_{10}[K_{a,NH_3}]$ at 298K		9.25
P_{atm}	Atmospheric pressure	bar	1.01325
p_{CO_2}	Partial pressure of CO_2	bar	-
p_{H_2O}	Partial pressure of H_2O	bar	-
p_{H_2}	Partial pressure of H_2	bar	-
p_{CO}	Partial pressure of CO	bar	-
p_{NH_3}	Partial pressure of NH_3	bar	-
$P_{headspace}$	Total gas phase pressure	bar	-
R	Gas Law Constant	L. bar. M^{-1}. K^{-1}	0.083
$X_{Biomass}$	Concentration of biomass	mole	0.0001
S_{CO_2}	Concentration of carbon dioxide	mole	-
$S_{HCO_3^-}$	Concentration of bicarbonate	mole	-
S_{IC}	Total concentration of carbon source	mole	-

S_{H_2O}	Concentration of water	mole	-
S_{H^+}	Concentration of hydrogen ion	mole	-
S_{NH_3}	Concentration of free ammonia	mole	-
$S_{NH_4^+}$	Concentration of ammonium cation	mole	-
S_{IN}	Total concentration of nitrogen source	mole	-
S_{Ac}	Concentration of acetic acid	mole	-
S_{UAc}	Concentration of undissociated acetic acid	mole	-
S_{Et}	Concentration of ethanol	mole	-
S_{H_2}	Concentration of hydrogen	mole	-
S_{CO}	Concentration of carbon monoxide	mole	-
T	Temperature	K	310.15
V	Volume of reactor	L	1
Y_1	Biomass yield (CO as electron donor)		0.126
Y_2	Biomass yield (H_2 as electron donor)		0.073
Y_3	Biomass yield (Transformation Ac to Et)		0.094

Appendix C Master thesis description

University College of Southeast Norway

Faculty of Technology, Natural Sciences and Maritime Sciences, Campus Porsgrunn

FMH606 Master's Thesis

Title: Modelling and simulation of syngas fermentation for the production of biofuels precursors

HSN supervisor: Carlos Dinamarca, Rune Bakke

Task background:
Acetogenic bacteria use the Wood–Ljungdahl pathway to oxidize H_2 and reduce CO/CO_2 producing an array of organic compounds as volatile fatty acids and alcohols. Such bacteria can be used as microbial biocatalysts for the production of biofuels precursors using synthetic gas as feed. Modelling such bioprocesses is a core activity to connect two apparently dissimilar research fields, biomass gasification and biogas. Products formation rates as limitations given by mass transfer phenomena are key issues in reactor design.

Task description:
The student needs to search in the literature for the relevant microbial catabolic pathways for H_2, CO_2 and CO consumption and biofuels (or biofuels precursors) formation. Build stoichiometrical relationships between reactant and products, search in the literature for the main kinetics parameters and modelling based on tree main overall process: 1) Bacterial growth, 2) Equilibrium phenomena and, 3) Transport process.

Student category: Preferably EET students

Practical arrangements: A parallel project consisting in the operation of a lab-scale reactor will be also perform. The student working in modelling is expected to get practical experience by involvement in the experimental work.

Signatures:

Student (date and signature): 01.02.2017

Supervisor (date and signature): 01.02.2017

01.02.2017

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