POLITECNICO DI TORINO

Corso di Laurea Magistrale in
Ingegneria per l'Ambiente e il Territorio

Tesi di Laurea Magistrale
"Biological Hydrogen Methanation (BHM):
experimental lab tests"

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A chi,
nonostante le avversità,
ha perserverato
e ci è riuscito.

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Abstract

Nowadays in Europe there is a growing attention on renewable energy sources (RES), especially solar and wind power. The increasing development of RES has put emphasis on the problem of surplus power production, due to the randomness of natural phenomena. At the same time, plenty of European Countries, such as Italy, are continuously encouraging the usage of biofuels, e.g., biomethane.

The aim of this thesis is to understand whether it is possible to produce biofuels in sustainable ways. Downstream of the Anaerobic Digestion, performed in Wastewater Treatment Plants, biogas is naturally produced by microorganisms. Therefore, this work focuses on the possibility to convert biogas or gas mixtures (carbon dioxide and hydrogen in ratio of 1:4) into biomethane by *Hydrogenotrophic* methanogens. These microorganisms use hydrogen and carbon dioxide for their metabolic activity, producing methane and water as reaction products. The process is known as Biological Hydrogen Methanation (BHM).

In literature there are many scientific papers that could help answering the former question. Indeed, the BHM has been studied for some years in the Northern Europe and researchers are constantly trying to find the best solution, in terms of productivity, gas quality, energy consumption and costs. As a result of the previous laboratory-scale and pilot-scale trials, discussed in the thesis, trickle bed reactors (TBR) and continuously stirred reactors (CSTR) seem to be the best configuration. This is evidenced by methane evolution rate (MER) and methane concentration in the final product. MER represents the net production of methane (CH_4) per unit volume of reactor, and it is measured as $L_{CH4}/(L_{Reactor}d)$, thus it is an indicator of the system efficiency. Both Peillex *et al*., 1988 and Voelklein *et al*., 2019 show great results performing a BHM with CSTR and working at mesophilic conditions; the former produces 289.8 $L_{CH4}/(L_{Reactor}d)$ with a CH_4 concentration of 97%; the latter reports a MER of 3.7 $L_{CH4}/(L_{Reactor}d)$ with 96% of methane. Burkhardt *et al*., 2015 shows that a TBR in thermophilic conditions can produce 1.5 $L_{CH4}/(L_{Reactor}d)$ with 98% of CH_4.

The research challenge is to determine the feasibility of BHM performed with a TBR. Therefore, a start-up trial is necessary to understand the behaviour of microorganisms and to estimate the MER. The start-up is performed in a CSTR working in Fed-Batch conditions. The two trials have the same reactants: primary sludge and H_2/CO_2 gas mixture. The former, coming from a local Wastewater Treatment Plant, is used as source of biomass; the latter, in ratio of 1:4 is the substrate. Mathematical models based on Monod's theory can be used to design the experimental phase; the model is useful to estimate the growth of microorganisms, which is related to the consumption of substrate inoculated in the reactor.

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Preface

How many times do media warn populations of the lack of fossil energy sources? How long do Nations compete for natural gases and petroleum supply? Other energy sources are available in nature: some of them are easily obtainable, e.g., solar and wind power, while others can be artificially produced, starting from waste product e.g., biofuels. Biological Hydrogen Methanation (BHM) is a clear example of a natural process, that can be enhanced in order to produce biomethane. Biomethane is a gas fuel originated from the metabolic activity of Hydrogenotrophic methanogenetic bacteria. These microorganisms use hydrogen (H_2) and carbon dioxide (CO_2) as a source for their metabolism and growth, producing methane (CH_4) during their respiration. BHM can be performed either downstream Anaerobic Digestion (AD) in wastewater treatment plants, or in a secondary plant. In both cases sludge can be used as biomass, i.e., as source of methanogenic microorganisms. The success of the process is due to the capacity of the bacteria to produce as much methane as possible, depending on the operational conditions they live in, e.g., temperature and pressure, pH, etc. Moreover, the CH_4 production is affected by the nutrient supply, which is mainly H_2 and CO_2, but it can also include other supplements, such as inorganic salts or organic compounds. The object of this thesis is to understand whether biomethane can be produced at laboratory scale, using primary sludge as biomass source and a gas mixture of CO_2 and H_2 (ratio 1:4) as substrate.

The issue of this work is very interesting from an engineering point of view: it comprehends biological, chemical, environmental, and practical considerations. BHM is an innovative process that could allow to produce a natural fuel, by using substrates that are already present as waste products of other industrial treatments. Thus, the so produced biomethane does not impoverish natural methane sources.

BHM has been deeply addressed in literature from a laboratory scale point of view. Thus, scientific papers have been compared in order to better understand the state of the art and the relationships between all the parameters that influence the final results. Indeed, the most important variables that have been taken into account are temperature, pressure, pH, stirring systems, recirculation, CH_4 concentration in the final product and Methane Evolution Rate (MER). MER is probably the most important parameter because it is a productivity index, i.e., it expresses the methane produced per unit volume of reactor. The research has been proceeded with some laboratory tests performed in the DIATI Biological Laboratory of the Politecnico di Torino. During the trials, CH_4 production has been monitored day by day, with different reactor configurations.

This work is structured in four chapters. The first chapter introduces the thesis subject: it starts with a definition of biogas, its role in Italian legislation and its treatments. Then, Power to Gas (PtG) technology is introduced as a sustainable way to produce a storable gas from electricity. Indeed, PtG is composed by two main processes: electrolysis and methanation; the first one uses the surplus of power from renewable energy sources to produce hydrogen; the second one is the methane production process. Methanation can be either chemical or biological and the second one is the main subject of the subsequent paragraphs and chapters. The second chapter describes the state of the art of biological methanation: it deeply resumes all the characteristic parameters that influence the process and the metabolic activity of methanogenic archaea. Then, a comparison between some laboratory scale projects is reported, to better understand which combination of parameters corresponds to higher MER and CH_4 concentrations. The third chapter relates to the experimental trial: it depicts the settlements of two different configuration for the same process.

Also, the first configuration has been studied in two different times, with some changings in volumes. The fourth and last chapter reports the conclusions of the research work, in which results are described and trial limits are explored, in order to overcome them with further studies and trials.

The experimental setup has given promising results, which can be compared to recent laboratory trials discussed in literature.

1 Introduction

1.1 Biogas

Biogas consists of a gas mixture, produced as a result of a biological mediated process, known as Anaerobic Digestion (DA). This consists of a complex biological process, during which organic matter is digested in a reactor, producing biogas; the process is composed of four phases, carried out by a complex microbial community: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The produced gas is considered one of the best media for the transport of renewable energy, as assessed by Fu *et al.* [1].

The produced gas mixture is mainly composed of methane (CH_4), in percentages ranging from 50% to 70% and carbon dioxide (CO_2), in concentrations between 30% and 50%. Biogas may also contain other compounds, considered as pollutants:

- Nitrogen (N_2): 0-3%.
- Vapour water (H_2O): 5-10%.
- Oxygen (O_2): 0-1%.
- Hydrogen sulphate (H_2S): 0-10000 ppmv.
- Ammonia (NH_3): 0-200 mg/m^3.
- Siloxanes: 0-41 mg/m^3.

The compounds listed above may adversely affect biogas quality. For instance, the presence of CO_2 and N_2 may strongly influence the lower calorific value of the gas; methane, in fact, has an energy content of 36 MJ/m^3; on the other hand, a biogas mixture with a methane content of 60-65%, presents values close to 20-25 MJ/m^3. Furthermore, ammonia and hydrogen sulphate are extremely corrosive and can cause damage to heat and power production units, as well as metal parts; this is due to the emission of sulphur dioxide (SO_2) produced after combustion.

Therefore, removing these undesirable compounds is necessary to increase the biogas quality; this activity can be exploited by a wide range of treatments, which can be summarized as follows:

- *Biogas cleaning*, which includes the removal of hazardous compounds (mainly H_2S);
- *Biogas upgrading*, which leads to an increasing of lower calorific value, in line with fuel quality standards.

Afterwards the improvement processes, if the purified biogas reaches concentrations of methane equal to or greater than 95%, it is named *biomethane*. If this condition is respected, in fact, the gas has features similar to natural gas.

1.1.1 Italian legislation on biofuels – Definitions and Incentives

The Italian legislation, according to the *D.Lgs 3 marzo 2011, n. 28*, fixes the minimum amount of renewable sources for transports at 10%. The decree also promotes biomethane as a source of energy for the transport sector and equates it with other biofuels.

Art. 1 of the *DM 2 marzo 2018* defines *biomethane* as the fuel obtained from biogas, following appropriate physico-chemical treatments, which respects the characteristics set by the Authority for Electricity, Gas and Water Service, now the Regulatory Authority for Energy, Networks and the Environment. The so defined gas is suitable for compression for subsequent injection into the natural gas distribution network and for subsequent uses. In addition, the decree states that the word *biomethane* is also referred to the fuel produced as a result of the methanation of hydrogen obtained from renewable sources and CO_2 present in biogas for the production of biomethane or produced by biological fermentation processes. The *DM 2 marzo 2018* promotes the usage of biomethane and other advanced biofuels in the transport field. Renewable sources are favored by Italian legislation, indeed there are incentives for subjects which are obliged to release for consumption of biofuels (Obliged Subjects). The aim is also to promote the reconversion of biogas plants and to produce other advanced biofuels, apart from biomethane.

For producers of biomethane released for consumption in transport, through road, motorway or private distribution plants, the release of Certificates of Release for Consumption (CIC – Certificati di Immissione in Consumo) is foreseen. It is calculated according to the procedures of the GSE (Gestore Servizi Energetici).

For producers of advanced biomethane a value of 375€ per each CIC is recognized, also taking into account any surcharges provided for in the quantification of the titles due; this incentive lasts 10 years. Subsequently, the only right is the release of the CICs, that can be sold to other employers. Moreover, the GSE can retire the advanced biomethane, even in partial quantities, paying the 95% of the average cost of a month registered on the natural gas market. The Table resumes the incentives and the main incomes.

Table 1. 1 Incentives for biofuels production, according to the Italian legislation.

Type	Incentive	Selling incomes	Duration
Biomethane	CIC + surcharges for raw materials	Biomethane market	Facility life
Advanced biomethane	375€/CIC + surcharges for pertinent facilities	Either biomethane GSE retention, or market biomethane	At least 10 years

1.1.2 Biogas upgrading technologies

Most of the biomethane plants in Europe are located in Germany, while other northern European countries are building facilities suitable for biomethane production (Figure 1. 1). Currently, there are many biogas improvement technologies under development (Figure 1. 2).

From 2018 to 2020, the number of biogas upgrading plants in Europe almost doubled from 483 in 2018 to 729 in 2020 (51% increase). Germany is still the country with the highest number of plants (232), followed by France (131) and the United Kingdom (80). Sweden uses even more biogas as a vehicle fuel than natural gas (Fu *et al.* [1]).

Biogas improvement technologies are mainly classified in physical and chemical technologies and biological technologies.

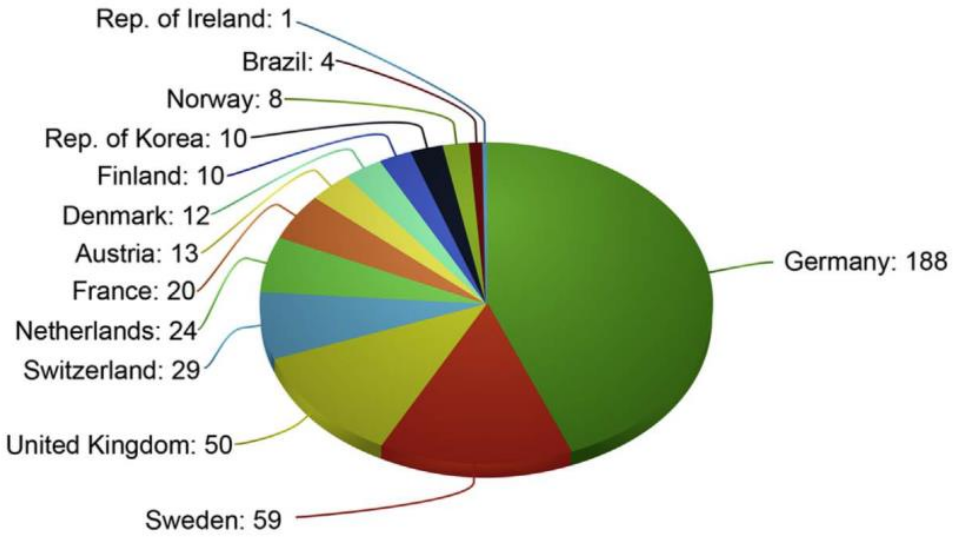

Figure 1. 1 Existing biomethane plants (I. Angelidaki et al. [2]).

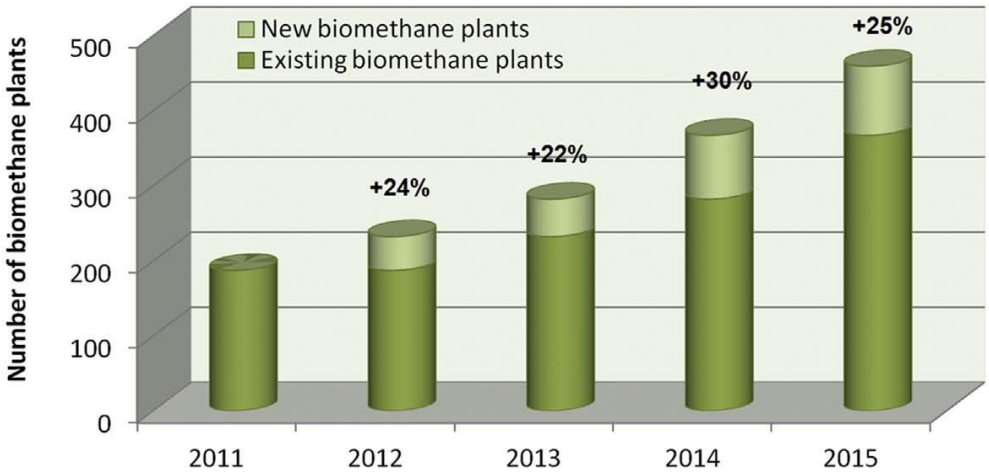

Figure 1. 2 Operative biomethane plants: time developing (I. Angelidaki et al. [2]).

1.1.2.1 Physical and chemical technologies

This type of upgrading technology mainly includes adsorption, absorption and membrane separation processes; other technologies are still under development as they include the use of cryogenic processes or chemical hydrogenation. Generally, these methods are able to achieve methane recovery > 96%, with optimal combinations of temperature, pressure and addition of chemicals if necessary.

Physical/chemical technologies have the advantage of being highly selective, effective and ensuring high methane content in the final product. However, they have high investment costs, as well as high energy demand; among other disadvantages there is the absence of a real disposal method for the removed CO_2, which is released into atmosphere, contributing to global warming; lastly, sometimes these technologies require the use of toxic substances.

1.1.2.2 Biological technologies

These technologies are mainly classified in chemoautotrophic and photosynthetic; they have been largely tested in laboratories and they are currently at the early stage of full-scale implementation.

a) Chemoautotrophic methods

They exploit the action of methanogenic hydrogenotrophic microorganisms, which use H_2 to convert CO_2 into CH_4; the process is based on the following reaction (1.1):

$$4H_2 + CO_2 \rightarrow CH_4 + H_2O \qquad \Delta G^0 = -130.7 KJ/mol \tag{1.1}$$

The hydrogen required for the reaction must come from renewable sources to consider the biogas improvement process itself as renewable. Therefore, the energy needed to hydrolyze water in order to produce hydrogen must come from renewable sources.

This process is able to obtain methane recovery values between 96 and 99%, depending on the type of performed process (in-situ, ex-situ or hybrid - Figure 1. 3), on the type of used reactor and on the most advantageous combination of pressure and temperature.

b) Photoautotrophic methods

These are photosynthetic methods, catalyzed by phototropic organisms such as algae; they can occur in open or closed photobioreactors. During the reaction, photosynthetic plants or microorganisms use water and solar radiation to reduce CO_2 in chemical energy in the form of carbohydrates. Methane recovery is around 97% and the variations depend on the used reactor and the chosen algal species.

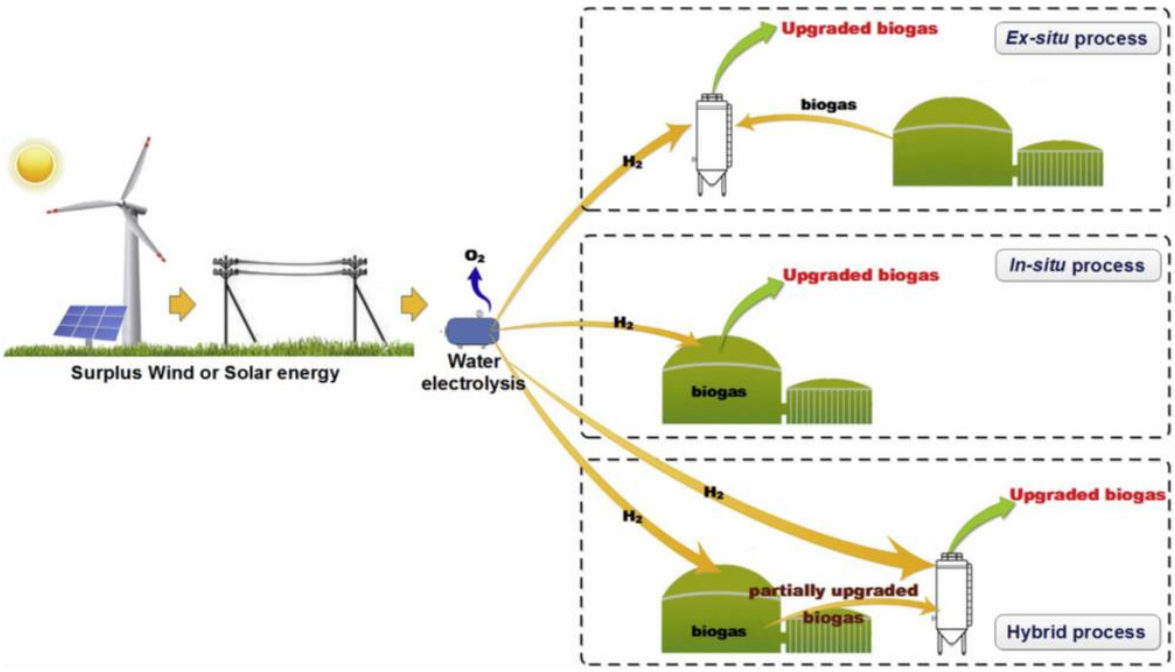

Figure 1. 3 Biological biogas upgrading based on hydrogen methanation; in-situ, ex-situ and hybrid configurations (Angelidaki et al., 2018 [2]).

1.2 Power to gas (PtG)

As mentioned in the previous chapter, some biological processes for the improvement of biomethane require the use of H_2 for the conversion of CO_2 into CH_4. Upgrading technologies aim is to improve biogas quality and its energy power, in order to reduce the extraction of natural gas; for this reason, these technologies must be green. It is therefore unthinkable to use non-renewable sources to produce the necessary hydrogen.

One of the main limitations of RES such as photovoltaic and wind energy lies in their electricity generation profile, which fluctuates over time. The strong development of these technologies in Europe has led to an increasing demand for new solutions for electricity storage. Analyzing the German case, mentioned by Jürgensen *et al.* [3], it can be observed that the electric power surplus produced by renewable energy plants has more than tripled from 2010 to 2011, i.e., from 127 GWh (2010) to 421 GWh (2011). A substantial increase in these values is expected in the upcoming years, concurrently with the development of renewable energy production technologies.

In this regard, Power to Gas (PtG) technologies become the main characters, allowing electricity to be transformed into gas that can be stored for long time periods. The two processes that characterize these technologies are water electrolysis and methanation.

1.2.1 Electrolysis

Electrical energy is transformed into chemical energy through the electrolysis of water: water is divided into its two components, hydrogen and oxygen, through the application of an electrical potential in two electrodes; in particular, hydrogen is formed at the cathode and oxygen at the anode. The electrolyser is made up not only of the two electrodes, but also of an electrolyte and a diaphragm. the former has the task of conducting the ions, the second is an electrical insulator and keeps the two gas flows separate; the separation is necessary to prevent the generation of a flammable gas mixture.

Hydrogen plays a fundamental role in the process, as it is used as an energy carrier; however, it has also some disadvantages due to its low volumetric density of energy and the lack of existing infrastructure for its storage and use.

1.2.2 Methanation

PtG technology can be implemented in two different systems: catalytic/chemical methanation and biological methanation with hydrogen (Biological Hydrogen Methanation – BHM). Both respond to the strongly exergonic Sabatier reaction (1.2):

$$4H_2(g) + CO_2(g) \rightarrow CH_4 + 2H_2O \qquad \Delta H^0 = -165\ \text{KJ/mol} \tag{1.2}$$

The biogas produced downstream of AD of waste or sewage sludge can be a very convenient resource for the methanation process. This represents an excellent solution for small PtG plants located near the natural gas network.

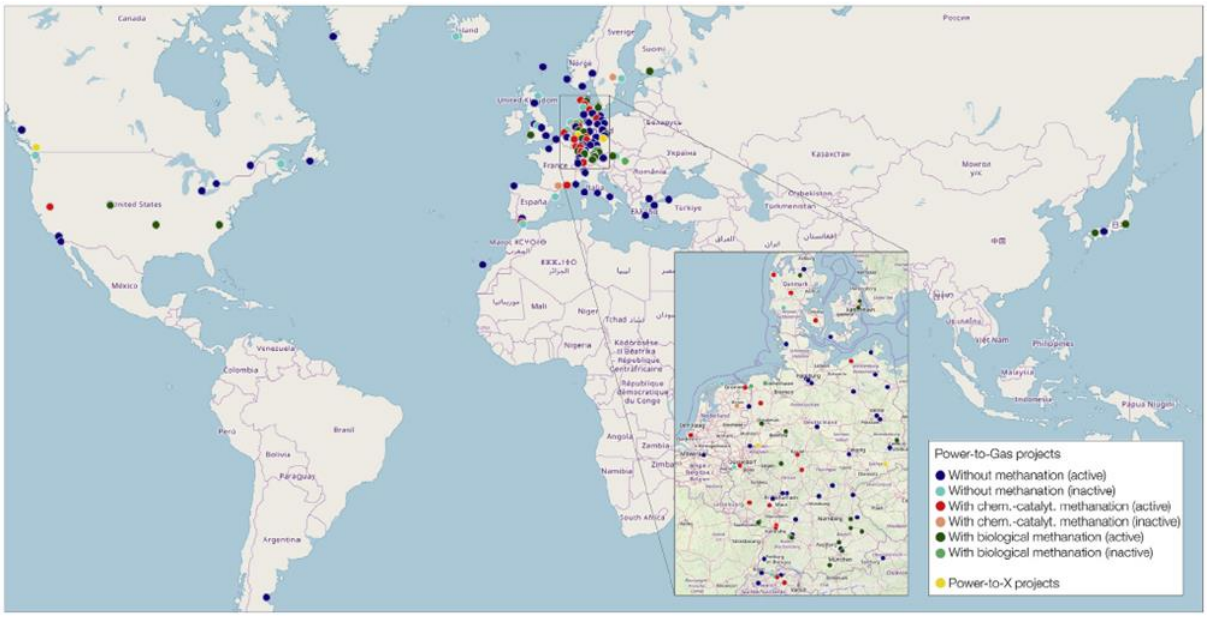

Figure 1. 5 PtG projects in the world; a distinction is made according to whether hydrogen or methane is produced and whether they are active or inactive. Dark green: PtG with active CO_2 methanation. Light green: PtG with biological CO_2 methanation, inactive. Red: PtG with chemical CO_2 methanation, active. Orange: PtG with chemical CO_2 methanation, inactive. Dark blue: PtG without methanation, active. Light blue: PtG without methanation, inactive. Yellow: Power to X projects (Thema et al., 2019 [4]).

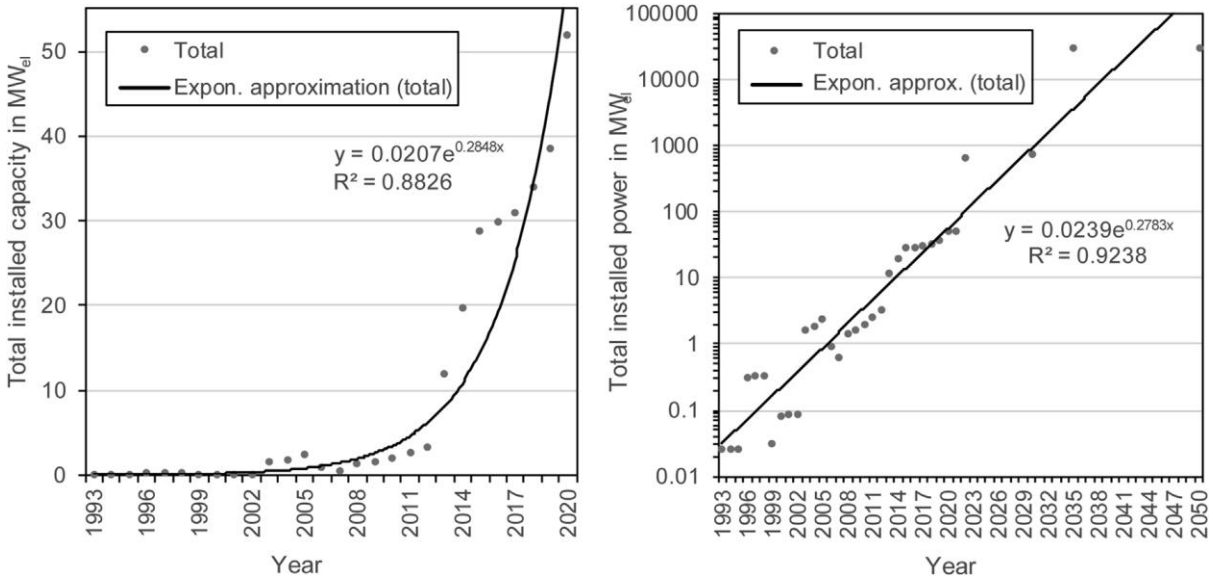

Figure 1. 4 Worldwide trend in total installed power for projects in the medium term, from 1993 to 2020 (left) and in the long term, from 1993 to 2050 (right). The approach excludes electrolysis-free methods, and the 2018 values are project-based (Thema et al., 2019 [4]).

1.2.3 Power to Gas worldwide

Figure 1. 5 shows the projects analyzed in 2019 by Thema *et al.* [4] worldwide. More than half of these projects (57%) focus exclusively (or have focused) on hydrogen production, storage and

use. The rest are focused on the combined or exclusive production of carbon dioxide. The methanation processes are half biological and the other half is chemical methanation.

It can be observed that most of the plants are located in Europe and, in detail, in Germany, Denmark and Norway. In addition, Germany has the majority of the installed capacity, with a value of almost 40 MW_{el}. Denmark follows with a capacity of more than 20 MW_{el}.

1.2.4 Future scenarios

Since the early 1990s the power installed in PtG projects has continuously increased and this growth has followed an almost exponential trend until today. Looking in detail at the three-year period 2012-2015, the graph on the left in Figure 1. 4 shows an intense growth in the number and size of plants. Future forecasts include a further increase in the exponential trend; indeed, as shown in the right graph of Figure 1. 5, and as observed by Thema *et al.* [4], new projects could be developed in the coming years, most of which are located in Germany, Denmark, the Netherlands and Hungary.

1.3 Thermochemical or catalytic methanation

Catalytic processes are carried out in the temperature and pressure ranges of 250-400 °C and 1-30 bar. Sabatier reaction (1.2) is mainly catalyzed by nickel or ruthenium compounds. Since the reaction is highly exothermic, particular attention must be paid to the reactors; indeed, they should be maintained at appropriate temperature values. The two main methods of temperature control are multi-tubular cooled beds and adiabatic beds with partial recirculation of the cooled reactor effluent. The objective of these methods is to maintain the temperature within the operating range of the catalysts.

The obtained gas must be technically water-free, i.e., dryers are used. Downstream of drying process, the dried gas can be brought to the required pressure value for feeding into the distribution network.

1.3.1 Cooled multi-tubular reactor (shell-and-tube)

Figure 1. 6 outlines the process involving a single-stage reaction and the used instruments. The reactor (REAC) has internal tubes containing the catalyst and it is cooled by a fluid with initial flow f0. Downstream of the reactor there is a condenser (VSSL) which removed the water; then, a splitter (SPLT) divides the gas and part of it is recirculated (R), preheated and finally mixed (MIX) with the biogas entering the process (N) to re-enter the reactor.

The reactor performance depends on the achieved cooling, in order to reach the optimum temperature profiles, i.e., those that allow a shorter reactor length: the heat transfer of the fixed beds must ensure adequate dissipation of reaction heat.

1.3.2 Tubular adiabatic reactors (fixed bed)

The process (Figure 1. 7) takes place in two stages and in two different reactors (REAC-1 and REAC-2) by adiabatic way, i.e., U=0. Downstream of each reactor there is a capacitor (VSSL-1 and VSSL-2); the first shifts the balance of the reaction towards the products, reducing the flow rates. Subsequently, the fluid is heated (XCHT) and part of it is recirculated (R), in order to dilute the

reagent and control the reaction from a thermal point of view. The second bed is necessary due to the thermodynamic limitations of the methanation reaction at high temperatures.

The cooled reactor is able to increase the methane yield by about 40% compared to the adiabatic bed (Gutierrez, *et al* [5]). The theoretical reactors, on the other hand, have efficiencies of 286% and 437% respectively: thermal optimization is a powerful means of intensifying the process and minimizing reactor size.

A biogas that is already rich in CH_4, of course, increases the reactor efficiency and reduces the flow rates; on the other hand, recirculation is not as favorable as in the case of a cooled bed.

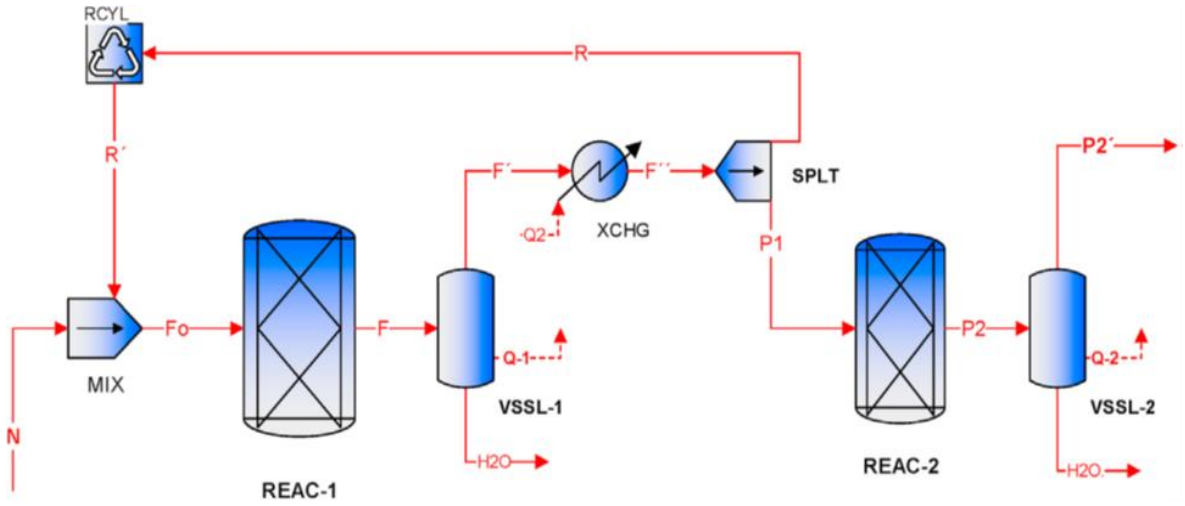

Figure 1. 6 Methanation with two adiabatic beds, gas recycling and water condensing (Gutiérrez et al, 2020 [5]).

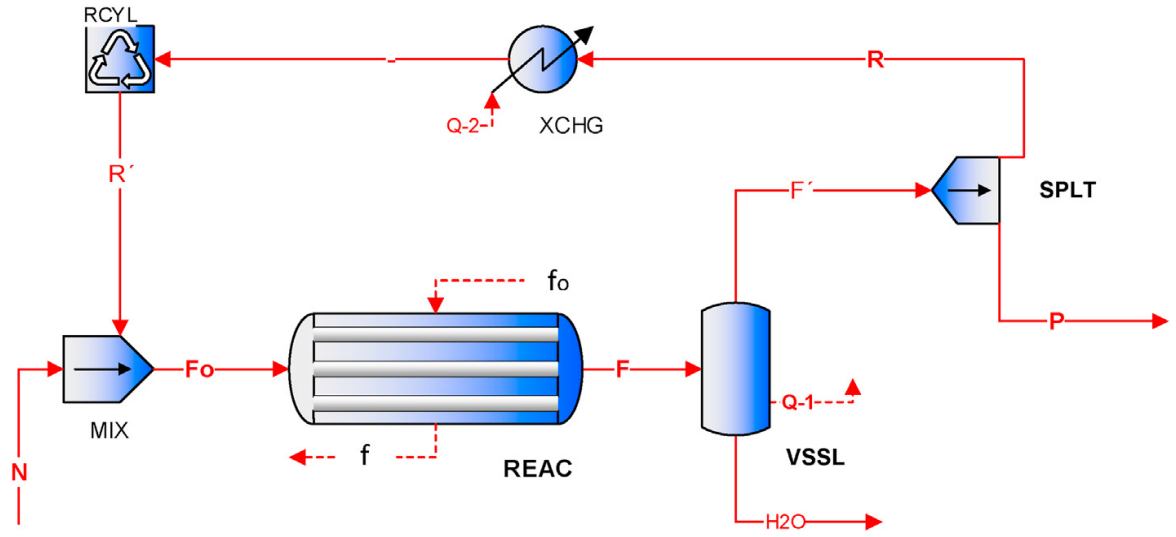

Figure 1. 7 Methanation with tubular reactor, gas recycling and water condensation (Gutiérrez et al, 2020 [5]).

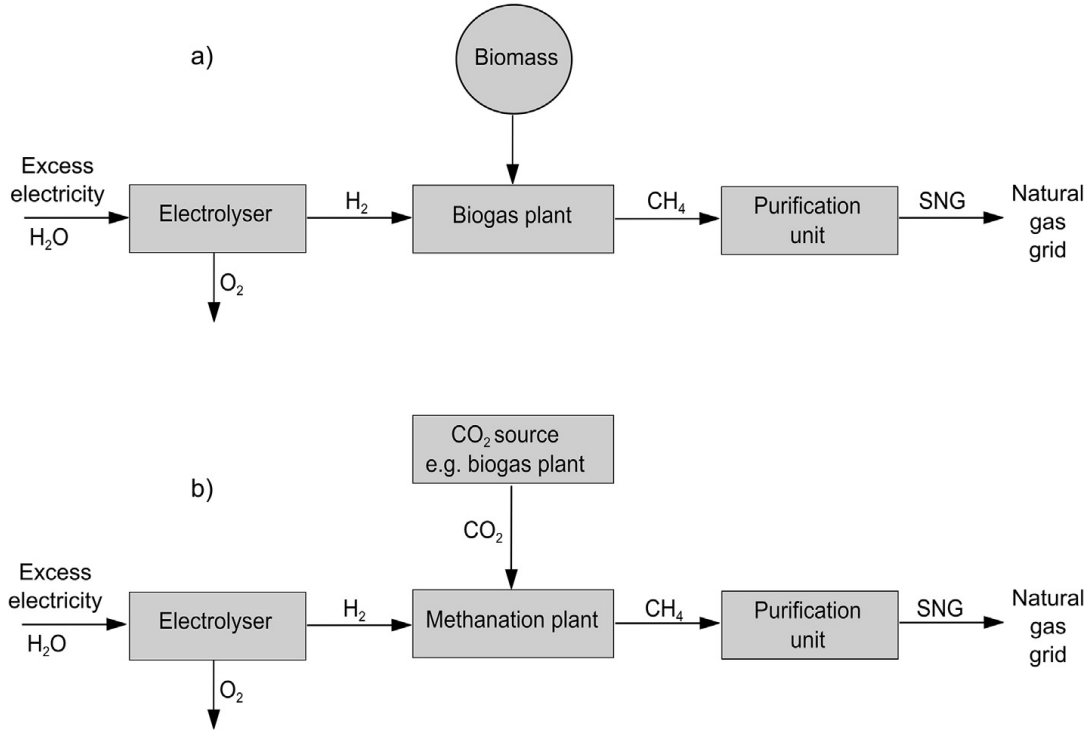

Figure 1. 8 a) BHM process in-situ; b) BHM process ex-situ (Lecker et al., 2017 [6]).

1.4 Biological methanation

As previously mentioned, biological methanation also takes place according to the Sabatier reaction (1.2), which refers to the reaction of 4 moles of hydrogen and 1 mole of carbon dioxide, producing 1 mole of methane and 2 moles of water. As can be seen, the reaction has a negative Gibbs free energy value (ΔG^0=-165 KJ/mol), under standard conditions of temperature and pressure; this means that the reaction is exergonic, and it occurs spontaneously from left to right.

The process is catalyzed by methanogenic hydrogenotrophic archaeobacteria. The process can be carried out in any biogas plant and sees the optimum temperature range for microorganisms as between 15 °C and 98 °C.

The process can be carried out either in in-situ or ex-situ configuration (Figure 1. 8) or hybrid, depending on where the hydrogen is injected compared to the anaerobic digester. In the former case, H_2 (preferably produced by electrolysis using surplus renewable energy) is injected together with an organic substrate directly into an anaerobic digester. The degradation phase of the substrate (hydrolysis and acidogenesis) provides for the formation of intermediate products, such as volatile fatty acids (VFA) and precursors for methanation, such as carbon dioxide.

On the other hand, the ex-situ methanation occurs with the parallel injection of H_2 and CO_2 into the reactor, with a stoichiometric ratio of 4:1; the system also requires the addition of essential nutrients and hydrogenotrophic methanogenic bacteria.

1.4.1 Microbiology of methanation: *Methanogens*

Methanation by degradation of organic compounds is carried out by different groups of microorganisms, which live in symbiosis in a single environment. These are the methanogenic bacteria, characterized by a high physiological specialization and a strong anaerobic character. Methanogens belong to the *Euryarchaeota* of *Archeobacteria*, and they can be classified into acetogenic and hydrogenotrophic, i.e., acetate or hydrogen consumers, respectively.

According to Burkhardt *et al.* [9], almost all species are able to produce methane from hydrogen and carbon dioxide, while only *Methanosaeta* spp. deal exclusively with the conversion of acetate into methane and CO_2.

In AD processes methanogens with different morphology, such as rod, cocci and spiral, have frequently been found; these have the same characteristics (Liu *et al.*, 2011):

1. Extremely low growth rate: *Methanosaeta*, for example, duplicate in 4-9 days.
2. They are strictly anaerobic: they cannot survive exposure to oxygen or air.
3. They use simple compounds as sources of nutrition.
4. They live in a neutral or weakly alkaline environment.
5. Biogas is their main metabolic product.

1.4.1.1 Methanogens metabolism

Living organisms utilize nutrients not only to provide the precursors of all the components of a cell, but also to generate the energy needed for biosynthetic and other endergonic processes. The precursors of the cells are produced during the degradative metabolic pathways known as "catabolic" routes; on the other hand, the biosynthetic processes are referred to as "anabolic" reactions. The metabolic link between these processes is given by the central metabolism pathways, whose reactions serve as the major routes of energy generation. Therefore, during the catabolic metabolism free energy is produced and the main reaction product is methane. On the contrary, the anabolic route needs energy to occur.

From a purely engineering point of view, methanogenic bacteria can be classified into hydrogenotrophic and acetoclastic methanogens. Hydrogenotrophic methanogens are fundamental for the stability of the of process of methanation because they metabolize H_2 and CO_2 to methane; they are able to maintain hydrogen to concentrations that allow stable acetogenesis, which is made by syntrophic acetogenic microorganisms. They can use hydrogen not only to reduce CO_2 according to the (1.3), but also to utilize CO as in the (1.4) (Guneratnam *et al.*, [11]):

$$4H_2 + CO_2 \rightarrow CH_4 + H_2O \qquad \Delta G'_0 = -135.6\ \text{KJ} \tag{1.3}$$

$$3H_2 + CO \rightarrow CH_4 + H_2O \tag{1.4}$$

Acetotrophic (acetoclastic) methanogens produce methane and carbon dioxide from acetic acid, as a result of their metabolic activity. They are influenced by the presence of NH_3 and volatile fatty acids (VFA). They respond to the reaction (1.5), according to van Lier *et al.* [8]:

$$CH_3^-COO^- + H_2O \rightarrow CH_4 + HCO_3^- \tag{1.5}$$

1.4.1.2 Most significant parameters for Methanogens growth

According to Liu *et al.* [10], the parameters that mainly affect methanogenic microorganisms' metabolic activity, thus their growth, are oxygen content, temperature, alkalinity, C/N ratio, the presence of toxic compounds, the entity of mixing and the inoculation. Table 1. 2 summarises the basic characteristics of certain methanogens, which include specific substrates and requirements for the cultivation conditions in accordance with Zabranska and Pokorna [16].

Table 1. 2 Characteristics of some methanogenic species (Zabranska and Pokorna [16]).

Species	**Substrate**	**Optimal temperature (°C)**	**Optimal pH range**
Methanobacterium bryantii	H_2/CO_2	37	6.9-7.2
Methanobacterium formicicum	H_2/CO_2, formate	37-45	6.6-7.8
Methanobacterium thermoalcaliphium	H_2/CO_2	58-62	8.0-8.5
Methanothermobacter thermoautotrophicum	H_2/CO_2	65-70	7.0-8.0
Methanothermobacter wolfeii	H_2/CO_2	55-65	7.0-7.5
Methanobrevibacter smithii	H_2/CO_2, formate	37-39	-
Methanobrevibacter ruminantium	H_2/CO_2, formate	37-39	-
Methanothermus fervidus	H_2/CO_2, formate	83	< 7
Methanothermococcus thermolithotrophicus	H_2/CO_2, formate	65	-
Methanococcus voltae	H_2/CO_2, formate	35-40	6.0-7.0
Methanococcus vannielli	H_2/CO_2, formate	65	7.0-9.0
Methanomicrobium mobile	H_2/CO_2, formate	40	6.1-6.9
Methanolacinia paynteri	H_2/CO_2	40	7.0
Methanospirillum hungatei	H_2/CO_2, formate	30-40	-
Methanosarcina acetivorans	Methanol, acetate	35-40	6.5
Methanosarcina barkeri	H_2/CO_2, methanol, methylamines, acetate	35-40	5.0-7.0
Methanosarcina mazei	Methanol, methylamines, acetate	30-40	6.0-7.0
Methanosarcina thermophile	H_2/CO_2, methanol, methylamines, acetate	50	6.0-7.0
Methanococcoides methylutens	Methanol	42	7.0-7.5
Methanosaeta concilii (soehngenii)	Acetate	35-40	7.0-7.5
Methanosaeta thermophila	Acetate	55-60	7.0

a) Oxidation-reduction potential

Since methanogens are strongly anaerobic, even oxygen concentrations in traces could inhibit their activity or even kill them. Therefore, in order to maintain adequate activity levels, the redox potential should be low, typically in the range of -400 to -150 mV.

b) Temperature

Gas production generally increases with increasing temperature. Three temperature ranges can be identified, corresponding to three different conditions: below 25 °C psychrotrophic conditions are obtained; average values, between 25 and 45 °C, identify mesophilic conditions; temperatures between 45 and 65 °C correspond to thermophilic conditions. The best methane production was found between 35 and 40 °C and at about 55 °C. This is because of the increased growth rate: thermophilic methanogens have a growth rate which is 2-3 times higher than mesophilic methanogens.

c) pH

Anaerobic microorganisms live in environments with a pH between 6.8 and 7.5 (Liu *et al.* [10]). Even small pH changes can significantly influence activity and growth of methanogens: for values below 6 or above 8, biogas production is generally inhibited or even stopped. However, some methanogens still work well in environments with pH between 5.5 and 9.5.

d) C/N ratio

The most suitable ratio of carbon and hydrogen for the anaerobic digestion process is 20-30. In order to obtain an appropriate C/N ratio value, it is preferable to mix different raw materials entering the digester.

e) Inhibitors

Raw materials used to feed digesters may contain toxic substances for microorganisms; for instance, organic waste from livestock farms may contain pesticides, heavy metals and disinfectants, which limit the growth and metabolism of archeobacteria.

f) Mixing

Mixing is essential, as it allows the substrates to be supplied more efficiently to microorganisms, dilute toxic substances, equalise the pH of the solution and the temperature, prevent stratification and the formation of preferential pathways in the digester.

g) Inoculums

The process activation is speeded up by the introduction of inoculations containing active microorganisms specialised in the production of methane into the digester; satisfactory results are observed after a supplement of 20-30%. Without this, it would take a very long time to enrich the colony with microorganisms. The volume of the inoculum should not exceed 10% of the total operating volume of the reactor.

1.4.2 In-situ biological methanation

Biogas production in the in-situ configuration is marked by the typical phases of anaerobic digestion (Figure 1. 9):

a) *Hydrolysis*: the complex organic matter, formed by insoluble biopolymers, is decomposed by the fermenting bacteria into simpler substances, such as soluble organic compounds.

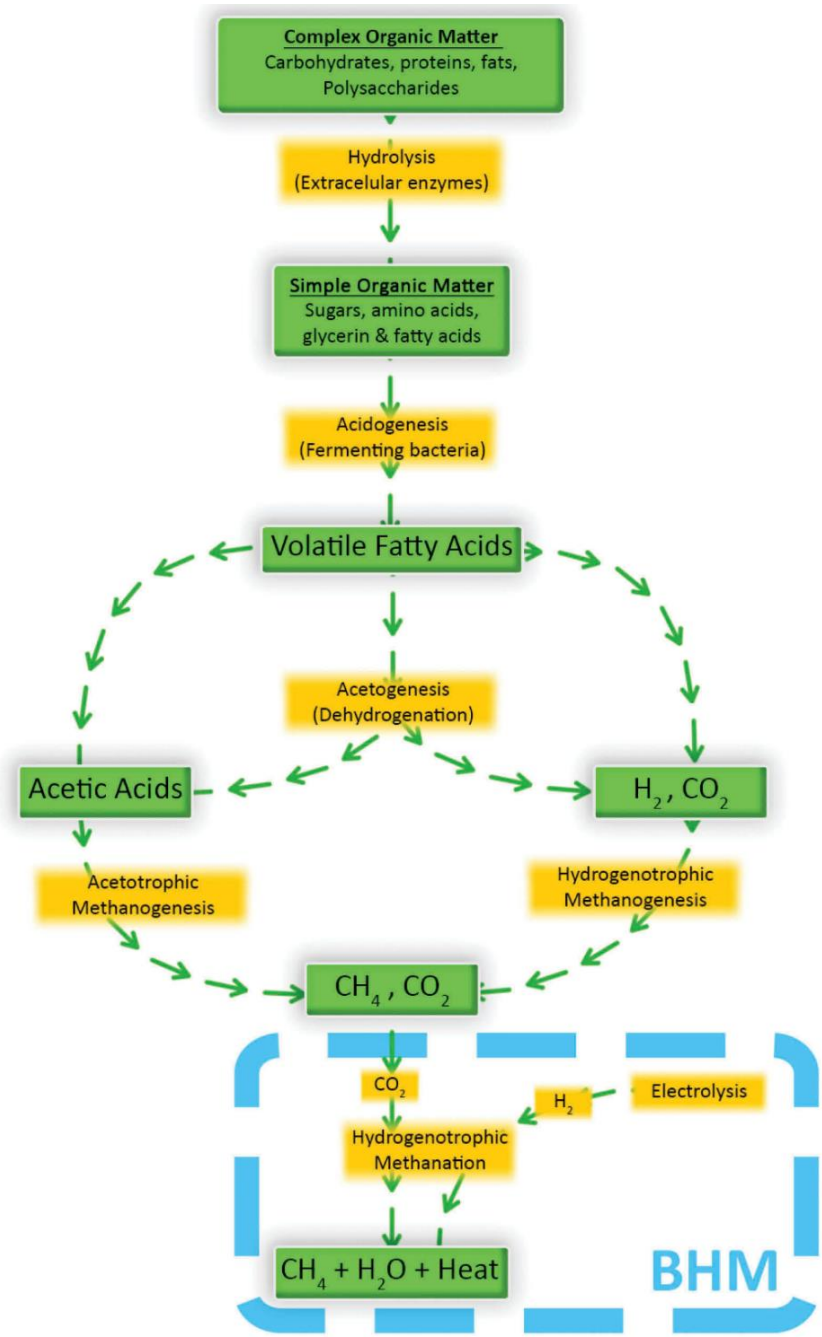

Figure 1. 9 Methane production by means of AD and Biological Hydrogen Methanation – BHM (D. Rusmanis et al., 2019 [7]).

b) *Acidogenesis*: soluble compounds are degraded into volatile fatty acids (VFA), carbon dioxide and hydrogen.

c) *Acetogenesis* (production of intermediate acids): the digestion products are converted into acetate, hydrogen and carbon dioxide.

d) *Methanation*: acetate, hydrogen plus carbonate, formate or methanol are converted to methane and carbon dioxide.

Acetogenic bacteria and methanogenic bacteria coexist in the system. The former produce hydrogen with their metabolic activity, while the latter consume hydrogen for the production of

methane. The degradation of fatty acids and alcohols depends on organisms such as methanogens, which sweep away electrons. When H_2-producing organisms can only grow and reproduce in the presence of microorganisms that consume H_2 for their metabolic activity, syntrophic association takes place. When, on the other hand, H_2 formation and consumption occur simultaneously, the phenomenon is known as interspecies hydrogen transfer.

Table 1. 3 Stoichiometry and free energy variations (ΔG0') for some acetogenic reactions, at natural pH values, temperature of 25 °C and pressure of 1 atm (van Lier et al., 2008 [8]).

Compound	Reaction	$\Delta G^{0'}\,(kJ/mol)$
Lactate	$CH_3CHOHCOO^- + 2H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 2H_2$	-4.2
Ethanol	$CH_3CH_2 + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$	+9.6
Butyrate	$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$	+48.1
Propionate	$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$	+76.1
Methanol	$4CH_3OH + 2CO_2 \rightarrow 3CH_3COO^- + 2H_2O$	-2.9
Hydrogen-CO_2	$2HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + 4H_2O$	-70.3
Palmitate	$CH_{3\_}(CH_2)_{14\_}COO\_ + 14H_2O \rightarrow 8CH_3COO^- + 7H^+ + 14H_2$	+345.6

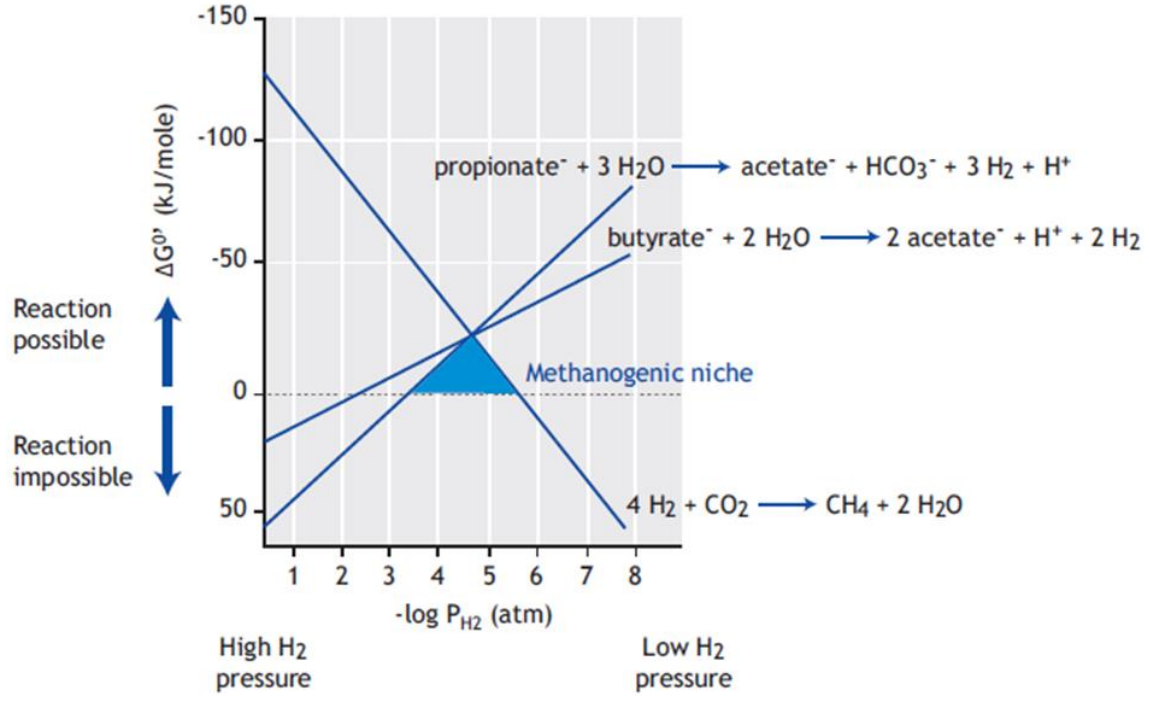

Figure 1. 10 Variation of Gibbs free energy as a function of the partial pressure of hydrogen (van Lier et al., 2008 [8]).

Propionate and butyrate are the most important acetogenic substrates, as they are key reaction intermediates in the AD process. Other compounds converted to acetate by homacetogenesis are lactate, ethanol, methanol, hydrogen and carbon dioxide. Acetogenic bacteria are mandatory

hydrogen producers, and their metabolism is inhibited by hydrogen and results from the stoichiometric conversion reaction; for propionate, for example, it is obtained:

$$\Delta G' = \Delta G'_0 + RT\ln\frac{[\text{Acetate}]\cdot[CO_2]\cdot[H_2]^3}{[\text{Propionate}]} \tag{1.6}$$

From (1.6) it emerges that some acetogenic reactions do not occur naturally under standard conditions, because they have a positive Gibbs free energy value, resulting in an energy efficiency of the bacteria below zero (Table 1. 3). Pressure can also influence bacteriological activity and associated reactions; under stable digestion conditions, acetogenesis reactions remain endogenous and partial hydrogen pressure is very low (< 10^{-4} atm, van Lier *et al*. [8]). This condition is guaranteed by methanogenic microorganisms, which absorb hydrogen and use it so quickly that the partial pressure is reduced; the latter remains at values adequate to guarantee the acetogenic reactions.

Figure 1. 10 shows how the partial pressure, expressed in logarithmic terms, influences Gibbs' free energy variation. The addition of external hydrogen in the system causes an increase in pressure, with a consequent alteration of the process balances and an increase in Gibbs' free energy variation: homoacetogenesis is stimulated, but hydrogenotrophic methanogenesis is limited. The optimal conditions of the system are characterized by the area that in Figure 1. 10 is defined Methanogenic nicke, corresponding to a partial hydrogen pressure range of 10^{-6}-10^{-4} atm.

Methanogenesis is the final stage of AD, carried out by methanogenic bacteria.

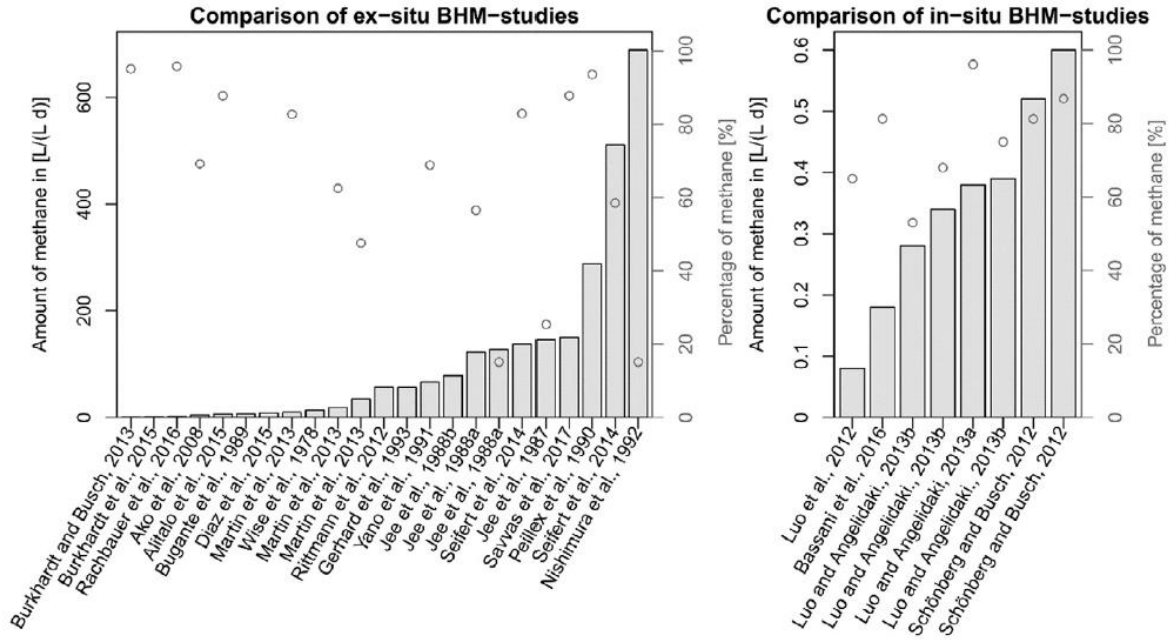

Figure 1. 11 Comparison between in-situ and ex-situ systems. Left, injection of a mixture of H_2-CO_2 for hydrogenotrophic methanogens; right, injection of H_2 into a continuous fermenter. The bars indicate the methane yield (L L^{-1} d^{-1}) and the circles indicate the content (%) of CH_4 (B. Lecker et al. [6]).

1.4.3 Comparison between in-situ and ex-situ methanation

Expanding an existing biogas plant to make in-situ carbon dioxide methanation represents a reduction in investment costs, compared to an ex-situ reactor. However, this second option reduces many of the difficulties encountered during AD, both from a mechanical and biological point of view.

The hydrogen injected during in-situ methanation must be constantly monitored and the quantities must be adapted to CO_2 production in the digester: additional costs are required for the installation of instruments for measuring and monitoring gas concentrations.

From a production point of view, however, the yield of methane (MER) in an in-situ system is very low, compared to an ex-situ reactor: studies carried out between 1992 and 2013 reported MER values between 0.08 and 0.39 $L/(L_Rd)$ for in-situ processes and values between 0.37 and 688.6 $L/(L_Rd)$ for ex-situ processes (Figure 1. 11).

The most frequently encountered problem in both configurations is the low gas-liquid mass transfer value of hydrogen; this can be increased using more efficient gas diffusors or different reactor configurations.

2 State-of-the-art technology of BHM

Most of the literature dealing with biological methanation is of recent publication; several authors have carried out a series of projects on laboratory scale, with different operating modes, different reactors (type and volumetry), but also different thermodynamic conditions. It is appropriate to make a comparison between these projects, in order to understand the best configuration for the production of organic methane, also considering the different origin of the digestate.

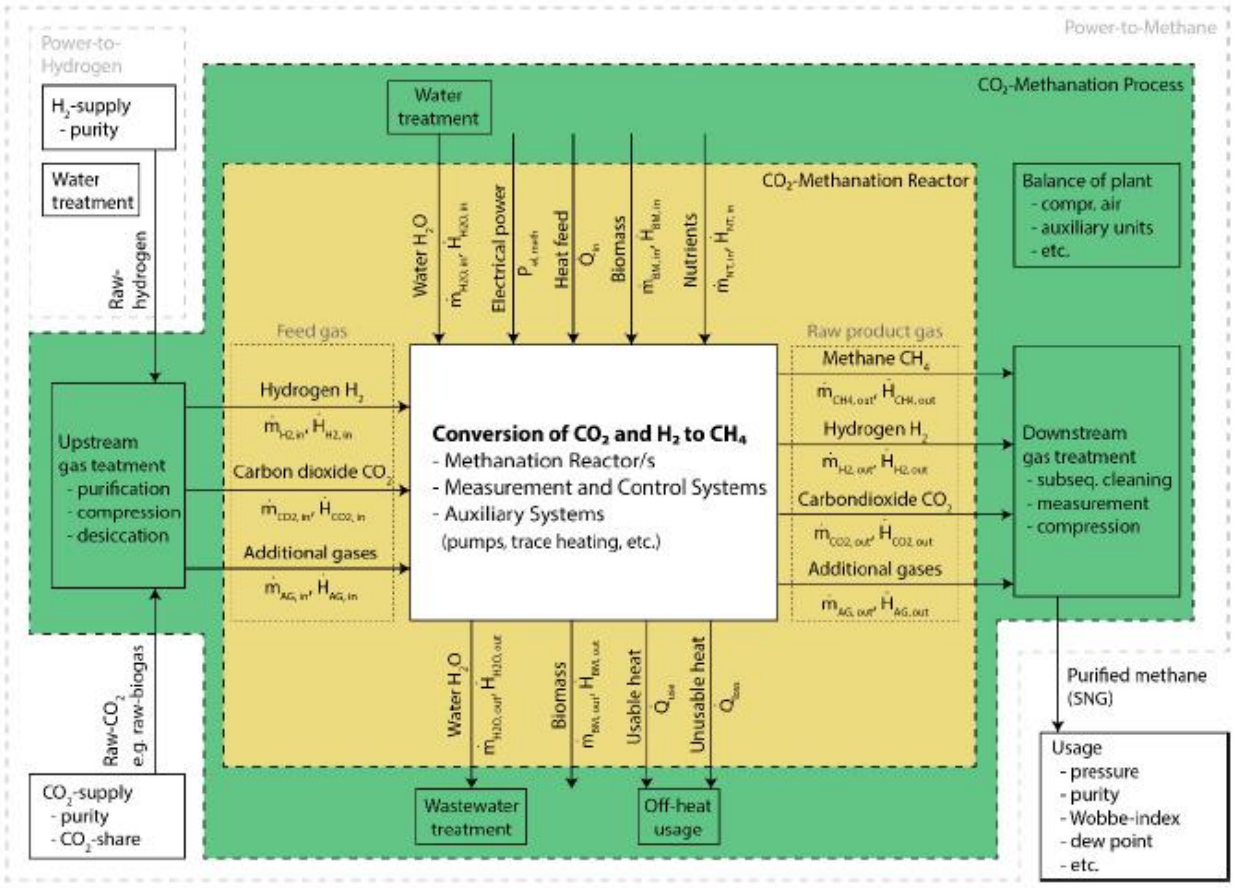

Figure 2. 1 Boundaries and mass and energy streams of the systems CO2-Methanation reactor (yellow), CO2-Methanation process (green), Power-to-Hydrogen and Power-to-Methane (Thema et al. [18]).

2.1 System boundaries and mass balances

Thema *et al.* [18] defined the boundaries of the entire technology, in order to standardize the biological methanation. The methanation step presents two system boundaries (Figure 2. 1): the "CO_2-Methanation reactor" and the "CO_2-Methanation process".

2.1.1 CO_2-Methanation reactor

The "CO_2-Methanation reactor" summarizes all the components of the reactor (the yellow rectangular in Figure 2. 1). It is the innermost part of the methanation system; it includes the methanation reactor, the measurement control systems of the reactor and all the potentially required components (e.g., pumps, systems for cooling/heating, stirrers etc.).

Equations from (2.1) to (2.3) are the mass and energy balances for the system and they derive from Figure 2. 1 (Thema *et al.* [18]):

$$\dot{m}_{H_2,in} + \dot{m}_{CO_2,in} + \dot{m}_{BM,in} + \dot{m}_{AG,in} + \dot{m}_{H_2O,in} + \dot{m}_{NT,in} = \dot{m}_{CH_4,out} + \dot{m}_{H_2,out} + \dot{m}_{CO_2,out} + \dot{m}_{AG,out} + \dot{m}_{H_2O,out} + \dot{m}_{BM,out} \quad (2.1)$$

$$\dot{H}_{H_2,in} + \dot{H}_{CO_2,in} + H_{BM,in} + \dot{H}_{AG,in} + \dot{H}_{H_2O,in} + \dot{H}_{NT,in} + P_{el} + \dot{Q}_{in} = \dot{H}_{CH_4,out} + \dot{H}_{H_2,out} + \dot{H}_{CO_2,out} + \dot{H}_{AG,out} + \dot{H}_{H_2O,out} + \dot{H}_{BM,out} + \dot{Q}_{out} \quad (2.2)$$

$$\dot{Q}_{out} = \dot{Q}_{loss} + \dot{Q}_{use} \quad (2.3)$$

The major advantage of biological vs. chemical methanation is the tolerance of system towards impurities in the feed gases; however, in a such defined system this issue is not revealed.

2.1.2 CO_2-Methanation process

The "CO_2-Methanation process" boundary system (the green box in Figure 2. 1) is an extension of the "CO_2-Methanation reactor" system boundary. It includes all the necessary peripheric treatments and instruments e.g., water and wastewater treatments, pre- and post- treatment of feed and product gases.

2.2 Characteristic parameters

An overview of all the parameters that influence the CH_4 production process, according to current literature, have been listed, defined, and compared in different conditions as follows.

2.2.1 Reactor type

Currently, there are various types of biological methanation systems, based on different reactor systems. Applied systems are very assorted, as they range from continuous stirred tank reactors (CSTR), trickle-bed reactorr (TBR) to bubble column reactors (BCR), and also membrane reactors (MR). Thema *et al.* [18] gave an overview of the characteristics of different standard configurations (Table 2. 1), which will be further described.

2.2.1.1 Fixed bed reactors

Archaea attach locally to surfaces, thus forming a biofilm. The thickness of this layer can range from a few micrometres to a few millimetres. Immobilization technologies offer the advantage that the most specialized microorganisms do not get discharged from the system. Such biofilms

are used in high performance fixed-bed reactors; the substrate is fed to the system with the liquid phase in dissolved form. The biofilm and the liquid phase above the carrier surface form a two-phase system (Figure 2. 2). If the reactor contains also gaseous compounds, they must be solubilized in water before decomposition.

Table 2. 1 Overview of the mass transport potential and the energy consumption related to the mass transport in the TBR, CSTR, BCR, MR (Thema et al. [18]).

Parameter	Unit	TBR	CSTR	BCR	MR
Gas holdup ε_G (1)	-	0.75-0.98	0.05-0.3	0.02-0.4	
Liquid holdup ε_L (1)	-	0.5-0.2	0.7-0.95	0.7-0.95	
Effective surface area a_{eff} (1)	m^{-1}	60-640	100-1500	100-1000	70-180
Mass transfer coefficient k_L (2)	m/s	$0.4\text{-}2\cdot10^{-4}$	$0.3\text{-}4\cdot10^{-4}$	$1\text{-}4\cdot10^{-4}$	$1\text{-}10\cdot10^{-4}$
Volume specific power input p_{VR,k_La}	Wh/m^3	4.3	50	12.5-15.6	

(1), (2) See respectively Sections 2.2.10 and 2.2.9 for further information.

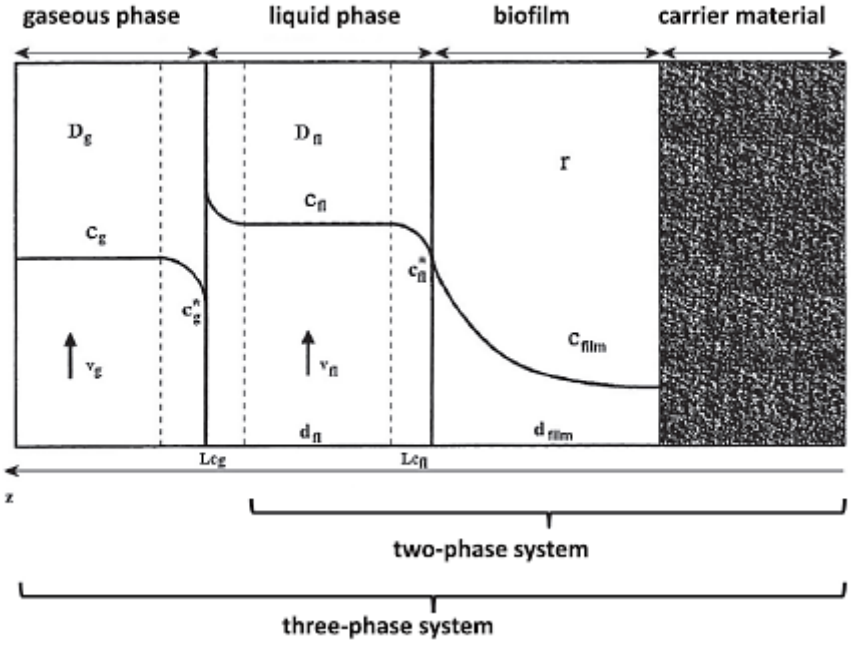

Figure 2. 2 The two-phase system in the liquid tensed fixed-bed reactor an the three-phase system in the trickle-bed reactor (Burkhardt et al. [9])

In traditional fixed-bed reactors the metabolism is limited, and the gas retention time is not sufficient. Bubbles formation in water does not let hydrogen and carbon dioxide solubilize. Thus, the mass transfer and the metabolic rate are strongly reduced, because of the lack of surface (Burkhardt *et al.* [9]).

On the other hand, the trickle-bed reactor (TBR) presents a gas phase above the liquid phase: a three-phase system is present (Figure 2. 2), which provides a sufficient surface area. Thus, the

mass transfer increases, and the degradation rate is higher. Gas phase is the continuous phase, and the volume of the packing material is less than 10% and that is why the gas holdup is higher with respect to CSTR and BCR. Since the liquid is pumped to the top of the column, no other additionally energy has to be spent for the dispersion of the liquid into droplets. Thus, the energy demand of a TBR is considerably lower.

2.2.1.2 CSTR

One of the most common problems during biological methanation is due to the solubility of hydrogen in the liquid phase. Mechanical mixing can overcome this problem when it occurs at high speeds; one of the simplest methods to promote hydrogen solubilization is to use CSTR type reactors with speeds up to 1500 rpm, at laboratory scale; for commercial scale reactors it is not uncommon to have mixing speeds of 60 rpm. The CRST reactor must be narrow and high, in order to increase the contact time with the culture of methanogenic microorganisms (Guneratnam *et al.* [11]).

2.2.1.3 BCR

Bubble column reactors (BCR) are used to generate and control gas-liquid chemical reactions. They are characterized by a cylindrical shape and they are filled with liquid. The gas is injected at the bottom of the liquid. As in the CSTR, in the BCR the continuous phase is liquid, and the microorganisms are suspended in it. The gas phase is dispersed in the liquid phase in form of bubbles; therefore, the gas holdup is lower than the TBR one.

Due to the dispersal of the gas phase into small bubbles, the effective surface area is higher, as in CSTR.

2.2.1.4 HFM - Hollow Fiber Membrane

Membrane reactors (HFM - Hollow Fiber Membrane) are based on the use of ceramic membranes, which act as a barrier between the liquid inside the reactor and the gas supplied from outside; the membrane comprises several fibres, containing small pores into which the gas is forced to pass, spreading directly into the surrounding liquid. This type of membrane ensures that the gas-liquid mass transfer is instantaneous; however, the flow rates in the system are affected by the porosity and relatively small area of the membranes themselves.

A classic disadvantage of using membranes is fouling, i.e., the formation of a biofilm on their surface, which reduces their operational efficiency.

2.2.2 Reactor construction materials

The construction material of a chemical reactor is chosen based on the chemical, mechanical and thermal stresses to which it will be subjected during the process; the most frequently used materials are non-alloy and low alloy carbon steels, stainless steels, glass, plastic materials, ceramic materials.

Pressure and storage vessels not subject to excessive corrosion are mainly made of non-alloy and weakly-alloy carbon steels with a percentage of carbon up to 0.25% by weight; these have good ductility, wide diffusion, and low cost.

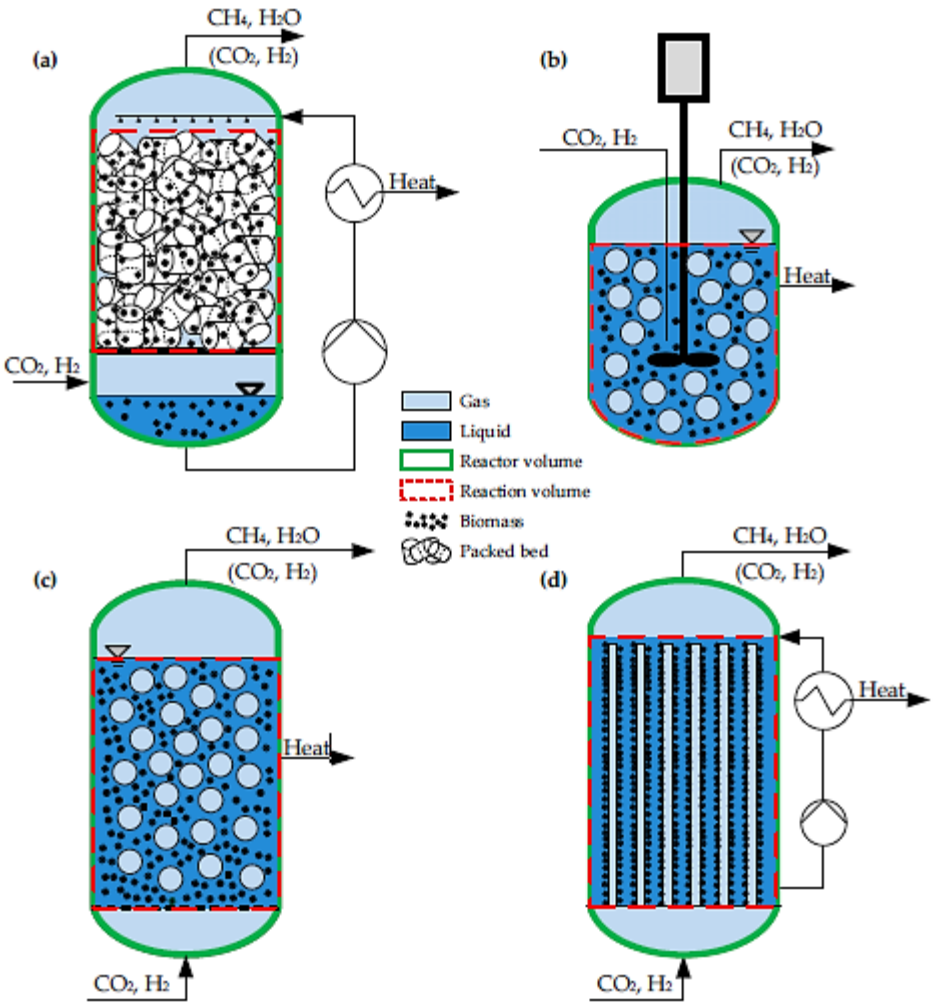

Figure 2. 3 Schematic flow diagram of a TBR (a), a CSTR (b), a BCR (c) and a MR (d) for biological methanation (Thema et al. [18]).

Stainless steels are iron based and contain chrome in variable percentages (12-30%), nickel up to 30% and other elements in smaller quantities. They are very resistant to heat and corrosion. Higher percentages of chromium guarantee higher corrosion resistance.

Glass has excellent acid resistance, excellent chemical inertia and is non-toxic. It is, however, brittle and sensitive to thermal shock. The combination with plastic or metallic materials improves its mechanical properties.

Plastics are mainly polymers: PE (polyester), PET (polyethylene terephthalate), PP (polypropylene) and PVC (polyvinyl chloride). These have good chemical resistance, but low mechanical resistance, especially at high temperatures, which limits their use in these fields.

Ceramic materials containing silica are attacked by hydrofluoric acid, even at low temperatures and with diluted solutions. Graphite and alumina are well resistant to solutions with HF content below 60% and at temperatures below their boiling temperature as reported in [12].

2.2.3 Reactor volume

According to Thema *et al.* [18], the reactor volume (V_R) is the sum of the volume of all sections within the reactor; they include, e.g., head space, sump, liquid, and internal components, as outlined in Figure 2. 3. The liquid volume only comprises the liquid present within the reactor during operation and it includes the volume of suspended biomass and solids. The gas volume is referred to the total volume of gaseous phase within the reactor volume. Finally, the reaction volume is the volume in which the reactions take place. The packing volume is referred exclusively to the application of TBRs and it is the volume of the packing zone.

This parameter is extremely useful for the computation of the methane production, expressed in terms of Methane Evolution Rate (MER, see Section 2.2.4).

2.2.4 Methane evolution rate - MER

The methane evolution rate (MER) is a simple method for calculating the system performance. It refers to the volume of methane produced in the unit of time, as a function of the reactor volume and can be derived from (2.5):

$$\mathrm{MER} = \frac{\mathrm{Q_{CH_4,out}} - \mathrm{Q_{CH_4,in}}}{\mathrm{V_R}} \qquad [\mathrm{L/(\,L_R d)}] \tag{2.5}$$

In (1.9) $Q_{CH_4,out}$ (L/d) represents the volumetric flow rate of methane out of the reactor, $Q_{CH_4,in}$ (L/d) the volumetric flow rate of methane entering the reactor.

2.2.5 Retention time

The volume of the reactor directly influences another important design parameter: the retention time; this can be referred to liquid (HRT - Hydraulic Retention Time) or gas (GRT - Gas Retention Time). It is expressed as the ratio between the reactor volume (V_R) and the flow through it (Q):

$$\mathrm{RT} = \mathrm{V_R/Q} \qquad [\mathrm{d}] \tag{2.4}$$

The retention time expresses the permanence of the fluid in the reactor, in terms of days. The shorter the retention time, the more compact the system is and the shorter the liquid/gas paths.

2.2.6 pH

The pH can give rise to technical challenges, especially when working with in-situ technologies; due to the consumption of bicarbonate by hydrogenotrophic methanogenic microorganisms, in fact, increases in pH value can occur, which negatively affects the acetoclastic methanogens, causing a reduction in methane production efficiency.

Hydrogenotrophic methanogenic microorganisms are able to adapt to a wider pH range than acetoclastic, between 5.5 and 7.5, under the same thermophilic conditions. The species operating in ex-situ processes are mainly Methanothermobacter thermautotrophicus, Methanobacterium thermoalcaliphilum and Methanosarcina barkeri, which adapt at pH ranges of 7.0-8.0, 8.0-8.5 and 5.7-6.2 respectively.

2.2.7 Temperature

The temperature is related to the microbial growth rate (Figure 2. 4) and dissolution temperature. The optimal temperatures vary depending on whether the microorganisms used are thermophilic or mesophilic, in the ranges of 55-65 °C and 35-40 °C, respectively. Previous studies have reported as optimal temperatures for hydrogenotrophic methanogens: 55 °C, 65 °C and 70 °C; as the temperature increases, methanogenic activity increases.

At low temperatures, on the other hand, there are higher values of gas solubility, which translate into higher levels of diffusivity (D_L), linked to viscosity (μ) from the equation (2.6):

$$\frac{\mathrm{D_L}\mu}{\mathrm{T}} = \text{costante} \tag{2.6}$$

Lemmer and Ullrich [17] investigated four temperature levels between 40 and 55 °C using TBRs. The aim of their work was to observe the variations in the methane production and conversion of hydrogen and carbon dioxide at different temperatures. As shown in Table 2. 2, the conversion rate increased with temperature; this obviously leads to an improved gas quality.

Table 2. 2 Overview of the averaged operating parameters, flow rates and conversion, using TBRs (Lemmer and Ullrich [17]).

Aimed Temperature Level	**40**	**45**	**50**	**55**
Temperature [°C]	40.41±0.12	44.99±0.12	50.12±0.14	55.08±0.17
Pressure [bar]	5.21±0.03	5.22±0.02	5.21±0.03	5.20±0.04
pH	7.44±0.09	7.45±0.11	7.57±0.08	7.54±0.05
Flow H_2 [Lh^{-1}]	21.67	22.01	20.83	21.04
Flow CO_2 [Lh^{-1}]	5.46	5.59	5.27	5.29
CO_2 /H_2	1:3.96	1:3.94	1:3.95	1:3.97
MER	8.48±0.45	8.85±0.43	8.46±0.40	8.59±0.38
Retention time [h]	2.79	2.75	2.89	2.85
Conversion H_2 [%]	97.68±0.01	98.52±0.00	99.12±0.00	99.24±0.00
Conversion CO_2 [%]	96.42±0.00	97.51±0.00	97.88±0.00	98.10±0.00

2.2.8 Solubility

In order to be available to microorganisms, hydrogen has to cross the interface between the gas and the liquid phase. The aqueous solubility of most gasses is rather low, which limits the gas-liquid mass transfer and obstructs the performance of the bioreactor. The solubility of gases decreases with the temperature. As shown in Figure 2. 5, hydrogen has a very low aqueous solubility, compared to the ones of carbon dioxide, oxygen, and methane. Thus, at 50°C the solubility of these compounds in water is respectively about 0.8 g/kg, 0.014 g/kg, and 0.028 g/kg,

while hydrogen has a solubility of 0.0013 g/kg at the same temperature value. CO_2 solubility could be a limiting factor and it should be monitored, to not negatively affect the entire process.

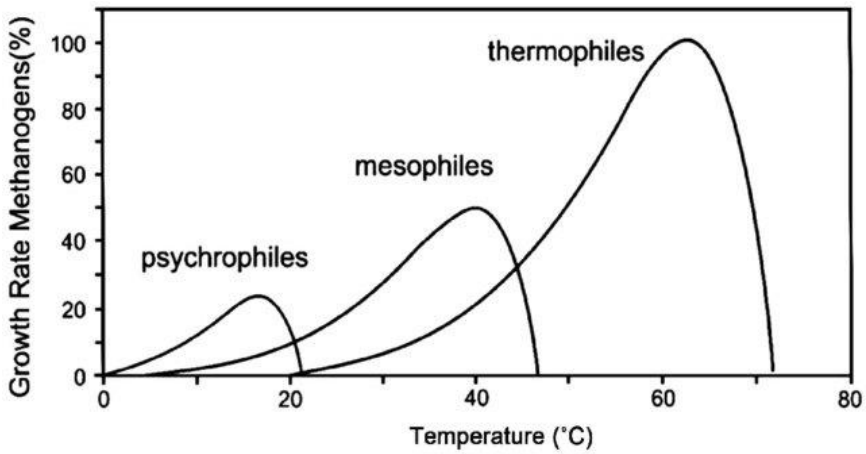

Figure 2. 4 Relative growth rate of methanogens depending on the temperature [21].

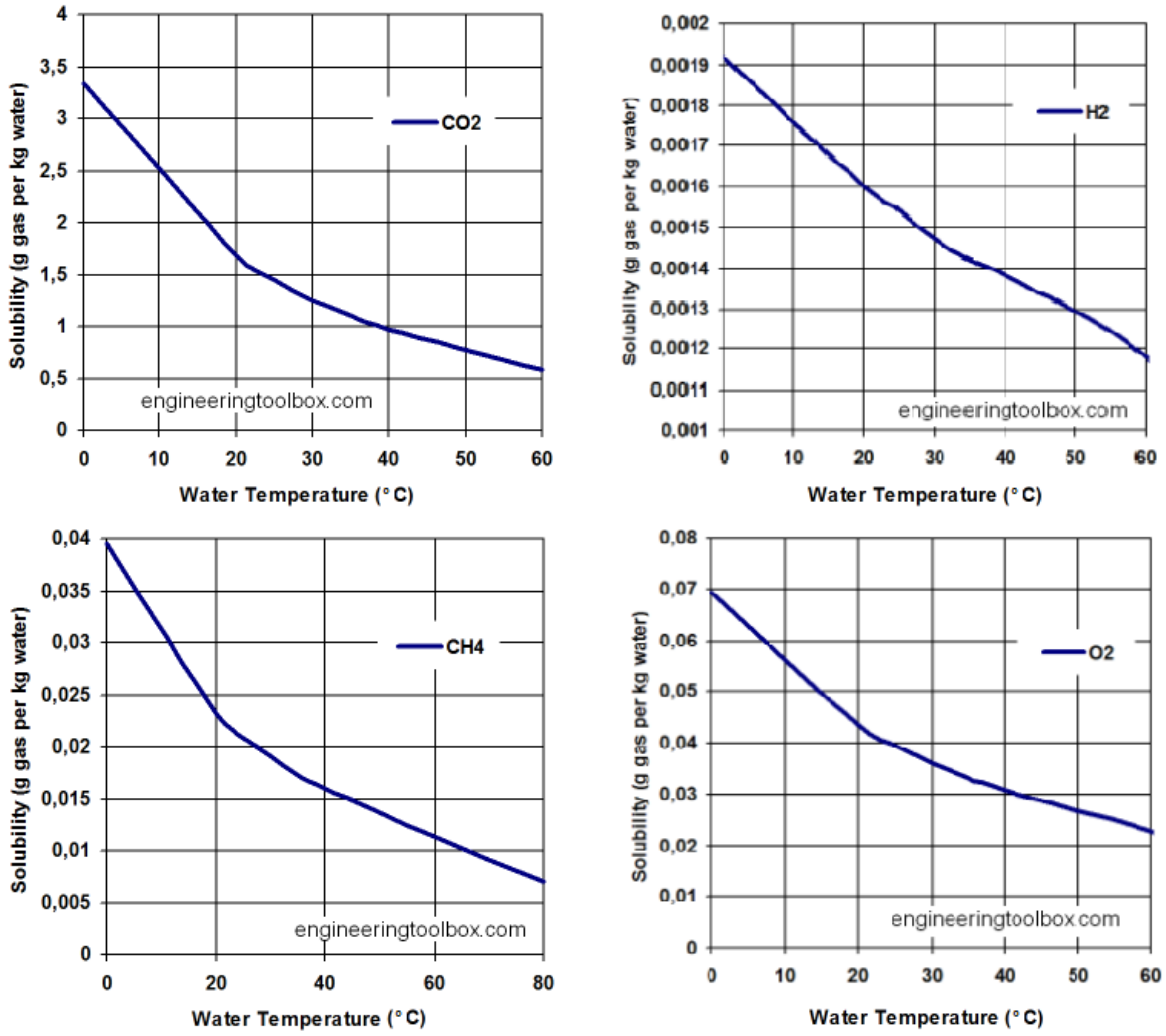

Figure 2. 5 Aqueous solubility of CO_2, H_2, CH_4 and O_2 (g gas per kg water) depending on the temperature (°C) (engineeringtoolbox.com).

2.2.9 Gas-liquid mass transfer rate

$D_L\mu$ and T influence a further operational parameter of biological methanation: the volumetric gas-liquid mass transfer coefficient, k_La (h^{-1}), which indicates the ability of the system to diffuse specific gases in a liquid. The gas-liquid mass transfer rate of hydrogen is described by Bassani's equation:

$$r_t = 22.4 k_L a\left(H_{2g} - H_{2l}\right) \qquad [LL^{-1}h^{-1}] \tag{2.7}$$

In (2.7) $r_t$is the gas-liquid mass transfer rate ($LL^{-1}h^{-1}$), 22.4 is the molar volume, H_{2g} (mol/L) is the concentration of hydrogen in gaseous phase and H_{2l} (mol/L) is the concentration of hydrogen in liquid phase. The coefficient k_La is a characteristic parameter of the reactor used, therefore it must be taken into consideration when choosing the reactor.

Table 2. 3 k_La values as a function of the inlet gas rate (Peillex et al. [13]).

	Straight blade impeller			**Rushton impeller**		
Stirring velocity (rpm)	320	660	1015	320	660	1015
Inlet gas rate [L/(L·min)]	0.65	0.8	2.4	0.67	1.7	2.1
CH_4 (%)	42	52	44	24	32	40
k_La	1200	1450	3550	1100	3250	3750

2.2.10 Gas and Liquid Holdup, Effective Surface Area

The holdup is referred to multiphase flows; it is the fraction of a particular fluid present in an interval of volume. The gas holdup represents the amount of gaseous phase V_G related to the reaction volume $V_{Reaction}$, according to (2.8). On the other hand, the liquid holdup describes the liquid volume related to the reaction volume, as showed in (2.9).

$$\varepsilon_G = \frac{V_G}{V_{Reaction}} \qquad [-] \tag{2.8}$$

$$\varepsilon_L = \frac{V_L}{V_{Reaction}} \qquad [-] \tag{2.9}$$

The effective surface area for gas-liquid mass transfer a_{eff} is the ratio of the specific surface area A_{spec} (m^3/m^3) within the reactor volume and the reaction volume (2.10). The A_{spec} for a TBR is the specific surface of the packing, while for CSTR and BCR it is the total surface of dispersed gas bubbles and for MR, it is the total active membrane surface (Thema *et al.* [18]).

$$a_{eff} = \frac{A_{spec}}{V_{Reaction}} \qquad [m^{-1}] \tag{2.10}$$

2.2.11 Stirring systems

The stirring instruments allow to reach the final production targets of the desired product, with good kinetics; moreover, they allow the respect of the safety conditions due to the heat exchange between the reagent system and the environment. The agitation influences the degree of mixing in the first analysis, which makes it possible to increase the number of molecules that come in contact with each other at the same time and, therefore, increases kinetics and process times. Kinetics in turn influences heat exchange: in the case of exothermic reactions, such as the one analysed in the case of methanation, as kinetics increases, the heat flow generated by the reagent system increases [12].

Agitators can be classified into three categories, depending on their operation: impeller systems, circulation pump systems and gas blowing systems.

The geometry of the agitator used influences the $k_L a$ coefficient; Peillex *et al.* [13] compared the Rushton turbine with a flat-blade mixer: the result was that, at the same speed of rotation (660 rpm), the Rushton type turbine allowed a $k_L a$ value 124% higher than the blade turbine (Table 2. 3).

The increase in rotation speed has positive effects on microbial growth and methane yield, increasing the value of $k_L a$. This advantage is, however, accompanied by the resulting increase in energy consumption, which cannot be overlooked.

2.2.12 Heat exchange systems

The management of the heat to be supplied or disposed of during a process is one of the most problematic aspects. The simplest way of heat exchange is through the hot currents coming out of the reactor; however, this system is not sufficient, so additional systems are required.

Heat exchange equipment is classified according to heat transfer mode, number of fluids participating in the heat exchange, flow directions, heat exchange mechanism and construction. The main techniques used to subtract the reaction heat are [12]:

- Partial recycling of the cold product.
- External coating filled with water.
- External cooling jacket with condenser with vaporisable liquid.
- Internal serpentine.
- Cooling by external condenser with vaporisable liquid.

Recirculation is not recommended in case of highly exothermic processes.

2.2.13 Reactor operating pressure

Increases in operating pressure correspond to an increase in the solubility of gases in liquids and a reduction in the size of gas bubbles: the result is a greater area of contact between microorganisms and gaseous substrates. In addition, reactor pressurisation is advantageous

because it facilitates subsequent injection of the gas produced into the natural gas network, where higher pressure is required.

Table 2. 4 Growth media of methanogenic archaea in literature (Rusmanis et al. [7]).

	Shill *et al.*, 1996 (g/L)	Peillex *et al.* [13] (g/L)	Nishimura *et al.*, 1991 (g/L dist. H_2O)	Voelklein *et al.* [15] (g/L dist. H_2O)	Rachbauer *et al.*, 2016 (g/L)
Na_2SeO_3	$1.73 \cdot 10^{-4}$			$5 \cdot 10^{-3}$	$1.26 \cdot 10^{-4}$
Na_2W04	$2.94 \cdot 10^{-3}$				
NaCl	$58.4 \cdot 10^{0}$	$40 \cdot 10^{0}$	$6.1 \cdot 10^{-1}$	$6.1 \cdot 10^{-1}$	$300.0 \cdot 10^{-3}$
NH_4Cl	$6.419 \cdot 10^{0}$	$2.5 \cdot 10^{-1}$	$1 \cdot 10^{0}$	$1 \cdot 10^{0}$	$300.0 \cdot 10^{-3}$
Nitrilotriacetic acid (NTA)	$2.29 \cdot 10^{-1}$				
$MgCl_2 \cdot 7H_2O$	$2.21 \cdot 10^{-1}$	$2.75 \cdot 10^{0}$			$100.0 \cdot 10^{-3}$
KH_2PO_4	$1.361 \cdot 10^{0}$	$3 \cdot 10^{-1}$	$3 \cdot 10^{-1}$	$3 \cdot 10^{-1}$	$408.0 \cdot 10^{-3}$
$CoCl_2$	$3.25 \cdot 10^{-4}$				
$CoCl_2 \cdot 6H_2O$				$6 \cdot 10^{-3}$	$1.0 \cdot 10^{-3}$
Na_2MoO_4	$5.15 \cdot 10^{-4}$				
$NiCl_2$	$6.48 \cdot 10^{-4}$				
$NiCl_2 \cdot 6H_2O$					$3 \cdot 10^{-4}$
$FeSO_4 \cdot 7H_2O$	$5.56 \cdot 10^{-2}$		$3 \cdot 10^{-3}$		
$Fe(NH_4)_2(SO_4)_2$		$2 \cdot 10^{-3}$			
$(NH_4)_2(SO_4)_2$			$3 \cdot 10^{-1}$		
$(NH_4)_2SO_4$				$3 \cdot 10^{-1}$	
KCl		$3.4 \cdot 10^{-1}$			
$CaCl_2 \cdot 2H_2O$		$1.4 \cdot 10^{-1}$	$8 \cdot 10^{-3}$		$110.0 \cdot 10^{-3}$
$MgSO_4 \cdot 7H_2O$		$3.45 \cdot 10^{0}$	$1.6 \cdot 10^{-1}$	$1.6 \cdot 10^{-1}$	
Resazurine			$1 \cdot 10^{-3}$		
$Na_2S \cdot 9H_2O$					$360.3 \cdot 10^{-3}$
$NaHCO_3$			$5 \cdot 10^{0}$		
Trace minerals soln.			$10 \cdot 10^{0}$		
Vitamins solution			$10 \cdot 10^{0}$		
Yeast extract			$2 \cdot 10^{0}$		
Trypticase			$2 \cdot 10^{0}$		
Conc. HCl					$1.0 \cdot 10^{-3}$
H_3BO_3					$5 \cdot 10^{-5}$
$ZnCl_2$					$7 \cdot 10^{-5}$
$CuCl_2 \cdot 2H_2O$					$5 \cdot 10^{-5}$
$MnCl_2 \cdot 2H_2O$					$2.0 \cdot 10^{-3}$
$H_{24}Mo_7N_6O_{24} \cdot 4H_2O$				$5 \cdot 10^{-3}$	
$(NH_4)Mo_7N_6O_{24} \cdot 4H_2O$					$1 \cdot 10^{-5}$
$AlCl_3 \cdot 6H_2O$					$9 \cdot 10^{-5}$
EDTA (Disodium salt)					$1 \cdot 10^{-3}$
$FeCl_2 \cdot 4H_2O$					$2 \cdot 10^{-3}$
$FeCl_3 \cdot 6H_2O$				$5 \cdot 10^{-5}$	
$Cl_2Ni \cdot 6H_2O$				$5 \cdot 10^{-3}$	

2.2.14 Biomass growth

Considering the stoichiometric ratio between hydrogen and carbon dioxide of 4:1, it has been seen that 6.4% of added CO_2 is used by microorganisms for their cell growth. There is, therefore, a close correlation between microbial growth and methane yield; where cell culture is not adversely affected by the gaseous substrate, biomass growth and methane yield grow rapidly in parallel.

2.2.15 Nutrients and other supplements

The microorganisms are cultivated in a nutrient solution, also known as growth medium. This has to recreate the natural habitat of the methanogenic archaea to allow the optimal growth conditions and performances. The addition of trace elements and nutrients favours the BHM, allowing the thriving of higher culture densities, thus increasing the overall performance of the reactor.

While performing a biological methanation, hydrogen and carbon as nutrients are provided as hydrogen gas and carbon dioxide. If the organisms grow autotrophically, which means that they use the carbon dioxide as a source for the synthesis of organic cell material, no other carbon compounds are needed for the methanogenic metabolism. Other elements are usually supplied in form of inorganic salts or organic compounds, e.g., sodium sulfide, Na_2S, is often used as a source of sulfur and can also serve as reducing agent (Thema *et al.* [18]). Sulfur is a necessary element for biosynthetic reactions and maintenance of a low redox potential (Rusmanis *et al.* [7]).

Table 2. 4 shows a comparison of the nutrient media supplied to microbes by different authors found in the literature (Rusmanis *et al.* [7]). This is due to the limitation in the availability and complexity of nutrient media.

The addition of external nutrients is necessary in ex-situ BHM, due to the lack of solid feed addition.

2.2.16 Gas injection systems

The gas injection system influences the size of the bubbles in the bioreactors. The use of HFM-type membranes has led to good results in previous studies regarding hydrogen injection; however, due to disadvantages such as increased gas inlet pressure and biofouling, these membranes have not been used for long-term experiments (Lecker *et al. [6]*). Other injection methods consist of ceramic diffusers, column diffusers, diffuser rings (perforated ring pipes).

The entry of hydrogen into the reactor can be regulated by the use of a peristaltic pump; this uses compression and decompression to move the gas through a pipe; it has no valves, seals and cable glands, which makes it economical from a maintenance point of view; the pipes that compose it allow the dosing of the fluid to be injected and have high resistance to abrasion.

2.2.17 Gas and biomass recirculation

Gas recirculation prolongs the contact time between the microorganisms and the gas, improves the addition of hydrogen and stimulates the conversion of substrates by methanogenic hydrogenotrophic microorganisms. The high speed of the gas through the reactor, however, can cause a reduction in the contact time between microorganisms and the gaseous substrate.

Recirculation allows for greater gas availability due to longer gas residence time and this process allows for better mixing.

Biomass recirculation takes place by means of microfiltration or centrifugation of the effluent released by the reactors; previous studies show that biomass recirculation allows to obtain a concentration of biomass in the reactor equal to 6 times what would be obtained with a classic stationary culture.

2.3 Laboratory and pilot scale projects: analysis of existing literature

Rusmanis *et al.* [7] made a comparison between different projects carried out so far. Table 2. 5 summarizes some of these among those with the highest percentage of methane gas production. Each project is identified with a number (N° R) to facilitate the subsequent analysis and calculation phases. It is also important to consider the hourly production of methane, in relation to the reactor volume (MER), to understand the extent of the process. The highlighted projects will be further investigated later.

Table 2. 5 Comparison between a few lab-scale projects analysed by Rusmanis et al. [7]

N°R	Ractor type	V_R (L)	HRT, GRT (h)	T (°C)	P (barg)	k_La (h^{-1})	H_2 rate [L/(L_Rd)]	CH_4 %	MER [L/(L_Rd)]	pH	Authors
						In-situ					
1	CSTR	1	8	55	1.5	7	1.872	94	0.5	7.8	Luo G., Angelidaki I., 2012
2	CSTR	1	4	55	1.5	11	3.6	95	0.9	7.8	
3	CSTR	1	2	55	1.5	20	14.4	90	1.6	7.8	
4	CSTR	1	2	55	1.5	21	14.4	94	1.6	7.8	
5	CSTR	1	1	55	1.5	40	14.4	91	3.2	7.8	
6	HFM	1	45.89	55	0.56		1.44	90	0.9	7.9	[14]
7	HFM	1	28.7	55	0.75		1.728	96	0.9	8.31	
						Ex-situ					
8	CSTR	1.5	0.025	65	0		1195.2	90	270.1	6.8	[13]
9	CSTR	1.5	0.03	65	0		1195.2	97	289.8	6.8	
10	CSTR	9.5	24	55	0.2		15.408	96	3.7	8.5	[15]
11	TBR	88	4	37	0		4.896	98-100	1.2	7.3	[9]
12	TBR	88	4	37	0		6.048	98	1.5	7.3	
13	TBR	7.54	3	37	0		5.76	98	0.9	7.5	Rachbauer et al, 2016
14	TBR	7.54	3.2	37	0		5.472	97	0.8	7.5	
15	HFM	31	1.21	55	0	205	25.056	82	5.8	7.2	Diaz *et al.*, 2015

The methane evolution rate is useful not only to assess the amount of methane produced, but also to get an idea of the thermal exchanges that take place inside the reactor. As has been anticipated in the previous paragraphs, in fact, Sabatier's reaction is strongly exergonic: the reactor sees a heat production inside it. The value of this production is important for the safety of the process itself, but also for the energy to be supplied to keep the reactor at the temperature necessary for the reaction to take place. In addition, it may be interesting to see whether this generated heat output can be used for other purposes, such as heating sewage sludge in a wastewater treatment plant.

The heat output per unit volume of the reactor, produced during the hydrogen and carbon dioxide methanation process, is calculated using the equation (2.11):

$$P_t = \frac{\Delta H^0 \cdot MER}{v_{mol} \cdot 86.4} \qquad \left[\frac{W}{L_R}\right] \tag{2.11}$$

In (2.1) the thermal power P_t is expressed in W/L_R; ΔH^0 is the enthalpy referred to the Sabatier reaction and it is equal to -165 kJ/mol; MER is expressed in $L_{CH_4}/(L_R d)$ represents the volume occupied by an ideal gas mass and is equal to 22.4 L_{CH_4}; 86.4 is the conversion factor. Considering the parameters shown in Table 2. 5, Table 2. 6 contains the thermal power values calculated for the projects analyzed.

Table 2. 6 Evaluation of the heat power (P_t) produced during Sabatier reaction.

N° R	MER [$L_{CH4}/(L_R d)$]	P_t [W/L_R]
1	0.5	$4.26 \cdot 10^{-2}$
2	0.9	$7.67 \cdot 10^{-2}$
3	1.6	$1.36 \cdot 10^{-1}$
4	1.6	$1.36 \cdot 10^{-1}$
5	3.2	$2.73 \cdot 10^{-1}$
6	0.9	$7.67 \cdot 10^{-2}$
7	0.9	$7.67 \cdot 10^{-2}$
8	270.1	$2.30 \cdot 10^{+1}$
9	289.8	$2.47 \cdot 10^{+1}$
10	3.7	$3.15 \cdot 10^{-1}$
11	1.2	$1.02 \cdot 10^{-1}$
12	1.5	$1.28 \cdot 10^{-1}$
13	0.9	$7.67 \cdot 10^{-2}$
14	0.8	$6.82 \cdot 10^{-2}$
15	5.8	$4.94 \cdot 10^{-1}$

As previously mentioned, Table 2. 5 contains some of the laboratory scale biomethanation projects carried out in recent years, which have a higher concentration of methane than the

others. However, this parameter cannot be taken into consideration without also evaluating the methane production rate. Therefore, among the listed projects, it has been chosen to investigate those with the best combinations of CH_4 and MER content.

Among the in-situ processes Luo and Angelidaki [14] have realized a project with an HFM type reactor, with in-situ configuration and methane yield of 0.9 $L_{CH_4}/(L_R d)$ and concentration of 96%.

As far as ex-situ configurations are concerned, the most significant project is the one carried out by Peillex *et al.* [13] which, with a methane percentage of 97%, reached a MER value of 289.8 $L_{CH_4}/(L_R d)$, using a CSTR reactor. Voelklein *et al.* [15] realized a lab scale project with methane production of 3.7 $L_{CH_4}/(L_R d)$ and concentration of 96%. Finally, Burkhardt *et al.*, 2015 [9] reached through a TBR reactor a concentration of 98% with MER of 1.5 $L_{CH_4}/(L_R d)$.

An interesting project at pilot-scale was carried out by Strübing *et al.* [19], that studied a trickle bed reactor in thermophilic and anaerobic conditions. In fact, at the end of the work (after 313 days), they found a final product with 98.5% of methane and a MER of 15.4 $L_{CH_4}/(L_R d)$.

Materials, operating methods and parameters are explained below, as well as some of the encountered problems in the mentioned projects.

2.3.1 Materials and methods

2.3.1.1 HFM reactor with in-situ configuration (Luo and Angelidaki, [14])

The system created by Luo and Angelidaki consists of two identical CSTR reactors (A and B) with a capacity of 600 mL in the preliminary phase the reactors were inoculated with digested manure and fed with a mixture of manure and whey from cattle, under thermophilic conditions and with HRT equal to 15 d. The use of these co-substrates is necessary to maintain favourable acidity conditions for AD. The reactors are mixed by means of a magnetic mixer with a rotation speed of 150 rpm and are fed once a day.

The HMF module was installed after 1.5 months in the A reactor; the HMF module consists of a beam of 400 membranes and is of the Mitsubishi Rayon brand, model MHF 200TL. The total surface area of the model covers an area of 713 cm^2. Hydrogen was pumped into the membrane module by a gas bag with a capacity of 2 L through a gas-tight neoprene tube. The daily flow rate of hydrogen was calculated from the difference between the initial and residual hydrogen in the gas bag using a gas-tight syringe (100 mL). Due to the speed variations of the peristaltic pump (2, 3, 4 rpm) different H_2 flow rates were obtained. A gas pressure meter made it possible to monitor the pressure inside the membrane.

2.3.1.2 Batch reactor with ex-situ configuration (Voelklein *et al.* [15])

The methanation takes place in a silage fed digester. The system, as shown in Figure 2. 6, consists of a Batch ex-situ type reactor made of stainless steel; this has a volume of 9.5 L with a diameter of 0.15 m and a height of 0.6 m. The quantities of gases injected into the reactor (hydrogen, carbon dioxide and methane) have been measured by means of a Ritter drum gas meter, model TG5/5, resistant to highly corrosive gases and capable of measuring even very low flow rates. The gas is stored in a gas bag with a capacity of 100 L before the gas is recirculated through a ceramic gas diffuser. Quantification of the output gas is carried out by means of a Ritter drum gas meter, model TG5/5 and a Hewlett Packard gas chromatograph (HP6890). Stirring was not necessary in the batch ex-situ experiment.

The process was carried out under thermophilic conditions (55 °C) and ambient pressure. It was divided into three major stages: Batch ex-situ (BES 1 and BES 2) with hydrogen and carbon dioxide injection, continuous ex-situ with hydrogen and carbon dioxide injection and continuous ex-situ with hydrogen, carbon dioxide and methane injection. The focus of this paragraph is the first stage, since the other two investigated the impact of a steady gas injection and release, whereas the BES reactor is fed once a day for a 24-hour upgrading period. Gases were introduced and recirculated through a ceramic gas diffuser after compression to 2 bar. The gas residence time has a value of 24 h. The values in Table 2. 4 are referred to BES 2.

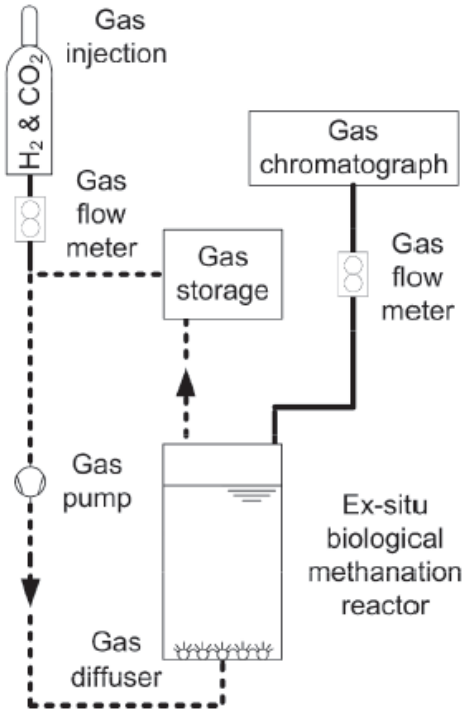

Figure 2. 6 Schematic of the project layout for the Batch ex-situ reactor (Voelklein et al., 2019).

2.3.1.3 CSTR reactor with ex-situ configuration (Peillex *et al.* [13])

The process was carried out at 65 °C and mediated by Methanococcus thermolithotrophicus microorganisms, inside a fermenter built in the laboratory; this has a capacity of 1.5 L and is made of glass; it has an antifoam control instrument, a pH meter, a Teflon porous disc, to facilitate the entry of bubbles in the middle, an impeller above the disc. A Rushton type mixer has been chosen for mixing. The fermenter was equipped with a level regulator and has a single outlet for liquid and gas. Before being filled with the substrate and inoculum, the reactor was sterilised and gassed with a mixture of H_2/CO_2.

After the microbial growth had reached an optical density of 2, the substrate was continuously renewed using a peristaltic pump with a rate ranging from 0.08 h^{-1} to 0.22 h^{-1}. The gas flow rate coming out of the reactor was variable in the range of 6-12 L/h.

Measurements were made to assess microbial growth and concentrations of CH_4, CO_2 and H_2. In the first case the cell mass (dry weight) per mole of methane was measured under optimal growth conditions. A gas chromatograph with argon as carrier gas and a thermal conductivity detector were used to measure the gases.

2.3.1.4 TBR reactor with ex-situ configuration (Burkhardt *et al.* [9])

The reactor, shown in Figure 2. 7, consists of a percolator filter, filled with Bioflow 40 produced by the RAUSCHERT company. The microorganisms were immobilized using digested sludge from a local wastewater treatment plant.

The metabolic activity of the microorganisms takes place only in the liquid phase: it was necessary to continuously wet the packing material and, consequently, a continuous trickling flow in the filter was necessary. The process flow was recirculated with a constant flow rate of 10.7 $Nm^3/(m^3_{FB}/d)$. The reactor was fed directly through the injection of a defined volume of H_2/CO_2, through a gas bag with a capacity of 20 L, produced by TESSERAUX. The injection of the gas and its consequent circulation in the reactor was possible thanks to the use of a variable performance gas-tight pump. The reactor was completely mixed. As for the feed gas, a small portion of the injected carbon dioxide is consumed by microorganisms as a source of carbon for their metabolic growth; hydrogen, on the other hand, is always present in large quantities.

During the production of methane there has been a reduction in volume and throughout the process samplings have been taken to observe the composition of the gas. The productivity of the gas under standard conditions, the temperature and pressure of both the influent and the effluent were measured. Table 2. 7 summarizes the main design parameters.

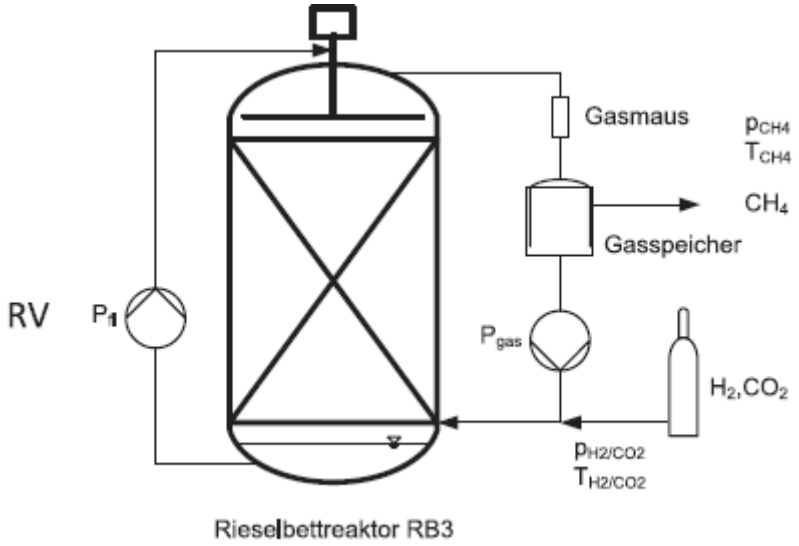

Figure 2. 7 Schematic representation of the trickle bed reactor (TBR) for the conversion of gaseous substrates into methane (Burkhardt et al., 2015).

Table 2. 7 Project parameters (Burkhardt et al. [9]).

Substrate	H_2/CO_2 (g)	*Shape factor l:d*	1:0.81
Operative mode	Batch	*Packing material*	Bioflow 40
Trickle bed filter volume	26.8 L	*Recycling rate*	10.7 $Nm^3/(m^3_{FB}/d)$
Process water volume	5 L	*Inlet flux*	2.3-11.6 $Nm^3/(m^3_{FB}/d)$

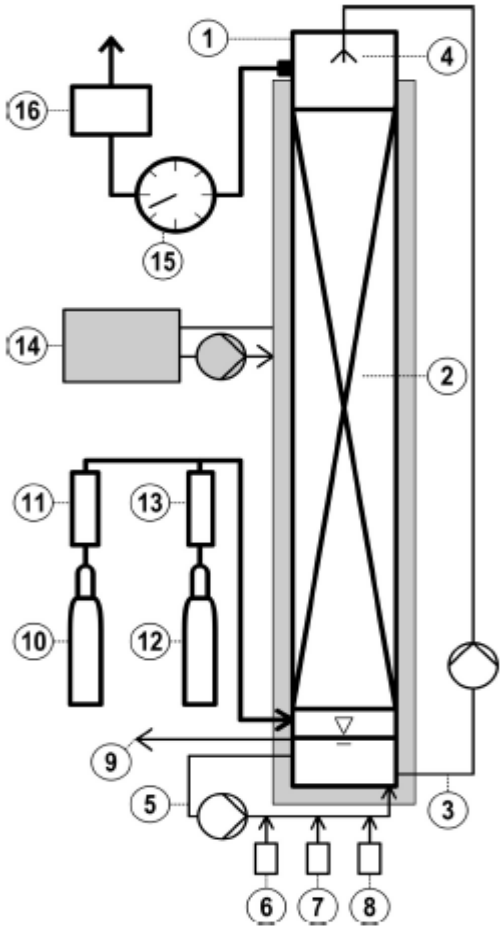

Figure 2. 8 Schematic of the TBR system utilized by Strübbing et al. [19]. (1) TBR, (2) packed trickle bed, (3) trickling liquid circuit, (4) spraying nozzle, (5) liquid recirculation, (6) pH buffer solution, (7) sulfide solution, (8) trace element solution, (9) excess liquid withdrawal, (10) H_2 gas bottle, (11) H_2 mass flow controller, (12) CO_2 gas bottle, (13) CO_2 gas controller, (14) thermostat, (15) drum gas counter, (16) gas analyser.

Table 2. 8 Technical parameters of the pilot-scale project by Strübbing et al. [19].

Operative mode	Batch	*Substrate*	H_2/CO_2 (g)
Operative temperature	55±1 °C	*Buffer solution*	K_2HPO_4
Shape factor l:d	7.4	$V_{Trickle\ bed}/V_{Liquid}$	5.48
Trickle bed filter volume	58.1 L	*Trickling liquid volume*	10.6 L
Hydrogen gas feed rate	1.7-62.1 $m^3_{H_2}/(m^3_{trickle\ bed}\cdot d)$	*Trickling liquid circulation rate*	10 L/h
Packing materials	RFK 25 L (lower); Hel-X bio carrier HXF12KLL (upper)		

2.3.2 Encountered problems

In the study of Luo and Angelidaki [14], during the first phase of work (20 days), there was an increase in H_2 pressure, from 0.23 to 0.32 bar inside the HFM module. This increase might be

caused by the formation of the biofilm on the HFM surface, which increased the resistance of H_2 diffusion from inside of the hollow fiber to the liquid. Also, SEM was able to observe the presence of the biofilm. When using a membrane biofilm reactor, biofilm formation is very important to retain the microorganisms. However, this study did not require biofilm formation, since there was already sufficient microbial activity in the liquid; thus, the biofilm formation decreased the efficiency of the hydrogen supply. Moreover, increased pumping speed was needed and thereby the energy consumption should be increased to maintain a constant supply of H_2 to the reactor. The problem of increasing the H_2 flow rate is followed by the importance of controlling the pH and maintaining it below 8.0 using on-line pH control.

In the experiment of Voelklein *et al.* [15], hydrogen in BES 1 was injected for a 24-hour period with a rate of 7.3 L H_2 $L_{VR}^{-1}d^{-1}$; hydrogen was completely consumed, resulting in a MER of 1.7 $L_{CH4}/(L_Rd)$ and methane concentrations as high as 92%. However, total gas conversion was not achieved, due to the premature depletion of hydrogen in the pre-configurated gas mixture. The problem was solved doubling the hydrogen loading to 15.4 L H_2 $L_{VR}^{-1}d^{-1}$ in BES 2, resulting in a MER equal to 3.7 $L_{CH4}/(L_Rd)$ with methane concentrations of 96%.

Peillex *et al.* [13] noticed that the initial growth was inhibited by a high gas flow rate; thus, it was necessary to progressively regulate the gas flow input.

In the test of Burkhardt *et al.* [9], the increase in the gas influent flow rate led to a breakthrough of hydrogen and carbon dioxide in the effluent flow, which implies an incomplete conversion in the reactor. This problem was solved thanks to the circulation. A retention time of 2.25 h led to a H_2 degradation rate of 80-95% and a subsequent methane concentration of 90%. The methane concentration increased to 94-97.9% after a retention time of 4 h. About 1.5% of volume in the effluent is occupied by carbon dioxide, that remains from a small biogas formation through the degradation of the organic matter contained in the process liquid. There is also a small percentage of nitrogen (about 2.5%) from the initial phase of the reactor.

A common problem reported by Thema *et al.* [18] is related to the formation of foam due to high cell densities of methanogenic archaea in the liquid volume V_L; at high gas throughputs, this phenomenon can cause plugging of gas and condensate pipelines or pumps and thereby damage the reactor and downstream equipment. Antifoam agents can be used in order to prevent foam-formation, e.g., oils, fatty acids, or esters.

Strübing *et al.* [19] observed a declining of the gas conversion from day 25 until day 47; this reduction could have been the lack of trace elements, mass transfer limitations, and an insufficient sulfur/sulfide supply. The first option was avoided by the continuous supplementation with trace element stock solution starting from day 39. In order to prevent mass transfer limitations, the hydrogen feed rate was reduced and kept constant in time. At day 47, a $Na_2S{\cdot}9H_2O$ solution was added, in order to increase the sulfide concentration to 0.3 mM in the trickling liquid; a sample taken at day 47, in fact, measured a sulfide concentration below 0.2 mM. After the injection, methane concentration immediately rose from 55% to 96%. This is an evidence of the importance of sulfur for the metabolism of methanogenic archaea. Moreover, Strübing *et al.* [19] confirmed the role of pH in the process: pH values below 6.2 caused a decline of gas conversion from 98% at day 63 to 85% at day 65, in accordance with previous studies. After increasing the pH value to 7.0 with the addition of NaOH and K_2HPO_3 buffer solution, the gas conversion recovered. After the increase in the gas feed rate, the excessive CO_2 dissolved in the reactor. Moreover, an increased metabolic water production could have diluted the trickling medium, thus reducing the buffer

capacity. Finally, homoacetogenesis produce acetate. The three listed factors may be the cause of the decrease in pH.

3 Biomethane production: the laboratory trial

As evidenced in Section 2, the TBR type reactor can be a simple solution for the realization of a BHM at laboratory scale. In this way the contact between microorganisms, liquid phase and gas phase can be easily controlled and increased, with respect to the other reactor types. Using a TBR with a proper packing material makes it possible to switch from a suspended biomass to an attached biomass system; thus, the formation of a layer of microorganism onto the packing material guarantees better growth rates (as previously explained in Section 2.2.1.1).

Nevertheless, the microbial growth rate is difficult to estimate, and it is one of the most important parameters affecting BHM. Thus, the aim of this chapter is to describe all the activities of the preliminary phases of the experimental laboratory trial, further expressed as *Start-up*, during which the microbial colony has been fed. Furthermore, during this period, some operational parameters have been changed day by day, in order to reach an optimization of the configuration. Other parameters have been monitored, to compute the effective methane production (MER) and the system efficiency. The Start-up process has been performed in a CSTR working in Fed-Batch conditions.

3.1 Materials and methods

The system has been studied in two different configurations, in order to find the combination which better optimizes the process. Figure 3. 1 outlines and makes it possible to compare the two configurations. Configuration 1 has also been studied in two different phases, which mainly differ in the reaction volume; the former is also the initial phase of the trial, while the latter is chronologically located after Configuration 2.

As previously mentioned, the Start-up system is performed with a CSTR, which works in Fed-Batch conditions. This type of operational mode consists in a system which has an open inlet and a closed outlet. It combines both continuous and discontinuous fermenters advantages and it is very common in biomass production processes. Nutrients are added in discontinuous way with low rates.

The reactor is a 2.6 L Schott bottle, filled with primary sludge, coming from the Wastewater Treatment Plant located in Castiglione Torinese (TO) and managed by SMAT S.p.A.; sludge coming downstream a primary settler is reach in hydrogenotrophic methanogenic bacteria, so it can be used as source of biomass. An electrical resistance heats the reactor, and a temperature-controlled thermostat by Inkbird ensures that the reactor remains at 38 °C. The process is performed at mesophilic conditions, in order to maintain the same conditions of the WWTP. The sludge is recirculated by means of a SEAFLO membrane pump with speed regulation; recirculation guarantees the continuous mixing of the biomass and enhances the contact with the gas phase; to maximise mixing, the sludge is collected from the bottom of the CSTR, and it is re-injected above the liquid level.

A gas mixture made of CO_2 and H_2 in a ratio of 1:4 is injected in the CSTR by means of a porous stone located in the lower part of the bottle. Thus, the gas mixture represents the principal

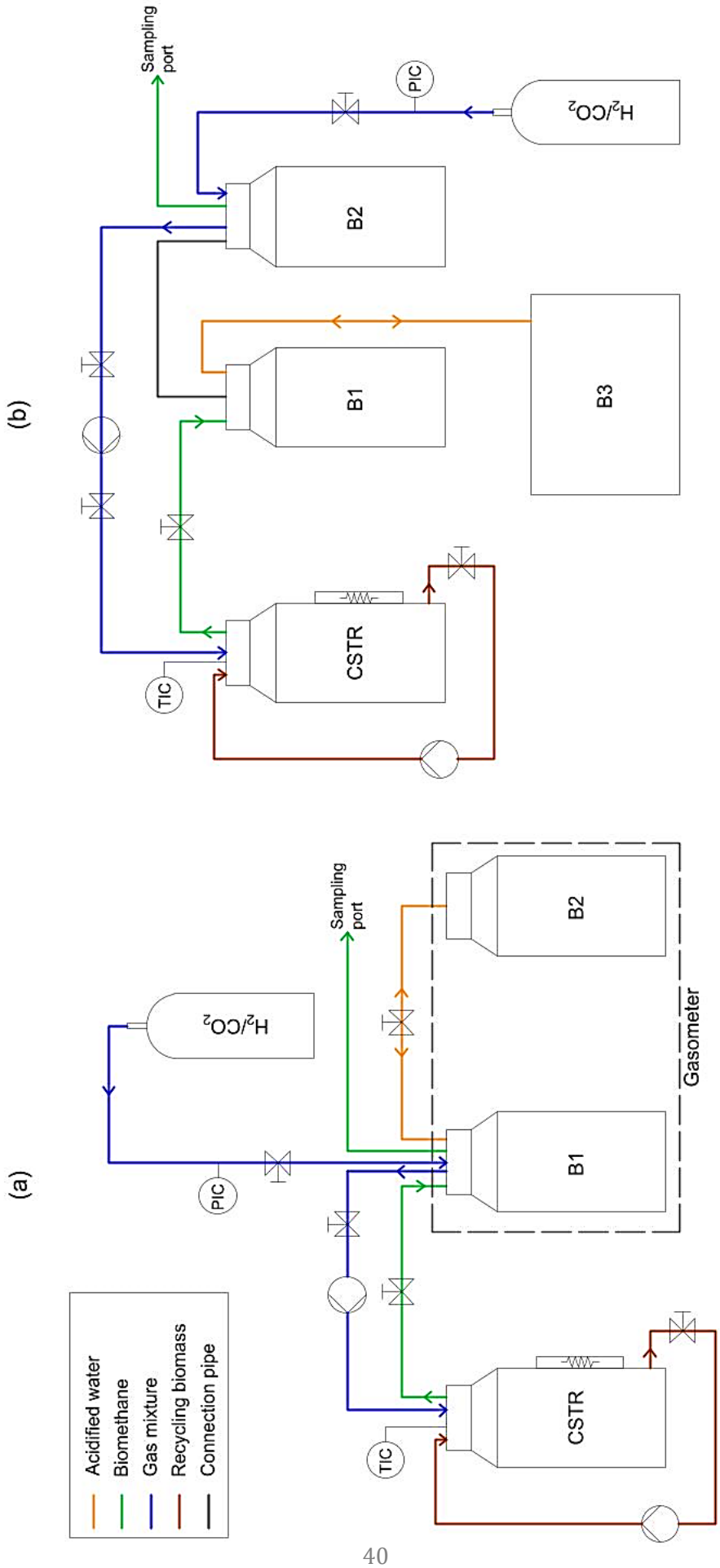

Figure 3. 1 Schematic of Configuration 1 (a), and Configuration 2 (b) for the biomethanation system.

nutrient for microorganisms, which consume it to produce methane. The porous stone has the aim of increasing the surface contact between the inoculated gas mixture and the sludge, which is in liquid phase. Before being injected, the gas mixture is pumped by means of a second membrane pump.

A small amount of gas (0.5 L) is extracted from a sampling port and analysed by the BIOGAS 5000 gas analyser (Geotech). The instrument computes the gas composition in terms of produced CH_4, residue H_2 and CO_2 and O_2. Since the process must be anoxic, it is very important to evaluate oxygen in the gas mixture. After sampling, the system is fed by injecting the H_2/CO_2 mixture, reaching a defined gas level, which changes from Configuration 1 to Configuration 2. A mass balance is useful to compute the effective methane production.

Table 3. 3 resumes the common features and the utilised instruments for the Start-up trial. The two mentioned configurations differ not only in the number of elements, bus also in the way that the gas mixture enters the CSTR and in the operating volumes.

Table 3. 1 Common features of the Start-up system.

Operative mode	Fed-Batch	*Operative temperature*	38±1 °C
Reactor type	CSTR	*Substrate*	H_2/CO_2 (g)
Biomass	Primary sludge	*CO_2 (%)*	20
Gas analyser	BIOGAS 5000 (Geotech)	*H_2 (%)*	80

Table 3. 2 Comparison between volumes in Configuration 1 and Configuration 2.

Configuration		Sludge volume, V_{sludge} (L)	Operating volume (L)	Number of operating bottles	Gas volume after H_2/CO_2 injection (L)
1	1st period	1.4	5.3	2	3.2
	2nd period	1.0	5.3	2	4.1
2		1.0	7.9	3	4.6

3.1.1 Configuration 1

Figure 3. 1(a) and Figure 3. 2 show that the system is mainly composed by the CSTR already mentioned and two bottles (B1 and B2 in Figure 3. 1(a)). The system has been studied in two periods, later named as Period 1 and Period 2, characterised by different sludge volumes: during Period 1, the CSTR is filled with fresh sludge, in volume of 1.4 L, while in the second period the volume is decreased to 1.0 L, in order to maximise the available gas volume. It is important to highlight that sludge in Period 2 is not fresh, but it is the sludge resulting from Configuration 2; thus, microorganisms are supposed to be grown, with respect to a fresh sludge.

B1 is directly connected to the CSTR, to the gas cylinder containing the H_2/CO_2 gas mixture and to B2. B1 and B2 work as a gasometer, i.e., they are filled with acidified water, which is marked with methyl orange solution and is free to move from one bottle to the other, following gas volume variations due to methane production. Indeed, according to Sabatier's equation (1.2), five moles of reactants (1 mole of CO_2 and 4 moles of H_2) produce only 1 mole of CH_4, resulting in a volume

Figure 3. 2 Configuration 1.

Figure 3. 3 Configuration 2.

reduction. Thus, since B2 is filled with acidified water and its head space is made of air, the two bottles are at the equilibrium. This means that a volume reduction due to methane production will lead to an acidified water transfer form B2 to B1. Hence, water level in B1 will increase.

The so composed system is in equilibrium, also thanks to the continuous mixing between the three bottles. Table 3. 2 resumes the system characteristic parameters.

3.1.2 Configuration 2

This setting has a more complex configuration: as it outlined in Figure 3. 1 (b) and showed in Figure 3. 3, the system is composed by three bottles (CSTR, B1 and B2) and a tank (named B3 in Figure 3. 1(b)). The tank allows to work with larger gas volumes, avoiding uncontrolled water overflows. Indeed, B3 plays the same role of B2 in Configuration 1: it guarantees more available volume, thus means free increasements or decrements of the water level in B1, due to CH_4 production.

In this configuration, the gas mixture enters the system from B2, which is connected to the pump that blows the gas into the CSTR. The produced biogas passes from the CSTR to B1 and then it goes into B2, where it is daily sampled. The pipe that connects B1 and B2 also allows acidified water flows, in case of huge gas volume reductions.

The CSTR is filled with 1.0 L of primary sludge, as reported in Table 3. 2.

3.1.3 The role of acidified water

Section 2.2.8 listed solubility as one of the main influencing parameters for BHM. CO_2 solubility in water is relatively high, i.e., CO_2 solubilization in water at 38 °C is equal to almost 1 g_{gas}/kg_{water}.

CO_2 solubility is strictly connected to pH: higher CO_2 concentrations in water mean lower pH and vice versa. Indeed, when CO_2 dissolves in water, the carbonic acid system is created; it includes 4 chemical species: carbonate and bicarbonate ions (respectively CO_3^{2-} and HCO_3^-), carbonic acid in dissociated form (H_2CO_3) and dissolved carbon dioxide ($CO_{2,aq}$). In many cases it could also include exchange with gaseous carbon dioxide ($CO_{2,g}$). The reactions from (3.1) to (3.4) represent the relationships between these chemical species (Morse et al. [20]); these equations can be combined, retaining the Henry's Law constant, resulting in the (3.5) as follows:

$$CO_{2(g)} \leftrightarrow CO_{2(aq)} \tag{3.1}$$

$$CO_{2(aq)} + H_2O_{(l)} \leftrightarrow H_2CO_{3(aq)} \tag{3.2}$$

$$H_2CO_{3(aq)} \leftrightarrow HCO_{3(aq)}^- + H_{(aq)}^+ \tag{3.3}$$

$$HCO_{3(aq)}^- \leftrightarrow CO_{3(aq)}^{2-} + H_{(aq)}^+ \tag{3.4}$$

$$CO_{2(aq)} + H_2O_{(l)} \leftrightarrow HCO_{3(aq)}^- + H_{(aq)}^+ \leftrightarrow CO_{3(aq)}^{2-} + H_{(aq)}^+ \tag{3.5}$$

The (3.5) shows that increasing $CO_{2(aq)}$ of a system will produce a $H^+_{(aq)}$ for each $HCO^-_{3(aq)}$; moreover, for each $CO^{2-}_{3(aq)}$ that is produced, $2H^+_{(aq)}$ are produced (Morse et al. [20]). The presence of hydrogen ion is indicative of the deep relationship between carbonic acid and pH: it can influence this value and the buffer capacity in water.

The carbonic acid system is regulated by four main parameters: pH, alkalinity, CO_2 partial pressure (P_{CO_2}) and total CO_2 ($\sum CO_2$). The relative distribution of the chemical species of carbonic acid system is generally showed as a function of pH and fractional amount of chemical species. Figure 3.4 represents this relationship: $CO_{2(aq)}$ and HCO^-_3 have the same concentration at pH=pK_1 and HCO^-_3 and CO^{2-}_3 have the same concentration at pH=pK_2, where K_1 and K_2 are the equilibrium constants at 25 °C: K_1 is related to the combination of (3.2) and (3.3), while K_2 is the equilibrium constant of the (3.4). Adding HCO^-_3 in the solution when $CO_{2(aq)}$ and CO^{2-}_3 does not influence the pH.

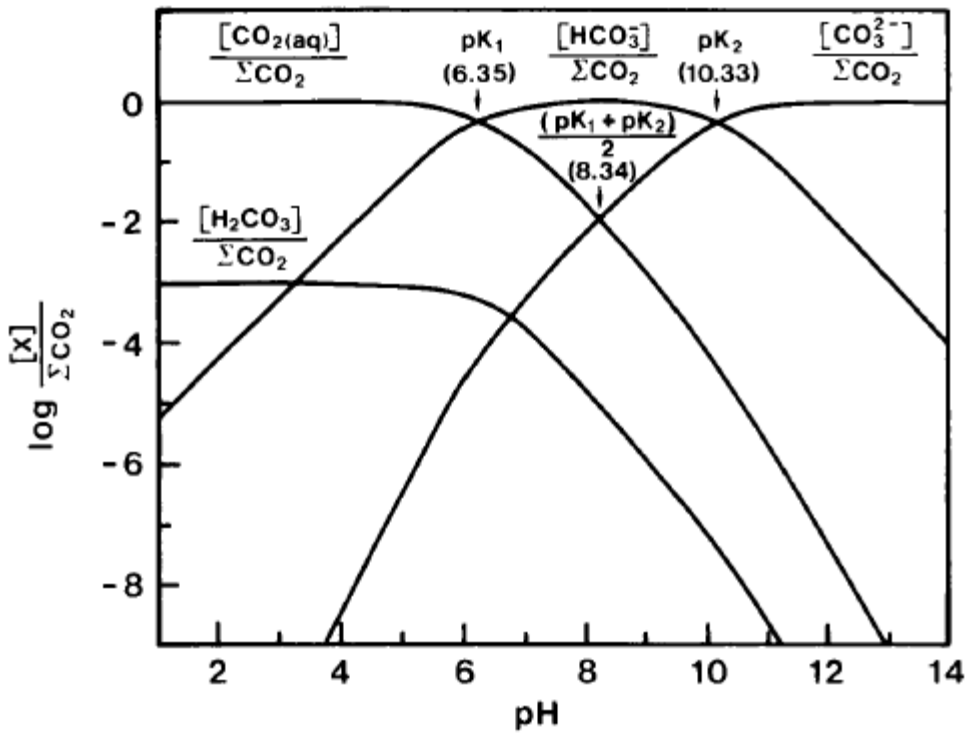

Figure 3. 4 Bjerrum diagram of carbonic acid solution. The pKi values are referred to T=25 °C (Morse et al. *[20]).*

The water below the H_2/CO_2 gas mixture in B1 contains HCl, which dissociates as follows:

$$HCl + H_2O \leftrightarrow H_3O^+ + Cl^- \tag{3.6}$$

HCl is a strong acid, thus a concentration of 10^{-3} M in water means pH = 3. The trial takes place in a laboratory in which other trials with specific needs are realised. For this reason, laboratory

temperature is about 27 °C. Van't Hoff equation defines the relationship between the equilibrium constants at different temperatures, as reported in (3.7):

$$\ln\frac{K_2}{K_1} = \frac{\Delta H^0}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \tag{3.7}$$

where $\Delta H^0 = 7646\ \mathrm{J/mol}$ is the heat of reaction, $R = 8.324\ \mathrm{J/(mol\ K)}$ is the gas equilibrium constant; $T_1 = 25\ °C$ and $T_2 = 27\ °C$. The equilibrium constant corresponding to 27 °C is expressed in the (3.8) as:

$$K_{1,27°C} = K_1 \exp\left(\frac{\Delta H^0}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)\right) = 4.538 \times 10^{-7} \rightarrow pK_{1,27°C} = 6.34 \tag{3.8}$$

$$pH < pK_1' \tag{3.9}$$

In a condition in which pH=3, CO_2 is present in the undissociated form.

In conclusion, by knowing the solution pH is possible to understand if CO_2 is present either in undissociated or in associated form and which are the concentrations of the single dissociated species. Moreover, acidified water is marked with methyl orange solution, which facilitates to detect pH variations and consequently understand in which form CO_2 is present in the bottles.

3.2 Results and discussion

The most important parameter that describes process efficiency is MER (see Section 2.2.4). As reported in (2.5), MER depends on methane flow rates. Nevertheless, gas injections and gas extractions are discontinuous, and they last form some seconds to some minutes, depending on the specific needs. This is due to reactor operational mode and for this reason, MER is computed using discrete values as reported in (3.17):

$$MER = \frac{V_{CH4,out} - V_{CH4,in}}{V_{sludge}\Delta t} \qquad \left[L_{CH4}/(L_{sludge}\,d)\right] \tag{3.17}$$

where $V_{CH4,out}$ (L) represents the volume of produced methane, evaluated by means of the measured gas concentration; $V_{CH4,in}$ (L) is the methane volume in the reactor after the H_2/CO_2 gas mixture daily injection; V_{sludge} (L) is the sludge volume, i.e., the volume in which methanation takes place; Δt (d) is the time between two subsequent gas samplings.

Comparing the system of this work with other similar trials, such as the one of Figeac *et al.* [22], it is expected to obtain MER of about 0.25 $L_{CH4}/(L_{sludge}\,d)$ at mesophilic conditions.

3.2.1 Configuration 1

3.2.1.1 Period 1

Period 1 is characterized by a fluctuating trend for MER and CH_4 concentration values. It is important to highlight that the early samplings of the trial have not been registered, because of a series of changings in system setting. Indeed, during the first days of trial, the gas mixture was injected in the CSTR by means of a peristaltic pump. Huge volume reductions were registered in bottle B1, with water retrieving from B2. A gas sample revealed that gas concentration was almost the same of the injected gas mixture, with a 5.7% O_2 concentration. This is an evidence of either gas leaks or air inlets in the system, thus peristaltic pump has been substituted with the membrane pump, because it was the weaker part of the configuration in terms of tightness.

Furthermore, initially CSTR contained 2 L sludge and it retrieved in B1, with the risk of compromising the acid-basic equilibrium of the system, thus damaging microorganisms. Sludge volume was then reduced firstly to 1.8 L and then to 1.4 L.

Analysing values in Table 3. 3. it can be observed a high MER in the first sampling (0.60 $L_{CH4}/(L_{sludge}d)$) corresponding to a satisfying methane concentration (52.3%). The day after a higher CH_4 concentration is registered, resulting in a lower methane production. The subsequent samplings register fluctuating methane concentration values, but MER is equal to 0.25±0.05 $L_{CH4}/(L_{sludge}d)$, as represented in Figure 3. 6. MER reduction in time may be caused by lack of available gas volume.

Table 3. 3 Results for Configuration 1 – Period 1.

Sampling day	Injected H_2 (L)	$V_{CH4,out}$ (L)	CH_4 (%)	Δt (d)	MER ($L_{CH4}/(L_{sludge}d)$)
1	2.65	0.84	52.3	1	0.60
2	1.60	1.04	64.6	1	0.25
3	1.60	0.66	41.2	0.25	0.30
4	1.20	0.74	46.0	0.71	0.20

Table 3. 4 Results for Configuration 1 – Period 2.

Sampling day	Injected H_2 (L)	$V_{CH4,out}$ (L)	CH_4 (%)	Δt (d)	MER ($L_{CH4}/(L_{sludge}d)$)
1	3.36	0.13	5.2	1	0.13
2	1.72	0.54	31.7	1	0.45
3	2.00	1.04	60.9	1	0.61
4	2.00	1.54	70.2	3	0.24
5	1.60	1.65	75.1	1	0.46
6	1.60	1.25	49.9	2	-0.01
7	1.28	1.68	67.0	1	0.83
8	1.36	1.30	76.5	1	0.16
9	2.00	1.42	83.3	1	0.12

3.2.1.2 Period 2

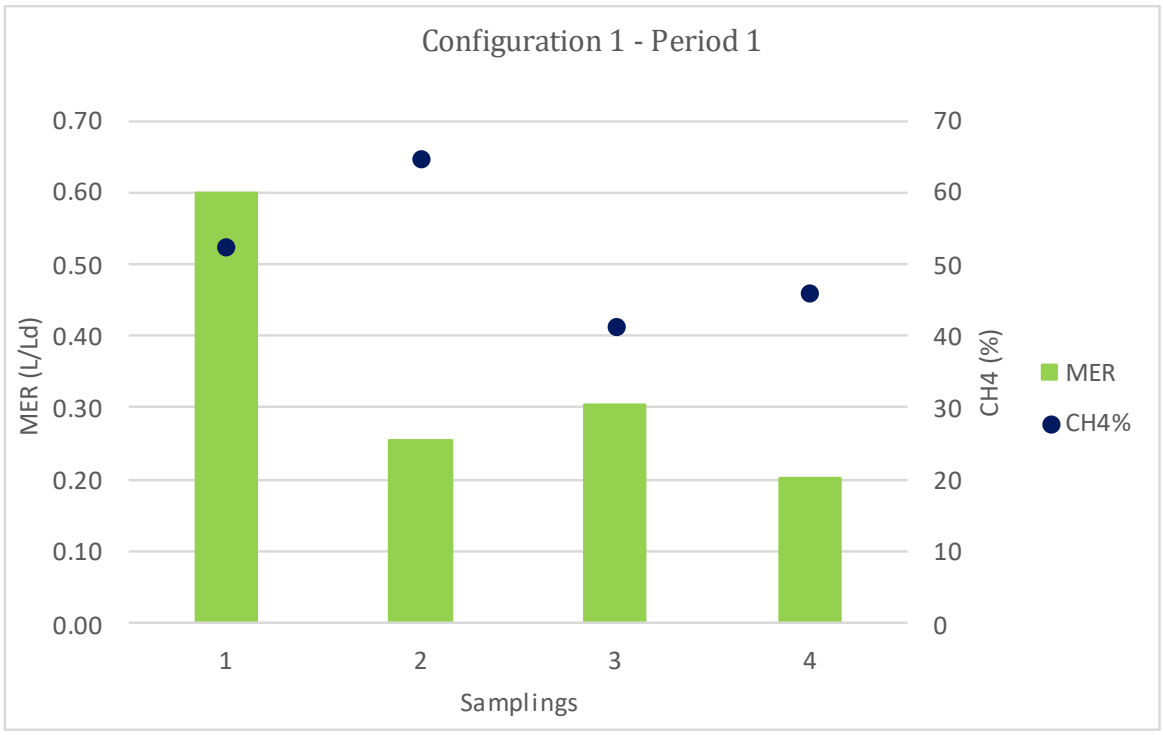

Figure 3. 6 Graphical representation of MER and CH4 concentration values in Period 1 of Configuration 1.

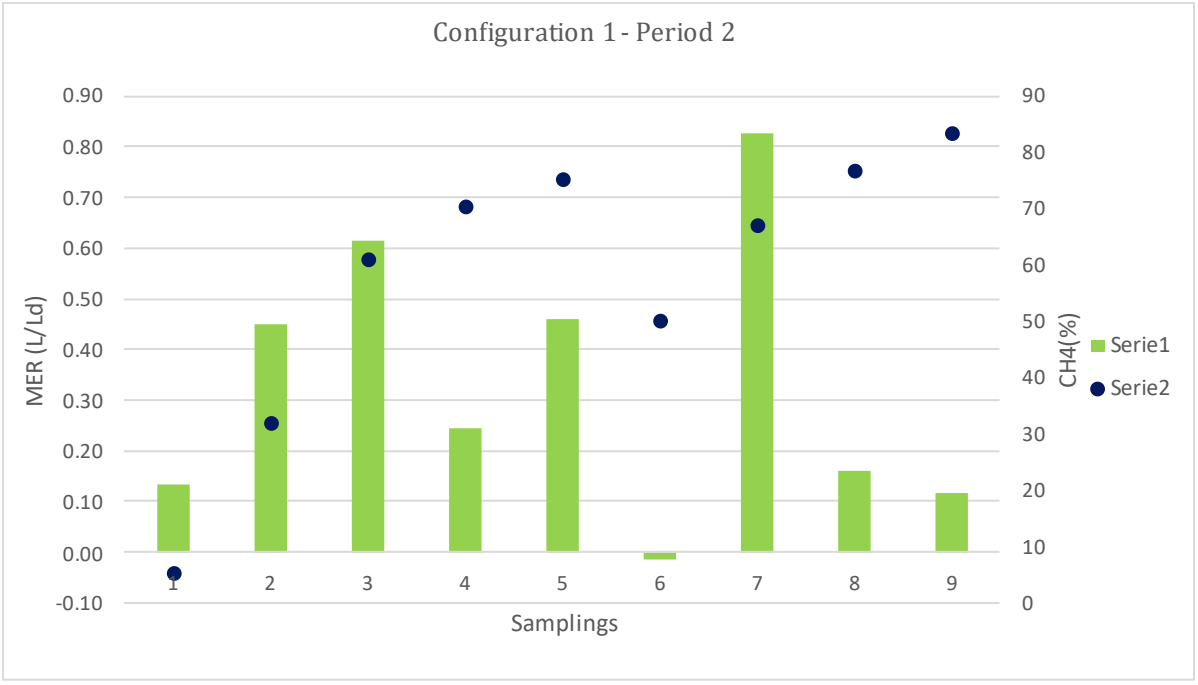

Figure 3. 5 Graphical representation of MER and CH4 concentration values in Period 2 of Configuration 1.

Figure 3. 5 shows that Period 2 has a more regular trend in the first sampling days with respect to Period 1. The initial CH_4 concentration is low, as reported in Table 3. 4, but this reflects Configuration 2 values. Indeed, sludge used in Period 2 of Configuration 1 is the same that was

cultivated in Configuration 2 and a CH_4 concentration of 5.2% is in line with the last measured value in Configuration 2 (see Table 3. 5 in Section 3.2.2). Subsequently, methane production increases in parallel with its concentration. Methane concentration continues increasing in time until Sampling day 5 (75.1%), while MER sees a reduction at sampling day 4. This reduction is caused by the shutdown of the gas recirculation pump during the weekend between Sampling day 3 and 4. This means that gas reached the CSTR by means of gas diffusion, thus in a slower way. At Sampling day 6 an anomaly occurs, with air infiltration in the system; hence, methane concentration rapidly decreases to 49.9%; also, MER decreases sharply, reaching a negative value, which clearly deviates from the objectives of the trial. Methane production starts increasing again by establishing the optimal conditions for methanogens and by re-injecting the nutrient gas mixture. At Sampling day 7 the maximum value of MER is reached (0.83 $L_{CH4}/(L_{sludge}d)$) and the maximum CH_4 concentration is measured Sampling day 9, with a value of 83.3%.

The availability of more nutrient for microorganisms and more gas volume has surely enhanced the process, as it can be seen by comparing values in Table 3. 4.

3.2.2 Configuration 2

The first sampling seems promising in terms of MER, reflecting Figeac *et al.* [22] results with a MER equal to 0.22 $L_{CH4}/(L_{sludge}d)$, as reported in Table 3. 5. This value corresponds to a low CH_4 concentration (4.8%), which is due to microorganism youth.

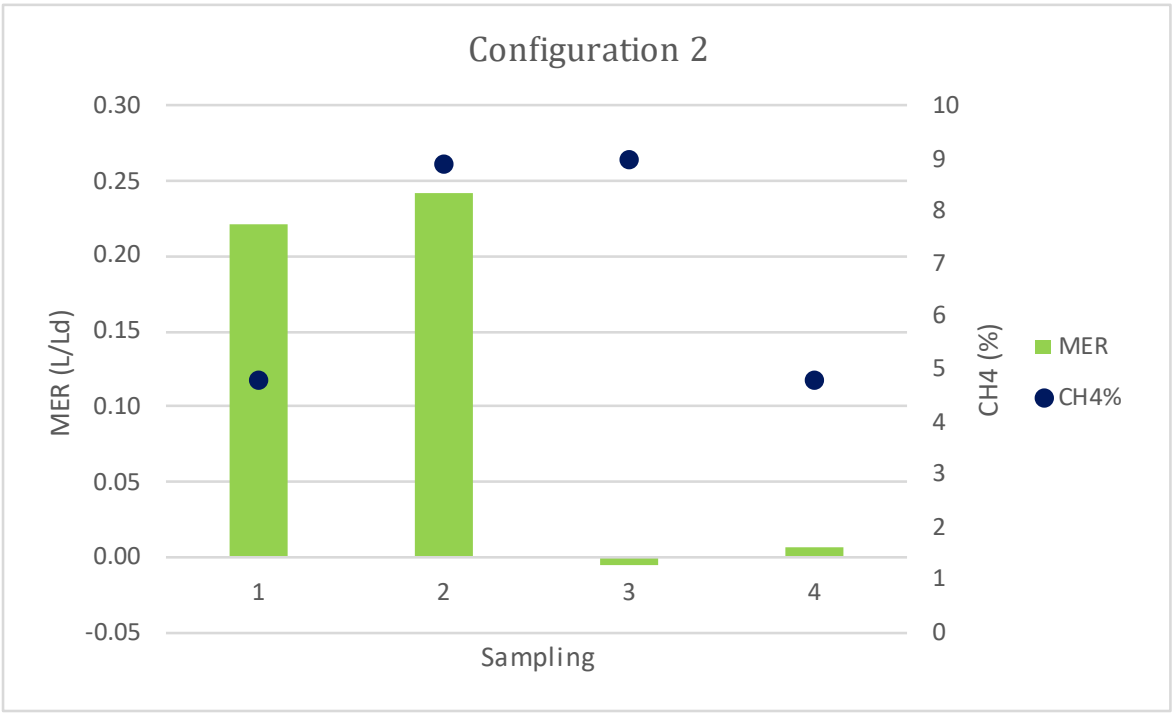

Figure 3. 7 Graphical representation of MER and CH4 concentration values for Configuration 2.

This configuration has an available gas volume larger than Configuration 1. Thus, methane concentrations are low since they are diluted with respect to the previous system. Moreover, the decreasing in CH_4 concentration can be caused by air infiltrations in the settlement. BHM is an anaerobic process and, as mentioned in Section 1.4.1 methanogenic bacteria cannot survive

exposure to oxygen or air. At sampling day 3, a concentration of 6.7% of oxygen is detected, resulting in a null value of MER (Table 3. 5). Furthermore, at the same sampling day, the lowest HCR is measured, i.e., methanogens are not properly consuming hydrogen to produce methane. Indeed, CH_4 concentration does not have a significant increasing, influencing in a negative way the subsequent sampling, as showed in Figure 3. 7.

Thus, a reduction in CH_4 concentration measurement can be caused either by inhibition of methanogenic archaea, or by dilution of produced gas in the mixture.

Table 3. 5 Results for Configuration 2.

Sampling day	**Injected H_2 (L)**	**$V_{CH4,out}$ (L)**	**CH_4 (%)**	**O_2 (%)**	**Δt (d)**	**MER (L_{CH4}/ ($L_{sludge}d$))**
1	3.76	0.22	4.8	1.1	1	0.22
2	0.40	0.38	8.9	1.9	1	0.24
3	0.64	0.25	9.0	6.7	3	0.00
4	1.84	0.18	4.8	1.5	1	0.01

4 Conclusions

The main objective of this thesis was to evaluate the possibility of producing a biofuel in a sustainable way, by utilizing sources that are already present in nature and without depleting natural fuel deposits. In detail, the aim was to determine whether it is possible to produce biomethane in a biological way, exploiting WWTP sludge as source of biomass and a H_2/CO_2 gas mixture as source of nutrients. A literature review already gives a positive answer to the previous questions. Indeed, a series of laboratory trials carried out in Europe by several authors have produced great amounts of biomethane (in the order of 90%) with great MER. This parameter be an indicator of the process yield and its values widely differ from one author to another. MER variations are due to the differences in trials, such as operating conditions, reactor type, type of biomass source and so on. Although a laboratory trial has been carried out to give more detailed answers to the previous questions. The trial has been performed with a CSTR reactor, in which a primary sludge has been used as biomass source. The setup described in Chapter 3 was used to cultivate hydrogenotrophic methanogenetic archaea and their activity has been monitored day by day.

4.1 Results interpretation

Two configurations have been studied, with a complex of three trial periods: two for Configuration 1 and one for Configuration 2. The various configurations have produced very different results, but always in line with predicted ones. Indeed, a MER of 0.25 $L_{CH4}/(L_{sludge}d)$ was expected to be obtained, based on the work of Figeac *et al.* [22]. Chronologically, the three trials are Configuration 1 – Period 1, Configuration 2, and Configuration 1 – Period 2. This explanation is necessary to deeply understand the evolution of the results.

Configuration 1 – Period 1 has satisfying results in terms of MER, reaching a maximum value of 0.60 $L_{CH4}/(L_{sludge}d)$ at Sampling day 1, in line with Luo and Angelidaki, 2012; the same gas sample has a concentration of 52.3% methane. However, this value corresponds only to the first sample, while others result in lower MER and lower methane concentrations, except for Sampling day 2, when CH_4%=64.6%. The reduction in MER and in methane concentrations may be due to inconsistent gas measurements. These inconsistencies depend in their turn on a lack of gas volume in the reactor, resulting in risks of acidified water drowning in the CSTR. Hence, the limiting factor for this trial may be the lack of volume available for the reaction and for gas exchanges.

Configuration 2 presents very low methane concentrations in the system, with MER in line with Figeac et al. [22] only for the first two sampling days. Indeed, the highest MER reached in this trial is 0.24 $L_{CH4}/(L_{sludge}d)$, with a CH_4 concentration equal to 8.9%. The day after, methane was only 9.0%, resulting in a much lower MER. From these results optimization was not reached, and gas volume may be the limiting factor again. Nevertheless, in this configuration oxygen has been found in high concentrations, altering microbial environment and, consequently, limiting methanogens metabolism.

Configuration 1 – Period 2 is the best setup in terms of MER and methane concentrations. Indeed, CH_4 concentration has continuously increased in time in the initial phase, reaching a value of 75.1% at Sampling day 5, then reducing to 49.9% and increasing again until Sampling day 9, when CH_4% = 83.3%. On the other hand, MER increased in the first 3 days, reaching a maximum of 0.61 $L_{CH4}/(L_{sludge}d)$, in line with Luo and Angelidaki, 2012, and decreased at Sampling day 4, due to the recirculation pump shut down during the weekend. In this configuration the maximum MER value is 0.83 $L_{CH4}/(L_{sludge}d)$ at Sampling day 7. The limiting factor in this kind of configuration could be microorganism's concentration in the liquid phase. Indeed, if bacteria grow up in a consistent way, their concentration in the sludge increases too much, limiting the gas-liquid mass transfer of nutrients, i.e., limiting both microbial metabolic activity and methane production. Hence, a way to enhance the process could be to dilute the sludge, in order to reduce methanogens concentration in the liquid phase.

The most promising values are those obtained in Configuration 1 – Period 1, which overcome the ones found by Figeac *et al.* [22] and are close to the ones retrieved by Luo and Angelidaki, 2012 and reported in Table 2. 5. As shown in Table 2. 5, MER values are higher, but the results are comparable, considering the differences in reactor volume and in the nutrient media injection rate.

4.2 Research limits and possibilities for improvement

As discussed in the previous section, the trials have some problems limiting the process. Some of these have been already solved, e.g., reducing sludge volume, avoiding air infiltrations etc.

Other limits may concern the gas-liquid mass transfer of hydrogen to the liquid phase. Hence, using a TBR instead of a CSTR could be a solution to solve this problem. Indeed, a TBR with a proper packing material could promote the contact between the sludge, i.e., microorganisms, and the gas mixture. In this way gas-liquid mass transfer could be enhanced and microbial metabolism could be positively affected. In fact, the so performed BHM with CSTR reaches promising MER value, but CH_4 concentrations that are still lower than law requirements. By improving the k_La, biomethane production can be increased in terms of methane concentration. The objective is to reach CH_4 concentrations of about 90% and more, to inject it in the existing methane distribution network.

Furthermore, a sustainability analysis should be done to evaluate investment costs, maintenance costs and energy consumptions. Indeed, it is important to ensure that energy consumption is not so high, otherwise the process cannot be considered as sustainable.

Glossary

Abbreviations

AD	Anaerobic Digestion
ADM1	Anaerobic Digestion Model No.1
BCR	Bubble Column Reactor
BHM	Biological Hydrogen Methanation
CIC	Certificati di Immissione in Consumo
CSTR	Continuously Stirred Tank Reactor
GES	Gestore Servizi Energetici
GRT	Gas Retention Time
HCR	Hydrogen Consumption Rate
HFM	Hollow Fiber Membrane
HRT	Hydraulic Retention Time
MB	Membrane Reactor
MER	Methane Evolution Rate
PtG	Power to Gas
RT	Retention Time
TBR	Trickle-Bed Reactor
VFA	Volatile Fatty Acids
WWTP	Wastewater Treatment Plant

Parameters

k_La	Volumetric gas-liquid mass transfer coefficient
r_t	Volumetric gas-liquid mass transfer rate
$\dot{m}$	Mass flow rate
P_t	Thermal Power
ΔH^0	Enthalpy of reaction
ΔG^0	Gibbs' free energy
D_L	Diffusivity
μ	Viscosity
ε_G	Gas holdup
ε_L	Liquid holdup
a_{eff}	Effective surface area
P	Pressure
T	Temperature
V_R	Reactor Volume

Chemical compounds

CH_4	Methane
CO	Carbon monoxide
CO_2	Carbon dioxide
CO_3^{2-}	Carbonate ion
HCO_3^-	Bicarbonate ion
H_2CO_3	Carbonic acid
H_2O	Water
H_2S	Hydrogen sulphate
N_2	Nitrogen
O_2	Oxygen
SO_2	Sulphur dioxide

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