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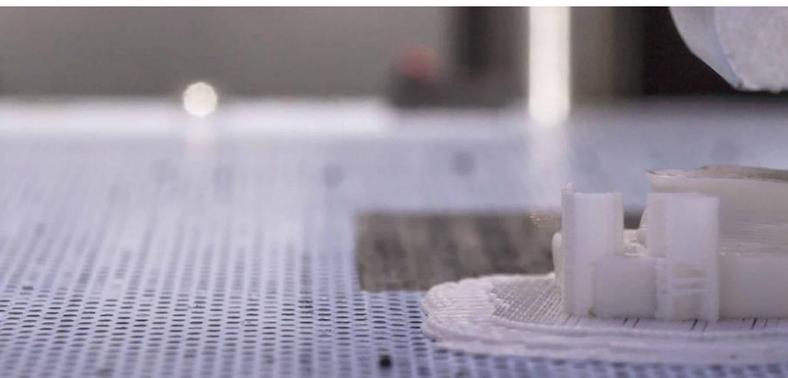
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Bachelor Integration Project for Industrial Engineering and Management



Development of a
multi-parallel
miniaturized



bioreactor system
for screening of
complex biological



processes using 3D
printing



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Title page

Development of a multi-parallel miniaturized bioreactor system for screening of complex biological processes using 3D printing

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Preface

This report is written for the Bachelor Integration Project concluding the Bachelor's degree programme of Industrial Engineering and management. The specialization followed is the Production Technology and Logistics track. This degree programme was facilitated by the University of Groningen.

This thesis is written with the help of many people. I want to briefly express my gratitude to some of them using this preface.

First of all, special thanks go out to my daily supervisor Spyros Achinas. The passion and enthusiasm you showed while helping me get acquainted with the laboratory and writing my thesis made the whole project very enjoyable. Thank you for your time and effort during all our meetings.

Secondly, I want to thank my first and second supervisor during this project: Gert-Jan Euverink and Gerald Jonker. Thank you for your advice and feedback. Throughout the whole process you kept me on track and helped me get the best out of myself.

Lastly, I want to thank my fellow groupmates and students. Thank you for your feedback during our meetings. Moreover, thank you for all the fun coffee breaks which helped put everything back into perspective and allowed for a good laugh during this project.

Abstract

The pressure on biochemical process development is increasing continuously. The resources as well as the time required for the development of such processes need to be reduced. Currently, the commercial pilot-scale bioreactors and bench-scale bioreactors are expensive, impractical and inefficient when used in a biological process screening system. Therefore, a system reducing the time and resources required is desired. The principles of miniaturization and parallelisation are used to facilitate this system. Parallelisation enables the testing of multiple process parameters simultaneously, reducing the time required, while miniaturization enables the parameters to be tested on a miniaturized scale, reducing the resources required. Different biochemical processes will require a different screening system. Therefore, the 3D printing principle is used to enhance the flexibility and modularity of the system. This enables the system to be modified easily, enabling the system to be applicable to a variety of biochemical processes, rather than one specific one. Thus, the goal is to develop a multi-parallel miniaturized bioreactor system for screening of complex biological processes, using the 3D printing technique.

The anaerobic digestion process was used as the complex biochemical reaction for the development of the system. Literature review provided functional requirements, which the individual reactors needed to fulfil. Based on these requirements, the applied systematic design approach generated an overview of the subfunctions, which the design needed to perform. Based on the subfunctions different working principles were formulated. The most suitable working principles were selected and, combined with the functional requirements, translated into a reactor design. This design was materialized using SolidWorks CAD software. The design was printed using a Formlabs Form2 stereolithography 3D printer.

The design was validated by conducting laboratorial experiments. Two designed miniaturized reactors, with a volume of 40 mL, were compared to two Eppendorf BioBLU® 0.3c Single-Use Vessels, with a volume of 400 mL. Both reactor systems were fed with a solution containing 0.003 g/mL milk powder. The miniaturized reactors were fed with a rate of 1 mL/day while the baseline reactors were fed with a rate amounting to 10 mL/day, corresponding with a hydraulic retention time of 30 days and an organic loading rate 0.1 g_{COD}/day for both systems. The monitored parameters were the biogas yield, pH, volatile fatty acid concentration, total alkalinity and chemical oxygen demand. Additionally, the 3D printing process was evaluated against injection moulding, casting and milling & turning using a qualitative comparison.

The experiment showed that the biogas production was similar for both the designed system as well as the baseline system. Based on the final design and the experimental results it can be concluded that combining the principles of miniaturization and parallelisation to develop a multi-parallel miniaturized bioreactor system for screening of complex biological processes using 3D printing is possible. Additionally, the 3D printing process shows potential with respect to the technical as well as the economic aspects of the evaluation. However, the costs of the photopolymer resin, which was used as fabrication material, as well as the printing time reduce the economic feasibility of the 3D printing technique.

Future research has been proposed to focus on solving problems entailed by small sample sizes. Additionally, the economic feasibility of the 3D printing process should be further investigated.

List of abbreviations

AD	anaerobic digestion
CSTR	continuously stirred tank reactor
COD	chemical oxygen demand
MEI	material energy information
TA	total alkalinity
TS	total solids
VFA	volatile fatty acid
VS	volatile solids

Units

g	gram
g _{COD}	gram chemical oxygen demand
L	litre
mg	milligram
mL	millilitre

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Biotechnological processes are present in many different types of industries and areas, often described by colours (DaSilva, 2004). Examples of these areas are health (red), food biotechnology (yellow), agricultural and environmental biotechnology (green) and coastal and marine biotechnology (blue). In this project, the anaerobic digestion process is considered as the biological system where multiple complex biochemical reactions occur. This process belongs to the green area.

The pressure to reduce development cost and accelerate the process development speed in pharmaceutical and biotechnological industries is ongoing and increasing (Bareither & Pollard, 2011). The identification of optimal parameters for these new biotechnological processes is a costly and time-consuming part of that development process, due to the high amount of combination possibilities of parameters (Kurzrock & Kress, 2014). Since determining these optimal parameters at manufacturing scale is not feasible and sustainable, development of miniaturized models that represent the performance of the commercial process are essential to achieve reliable process characterization (Li, et al., 2006). Thus, mass screening for the identification of optimal parameters is urgently needed for biotechnological process development (Kurzrock & Kress, 2014). Mini-scale bioreactors are useful as such a process screening method, where multiple formulations or conditions are screened, multi-parallel, to identify optimal parameters and conditions (Powell, 2006). When miniaturizing a bioreactor, the integration of engineering and biological principles is essential (Van't Riet & Tramper, 1991).

Biochemical reactions can differ significantly from each other. Therefore, a suitable process screening system can differ per specific reaction. This implies that the system is desired to be modular and flexible. Therefore, the 3D printing process is applied. 3D printing belongs to the additive manufacturing methods. 3D printing enables the construction of the design layer-by-layer rather than through moulding or subtractive techniques (Manyika, et al., 2013). Moreover, this technique provides high customizability while producing small quantities at relatively low cost, in a short time period (Berman, 2012). The Formlabs Vat Polymerisation platform Form 2 printer will be used (Formlabs, 2018).

This project aims to develop a system of 3D printed mini-bioreactors, which can be used for bioprocess screening. In order to validate the design, laboratorial experiments will be conducted. The anaerobic digestion process will be the biological reaction which will be studied in the lab. However, the outcome of this study is not limited to solely the anaerobic digestion process and can be used in wide range of alternative complex microbial reactions as a process screening method.

Firstly, the problem context will be discussed in chapter two. Thereafter, chapter three will focus on the design orientation, containing the goals and research questions of the project. Chapter four will discuss the methodology applied throughout the project. Thereafter, a literature review is discussed in chapter five. Based on that literature review, a list of functional

requirements is formulated which is used for the design process, which is described in chapter six. Thereafter, the final design will be validated using laboratorial experiments, described in chapter seven. Chapter eight will contain the discussion before conclusions can be drawn out of the obtained results. Lastly, chapter nine will provide the final conclusions.



The problem and its context this study considers is specified in the problem definition. A concrete problem statement is formulated. The problem context specified by defining a stakeholder analysis. Thereafter, the project is defined with respect to its environment by means of a system description. This chapter aims to define the main problem and the context of the problem.

2.1 Problem definition

Currently bioreactors occur in many different types. The sizes of these bioreactors can vary over several orders of magnitude, from mini-bioreactors (1-10 mL) to plant scale reactors (2-500 m³) (Modak, 2008). The relationship between the process information available (i.e. size) and the experimental throughput is visualized in Figure 1. This project focusses on the 'miniature bioreactors' since this is the smallest level which still provides measurable process data. The need for automated multi-parallel mini-bioreactor system is becoming more prominent (Kurzrock & Kress, 2014). It occurs that no device is capable yet of meeting all the challenges of miniaturizing large-scale processes while keeping the functionality of conventional bioreactors (Bareither & Pollard, 2011). As the bench-scale bioreactors are expensive, 3D printing can be considered as an alternative solution for the fabrication of miniaturized bioreactor systems. Also, as can be seen in Figure 1, the experimental throughput of miniaturized systems is higher compared to pilot-scaled systems. Thus, the smaller the reactor, the more efficient it becomes in terms of experimental throughput. This highlights the need for automated multi-parallel mini-bioreactor systems. The pilot-scale reactors are often considered impractical since they require more feedstock, space and energy than the mini-scale reactors. This makes the commercial bioreactors expensive, impractical and inefficient as a process screening method. Additionally, the current state of the art mini-bioreactors are not applicable to complex microbial systems (e.g. the biogas production by means of anaerobic digestion). Therefore, the problem statement can be formulated as:

Pilot-scale bioreactors and ultimately bench-scale bioreactors are expensive, impractical and inefficient when used as a biological process screening method.

The main problem owner of this problem is the head of the 'Products and Processes for Biotechnology in the Biobased Economy' research group from the engineering and technology institute Groningen (ENTEG), Prof. dr. G.J.W. Euverink.

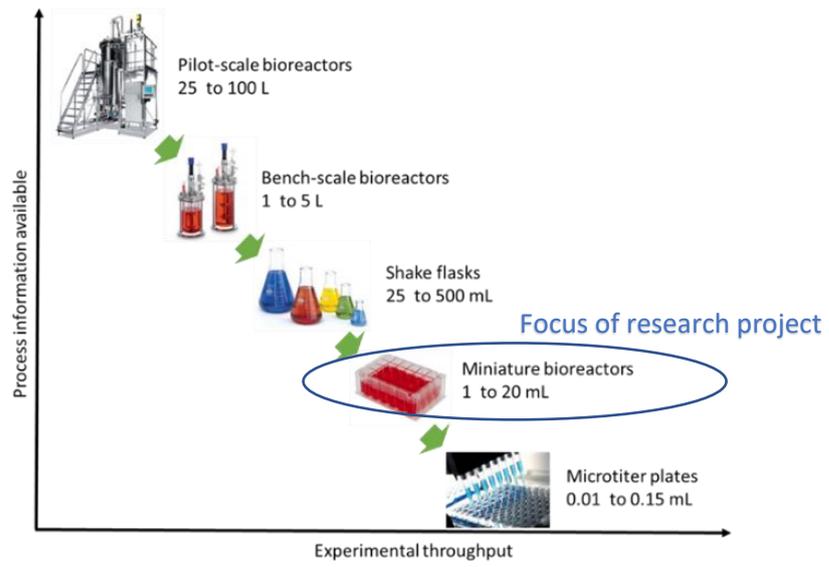


Figure 1: Visualization of relationship between availability of process information and experimental throughput. The focus of this project is on the 'miniature bioreactors' level.

2.2 Stakeholder analysis

The key player within this design project is the problem owner, Prof. dr. G.J.W. Euverink. He has the most influence, power and interest within this project (Jonker & Muñoz Arias, 2018). Additionally, Spyros Achinas, the daily supervisor of the bachelor IP is a stakeholder since he is, like the problem owner, interested in the outcome of the project. He is also considered as a key player in the project. Another stakeholder is Applikon Biotechnology, a company based in Delft. This company facilitates the funding of this project. Applikon Biotechnology is a world leader in developing and supplying advanced bioreactor systems for industrial biotechnology. Applikon can guide a customer from initial screening up to full-scale production, using the same platform (Applikon Biotechnology, 2019). The power of Applikon is relatively low, while its interest in the project is high. Lastly, researchers in academia can be interested in the outcome of the project since the process screening method can help them in their quest towards rigorous knowledge. The different stakeholders and their relations are visualized in Figure 2.

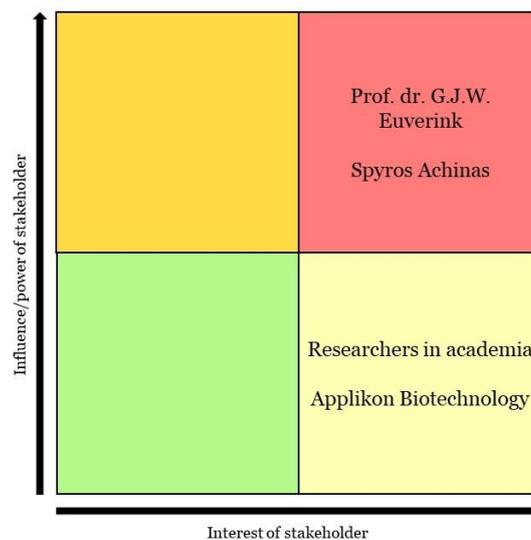


Figure 2: Stakeholder map with the key players, Prof. dr. G.J.W. Euverink and Spyros Achinas in the top right segment. They have the most influence and interest in the project. The researchers in academia and Applikon Biotechnology important to consider and meet their needs. They are placed in the bottom right segment.

2.3 System description

A system has an input, transformation process and an output. A system is defined by its elements, relationships and boundaries. An overview of the system can clarify the problem context and defines the elements and boundaries of the research (Jackson, 2003). A visualization of the system description is depicted in Figure 3. The inputs of a single bioreactor are defined as feedstock, heat and inoculum (substance containing bacteria). The transformation process occurs in the mini-bioreactor. It processes the inputs by means of anaerobic digestion into biogas and digestate (material remaining after the anaerobic digestion) which are the outputs of a single bioreactor. In the system, a certain number (n) of mini-bioreactors are placed in parallel which allows the system to be used for process screening. Each mini bioreactor is setup the same with exception of one predefined parameter. The performance of each mini-bioreactor is measured. By comparing the performances of the n reactors, the optimal parameters of a process can be defined. The 3D printing process is acting on the bioreactor. The scope of the project is solely on the biological reactor, as is also indicated in Figure 3. The ultimate goal is to commercialize the outcome of the design study, which highlights the efficacy of the project. Applikon Biotechnology is a company which provides these systems commercially for companies and research institutes.

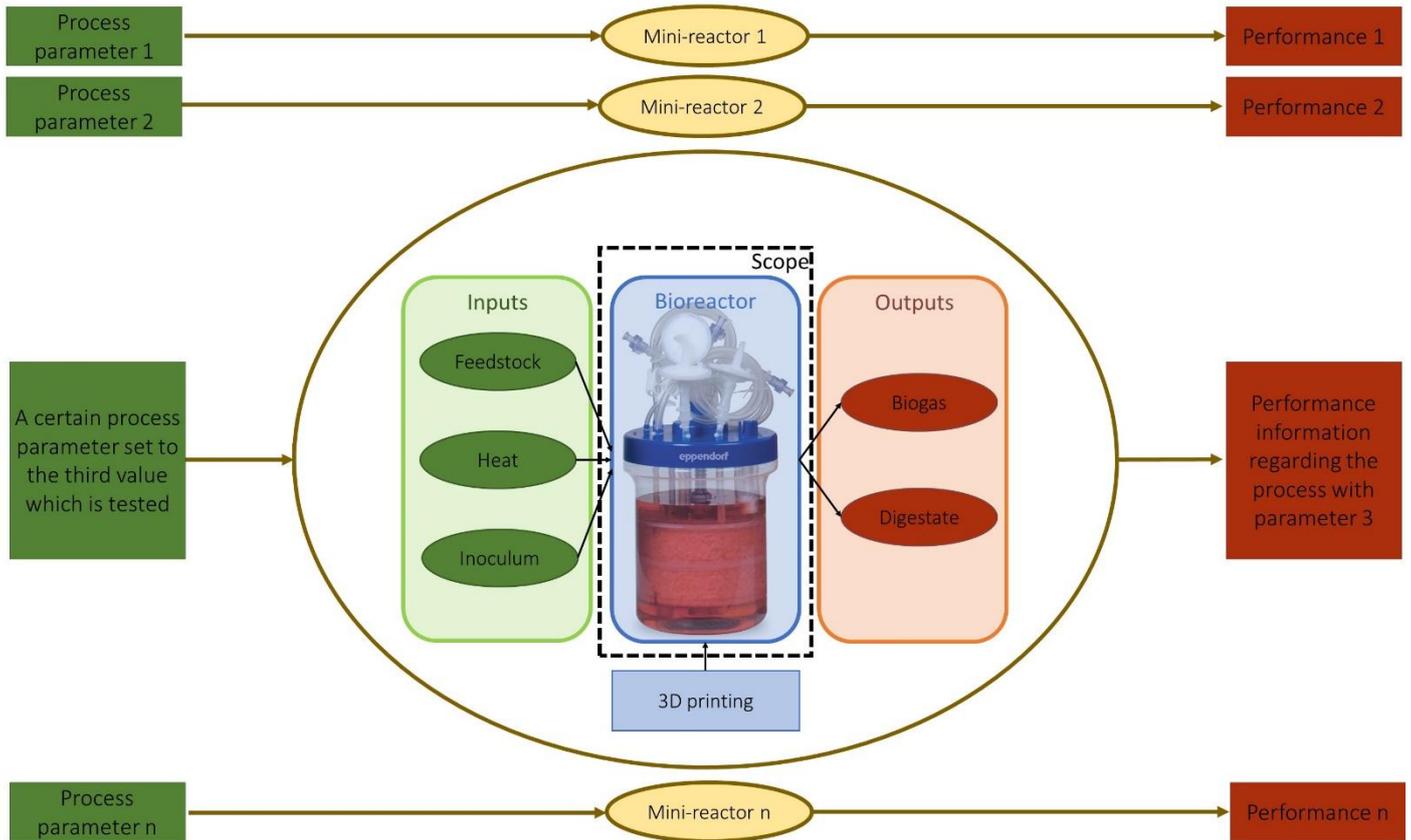


Figure 3: System description and scope of the project. The system consists of a process screening setup containing n mini-bioreactors. For each bioreactor a certain parameter is changed such that the optimal parameters for a process can be derived. The inputs of a single bioreactor are feedstock, heat and inoculum. The bioreactor processes the inputs by means of anaerobic digestion into biogas and digestate. The scope is on the bioreactor on which the 3D printing process is acting.



Using the problem definition, a goal for the project can be formulated. Based on this goal, the main research question is generated. The research question is separated into sub questions, which act as milestones towards the main question. This chapter aims to structure the research into separate parts, which together can answer the main research question.

3.1 Goal

Based on the previously formulated problem definition and problem statement, the goal of the project is defined as:

Develop a multi-parallel miniaturized bioreactor system for screening of complex biological processes that can perform as efficiently as larger, already applied and tested, commercial bioreactor screening platforms, using the 3D printing technique, within three months

3.2 Research questions

To overcome the formulated problem and fulfil the goal, certain questions need to be answered. The main research question of this project is:

Can 3D printing be used to develop a multi-parallel miniaturized bioreactor system for screening of complex biological systems that can perform as efficiently as already applied and tested commercial bioreactor screening platforms?

In order to be able to answer this main research question, firstly some general rigorous knowledge needs to be acquired. Knowledge about 3D printing techniques, complex biological reactions and bioreactors. Thereafter, it is required to translate this knowledge into certain parameters and conditions for the design. After a prototype of the design solution is produced, it is required to validate and test the system.

1. Which factors are crucial when fusing the 3D printing technique and bioreactors for complex microbial reactions when miniaturizing the system for process screening purposes?

Literature research will be conducted in order to answer this first sub question. This scientific knowledge will provide a crucial fundament for the later stages of the project. This literature research will primarily focus on review papers concerning bioreactors and anaerobic digestion. Search terms like 'review', 'anaerobic digestion', 'biogas' and 'miniaturization' will be applied.

2. How can the performance of the 3D printing process be measured and compared to conventional production techniques?

Again, literature research will be conducted to provide an answer to this sub question. Literature research will provide the required parameters which can be measured and the required methodology to evaluate these parameters. Performance is in this respect mainly defined in terms of costs and time. This literature study will focus on finding methods to evaluate production processes. Search terms such as 'production process evaluation' and 'manufacturing performance' will be used.

3. How can the determined factors be combined into a design for a miniaturized bioreactor system?

In order to answer this sub question, a concrete design needs to be formulated based on the derived design parameters and conditions. The engineering design process will be conforming to the guidelines of Pahl & Beitz (2007). These guidelines allow for a structured and step-by-step acquisition of a concrete design which can be used in later stages of the project.

4. How is the 3D printed, miniaturized bioreactor system performing compared to its largescale counterpart?

When the obtained design is produced using the 3D printing technique, its relative performance is evaluated against the commercial BioBLU® 0.3c Single-Use Vessel. The system performance is measured in the lab in terms of volume and yield. Additionally, the performance of the production process is compared to conventional production techniques.



This chapter aims to give an overview of the methodology applied. Firstly, the cycle choice is specified. The cycle choice positions the sub questions with respect to the research and the environment. Thereafter, the specific tools which are used to answer the different sub questions are specified in the materials and methods section. Thereafter, the risk analysis and the deliverable & validation of the project will be discussed.

4.1 Cycle choice

The cycles of Hevner (2007) are used to position the sub questions with respect to the research and the environment. The relevance cycle is used to initiate the design science research with an application context. It provides both the requirements for the research as the acceptance criteria for the ultimate evaluation of the project results. These requirements and acceptance criteria originate from stakeholders. The design cycle is used to set the goal and the methods/approaches used in the research. Additionally, it constructs a treatment which is transferred to the appliance context (Wieringa, 2014). For the design cycle the requirements from the relevance cycle and methods/theories from the rigor cycle are essential. The rigor cycle contributes to the general scientific knowledge base. This project requires a thorough understanding of anaerobic digestion, bioreactors, 3D printing as well as miniaturization principles. This knowledge is provided by the rigor cycle. The outcome of the project also contributes to the rigor cycle in terms of knowledge concerning the fusion of 3D printing and miniaturized bioreactor systems for screening of complex biological processes. The relations between the three cycles allow for transitioning between them in order to connect the outside world with generic knowledge and the artefact, which indicates the essence of design science research. A visualization of the three cycles as indicated by Hevner is stated in Figure 4.

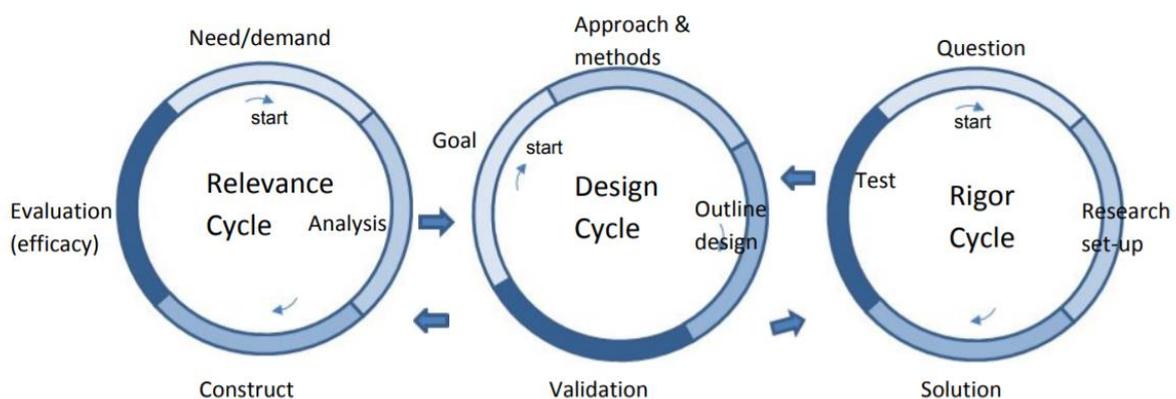


Figure 4: The three-cycle view of design science as described by Hevner (2007)

4.2 Materials and methods

4.2.1 Literature research

As introduced in the research questions and methodology section, literature research will be used to obtain knowledge concerning the 3D printing process, bioreactors and anaerobic digestion. Additionally, literature research will be used to determine information about conventional mini-bioreactor production processes in order to facilitate the benchmarking of the designed 3D printed production process for miniaturized bioreactor systems against the conventional ones. Literature research should include relevant descriptive theory and prior prescriptive knowledge such that it will create a fundamental knowledge base for the rest of the project (Gregor & Hevner, 2013).

4.2.2 Laboratorial experimentation

Additionally, lab experiments will be used to measure the performance of the design relative to the commercial bioreactor. An overview of the laboratorial test is depicted in Figure 5.

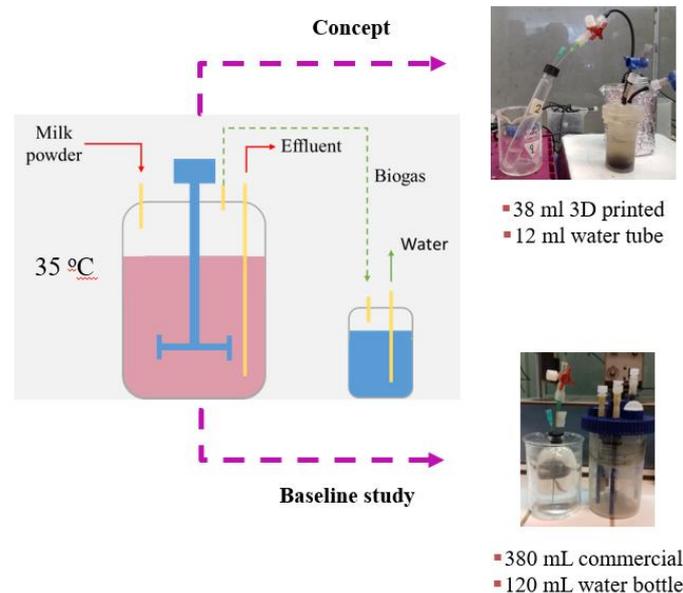


Figure 5: Visualization of the laboratorial experiment setup with as an example a downscaling factor 10. This experiment allows the comparison between the designed system and the commercial system. The pictures are from an older experiment.

4.2.3 Engineering design

The guidelines as described by Pahl & Beitz (2007) will be applied to generate a concrete design solution. The method consists of four main steps. In the first step, task clarification and planning, the design requirements and specifications are formulated. In the second phase, the conceptual design phase, these requirements are translated in so-called 'working principles' and ultimately in 'working structures' which solve the design problem. In essence, these principles and structures combine many different feasible solutions into one solution which promises to be the best option. Thereafter, in the third phase, the embodiment design phase, the selected working structure is materialized and shaped. The outcome of this third phase is thus a concrete design which is ready to be produced. The fourth phase, the detail design phase, covers the production aspect of product design. However, since in this project the production method is already selected, this phase is omitted. Together, the method enables a highly structured approach toward the design process. Within the engineering design the CAD software SolidWorks will be used to create and draw the design.

4.2.4 Benchmarking

Benchmarking techniques will be applied to compare the 3D printing process for miniaturized bioreactor systems for screening of complex biological systems to conventional production techniques. Benchmarking includes measurement, comparison, identification of best practices, implementation and improvement (Anand & Kodali, 2008). The data and information that is required for the benchmarking will be facilitated by literature research as well as data obtained from the 3D printing process.

4.3 Risk analysis

There are three major risks that need to be considered during this project. Firstly, there is a risk that the bioreactor fails due to the combination of 3D printing and miniaturization. The concept of miniaturization is theoretically never-ending. However, in practise problems might occur when something is designed too small when using 3D printing. Despite the unique capability of 3D printing in rapidly fabricating parts with complicated shapes, in practice, there are limitations such as surface quality and strength (Vaezi & Chua, 2011). The 3D printer can print a minimum layer thickness of 1 millimetre (Formlabs, 2018). If this limit is violated, the product can fail. Therefore, the mini-bioreactors should be designed such that the minimum layer thickness is not violated.

Secondly, the process performance will be measured in, among others, volume of biogas production. However, if the bioreactor is designed too small, the produced volume of biogas cannot be measured accurately anymore. This risk should be considered because it has direct influence on the validity of the project.

Lastly, there is a risk that the complexity of the anaerobic digestion process influences the results of the measurements. The major reason for variations is the complexity of the anaerobic digestion process where different mechanisms such as antagonism and synergism could significantly affect results (Chen, et al., 2008). There are millions of microbes that can interact with each other. This risk can be monitored and avoided by measuring parameters like pH, VFA's, TA and COD.

4.4 Deliverable and validation

This project aims to deliver a 3D printed miniaturized bioreactor system that can be used for process screening. Additionally, this project delivers a procedure describing how 3D printing can be used for miniaturized bioreactor systems. Therefore, this project contributes to the existing knowledge base as well as practical applications for companies (Gregor & Hevner, 2013) (Wieringa, 2014).

The project is validated by measuring different parameters of the biological process. The pH as indicated by Kim et al. (2002) and Jiang et al. (2012), VFA, TA and COD described by Weemaes et al. (2000), and temperature and biogas production as indicated by Chae et al. (2008) are measured. If all these variables are stable during a longer period, the reaction itself is stable (Ward, et al., 2008). If the reaction is stable, the obtained results are valid, and the conclusions drawn should hold.



The literature review section will provide the required background knowledge required for the later stages of the project. It aims to answer the first two sub questions as formulated in the research questions section. The chapter will start with the analysis of the different types of anaerobic digestors. Thereafter, the critical process parameters of anaerobic digestion are discussed as well as a short description of the process. Moreover, the technological facets which arise when the anaerobic digestion process is combined with miniaturization and 3D printing principles are discussed. Thereafter, the performance evaluation of the 3D printing process is analysed. Lastly, a short conclusion is given of the literature review. The goal of this review section is to formulate a list of functional requirements which will be the basis of later stages. The requirements are focussed on one reactor, placing multiple reactors in parallel will create the system.

5.1 Types of anaerobic bioreactors

Before discussing the crucial process and reactor parameters of AD, a distinction between the type of anaerobic bioreactors is made. There are two main types of reactors. Firstly, batch reactors, and secondly, continuous reactors (Bouallagui, et al., 2005). Depending on how the fermentation substrates will be fed into the fermenter, we speak about continuous and discontinuous processes (Kumar, et al., 2013).

5.1.1 Batch reactors

A batch reactor is an ideally stirred vessel in which a reaction occurs (Akker & Mudde, 2014). The feedstock is batch wise fed to the anaerobic digester which initiates the biogas production (Wellinger, et al., 2013). When the gas production decreases, and ultimately completely ceases, the reactor is opened, and half the feedstock is removed while the other half remains as inoculum for the next batch (Nizami & Murphy, 2010). Compared to other systems, batch digestion has the advantage of low operation costs, simplicity of reactor design and low costs of the mechanical technology behind it, and the disadvantage of high process energy consumption and maintenance costs (Al Seadi, et al., 2008).

5.1.2 Continuous stirred tank reactor

The continuous stirred tank reactor (CSTR) is an ideally mixed reactor which is characterized by an influx stream coming in to the reactor and an efflux stream exiting the reactor (Akker & Mudde, 2014). The volumetric flow rates of the influx and efflux are required to be equal in order to prevent the reactor from overflowing or running dry (Akker & Mudde, 2014). Unlike the batch reactors, continuous digesters can produce biogas without the necessity of interruption for the loading of new feedstock and the unloading of the digested effluent, which makes the biogas production constant and predictable (Al Seadi, et al., 2008). Different stirring methods can be applied, such as: horizontally rotating paddles (Rushton) and inclined paddle stirrers (Marine) (Wellinger, et al., 2013). In this research a continuous anaerobic digestion process is desired due to its continuity and stability which will be crucial for process screening purposes. Moreover, the reference reactor is also a continuous vessel.

5.2 Critical process parameters AD

Anaerobic digestion is the process in which microorganisms break down biodegradable material in the without the presence of oxygen (Torales, 2013). Anaerobic digestion is often considered to be a complex process, the digestion itself, which takes place under anoxic conditions, is based on a reduction process consisting of several biochemical reactions (Aslanzadeh, 2014). The biochemical reactions entail a series of metabolic stages. Namely, hydrolysis, acidogenesis, acetogenesis and methanogenesis (Surendra, et al., 2014). Different factors can affect this anaerobic digestion process. Factors could be physical, chemical, or biological (Adekunle & Okolie, 2015). The most important factors are: temperature, pH, volatile fatty acids, alkalinity, organic loading rate, retention time, C/N ratio, mixing and toxic compounds (Tabatabaei & Ghanavati, 2018). Additionally, reduction oxidation potential (redox potential) in the reactor has influence on the process (Zupančič & Grilc, 2012).

5.2.1 Temperature

The anaerobic digestion process is profoundly influenced by the temperature (Chen, et al., 2016). The process can operate within three temperature intervals: psychrophilic (15 ± 1 °C) mesophilic (30 °C to 45 °C) or thermophilic (45 °C to 80 °C) (Choorit & Wisarnwan, 2007) (PAS, B, 2010) (Tabatabaei & Ghanavati, 2018). The thermophilic condition reportedly entails the highest productivity due to various advantages such as: reduced retention time, improved digestibility, effective destruction of pathogens (Mao, et al., 2015). However, the ammonia toxicity is believed to intensify at this temperature, which can result in an unstable digestion process (Weiland, 2010). Other disadvantages of thermophilic AD are low-quality effluent, increased acidification and susceptibility to environmental conditions, larger investments, poor methanogenesis and higher energy input (Mao, et al., 2015). The mesophilic temperature interval is applied because it entails a more stable digestion process.

5.2.2 pH

Microbial growth in an anaerobic digester is largely dependent on the pH (Yang, et al., 2015). The different microbial groups which enable the AD process experience optimal growth under different pH values (Kundu, et al., 2017). For methanogens the optimum range varies from pH 6.5 to pH 8.2, with an optimal value of pH 7.0 (Lee, et al., 2009). When the pH level decreases below 6.6, the growth rate of methanogens is greatly reduced, and at pH levels higher or lower than the indicated interval, the activity of methanogenic bacteria is decreased (Zhang, et al., 2009). For acidogenesis the optimal pH value has been reported to lie in the interval of pH 5.5 and pH 6.5 (Kim, et al., 2003). This difference in optimal pH ranges is the reason why a two-stage AD is the preferred mode of operation (Mao, et al., 2015). In a two-stage AD process the acidogenesis and methanogenesis processes are separated (Chaikasem, et al., 2015). If single-phase operation is intended, the favourable pH range to partially meet the requirements of different microbial groups involved in the AD process would be between pH 6.8 and pH 7.4 (Fang & Liu, 2002). Therefore, a stable reactor pH between a pH value of 6.8 and a pH value of 7.4 is desired.

5.2.3 Volatile fatty acids

Volatile fatty acids are used as a substrate for the acetogenesis and methanogenesis stages of the anaerobic digestion process to produce the final product (Haugen, 2014). The concentration of VFAs is a good indicator for the stability of the anaerobic digestion (Møller, et al., 2004) (Tabatabaei & Ghanavati, 2018). When the bacterial activity of hydrolysis/acidogenesis is higher than the methanogenesis bacterial activity, the VFA concentration increases, which can eventually lead to irreversible acidification (Mao, et al., 2015). This will cause the pH to decrease, which will restrict the methanogenesis bacteria to convert the VFAs to methane (Jain, et al., 2015) (Drosg, 2013). This decrease in pH can be prevented by a sufficient buffer capacity in the reactor, providing the digester with tolerance

against high concentrations of VFAs, preventing pH drops (Boe & Angelidaki, 2006) (Falk, 2011) (Lahav & Morgan, 2004). The quantity of VFAs to the buffer capacity of carbonate in a digester is characterised by the VFA/TAC (total alkaline carbonate) ratio (Deublein & Steinhauser, 2011). This ratio is typically between 0.1 and 0.35 in anaerobic digesters (Esteves, et al., 2012) (Switzenbaum, et al., 1990).

5.2.4 Alkalinity

The alkalinity, also known as the buffer capacity of a reactor, is a typical fast indicator for monitoring the digestion process (Tabatabaei & Ghanavati, 2018). The buffer capacity contributes to the pH stability in the digester (Boe & Angelidaki, 2006). During the anaerobic digestion process, ammonia is formed during the processing of the substrate (Surendra, et al., 2014). Substrates rich in carbon have been proposed as a solution to reduce ammonia and other toxic substances (Rajagopal, et al., 2013) (Fitamo, et al., 2017). These substrates containing bicarbonate buffering capacity and high ammonia content generally maintain the pH and provide the digester with tolerance against high VFAs values, preventing pH drops (Boe & Angelidaki, 2006) (Falk, 2011) (Lahav & Morgan, 2004). Alkalinity of a biogas process can be measured both as total alkalinity as well as bicarbonate alkalinity. The bicarbonate alkalinity for stable processes usually varies in the range 3,000 - 15,000 mg HCO₃/litre (Schnurer & Jarvis, 2009). Additionally, the alkalinity can be described by the VFA to total alkalinity ratio (VFA/TAC), indicating the quantity of volatile organic acids to the buffer capacity of carbonate in a digester (Deublein & Steinhauser, 2011). In general, under appropriate conditions, this ratio is in between 0.1-0.35 for anaerobic digesters (Esteves, et al., 2012).

5.2.5 Organic loading rate

The organic loading rate (OLR) refers to the amount of organic fermentable biomass which is added to the digester on a daily basis (Drosg, 2013) (Schnurer & Jarvis, 2009). The OLR is expressed as the quantity of volatile solids which is fed per working volume of a digester per day (Esteves, et al., 2012). This is closely related to the chemical oxygen demand, which is defined as the total concentration of substances that can be oxidized biologically (Pisarevsky, et al., 2005). The optimum value of the OLR is dependent on the substrate and the operating temperature (Tabatabaei & Ghanavati, 2018). An OLR value which is above the optimal value for a digester can result in unfavourable changes in environment present in the reactor (Zhang, et al., 2014). The irreversible acidification of the anaerobic digester due to increased VFA concentration and decreased pH is likely (Palacio-Barco, et al., 2010) (Zhang, et al., 2013). On the other side of the spectrum, an OLR value which lies below the optimal determined OLR value for a digester, could entail starvation of the microbial populations, which consequently negatively influences the overall performance of the reactor (Jiang, et al., 2012).

5.2.6 Retention time

The hydraulic retention time (HRT) specifies the statistically average residence time of substrate in the digester (Falk, 2011). In other words, it is defined as “the time required to complete the digestion of organic materials introduced into a digester” (Tabatabaei & Ghanavati, 2018). It is closely linked to process parameters such as temperature and OLR, as well as substrate composition and microbial growth (Mao, et al., 2015) (Henze, et al., 2008). When the retention time is short, it is likely to entail washout of active microorganisms, while a long retention time requires a larger digester and therefore more investment and equipment costs (Sreekrishnan, et al., 2004). Thus, it is important to make sure that HRT is adjusted at the appropriate values for a given substrate fed into the digester (Drosg, 2013) (Wolf, 2013).

5.2.7 C/N ratio

The nutrient levels of a digestion substrate are reflected by the C/N ratio (Mao, et al., 2015). For microbial growth inside digesters, the availability of nutrients is crucial. An optimized C/N

ratio can prevent problems during the anaerobic digestion process, whilst ensuring availability of nitrogen required for microbial activity (Tabatabaei & Ghanavati, 2018). Both above- and under-optimal C/N ratios are related to adverse effects on the methane production rate (Tabatabaei & Ghanavati, 2018). The optimal C/N ratio has reported to lie in between 20:1 and 30:1, with a ratio of 25:1 being the most frequently used (Kigozi, et al., 2014) (Buekens, 2005) (Yen & Brune, 2007) (Zhang, et al., 2013).

5.2.8 Mixing

Mixing affects the formation, structure, and metabolism of the microbial populations involved in the anaerobic digestion process (Jiang, et al., 2016). It is essential for homogenizing the influent and guarantees an efficient contact between the substrates and the microbial biomass, which directly influences the reactor's performance (Lindmark, et al., 2014). Above-optimal mixing intensities can entail serious threats to the survival of the microorganisms (Deublein & Steinhauser, 2011).

5.2.9 Toxic compounds

Tabatabaei & Ghanavati (2018) state that the presence of toxic compounds could severely jeopardize AD process leading to substantial accumulation of volatile fatty acids which will lead to lower yields or sometimes complete inhibition of the process. The toxic compounds can be added to the digester via the feedstock or generated in the digester (Haak, et al., 2016) (Yang, et al., 2016) (Kwietniewska & Tys, 2014). Anaerobic microbial communities suffer from toxic compounds such as ammonia, oil and heavy metals. However, the specific resistance is dependent on different environmental conditions inside an anaerobic reactor (Al Seadi, et al., 2008).

5.2.10 Redox potential

The oxidation-reduction potential (redox potential) has been reported to be a useful monitoring parameter in anaerobic digestion (Wang, et al., 2006). Low redox potential is required in the anaerobic digester. Typically, a redox potential between -300 and -330 mV is necessary for optimal performance (Zupančič & Grilc, 2012). In order to achieve this redox potential, few oxidizing agents should be added, such as oxygen, nitrate, nitrite or sulphate (Deublein & Steinhauser, 2011).

5.3 Technological facets

In addition to the discussed anaerobic digestion process parameters, additional factors arise when the AD process is combined with miniaturization and 3D printing principles. The concept of miniaturization as well as the concept of 3D printing will be discussed. Additionally, regarding the complexity of the anaerobic digestion process, it is necessary for the construction material used, for the reactor, to be biocompatible (de Lemos Chernicharo, 2007). Fabrication materials and biocompatibility will therefore also be discussed. Lastly, when miniaturizing a reactor changes in fluid dynamics (Dietzel, 2016). Therefore, the fluid dynamics with respect to miniaturization are also discussed.

5.3.1 Miniaturization concept

The design of a bioreactor generally varies in shape, instrumentation and material properties, which has a direct influence on its performance (Lattermann & Büchs, 2016). A promising solution for optimization studies is the downscaling of bioreactors (Velez-Suberbie, et al., 2018) (Bartlett, 2002). The downscaled bioreactors aim to replace pilot-scale and ultimately bench-scale bioreactors (Hemmerich, et al., 2018). Currently, the environment in the lab-scale systems are negatively influenced by the lack of automated feeding, pH control and oxygen control (Nienow, et al., 2013) (Bareither, et al., 2013). Therefore, mini-bioreactors which run in parallel have become more attractive since they provide a clear process understanding during early stages of the process development (Betts, et al., 2006). Watts & Wiles (2007) and Betts & Baganz (2006) argue that mini-bioreactors have the capability to mimic the environment present in larger vessels.

In case of the AD process, miniature anaerobic digesters can serve as a screening tool for biogas production. A miniature AD system can monitor and control many parallel operating anaerobic digesters in order to deal with interchangeable process parameters at the same time. Additionally, the parallelization of a miniature AD system is advantageous for the visibility of operating conditions allowing detailed process insight (Hemmerich, et al., 2018).

Currently, no maximal size of a commercial miniaturized bioreactor system has been established. However, the operating volume preferably remains less than 50 mL. In Table 1 an overview is given of the state-of-the-art miniature bioreactor systems and their main characteristics. The control of miniaturized bioreactor systems remains very complex and lacks research. Due to the necessity of an anoxic environment, the downscaling of anaerobic digesters is more challenging compared to other bioreactors. In this project the desired reactor volume amounts to 40 mL.

When miniaturizing bioreactors for microbial processes, some dimensional conditions have been reported to be optimal. Firstly, the height/diameter ratio needs to be 2.2. Rushton stirring with an impeller diameter of 30% of the reactor diameter should be applied (Applikon academy, 2019).

Table 1: Overview of the state-of-the-art miniature bioreactor systems and their main characteristics.

Reactor volume	Application	Material	Mixing	Sensing	Reference
μ BR (150 μ L)	Microbial fermentation	PMMA, PDMS	Magnetic	pH, DO	(Szita, et al., 2005)
μ BR (150 μ L)	Fermentation	PDMS	Peristaltic oxygenating mixer	pH, DO	(Lee, et al., 2006)
μ BR (150 μ)	Cell cultivation	Plastic	Unknown	pH, DO, dCO ₂	(Maharbiz, et al., 2004)
Millilitre scale tank BR (10ml)	Mycelium forming	PEEK	Magnetic	pH, DO	(Hortsch, et al., 2010)
Millilitre scale BR (12ml)	Measuring power consumption/energy dissipation		magnetic	Torque, particle size	(Hortsch & Weuster-Botz, 2010)
Simcell™ (1ml)	Cell cultivation		Sparging	pH, DO	(Amanullah, et al., 2010)
MA (0.1-2.0ml)	Controlling cellular microenvironments	PDMS	Unknown	Flow velocity	(Figallo, et al., 2007) (Cimetta, et al., 2009)
ambr™ (15ml)	Cell cultivation		Sparging	pH, DO	(Janakiraman, et al., 2015)
ambr™ (15ml)	Cell cultivation		Sparging	pH, DO	(Delouvroy, et al., 2015)
Mini-bioreactor (30ml)	Mammalian cell culturing		Angled disc impeller	Temperature	(Bulnes-Abundis, et al., 2013)
BioREACTO R48 (8-15ml)	Parallel fermentation		Autoinduction impeller	pH, DO	(Nickel, et al., 2017)

5.3.2 Sensing capabilities of miniature bioreactors

Detailed monitoring of the state of a bioprocess online and in real time is inherently complex but is increasingly required to ensure the control and optimized use of raw materials, consistent quality of the final product, a reduction in wastes and process cycle time, replacement of costly and slow laboratory testing, and continuous learning, which opens up the possibility of bioprocess innovation (Larroche, et al., 2016). When the bioreactors are scaled down, the sensors are also required to decrease in size, to assure this detailed

monitoring of the bioprocess in the miniature bioreactor. A solution is integrating the sensor into miniature fabricated devices, which is indicated by Szita et al. (2005). Currently, most of these microfabricated devices with integrated sensing capabilities solely monitor just the basic culture conditions, such as temperature, oxygen levels, pH, and optical density (Zanzotto, et al., 2004) (Bower, et al., 2012). Besides sensors that are integrated into miniature bioreactors, there are other available micro-sensors that can be used. Examples are MEMS-based chemical concentration sensors (Sparks, et al., 2008), gold-plated microscopic electrode needle arrays (Harper, et al., 2009) and miniaturized gas sensors (Manyika, et al., 2013).

5.3.3 Fabrication materials for miniature bioreactor

The construction material of can influence the performance anaerobic digestion process (Ward, et al., 2008). Factors influencing the interaction between the microbial populations and the reactor surface are described by (Katsikogianni & Missirlis, 2004). These factors include chemical composition of the material (Cordero, et al., 1996) (Kiremitçi-Gümüşderelioglu & Peşmen, 1996) (Gottenbos, et al., 2000) (Tegoulia & Cooper, 2002) (Buczynski, et al., 2003) (Henriques, et al., 2004) (Speranza, et al., 2004), surface charge (Gottenbos, et al., 2000), hydrophobicity (Balazs, et al., 2003) and simply surface roughness or physical configuration (Scheuerman, et al., 1998). Additionally, the reactor surface should be corrosion resistant, because the anaerobic degradation of certain compounds can lead to the formation of highly aggressive by-products (Dietzel, 2016). Lastly, thermal properties of the construction materials should be considered, since the anaerobic digestion process can occur at different temperature intervals. In Figure 6 an overview is given of the fabrication materials and their advantages and disadvantages.

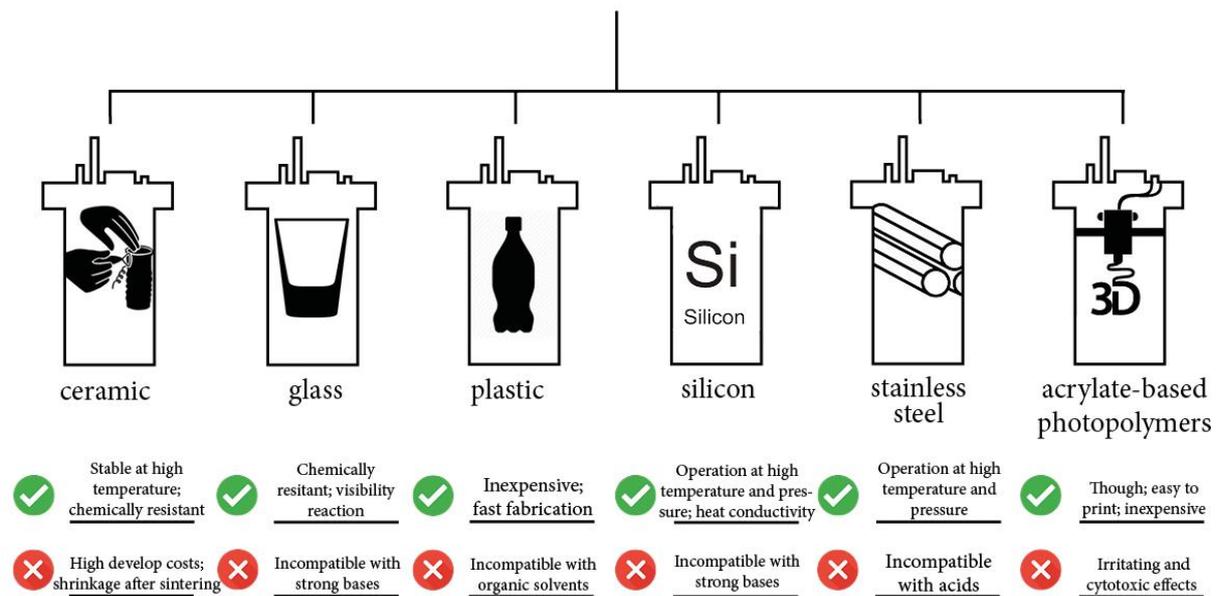


Figure 6: Overview adapted from (Dijkstra, 2018) visualizing the advantages and disadvantages per fabrication material (Knitter & Liauw, 2004) (Jain, et al., 2009) (Woolley & Mathies, 1994) (Mellors, et al., 2008) (Bens, et al., 2007) (Becker & Gaertner, 2001) (Becker & Gärtner, 2000) (Geyer & Seeberger, 2007) (Yen, et al., 2005) (Hessel, et al., 2004) (Ehrfeld, et al., 2000).

5.3.4 3D printing

3D printing, otherwise known as additive manufacturing, enables the construction of the design layer-by-layer rather than through moulding or subtractive techniques (Manyika, et al., 2013). Moreover, this technique provides high customizability while producing small quantities at relatively low cost, in a short time period (Berman, 2012). Different types of 3D printing techniques can be identified. Namely, selective laser sintering (SLS), direct metal laser sintering (DMLS), fused deposition modelling (FDM), stereolithography (SLA), laminated object manufacturing (LOM), and lastly, inkjet-bioprinting (Manyika, et al., 2013). The advantages and disadvantages of the different techniques, with exception of LOM due to its lack of precision, are outlined in Figure 7 (Olakanmi, et al., 2015) (Yan, et al., 2015) (Ning, et al., 2015) (Au, et al., 2014) (Liu, et al., 2015) (Zhang & Zhang, 2015) (Derakhshanfar, et al., 2018) (Manyika, et al., 2013). Currently, 3D printing is mainly used for medical purposes, but it has also gained the interest researchers operating in biotechnology (Ventola, 2014) (Bhattacharjee, et al., 2016). A variety of different materials used in 3D printing, such as plastic, stainless steel, ceramics, glass, paper, photopolymers, and living cells, enable opportunities for multiple applications (Chae, et al., 2015) (Ambrosi & Pumera, 2016) (Jain, et al., 2009) (Kotz, et al., 2017) (Selimis, et al., 2015) (Murphy & Atala, 2014).

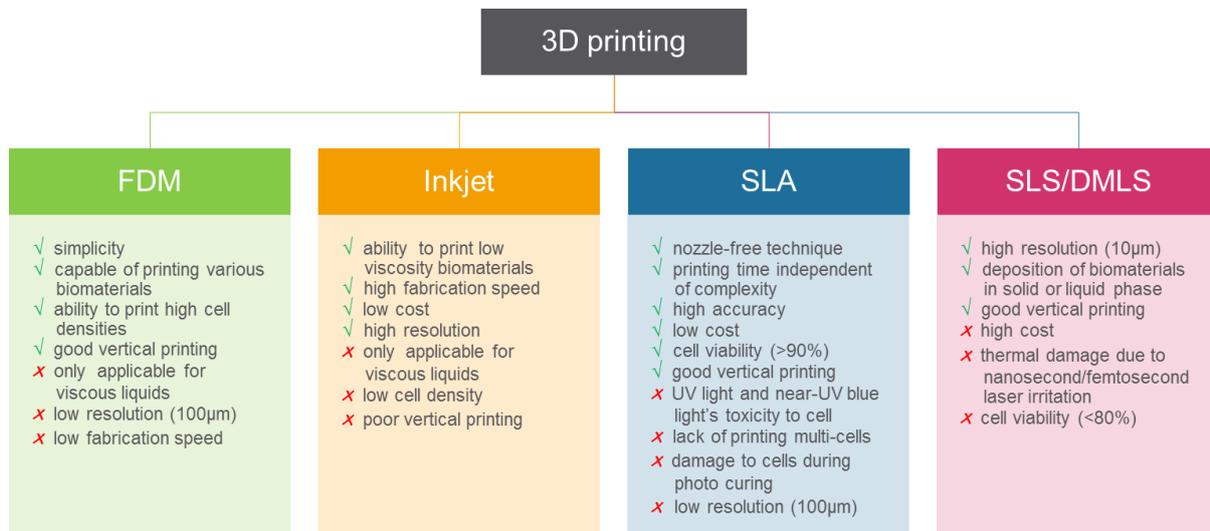


Figure 7: Overview with advantages and disadvantages of the different types of 3D printing.

5.3.5 Biocompatibility

Williams (1987) states that biocompatibility refers to the ability of a material to perform with an appropriate host response in a specific situation. However, this definition is argued to be so general and so self-evident that it is not of any real help in advancing knowledge of biocompatibility (Williams, 2008). Therefore, Williams (2008) redefined the definition of biocompatibility as the ability of a biomaterial to facilitate the most appropriate cellular or tissue response, while performing its desired function concerning a medical therapy, while optimising the clinically desired performance of the therapy, without drawing out any undesired local or systemic effects in the beneficiary or recipient of the therapy. Black (2005) separates the definition of biocompatibility into a host response and a material response. The host response is defined as: “the local and systemic response, other than the intended therapeutic response, of living systems to the material”, whereas the material response is defined as: “the response of the material to living systems” (Black, 2005). These definitions imply that biocompatibility phenomena associated with a biomaterial will vary depending on the application, meaning that biocompatibility is not a property of a material but of a

biomaterial-host system (Williams, 2018). Based on the generic biocompatibility pathways described by Williams (2014), three main biocompatibility goals are described. These goals are defined as defined as ‘defensive’, ‘target’, and ‘interfering’, as depicted in Figure 8.

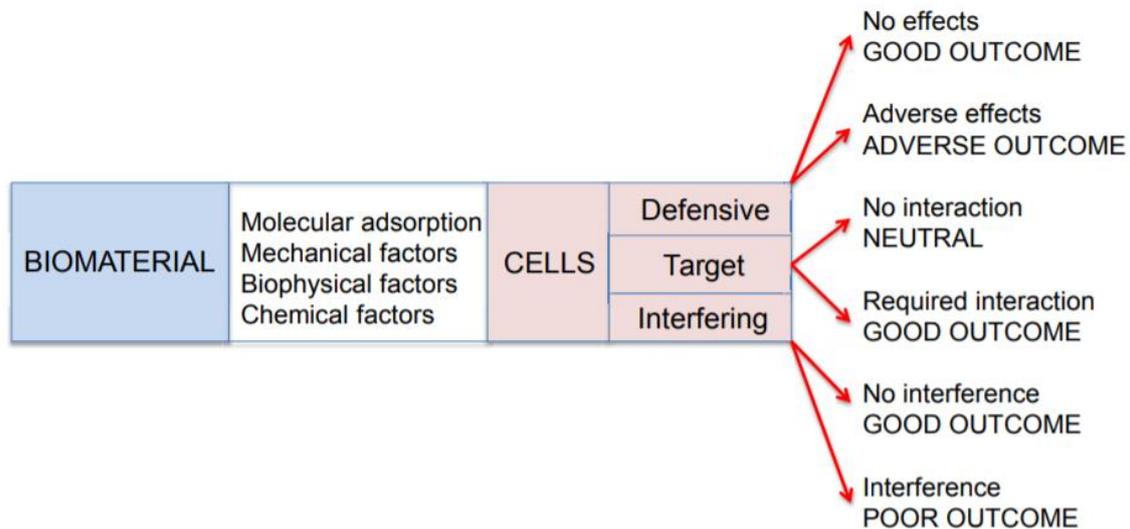


Figure 8: Summary of biocompatibility pathways involving interactions between biomaterial and the defensive, target and interfering cells, giving different clinical outcomes (Williams, 2014)

For this particular research biocompatibility is described by translating the three goals into specific requirements.

1. The material surface area of the anaerobic reactor is inert.
2. The material surface area of the anaerobic reactor is not biodegradable.
3. The material surface area of the anaerobic reactor does not adhere to bacteria.

5.3.6 Fluid dynamics

The flow characteristics in a miniaturized bioreactor differ significantly from those on the laboratory scale (Beebe, et al., 2002) (Tehranirokh, et al., 2013). At the microscale, different forces become dominant over those experienced in everyday life (Brody, et al., 1996). Because of scaling, it is often counterproductive to simply shrink an existing large device and expect it to function properly at the microlevel (Purcell, 1977). Therefore, new designs must be created in order to take advantage of forces that work on the microscale. Beebe, et al. (2002) describe different effects that become dominant in microfluidics including laminar flow, diffusion, fluidic resistance, surface area to volume ratio, and surface tension. The small dimensions of miniaturized bioreactor systems typically result in laminar fluid flow conditions of the fluid phase (Breslauer, et al., 2006) (Wu, et al., 2010) (Alam & Gernaey, 2012) (Dietzel, 2016). A cylindrical reactor design, which is common for CSTR's, enhances the predictability of the laminar flow dynamics (Tabatabaei & Ghanavati, 2018) (Beebe, et al., 2002). The rounding of the edges enables the flow to remain laminar and predictable, rather than becoming a random and complicated flow structure of interacting vortices (Brody, et al., 1996). In order to achieve homogenous conditions considering fluid dynamics different stirring mechanisms and pumps have been successfully integrated (Schäpper, et al., 2010) (Szita, et al., 2005) (Zhang, et al., 2006) (Lee, et al., 2006) (Lee, et al., 2011) (Park, et al., 2013) (Dietzel, 2016). Therefore, a cylindrical reactor shape with slightly rounded edges at the bottom of the reactor is chosen as the preferred reactor shape in this project.

5.3.7 Parallelisation

Gathering comprehensive process information concerning the development of a bioprocess requires several simultaneous experiments. Therefore, there is demand for cost-effective, parallel, and multiparametric systems with high-throughput (Zanzotto, et al., 2004). Parallelisation, in bioprocess development, signifies the practise where multiple reactors are employed side by side, thus in parallel (Powell, 2006). This utilization of multiple reactors in parallel, rather than one reactor which is used sequentially, increases the experimental throughput. Additionally, the time required for the process development will reduce (Kurzrock & Kress, 2014). By setting the tested parameter differently for each reactor, high experimental throughput is achieved regarding that parameter.

5.4 Performance evaluation 3D printing process

Pahl & Beitz (2007) distinguish two main types of criteria. Namely, technical and economic criteria. Both criteria consist of multiple aspects, which can be modified to the product or process under consideration. In general, the majority of the conventional bioreactors are fabricated using glass, plastic or metal (Eppendorf, 2018). The most generic and applicable manufacturing techniques with respect to these materials are injection moulding, casting and milling & turning (Kalpakjian, 2008).

The technical criteria cover the performance of the process with respect to the final desired product. The overall quality and lifespan are therefore included in the technical criteria aspects. Additionally, it is relevant how complex and accurate the process is. Thus, how complex the overall process of producing the product is and how detailed the product can be produced. Lastly, the maximum object size is included as a technical criteria aspect. Currently, the 3D printers entail a limited object size which can be produced. This should also be considered when evaluating the technical performance.

The economic criteria firstly consider the costs. The costs are separated into raw material costs, production costs and general overhead costs (Edmonds, et al., 2016). Additionally, the time required to produce one product is considered. Lastly, the flexibility, the flexibility the production process entails is considered in the economic criteria aspects.

The information for the 3D printing process can be obtained using the Formlabs Preform software. The software calculates the resources and time required per component. For the information considering the different production techniques, literature will be used.

5.5 Literature review conclusion

Based on the literature review, a list of functional requirements can be formulated. The conclusions based on the literature will be discussed. Using these conclusions, a list of functional requirements is formulated.

The best reactor type for this project is a CSTR due to its continuity and stability which will be crucial for process screening purposes. The volume of the reactor should amount to 40 mL. In order to ensure a homogeneous mixture, a cylindrical reactor shape is chosen with rounded edges at the bottom. The continuous process setup requires one influx port for feedstock, one efflux port for desired product and one efflux port for effluent. To ensure that the fluids can flow adequately through the ports, the diameter of the ports should have a minimum of 2 mm. For the gas, the minimum port diameter is 1.5 mm. The effluent outlet port should be located underneath the fluid surface. Specifically, at a height which is equal to 30% of the height of the reactor itself. Additionally, the reactor should be fitted with a marine stirring mechanism. This stirrer diameter should be 40% of the inner diameter of the reactor. The critical process parameters of anaerobic digestion entail some crucial reactor design requirements. Since the mesophilic temperature will be used for the reaction, the reactor should be resistant to this temperature range. The desired value of the pH in the reactor amounts to a value between pH 6.8 and pH 7.4. Therefore, the reactor should not degrade and function properly in this acidity environment. The combination of volatile fatty acids and the alkalinity highlights that the reactor should be able to hold a sufficient buffer capacity. However, this is mostly dependent on the contents of the feedstock and influx. The organic loading rate, retention time and the C/N ratio are determined by the contents of the influent and the effluent. Therefore, there are not specific reactor requirements based on those parameters. To ensure that the redox potential is between the specified range, the reactor needs to be able to provide a complete anoxic environment. This entails that the reactor should be sealed completely. The fabrication material used for the SLA 3D printing process, and ultimately the reactor, should not introduce any toxic compounds to the environment in the digester. Additionally, the material should be biocompatible as defined in the biocompatibility section. The sensor required to monitor the anaerobic digestion process needs to be fitted to the reactor. The pH meter has a diameter of 6.3 mm. Again, when fitting this measuring instrument, it needs to be sealed hermetically such that the anoxic environment is sustained.

Based on the literature review a list of functional requirements is formulated, which will be used for the system design.

- The reactor should be round
- The reactor should have rounded edges (inside) for fluid dynamical purposes (source)
- The height/diameter ratio should be 2.2
- The inner volume of the reactor should be 40 mL
- The reactor should have an influent inlet with a diameter of 2 mm which can be connected to a piece of tubing.
- The reactor should have an outlet for effluent with 2 mm in diameter, the depth of the effluent outlet should be $0.3 * \text{inner height}$
- The reactor should have an outlet for biogas with a minimum diameter of 1.5 mm
- The reactor should be mechanically stirred using a stirrer driven by an external motor
- The reactor should be airtight such that the anoxic environment can be sustained
- The reactor should have a port for the pH meter with diameter 6.3 mm
- The reactor should have a removable lid
- The reactor should be able to operate and function properly at mesophilic temperatures
- The reactor should be produced using (SLA) 3D printing
- The material surface area of the anaerobic reactor is inert.

- The material surface area of the anaerobic reactor is not biodegradable.
- The material surface area of the anaerobic reactor does not adhere to bacteria.
- The reactor should be able to function in a multi-parallel screening platform
- The minimal thickness of walls, edges and such should be 1 millimetre.
- Marine stirring should be used with diameter of $0.4 \cdot \text{reactor diameter}$.

Considering the 3D printing process evaluation, the criteria can be separated into a set of technical criteria and a set of economic criteria.

The technical criteria consist of quality, lifespan, process complexity, process accuracy and maximum object size. The economic criteria consist of raw material costs, production costs, production time, flexibility, general overhead costs.



The system design chapter will describe the process from requirements to final system design. The functional requirements and the system design will primarily focus on one reactor. Placing several reactors in parallel will create the system. The systematic design approach described by Pahl & Beitz (2007) will be used. Firstly, a material-energy-information diagram will be used to visualize the main function of the system with its in and outputs. Thereafter, the MEI diagram is expanded into a function structure describing all the subfunction of the system. Based on these subfunctions the working principles can be formulated. Thereafter, solutions for the working principles are generated. Lastly, the best option per working principle is selected and the final design is specified.

6.1 MEI diagram

The MEI diagram describes changes caused by a function to inputs such as energy, material and signals (Pahl & Beitz, 2007). For the 3D printed miniaturized reactor system, the main function is 'obtaining process information'. In order to perform this action, some inputs are necessary. Firstly, energy is required in the form of heat and electrical energy for the stirring. Secondly, material is required in the form of feedstock and inoculum, which will enable the microbial reaction. Lastly information is required in the form of signals switching the system on and off, setting the different process parameters like temperature, organic loading rate and mixing speed. These inputs are transformed, by the main function, to modified outputs. Firstly, not all of the heat will be utilized. Therefore, some energy in the form of heat is considered as an output of the system. The energy used for stirring will remain in the system and is therefore not included as an output. The microbial reaction will convert the feedstock, using the inoculum, into biogas and digestate. This biogas and digestate are considered as a materialistic output of the system. Lastly, the system will provide process information. This information output is crucial, since it enables the fulfilment of the main function. For the anaerobic digestion process, parameters measured include pH, biogas volume produced, alkalinity, chemical oxygen demand, and lastly the total amount of volatile fatty acids. The selection and justification of these parameters will be addressed in the experimental section. The MEI diagram is visualized in Figure 9.

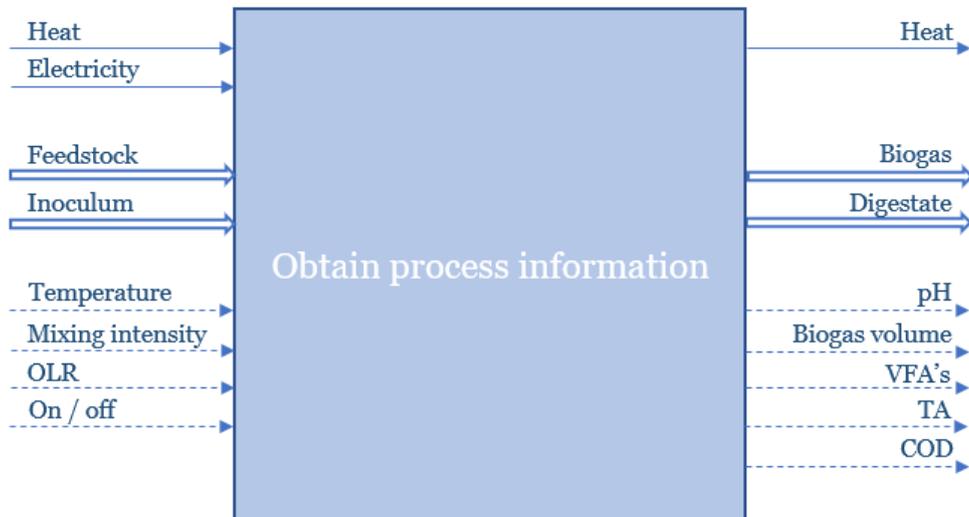


Figure 9: MEI diagram describing the in- and outputs of the main function of the system as well as the main overall function of the system.

6.2 Function structure

When the in- and outputs of the main function are defined, the function itself can be decomposed into different subfunctions. These subfunctions are based on the functional requirements derived in the literature review. All the subfunctions combined create the main function as specified in the MEI diagram. Visualizing all the subfunctions and their interrelations, within the main function, together with their in- and outputs creates a function structure. This function structure is used when developing working principles. The function structure for the 3D printed miniaturized bioreactor system is depicted in Figure 10.

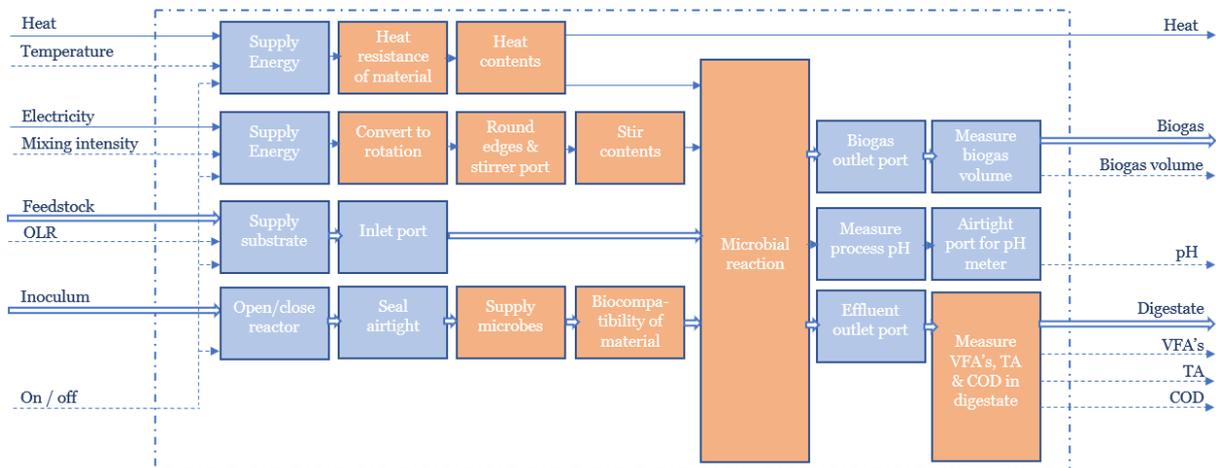


Figure 10: Function structure containing all the subfunctions which together enable the main function of the system. The specific material/energy/information input are also indicated per subfunction. The blue subfunctions indicate the presence of multiple possible solutions. The orange subfunctions indicate the solution has already been predetermined.

6.3 Working principles

For all the different subfunctions, as stated in the function structure, a suitable solution needs to be found. For some subfunctions the solution is already predetermined by the functional requirements set by the literature review. Additionally, some subfunctions only have one suitable solution. These subfunctions, and their solution, will be discussed first. Thereafter, the subfunctions which have multiple solutions are discussed. Per subfunction, the multiple solutions are discussed, which can later be used in the morphological overview.

6.3.1 Subfunctions with predetermined solutions

The subfunctions which already have predetermined solutions are: heat resistance of material, convert to rotational velocity, heat contents of reactor, rounded edges, supply microbes, stir contents of reactor, biocompatibility of fabrication material, microbial reaction and measure VFA's, TA and COD in digestate. These subfunctions are indicated in orange in the function structure. These subfunctions will be discussed and their solution will be specified in this subchapter.

6.3.1.1 Heat resistance of material

The material used in the 3D printer is called resin. Different types of resin can be used. The resin used for this project is 'Clear Resin – FLGPCL04' produced by Formlabs. The material has a heat deflection temperature of 73.1 °C at 66 psi (Formlabs, 2017). Therefore, the material will start deforming at a temperature of 73.1 °C when a pressure of 66 psi (4.5 bar) is exerted on the material. Since neither a temperature of 73.1 °C nor a pressure of 4.5 bar are reached during the microbial reaction, the material is expected to be resistant to the moderate heat exerted on the system.

6.3.1.2 Convert to rotational velocity

The contents of a reactor need to be stirred in order to obtain a homogeneous mixture. A design parameter was that a mechanical stirrer was used. The stirrer is powered by a 12V DC electric motor with model number: SKU212191. The motors were bought from an online retailer. The motor has an output rotational velocity of 300 RPM, which is sufficient for the stirring of the sludge. In Figure 11 a picture with a schematic is visualized. The dimensions of the motor are also added to the sketch.

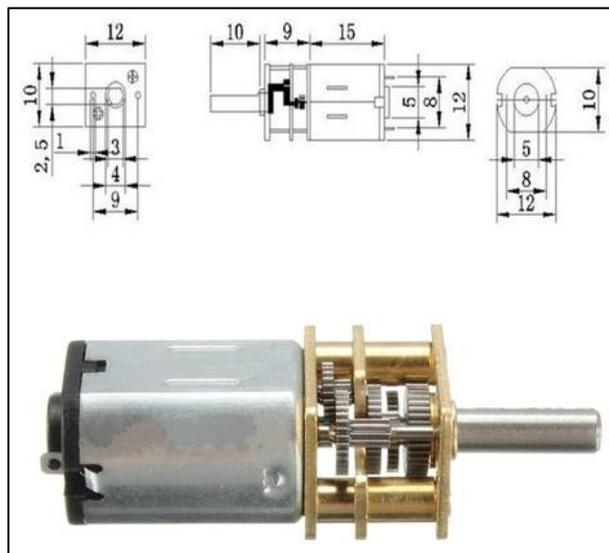


Figure 11: Picture and dimensional sketch of the SKU212191 12V DC 300 RPM motor powering the mechanical stirrer, thus providing the conversion to rotational velocity.

6.3.1.3 Heat contents of reactor

The contents of the reactor will be heated using convection. The mechanism supplying the thermal energy is not determined yet. However, regardless of the exact mechanism, the heat will be transferred to the contents of the reactor using convection. The exact mechanism supplying the thermal energy will be specified later.

6.3.1.4 Rounded edges

The rounded edges in the design can easily be created in SolidWorks. Therefore, there are no multiple solutions to this subfunction.

6.3.1.5 Supply microbes

The microbes are supplied by inoculating the reactor with sieved sludge. The sludge originates from a semi-continuous lab bioreactor treating cow manure.

6.3.1.6 Stir contents of reactor

The stirrer, stirring the contents of the reactor, will be powered by a small motor. In general, two types of stirrers can be used in bioreactors. Firstly, a marine impeller and secondly, a Rushton impeller (Applikon academy, 2019). The diameter of the stirring blades need to be 40% of the reactor diameter and 33% of the reactor diameter of marine and Rushton respectively. Additionally, it is important to ensure that the stirrer does not interfere with any in- and outlet ports. The specific stirrer design will be discussed later.

6.3.1.7 Biocompatibility of fabrication material

The resin used for fabrication is a photopolymer. A photopolymer is a polymer which changes its properties when it is exposed to UV-light. The material transforms from a liquid to a solid due to the forming of cross-links between monomers (Phillips, 1984). Therefore, the final printed product is a thermoset plastic. Since the biodegradability of thermoset plastics is low, the final product should not be biodegradable when the FLGPCL04 clear resin is used. Additionally, the cured resin is inert and when the reactor is stirred properly, the bacteria should not adhere to the reactor.

6.3.1.8 Microbial reaction

The microbial reaction functions as the transformation function of the inputs within the system. The anaerobic digestion process is used as this reaction. During the literature review the process and its important parameters are described.

6.3.1.9 Measure VFA's, TA and COD in digestate

The VFA's and the TA are measured using a Hach automatic titrator. Specifically, the TitraLab AT1000 automatic titrator was used. 5 mL of digestate is added to a beaker. Thereafter, the digestate is diluted with 50 mL of demineralised water. The solution is placed in the device, which then automatically measures the VFA's and TA of the solution. VFA/TA is in German literature also referred to as FOS/TAC. The titrator also uses this notation.

The COD is determined using Hach Lange LCK014 chemical oxygen demand cuvette tests. This type of test kit has a range between 1000 and 10000 mg/L. Firstly, 0.5 mL of digestate is added to a cuvette. This is done for each reactor separately. The chemicals inside the cuvette and the digestate are mixed. Thereafter, the cuvette is placed inside a Hach Lange HT200S digester, which heats the samples at a temperature of 175°C for 15 minutes. After the digestion, the samples are placed in a Hach Lange DR 3900 spectrophotometer, which measures the COD.

6.3.2 Subfunctions with multiple suitable solutions

The subfunctions which have multiple suitable solutions are: supply thermal energy, supply electrical energy, supply substrate, open / close reactor, airtight ports for in- and outlet and stirring, airtight seal when opening and closing reactor, measure process pH and measure biogas volume. These subfunctions are indicated by a blue colour in the function structure.

Solutions for these subfunctions are generated by brainstorming and studying other bioreactors.

6.3.2.1 Supply thermal energy

Water bath

A laboratory water bath can be used to heat samples and reactors. The reactors are placed in the bath and are thereby heated by the water. The reactors can be placed on a platform which is underneath the water surface, or the reactors can hang from a frame which keeps them in place. These water baths are often relatively large devices and therefore require a significant amount of work space.

Water jacket

An alternative to the water bath is the water jacket. A water jacket is a casting surrounding the reactor, filled with water. This entails a more complex reactor design. The water is pumped through the reactor. The pump enabling the water flow also provides the water with thermal energy. Designs for the pumping and heating device vary in terms of size and power.

Hot air oven

Hot air ovens can also be used to facilitate the reactor with its required thermal energy. These hot air ovens are based on the principle of forced air thermal convection. However, these ovens are typically used for relatively high temperatures. Additionally, the oven needs to be closed completely. This entails that all experimental equipment should also be fitted in the oven. This is not desirable and unpractical in terms of the laboratorial experiments.

Micro heaters

Micro heaters are small electrical devices that can be fitted in a reactor. The advantage of the micro heater is that they can be self-regulating. Therefore, the principle can ensure a very constant and accurate temperature. However, because the heaters are very small, they are not powerful enough to heat the sludge in the reactor. Moreover, the heaters would require a separate entry slot in the reactor. This entails challenges in sealing the heater port and preventing leakages.

Coil

The final principle that can be applied for the heating of the reactor is the coil principle. In essence, the coil is a tube which runs through the reactor. Through the tube, hot water is pumped which heats the contents of the reactor. The disadvantage is that more surface area is created in the reactor on which bacteria can adhere. Also, the complex coil structure can create some corners and areas which cannot be stirred probably. This can have negative effects on the performance of the reactor. Moreover, the coil is likely to interfere with in- and outlet ports and the stirring mechanism.

6.3.2.2 Supply electrical energy

Plug and socket

A very generic and simple option for the supply of electrical energy is using the main electricity grid. However, the motors used cannot be directly plugged in to the grid due to its high voltage. Therefore, transformers would be required.

Batteries

Batteries can be acquired easily in various sizes and types. The motor requires a 12V power source to perform optimally. However, a 9V battery will also supply a sufficient amount of energy. However, the output velocity will be slightly lower. The advantage of a battery is that it is portable. The main disadvantage is that the batteries need replacement when they have run out of energy.

Controller

A controller can be used to direct all motors at once. The downside is that these controllers are very complex and expensive. Currently, the university lab does not have a suitable controller for the specific type of motor used. Moreover, the controllers tend to be relatively large and heavy. This makes them impractical when they are applied in a miniaturized reactor system.

6.3.2.3 Supply substrate

Infusion pump

An infusion pump, or syringe pump, can be used to feed substrate to the reactor. The pumps can be set to a certain input velocity. The feedstock itself is placed in syringes, which are placed in the pump. The pump compresses the syringes which causes its contents to flow, via tubing, into the reactor. The compression rate can be adjusted to meet the requirements of the reaction. The advantage of this system is that it can feed the reactor continuously. Additionally, when the syringes are empty, they can be replaced easily. Cleaning the pump is not required since the feedstock and the pump itself do not interact.

Infusion controller

A slightly different variant of an infusion pump is an infusion controller. Infusion controllers are typically used in the healthcare sector for providing patients with their required drugs. The infusion controller however does interact with the feedstock. The feedstock can be added to the controller using infusion bags. Generally, infusion controllers are more accurate compared to infusion pumps. However, the controllers require regular cleaning. Additionally, the controllers are generally more expensive compared to the syringe pumps.

Effluent from large reactor with pump

In order to supply the reactor with fresh substrate and inoculum, a larger reactor system can be used. The effluent of that reactor can be used as feedstock for the smaller reactor.

6.3.2.4 Open / close reactor

Insertable lid

The first option for a lid is the insertable lid. This lid functions somewhat similar to a cork and bottle. The lid is slightly smaller in diameter than the vessel. Then, the lid can be placed in the vessel which. The disadvantage of this methodology is that the lid is not fixed in its position tightly. Especially when this principle is applied on a vessel which is used for microbial reactions. The pressure generated by the reaction can cause the lid to come off. However, the advantage is that the lid is very easy to design.

Lid with thread

The lid with thread is a very generic principle used for example bottles with plastic caps. The advantage of this method is that the lid can be tightened very tightly. This ensures that it remains fixed at its position. However, printing thread using stereolithography can prove to be difficult. Some trial and error is required to obtain the optimal thread dimensions for the vessel and lid.

6.3.2.5 Airtight ports for in- and outlet and stirring

Teflon tape

Teflon tape, or thread seal tape, is typically used for plumbing activities. Pipes can be connected to taps using thread. By placing tape on the thread before the pipes are connected, the thread is sealed. The advantage of this method is that the tape is very cheap and easily accessible. However, this tape slightly reduces the modularity of the design. When something is unscrewed again, the tape often needs replacing. Another advantage is that generally a lot of force is required to tighten the parts. When the parts are 3D printed and very small, the parts

may not be able to handle these forces. This should be taken into consideration when selecting this principle.

Butyl rubber and needle

In laboratories test tubes are often sealed from its environment using butyl rubber caps. Contents can be inserted or extracted from the test tube using needles. These needles can be inserted through the rubber. When the needles are removed, the rubber seals itself again and maintains the general seal of the outer environment. The disadvantage is that all the in- and out-puts need to be through a needle. This can be problematic when there are solids in the sludge or in the feedstock. For the stirring, a bigger hole is required. This is theoretically possible. However, the rubber will probably entail a lot of friction on the stirrer. This can cause problems regarding the power of the motors.

Tight thread

Another solution for sealing the ports is using a thread which is very tight. However, the force required to assemble the system will be large. Additionally, the fabrication of fine thread is difficult using 3D printing.

Rubber O-rings

Rubber O-rings are a well-known solution for sealing different parts in taps or pipes. The rings can be acquired in various types and sizes at a low price. The disadvantage of the rubber rings is that they require an edge where they can be placed in the design. Additionally, the rubber rings need to be fixed tightly to their place. Otherwise, leakage can occur. This can entail some complex design features. However, the rings have proven to be effective.

One component design

The most ideal method of sealing is creating the whole design in one component, which can directly be printed. For some ports this is possible. However, some modular parts are required. For example, the stirrer needs to be able to turn freely with respect to the reactor. Therefore, this principle is not applicable to all ports.

6.3.2.6 Airtight seal when opening and closing reactor

Rubber O-rings

As discussed at section 6.3.2.5, the rubber rings are likely to entail some complex design features. However, the principle is very effective and cheap.

Teflon tape

As discussed at section 6.3.2.5, the Teflon tape will reduce the modularity of the design. However, the tape is often effective and very cheap.

Tight thread

As discussed at section 6.3.2.5, the tight thread can cause assembly issues. Moreover, using 3D printing for fabricating thread is not ideal.

6.3.2.7 Measure process pH

pH meter (online)

Online pH meters are devices which continuously monitor and measure the pH. This online measuring does not require sampling. However, a disadvantage of this principle is that the reactor needs to be modified such that a pH meter can be fitted in. Additionally, these online pH meters are relatively expensive. However, they are also very accurate. Therefore, the online pH meters are very suitable for this reactor concept.

Taking samples (offline)

The counterpart of online measuring is offline measuring. This method requires sampling and is discontinuous. The samples can be inspected using a digital pH meter or strips of pH paper.

The strips of paper are cheap. However, they are also inaccurate compared to the digital meters. However, when a digital meter is used, it might be preferable to insert the pH meter in the reactor such that it becomes online. That will entail more process data and information.

6.3.2.8 Measure biogas volume

Gas counter

A gas counter is often used in industry or in setups where the produced volumes of gas are relatively large. Moreover, in households this device is also used to measure the amount of gas which is used by a household. These devices are at small scale not accurate. However, the devices are relatively expensive. The measurements are continuous and therefore do not require sampling.

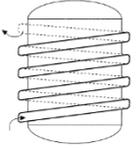
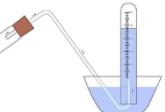
Water displacement

In laboratories the principle of water displacement is used very often. The produced gas is, due to the pressure difference, pumped into a test tube. The test tube is sealed from the environment. However, there is an outlet for the water in the test tube. Because the pressure in the test tube increases due to the gas being pumped in, the water will flow out of the test tube. By monitoring the level of the water surface, the volume of gas can be accurately measured at very small volumes. Additionally, the measuring setup is continuous, does not require samples, and is cheap to set up.

6.4 Morphological overview of working principles

A morphological overview is depicted in Table 2, to give an overview of the possible solutions per subfunction. This overview provides a systematic approach for combining solutions and deriving working structures.

Table 2: morphological overview with in the left column the subfunctions with multiple suitable solutions and in the remaining columns the different solutions

Solutions Subfunctions		Solutions				
		1	2	3	4	5
A	Supply thermal energy	Water bath 	Water jacket 	Hot air oven 	Micro heaters 	Coil 
B	Supply electrical energy	Plug & socket 	Battery 	Controller 		
C	Supply substrate	Infusion pump 	Infusion controller 	Effluent & pump 		
D	Open / close reactor	Insertable lid 	Lid & thread 			
E	Airtight ports for in- and outlet and stirring	Teflon tape 	Butyl rubber and needle 	Tight thread 	Rubber O-ring 	1-component design 
F	Airtight seal when opening and closing reactor	Rubber O-ring 	Teflon tape 	Tight thread 		
G	Measure process pH	Online 	Offline 			
H	Measure biogas volume	Gas counter 	Water displacement 			

6.5 Selection of conceptual design

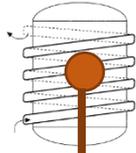
In order to create a design which fulfils all the functional requirements, is able to carry out all the subfunctions and therefore ultimately the main function, a set of working principles need to be selected. The working principles are selected using a selection chart as described by Pahl & Beitz (2007). The chart, Table 3, assesses the working principles with multiple solutions based on compatibility, whether it fulfils the demands of the requirements list, realizability, costs, whether it incorporates direct safety measures and lastly, designer preference. Each working principle is referred to using a letter-number combination which is introduced in the morphological overview depicted in Table 2.

Table 3: Selection chart evaluating each working principle, identified by their letter-number combination, with respect to compatibility, whether it fulfils the demands of the requirements list, realizability, costs, whether it incorporates direct safety measures and lastly, designer preference

University of Groningen		SELECTION CHART for miniaturized bioreactor							Part: 1	Page: 1
Enter solution variant (Sv):	Solution variants (Sv) evaluated by							DECISION		
	SELECTION CRITERIA							Mark solution variants (Sv)		
	(+) Yes (-) No (?) Lack of information (!) Check requirements list							(+) Pursue solution (-) Eliminate solution (?) Collect information (re-evaluate solution) (!) Check requirements list for changes		
	Compatibility assured									
	Fulfils demands of requirement list									
	Realisable in principle									
	Within permissible costs									
	Incorporates direct safety measures									
	Preferred by designer									
			A	B	C	D	E	F	G	Decision
		Remarks								
A1	1	+	+	+	+	+	-	Requires a lot of space and a separate heating element	-	
A2	2	+	+	+	+	+	+	Requires a separate heating and pumping mechanism	+	
A3	3	-	+	+	-	+	-	Is not available at university and the reactors cannot be connected properly	-	
A4	4	-	-	-	+	+	-	Require a separate entry point in the reactor and are not powerful enough	-	
A5	5	+	+	-	+	+	-	Likely to interfere with inlet and outlet ports	-	
B1	6	-	+	-	+	+	-	Not directly connectable to the SKU212129 motors	-	
B2	7	+	+	+	+	+	+	Directly connectable to motors and portable	+	
B3	8	+	+	+	-	+	-	Very expensive and large	-	
C1	9	+	+	+	+	+	+	The university already has multiple infusion pumps which can be used	-	
C2	10	+	+	+	-	+	-	The infusion controllers need to be purchased	-	
C3	11	+	+	+	-	+	-	The principle is very good, however the setup will require a lot of time and resources	-	
D1	12	-	+	+	+	+	-	The pressure inside the vessel can cause it to pop of during the digestion process	-	
D2	13	+	+	+	+	+	+	Ensures a good and tight seal	+	
E1	14	+	+	+	+	+	-	Not very durable. Tape needs to be replaced often	-	
E2	15	-	+	-	+	+	-	Hard to integrate in reactor	-	
E3	16	-	+	-	+	+	-	Hard to fabricate using 3D printing. Assembling and disassembling requires much force	-	
E4	17	+	+	+	+	+	+	Requires edges where they can be placed	+	
E5	18	-	-	-	+	+	-	Not possible, some parts are required to be removable.	-	
F1	19	+	+	+	+	+	+	Requires edges where they can be placed	+	
F2	20	+	+	+	+	+	-	Not very durable. Tape needs to be replaced often.	-	
F3	21	-	+	-	+	+	-	Hard to fabricate using 3D printing. Assembling and disassembling requires much force	-	
G1	22	+	+	+	+	+	+	Requires more investment, but enables a continuous monitoring of the process	+	
G2	23	-	-	-	+	+	-	Requires sampling and is less accurate than online measuring	-	
H1	24	+	+	+	-	+	-	Expensive and large	-	
H2	25	+	+	+	+	+	+	Simple, cheap and sufficiently accurate	+	
Date: May 10 2019		Jorn-Ids Heins								

As can be deduced from the selection chart, a preferred working principle has been selected per subfunction. This creates a design path which is visualized in the adapted morphological overview in Table 4.

Table 4: Morphological overview showing the selected solutions, based on the selection chart, per subfunction. Thus, indicating the design path which will be followed.

Solutions		1	2	3	4	5
Subfunctions						
A	Supply thermal energy	Water bath 	Water jacket 	Hot air oven 	Micro heaters 	Coil 
B	Supply electrical energy	Plug & socket 	Battery 	Controller 		
C	Supply substrate	Infusion pump 	Infusion controller 	Effluent & pump 		
D	Open / close reactor	Insertable lid 	Lid & thread 			
E	Airtight ports for in- and outlet and stirring	Teflon tape 	Butyl rubber and needle 	Tight thread 	Rubber O-ring 	1-component design 
F	Airtight seal when opening and closing reactor	Rubber O-ring 	Teflon tape 	Tight thread 		
H	Measure process pH	Online 	Offline 			
I	Measure biogas volume	Gas counter 	Water displacement 			

6.6 Concept specification

The selected working principles are translated to a concrete design which fulfils all functional requirements. The design is created using SolidWorks. The technical drawings, also produced using SolidWorks, are added to Appendix I. The reactor design consists of 6 separate parts.

1. Reactor vessel
2. Reactor lid
3. Marine stirrer
4. Motor to stirrer connection
5. Stirrer nut
6. pH meter nut.

The vessel is used for holding the contents used for the microbial reaction. Additionally, the double walled design allows for water to be pumped through the walls of the reactor, enabling the heating of the vessel. The vessel is fitted with thread which can be used to connect the vessel with the lid. An edge is present where a rubber ring can be placed to ensure a proper seal. The lid can be screwed to the vessel. The lid is fitted with a support structure in which the motor can be placed. This ensures that the motor is perfectly above the centre of the vessel and the stirrer can be fitted properly. The lid has three ports for in- and outlet streams. Firstly, an outlet port for the produced biogas. This port is located underneath the support structure for the motor. Secondly, an outlet port for effluent is present. This port has an extension which reaches underneath the surface of the contents in the reactor. The last port is used for feeding the reactor with substrate. This port has a short extension from which the feedstock can drip into the reactor. Moreover, the lid is fitted with two additional ports. One port is perfectly in the centre of the reactor. Through this port the stirrer can be inserted. The other port is the largest port of the reactor. This port enables a pH meter to be placed inside the reactor. Both ports are sealed using rubber rings. Two nuts are used to fix the rubber O-rings in place. The nuts can be slid into the hole using two small shafts which are present on the nuts. The orientation of the path the shafts follow changes from vertical to horizontal when the nuts compress the rubber O-rings sufficiently. This change in orientation allows the nuts to be 'screwed' in the lid and remain properly fixed. The stirrer is a marine type stirrer which can be connection via a connection part to the motor. The final part of the reactor is that small part connecting the stirrer shaft to the motor. A picture of the final reactor design is visualized in Figure 12. Additionally, an assembly drawing is also added to Appendix I.

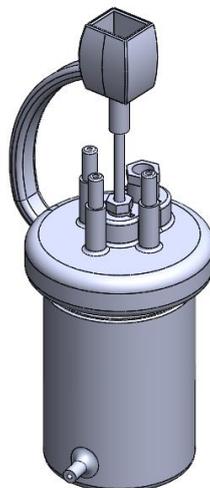


Figure 12: Visualization of final reactor design extracted from SolidWorks.

The bioreactor system is obtained by placing multiple reactors in parallel. For the validation of the reactor, and therefore the system, a system containing four reactors is chosen. A platform was created which can be used to neatly fix the reactors. However, this platform is optional and can be modified to the specific system requirements. The platform can be fitted with test tubes, used for the experimental phase. Moreover, the platform is fitted with a small box, which can be used to store batteries and cables for the stirring motors. A visualization of the platform is depicted in Figure 13. Since the platform is optional and not relevant for the performance of the system, the specific drawings of the platform are not included.

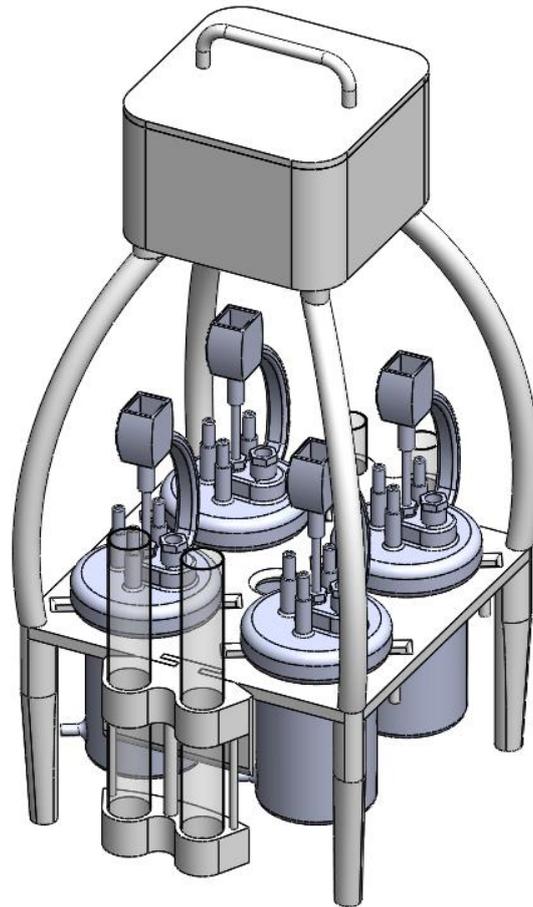


Figure 13: Complete assembled bioreactor system used for the laboratorial experiments. The system consists of four bioreactors and a platform.

The reactors and the platform are produced using the Formlabs Form2 3D printer. The system was assembled and fitted with all the rubber rings, motors, tubes and the pH meters. This assembled system is used for the experimental phase in order to validate the design. The experimental setup will be further discussed in the laboratorial experiment section.



This chapter aims to validate the design concept which resulted from the system design phase. Firstly, the laboratorial experimentation will be discussed. The setup will be specified, and the analytical methods used will be explained. Thereafter, the results of the experiment will be stated. Secondly, the evaluation of the 3D printing will be discussed. The setup will be explained followed by the results.

7.1 Laboratorial experiment

In order to validate the concept, laboratorial experimentation will be used. Firstly, the experimental setup will be explained. All the equipment will be discussed, and the general procedure will be elaborated.

7.1.1 Experimental setup

For the experiment, the anaerobic digestion process is studied. For the process sieved sludge is added to the reactor systems as inoculum. Thereafter, milk powder, which contains glucose, is added as feedstock. The milk powder was dissolved in demineralized water. Per mL of water, 0.003 g of milk powder was added. This solution is based on a hydraulic retention time of 30 days and organic loading rate of 0.1 g COD per day. This corresponds with a feeding rate of 10 mL per day for the baseline reactors and 1 mL per day for the miniaturized reactors. The effluent removal rate is also 10 mL and 1 mL per day for the baseline and miniaturized reactors respectively.

In the experiment, the bench scale Eppendorf BioBLU® 0.3c Single-Use Vessel with a volume of 400 mL will be compared to the 3D printed miniaturized concept. The baseline concept was stirred using an ABI motor and a serpentine belt. The motor is controlled using a Gossen motor controller. The system is heated using a water bath. The water is heated using Julabo water heating and pumping system. The experiment will contain 2 miniaturized reactors and 2 Eppendorf reactors.

All the reactors will be fed using tubes, pumps and syringes. The tubes have an inner diameter of 3mm and a wall thickness of 1 mm. The syringes are manufactured by Terumo. The syringes have a volume of 12 ml and 6 ml for the Eppendorf reactors and the 3D printed miniaturized reactors respectively. The syringes are placed in infusion pumps. The pumps are made by Inacom instruments and they are slightly modified such that each pump can hold 4 syringes. The specific pump used is the NE-1000X model.

The 3D printed reactor system is heated by using a heated water flow. The water is heated and pumped using the Haake D8 Fisions water bath produced by Haake Technik GmbH. Tubes are used to connect the water bath to the reactors. The tubes have a 3mm inner diameter and a wall thickness of 1mm.

A controller is used to monitor the pH of the 3D printed reactors as well as the Eppendorf reactors. The Consort C3060 multi parameter analyser was used. The controller has in total 4 ports for pH meters and 4 ports for Redox potential meters. Only the 4 pH ports will be used.

2 for the 3D printed system and 2 for the reference system. The pH electrodes used to measure the pH are manufactured by VWR. The electrodes can operate in a temperature range from 0 – 100 °C, a pressure range from 0 – 7 bar and have a response time which is less than 1 second (VWR international, 2019). Therefore, the electrodes are suitable for this experiment.

In order to measure the water displacement, i.e. biogas volume, different test tubes are used. For the 3D printed systems test tubes of 7.5 ml and 15 ml are used. For the bench scale Eppendorf system tubes of 150 ml are used. The tubes are fitted with a rubber lid, which seals them. Thereafter, a needle is inserted which enables the inflow of biogas. Another needle is inserted, underneath the water level, which allows the water to escape from the test tube when the pressure, due to biogas production, increases.

In order to remove effluent from the reactor, the same mechanism, which is also used for influent, is used. The same syringes, tubes and pumps are used. However, the pumps are now set to retract fluid from the system, instead of inserting it. This allows for a continuous flow through the reactor systems while maintaining the level working volume. A visualization of the experiment is depicted in Figure 14.

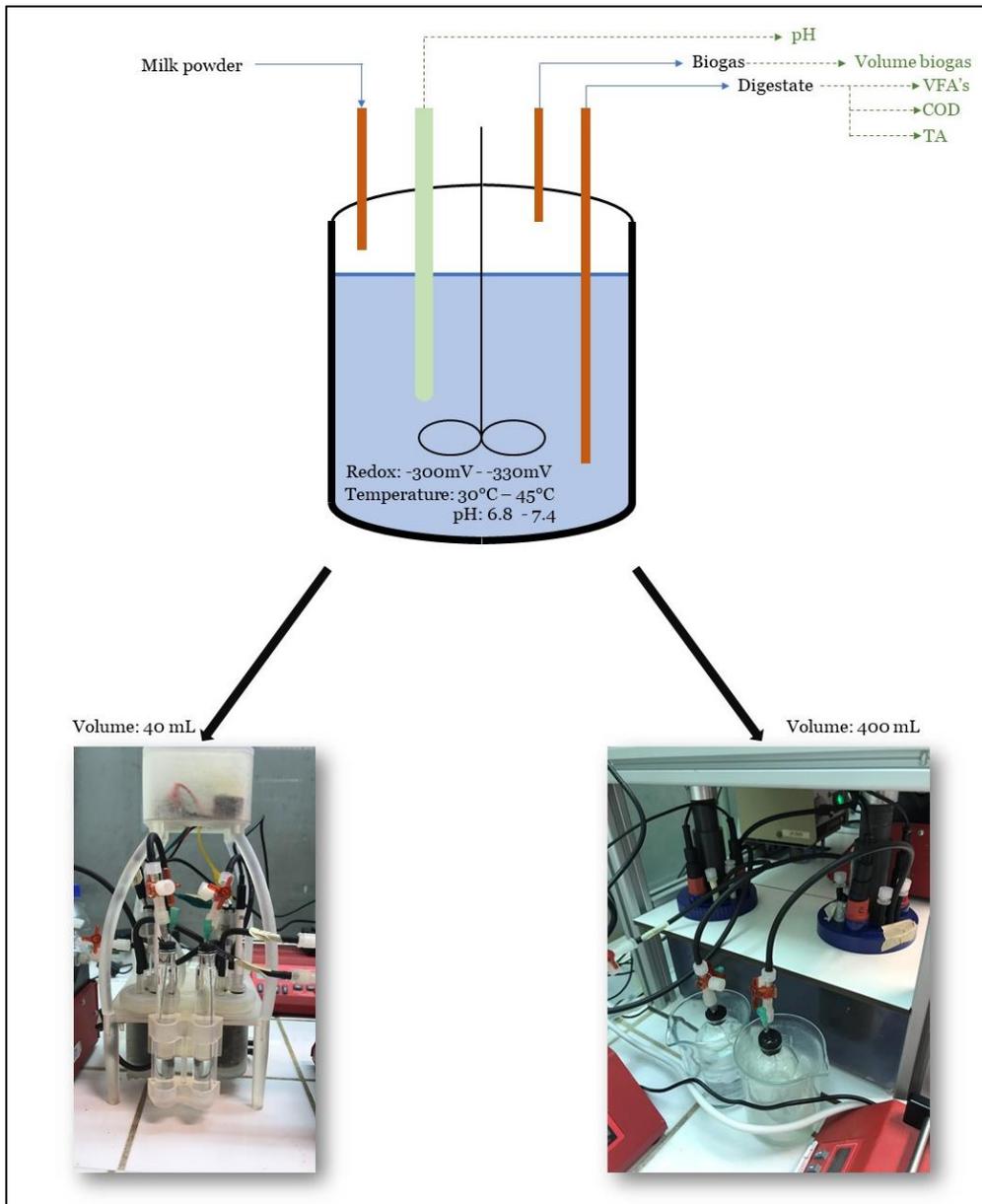


Figure 14: Visualization of the laboratorial experiment. The parameters for miniaturized system and the baseline system will be set the same. The difference is a size of the miniaturized system. Which is a factor 10 smaller compared to the baseline system.

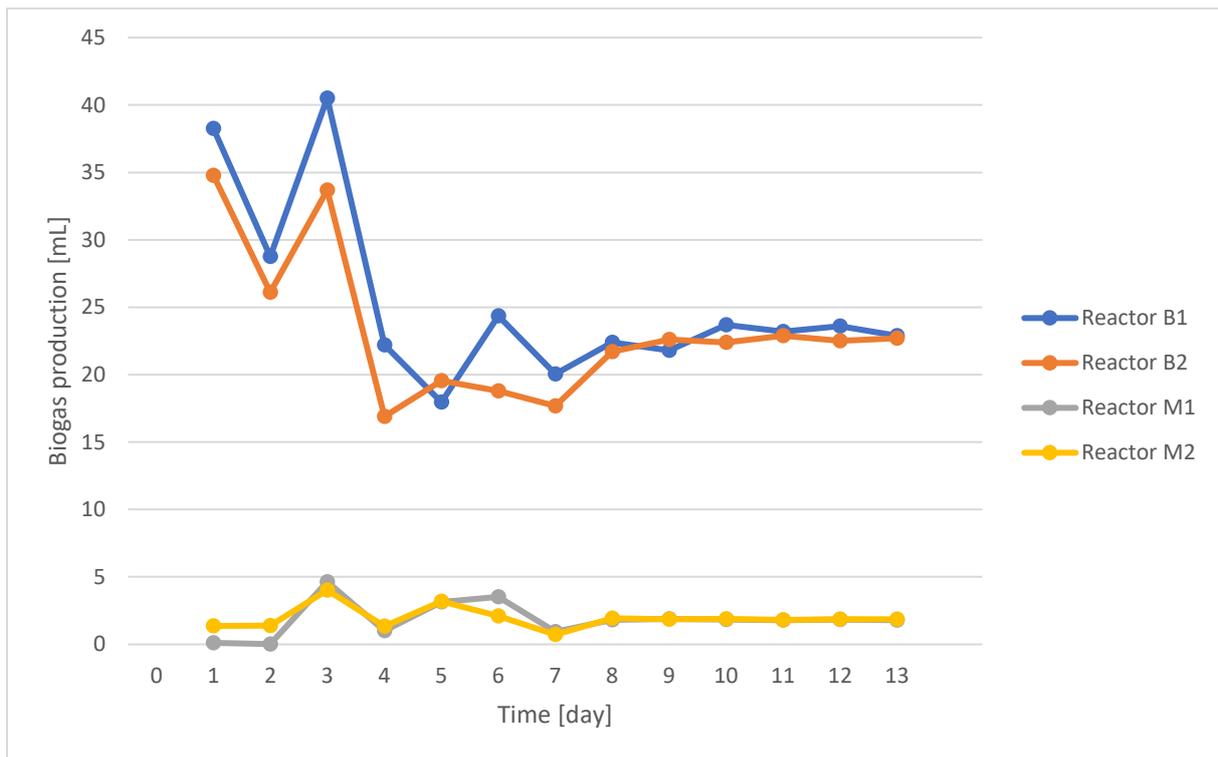
7.1.2 Analytical methods

Different process parameters are measured during the experiment. In order to determine whether the process is stable and steady state, the pH, VFA concentration, ammonia concentration (total alkalinity) and the biogas yield need to be measured (Hansen, et al., 1998). Steady state is defined as the situation when these parameters are constant over a time period of 10 days. Additionally, the and chemical oxygen demand is monitored to obtain more information concerning the digestion performance in the reactor. The pH and biogas production will be measured daily. The VFA concentration and total alkalinity will be measured when there is a sufficient amount of effluent available. The COD is measured every two days. However, this is also dependent on the availability of the test kit.

7.1.3 Results

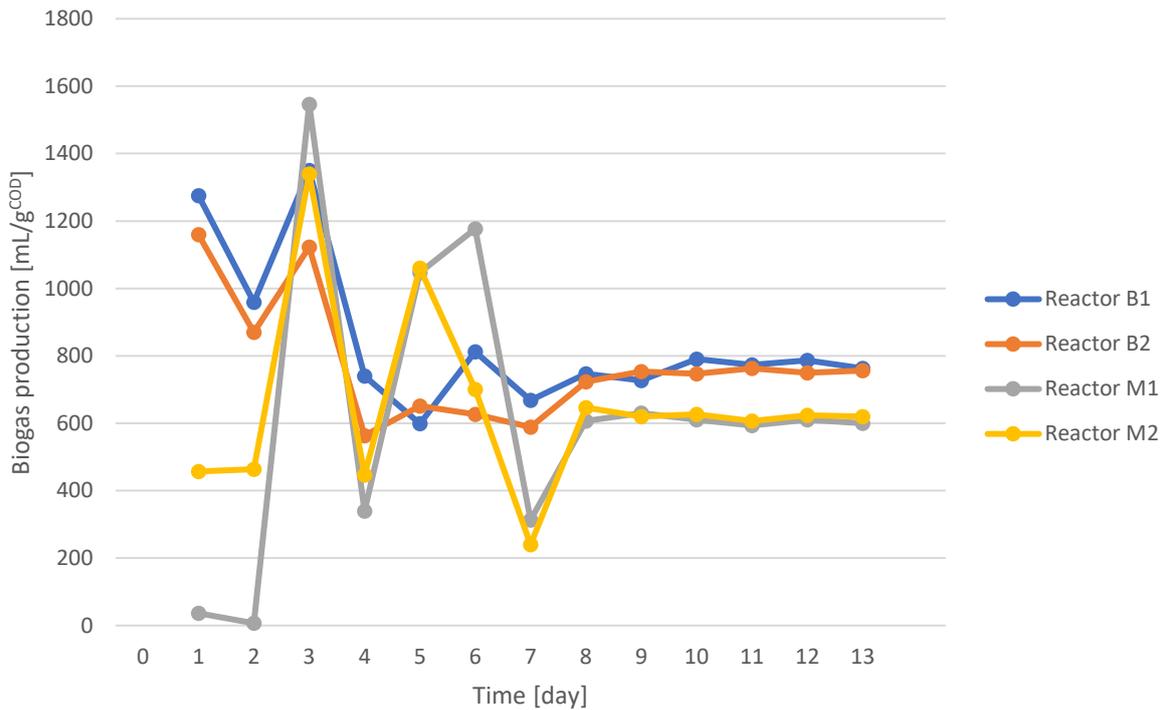
7.1.3.1 Biogas production

The biogas production was measured using the principle of water displacement. Graph 1 shows the biogas production in mL with respect to the time. The baseline reactors are indicated by B1 and B2 while the miniaturized reactors are indicated by M1 and M2. The biogas production of the baseline reactors is higher compared to the miniaturized reactors. A rough estimation shows that the biogas production of the baseline reactors is approximately a factor of 10 higher compared to the miniaturized reactors. This is intuitively correct, since the working volume of the baseline reactors is also a factor of 10 higher.



Graph 1: Biogas production in mL per day for both the miniaturized reactors (M1 and M2) as well as the baseline reactors (B1 and B2).

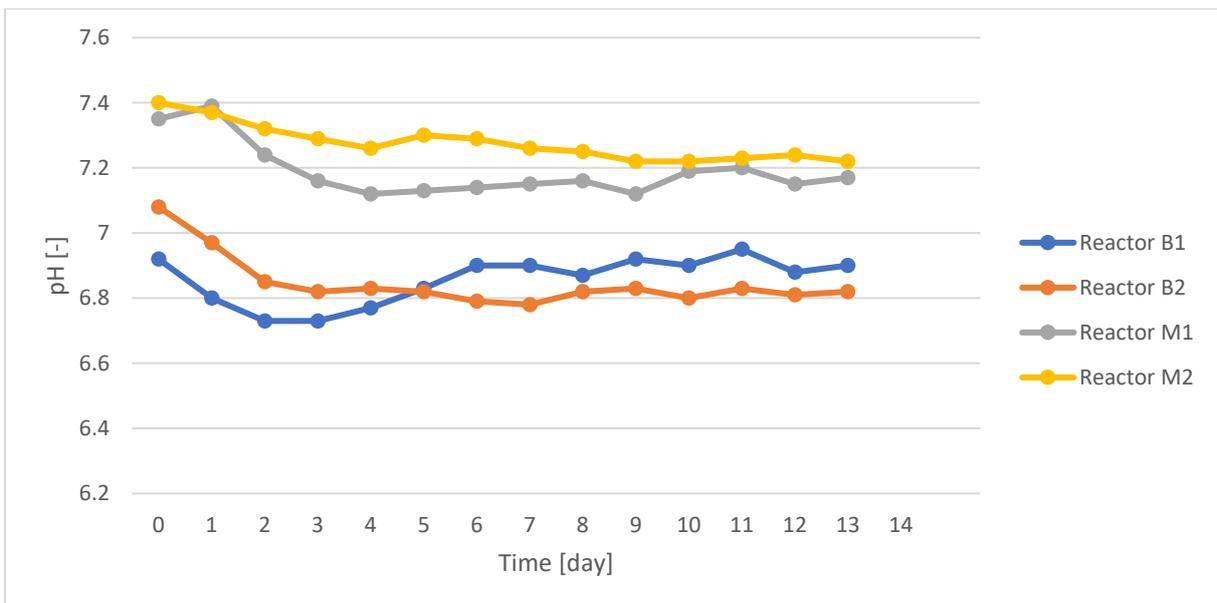
In order to accurately compare the biogas production, the volume of biogas produced is divided by the COD added per day. This allows for a comparison of biogas production without the volume of the reactor being influential. Graph 2 shows the biogas production in mL per g COD added with respect to time. The results are comparable. The biogas production of the miniaturized system is less stable than the larger reactors. But on average both the baseline as well as the miniaturized system produce a similar amount of biogas per day.



Graph 2: Biogas production in mL/g_{COD} per day for both the miniaturized reactors (M1 and M2) as well as the baseline reactors (B1 and B2).

7.1.3.2 pH

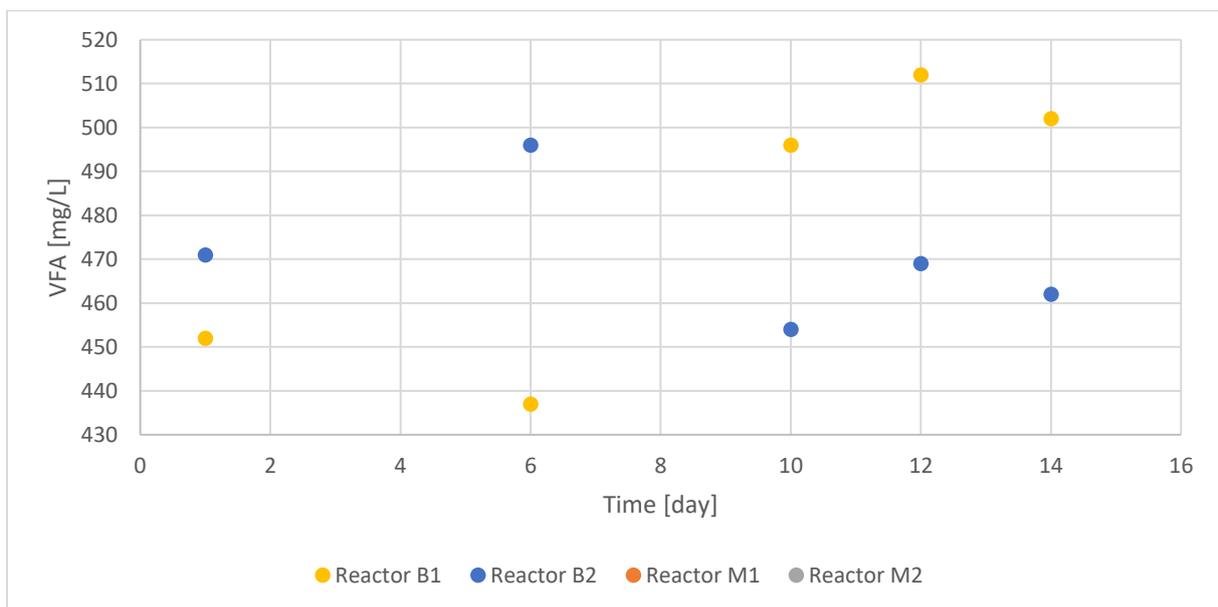
The pH is generally a good indication of the behaviour and stability of the anaerobic digestion process. The pH of both baseline reactors and the miniaturized reactors are plotted in Graph 3. The pH in the miniaturized reactors is slightly higher compared to the baseline reactors. For all four reactors, the pH remains within the specified optimal range. Moreover, no explicit fluctuations are present. Thus, the pH is relatively stable over time. This indicates a stable anaerobic digestion process.



Graph 3: The pH per day for both the miniaturized reactors (M1 and M2) as well as the baseline reactors (B1 and B2).

7.1.3.3 Volatile fatty acids

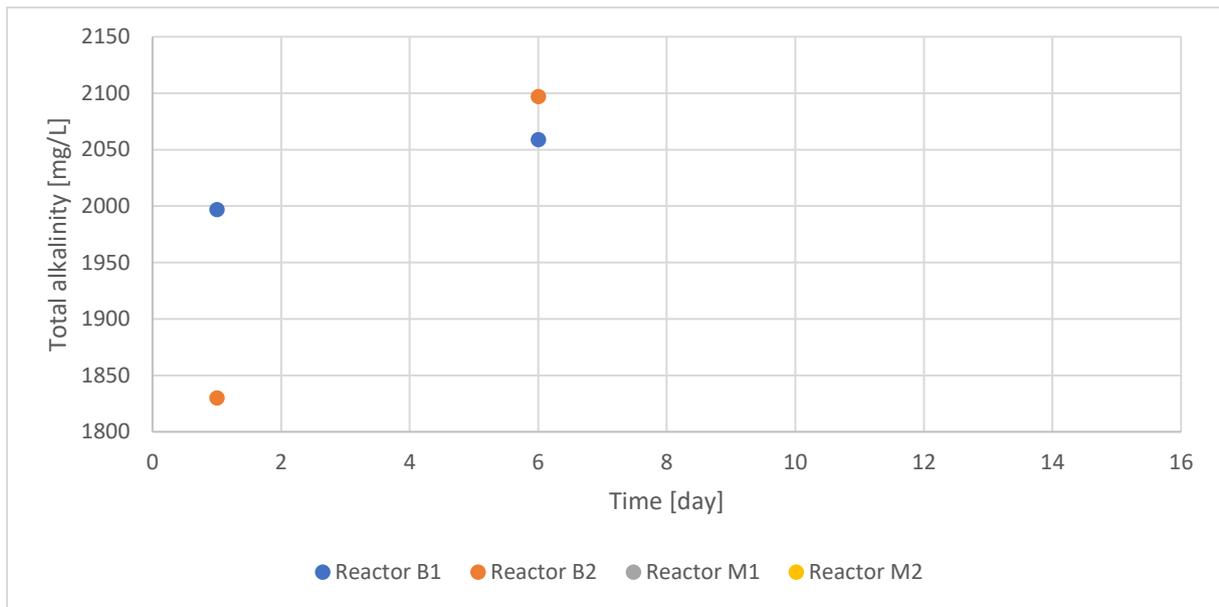
The amount of VFA's in the effluent of the reactor was monitored. The concentration of VFA's in the reactor is an indication of the stability and performance of the reactor. When the concentration increases excessively, the contents of the reactor will acidify and reduce the performance of the overall anaerobic digestion process. When the concentration of VFA's is stable, it indicates a balance between the hydrolysis & acidogenesis stage and the acetogenesis & methanogenesis stage of the digestion process. This balance enhances the overall performance of the reactor. Graph 4 shows the VFA concentration with respect to the time. The amount of data, especially for the miniaturized reactors, is low. For the measuring of the VFA concentration, 5mL of effluent is required. Since the miniaturized reactors only provide 1 mL of effluent per day, the determining of the VFA concentration was not possible on a daily basis. Diluting the 1 mL of effluent resulted in invalid measuring results since the VFA concentration was too low for the titrator to detect.



Graph 4: The concentration of volatile fatty acids in mg/L per day for both the miniaturized reactors (M1 and M2) as well as the baseline reactors (B1 and B2). Data was missing due to insufficient sample size concerning the miniaturized reactors.

7.1.3.4 Total alkalinity

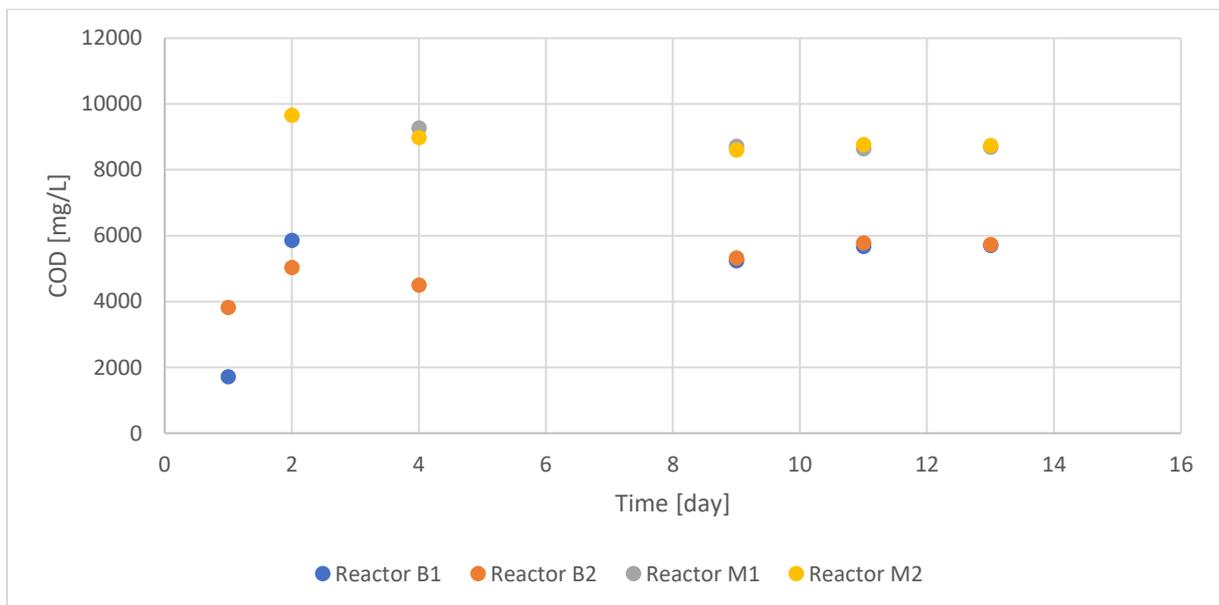
The total alkalinity was monitored in order to obtain data regarding the buffer capacity of the reactor. The buffer capacity of a reactor ensures a stable pH during the anaerobic digestion process. That is due to the ability of alkaline compounds to neutralize acids and prevent pH drops. Graph 5 shows the total alkalinity in mg/L with respect to the time. Similarly, to the VFA measurements, the number of data points for the miniaturized system is low. Because the same measuring procedure applies, the sample size of the miniaturized reactors was too low to obtain valid measurements.



Graph 5: The total alkalinity in mg/L per day for both the miniaturized reactors (M1 and M2) as well as the baseline reactors (B1 and B2). Data was missing due to insufficient sample size concerning the miniaturized reactors.

7.1.3.5 Chemical oxygen demand

The chemical oxygen demand was monitored to obtain information concerning the conversion rate of feedstock into biogas. As can be derived from Graph 6, the COD is generally higher in the digestate of the miniaturized system. Moreover, the COD is rather stable for the miniaturized system. The COD values for both miniaturized reactors are relatively similar. For the baseline system, the COD is less stable during the first few days since the digestion process was started. The COD values become stable for both systems after approximately one week.



Graph 6: The chemical oxygen demand in mg/L per day for both the miniaturized reactors (M1 and M2) as well as the baseline reactors (B1 and B2).

7.2 3D printing process evaluation

7.2.1 Setup

The 3D printing process will be monitored using the Formlabs printing software. This software keeps track of the resources used and time required for each component. Additionally, the end product will be assessed qualitatively. The 3D printing process will be evaluated against other general production techniques. Namely, injection moulding, casting and milling and turning. Injection moulding is mainly for plastics. Casting can be applied for both plastics and metals. Milling and turning is primarily used for metals. In order to enable the comparison between qualitative and quantitative aspects, each aspect is rated on a scale ranging from 1 to 10. The aspects are divided into two broad categories, namely technical criteria and economic criteria (Pahl & Beitz, 2007). The technical criteria are: quality, expected lifespan, process complexity, process accuracy, maximum object size. The process accuracy entails the precision of the manufacturing technique while the maximum object size entails the maximum component size which can be produced. The economic criteria that will be applied are: raw material costs, production costs, production time, general overhead costs and flexibility.

7.2.2 Results

For each complete reactor approximately 75 mL of photopolymer resin was required, which corresponds to approximately €10, -. To print one complete set, approximately 12 hours was required. The end product needed to be cleaned using isopropyl alcohol. Thereafter, the product needed to be cured, using UV-light for two hours. In total, the production time for one reactor set amounts to approximately 15 hours.

Table 5 shows the assessment of the production techniques based on the technical criteria. The total score for each production process, as indicated by R_t , is the ratio between the obtained score and the highest possible score. This technical score is located in the final row of the table. Same goes for Table 6 regarding the economic criteria. The total economic score, as indicated by R_e is depicted in the final row of the table.

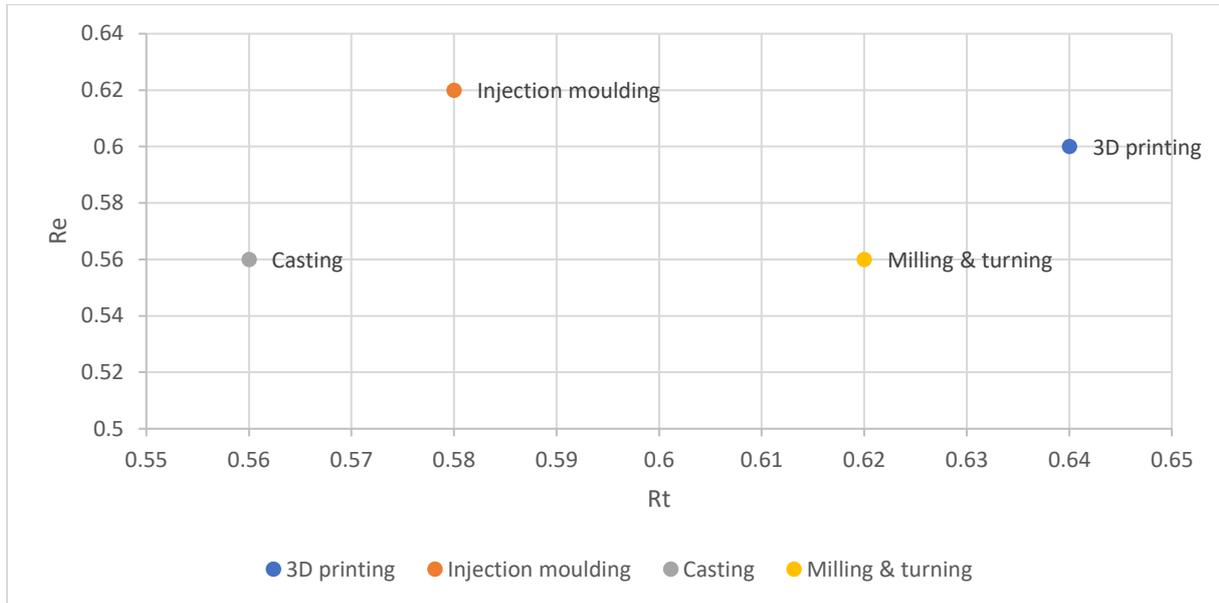
Table 5: Classification table regarding technical criteria, including the final technical score, R_t , for each production technique.

Technique \ Technical criteria	3D printing	Injection moulding	Casting	Milling & turning
Reference	(Manyika, et al., 2013) Preform FormLabs software	(Heinzel, et al., 2004) (Kalpakjian, 2008)	(Chougule & Ravi, 2006) (Kalpakjian, 2008)	(Shunmugam, et al., 2000) (Hui, et al., 2001) (Kalpakjian, 2008)
Quality	7	7	6	8
Lifespan	5	5	5	7
Process complexity	10	5	5	2
Process accuracy	7	5	5	7
Maximum object size	3	7	7	7
Total	32	29	28	31
R_t	$\frac{32}{50} = 0.64$	$\frac{29}{50} = 0.58$	$\frac{28}{50} = 0.56$	$\frac{31}{50} = 0.62$

Table 6: Classification table regarding economic criteria, including the final technical score, R_e , for each production technique.

Technique \ Economic criteria	3D printing	Injection moulding	Casting	Milling & turning
Reference	(Manyika, et al., 2013) Preform FormLabs software	(Heinzel, et al., 2004)	(Chougule & Ravi, 2006)	(Shunmugam, et al., 2000) (Hui, et al., 2001)
Raw material costs	4	10	7	8
Production costs	10	8	8	4
Production time	3	7	4	6
Flexibility	10	1	1	3
General overhead costs	3	5	5	7
Total	30	31	28	28
R_t	$\frac{30}{50} = 0.6$	$\frac{31}{50} = 0.62$	$\frac{28}{50} = 0.56$	$\frac{28}{50} = 0.56$

Both the total technical score as well as the total economic score per production technique are plotted against each other in Graph 7. On the x-axis, the R_t is plotted while on the y-axis the R_e is plotted.



Graph 7: Overview of the total score per production technique with respect to each other.



This chapter is used to discuss what the obtained results mean. The chapter aims to reflect on the influence of the selected methodology on the results of the research. Before conclusions can be drawn out of the results, the findings will be discussed. Additionally, the limitations of the study are discussed, and further research is suggested.

The experimental data shows that the miniaturized design is able to mimic the performance of the baseline system. However, the biogas production was slightly fluctuating over time. This could be caused by inadequate feeding. Because the feeding rate was only 1 mL per day, the mixture containing the feedstock became clouded over time. Therefore, the reactor was possibly not fed with an adequate homogeneous mixture containing $0.003 \text{ g}_{\text{COD}}/\text{mL}$. Concerning the larger baseline system this problem was less prominently present because the feeding rate was a 10 times larger.

Additional experiments are required to validate the utilization of 3D printed miniaturized reactors in a wide range of applications. Other microbial reactions should be studied to further validate the application of 3D printing. Additionally, different parameter settings and configurations need to be studied for different biochemical reactions occurring in the designed system.

When starting up the system, the reactors were inoculated and injected with nitrogen, to obtain an anoxic environment. The nitrogen was pumped in via the effluent port. When all the other ports were closed, the nitrogen could not be pumped in, indicating pressure was built up, which indicates the reactor was completely airtight.

Due to the low effluent removal rate of 1 mL/day for the miniaturized reactors, the sample size was too small for obtaining accurate measurements. Thus, due to the miniaturization principle, the sample size of the designed reactors was too small for reliable and accurate data. However, based on the experimental validation it can be concluded that the miniaturized system can mimic the performance of the larger baseline system.

While connecting all the tubing to the different ports of the miniaturized reactors and the baseline reactors, it was practically impossible to create an equal tubing length for each port. Therefore, the obtained data can slightly differ from the exact results. That is mainly due to the possible pressure differences and the possibility of effluent being influenced by the time it remained in the tube, before it was tested.

The results of the 3D printing process evaluation indicate that 3D printing is a suitable option for the development of a process screening system. However, it should be noted that the methodology used is qualitative. Therefore, the results are vulnerable to opinions of stakeholders in the study. However, while the results might not be exact, the results are valuable as an indication.

Further research should be conducted in order to solve the problem entailed by small sample sized, which is a consequence of miniaturization. Different sensors of measuring techniques

should be developed which can accurately relevant parameters using small sample sizes. Moreover, the feasibility study regarding the 3D printing process evaluation should be conducted more elaborately in further research to obtain more information regarding the exact feasibility of the 3D printing technique with respect to other production techniques. Alternatives speeding up the printing process or reducing the resin costs could increase the economic potential of the 3D printing procedure.



In order to determine whether the 3D printing principle can be used to develop a flexible and modular screening system for the analysis of complex microbial reactions, a multi-parallel miniaturized bioreactor system was developed, using 3D printing, in this study. Based on literature findings, a list of functional requirements was generated which the design needed to comply with. Based on this list of functional requirements, the design phase was initialized. The main function of the design is to obtain process information. Separating that main function into basic subfunctions, and formulating solutions for the different subfunctions generated the different working principles. After selecting the best option, of the working principles, for each subfunction, the design concept was specified. This concept was materialized using SolidWorks CAD software. The design was produced using a Formlabs Form2 stereolithography 3D printer which uses FLGPCLO4 photopolymer resin as fabrication material. Additionally, a platform was designed, and 3D printed, which can hold four of the designed miniaturized reactors.

In order to validate the design, a laboratorial experiment was conducted. Two 40 mL miniaturized reactors were compared to two 400 mL Eppendorf BioBLU® 0.3c Single-Use Vessels. The monitored parameters were biogas production, pH, total alkalinity, chemical oxygen demand and the concentration of volatile fatty acids. The reactors were fed inoculated with sieved sludge originating from a semi-continuous lab reactor treating cow manure. The reactors were fed with solution containing 0.003 g milk powder per mL, which corresponds with 0.003 g_{COD}/mL . The feeding rate was set at 10 mL/day and 1 mL/day for the baseline study and the miniaturized system respectively, corresponding with a hydraulic retention time of 30 days and an organic loading rate 0.1 g_{COD}/day .

The experiment showed that the biogas production in mL per g_{COD} was on average similar for both the designed system as well as the baseline system. The biogas yield in the miniaturized system was slightly fluctuating, indicating that the anaerobic digestion process was not completely stable. The pH was stable and within the desired range of 6.8-7.4, for both the miniaturized as well as the baseline system. The COD contained by the effluent of the miniaturized reactors was higher compared to the COD in the digestate of the baseline reactors, indicating the feedstock was not utilized to its full potential. The values for the VFA concentration and total alkalinity indicate a stable digestion process for the baseline study.

It can further be concluded that the designed system fulfils all the functional requirement set by the literature review. The anoxic environment was sustained because no leakages occurred and the pH as well as the biogas production were relatively stable. Additionally, using the same reasoning, it can be concluded that the design functions properly at mesophilic temperatures.

Based on the final design and the experimental results it can be concluded that combining the principles of miniaturization and parallelisation to develop a multi-parallel miniaturized bioreactor system for screening of complex biological processes using 3D printing is possible.

The 3D printing process shows potential based on the results of the 3D printing process evaluation. With respect to the technical criteria, the 3D printing principle is most suitable, relative to other conventional production techniques, with respect to the development of a

multi-parallel miniaturized bioreactor system for screening of complex biological processes. Additionally, with respect to the economic criteria, the 3D printing shows potential. However, due to the costs of the photopolymer resin and the time required for the printing itself the economic viability is not optimal. However, combining both the technical as well as the economic aspect the 3D printing process proves to be the best solution for developing a multi-parallel miniaturized bioreactor system for screening of complex biological processes.

Future research has been proposed to focus on solving problems entailed by small sample sizes. Additionally, the economic feasibility of the 3D printing process should be further investigated.

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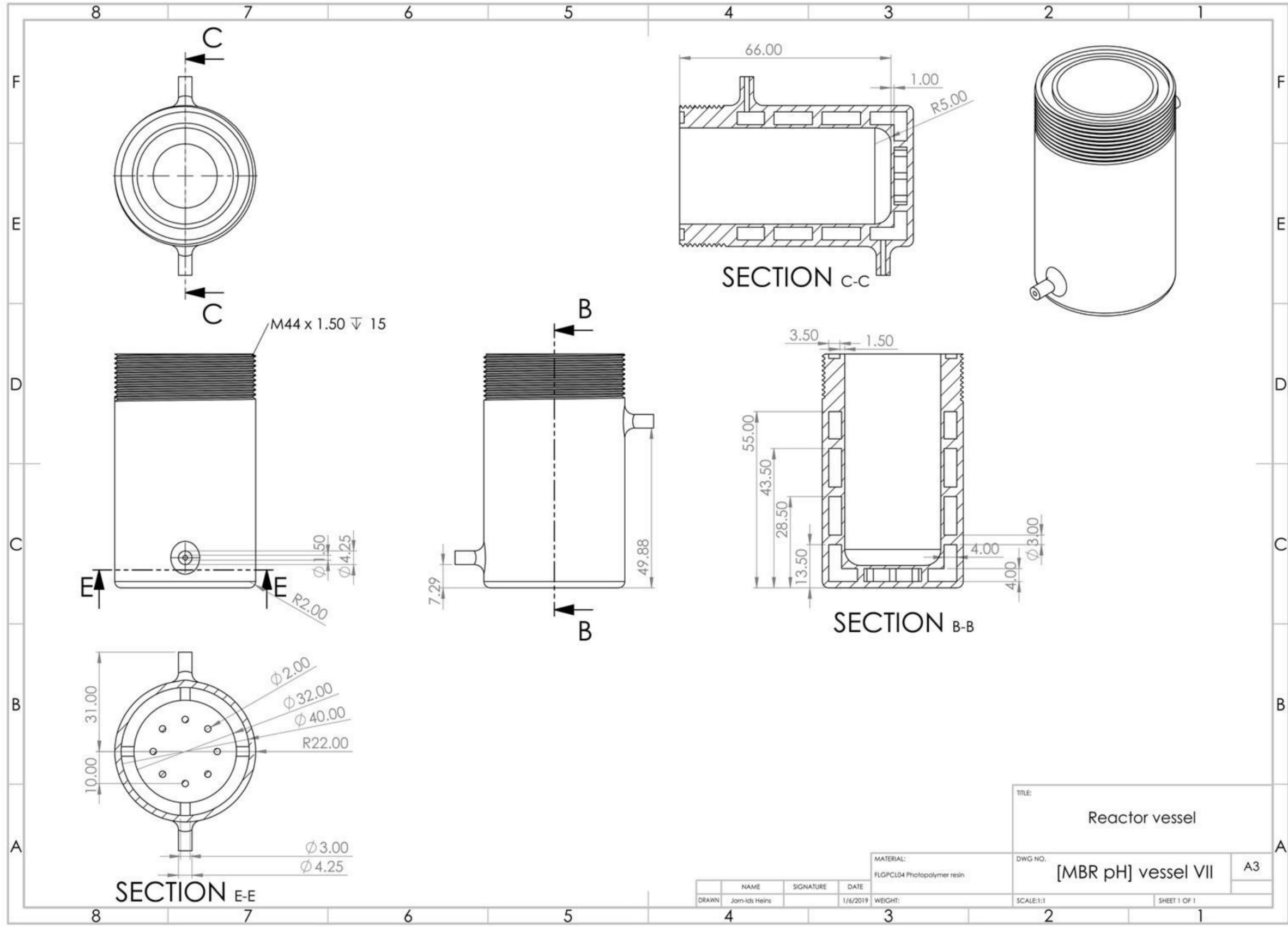
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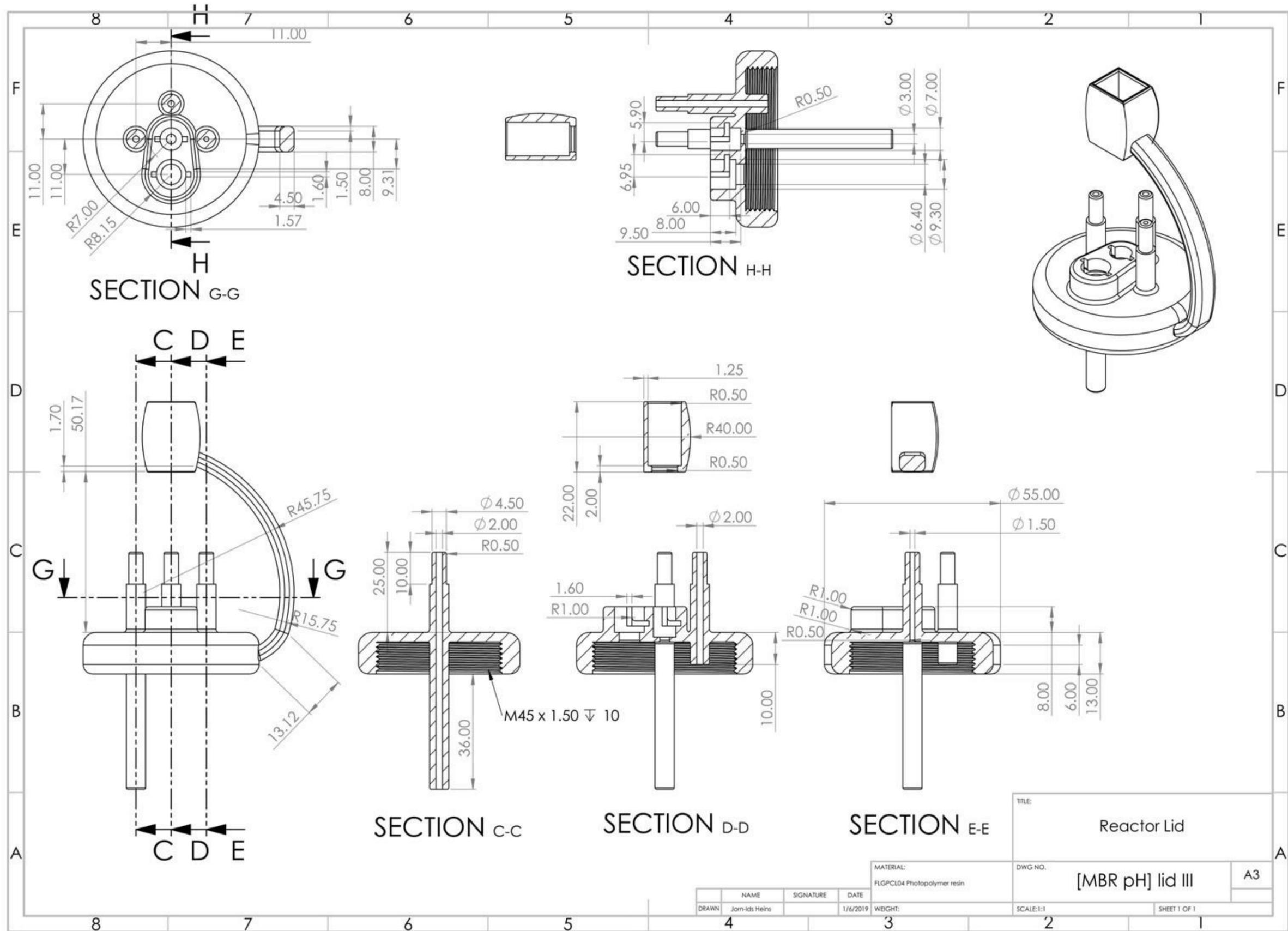
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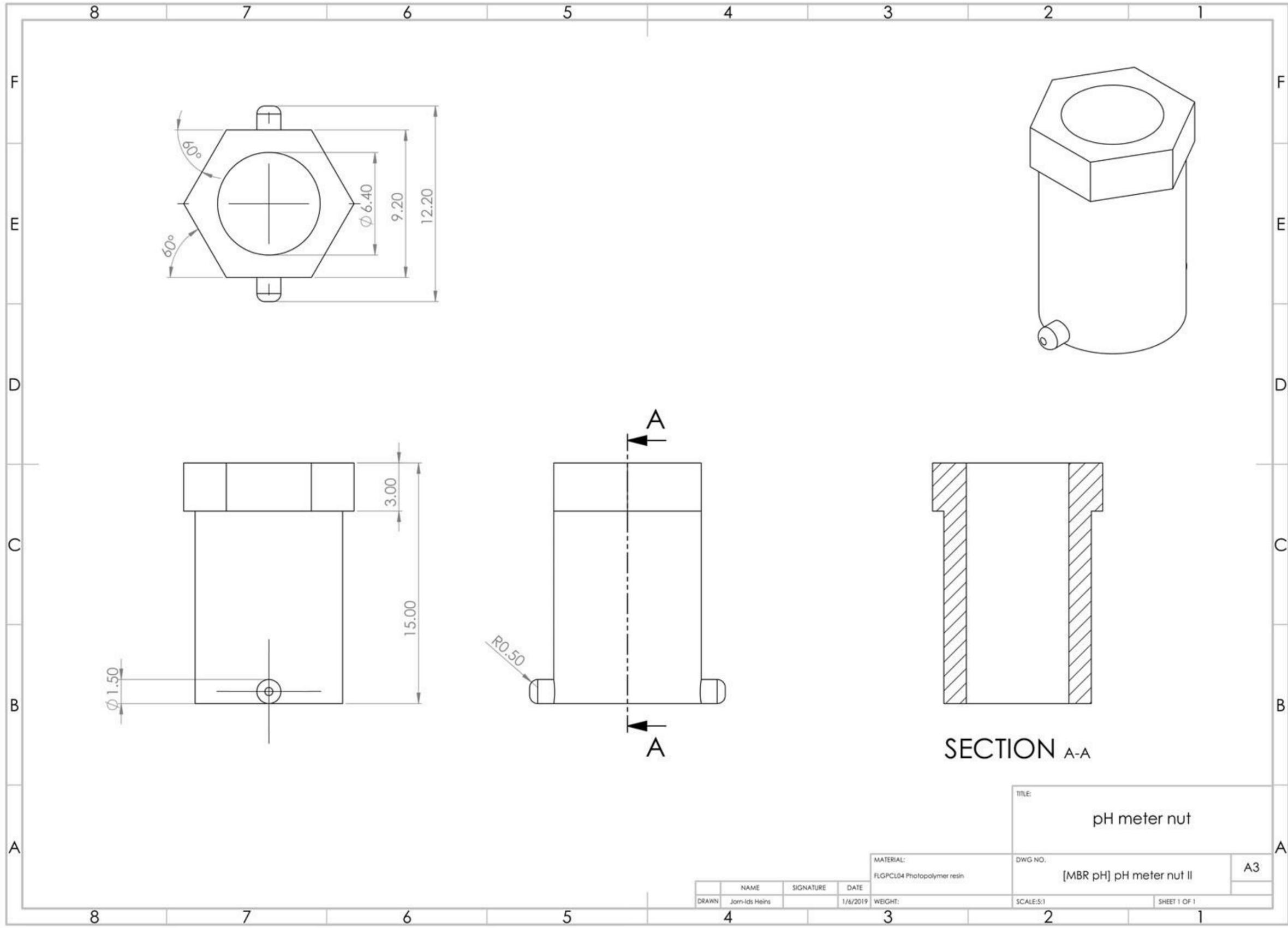
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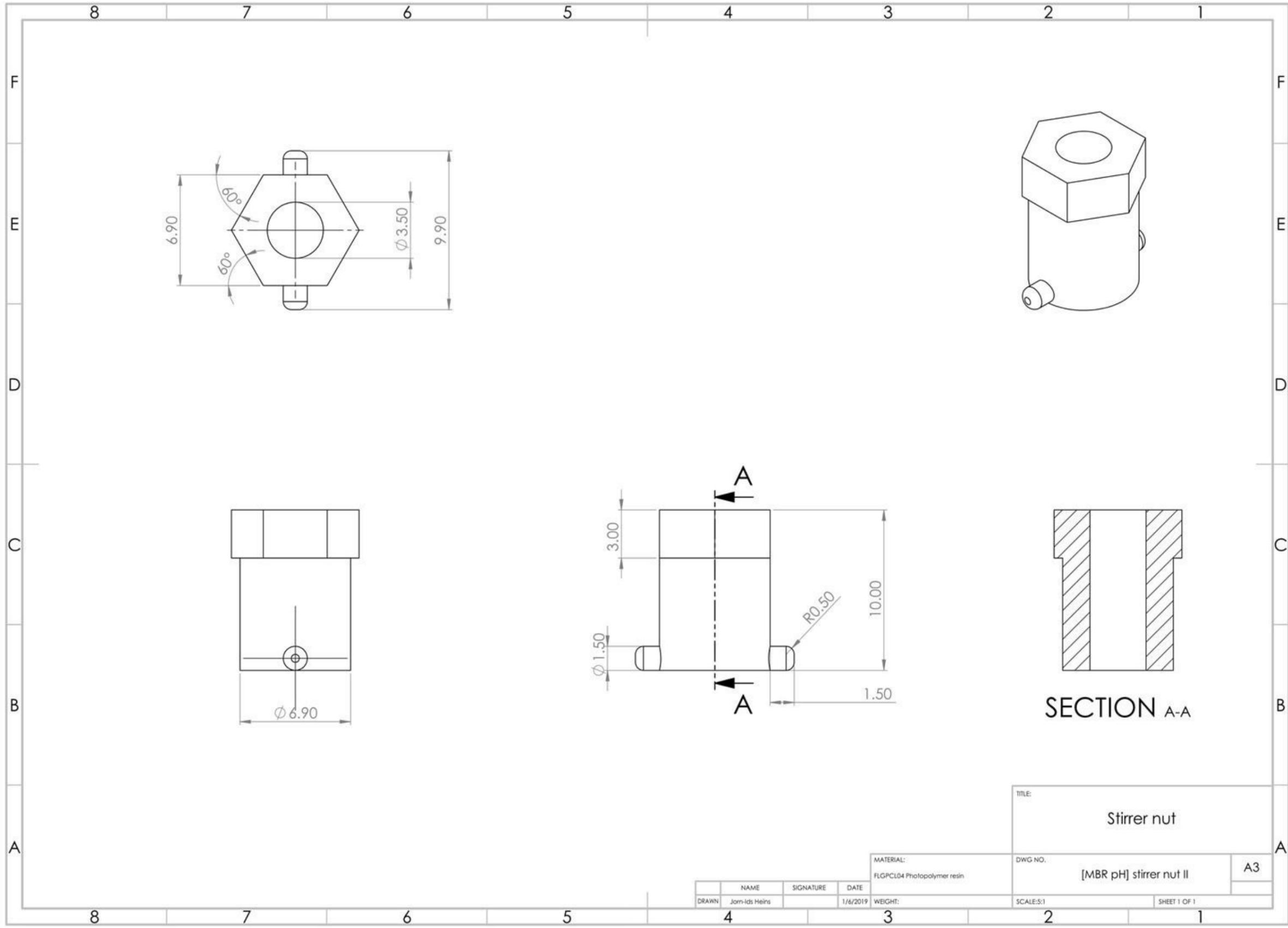
Appendix I

1. Reactor vessel	[MBR pH] vessel IV	62
2. Reactor lid	[MBR pH] lid III	63
3. pH meter nut	[MBR pH] pH meter nut II	64
4. Stirrer nut	[MBR pH] stirrer nut II	65
5. Marine stirrer	[MBR pH] stirrer marine	66
6. Motor to stirrer connection	[MBR pH] motor to stirrer connection	67
7. Reactor assembly	[MBR pH] Assembly III	68

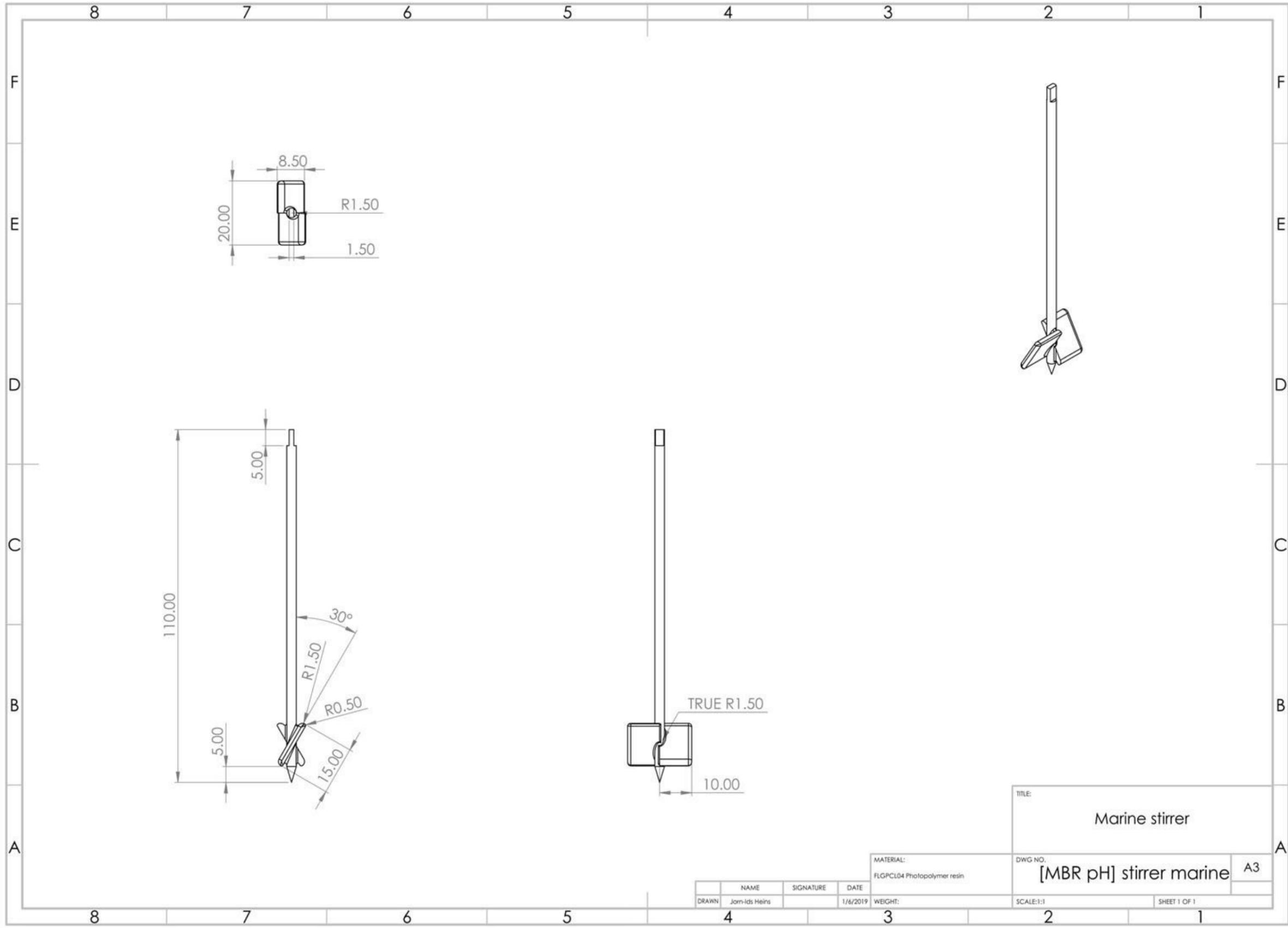




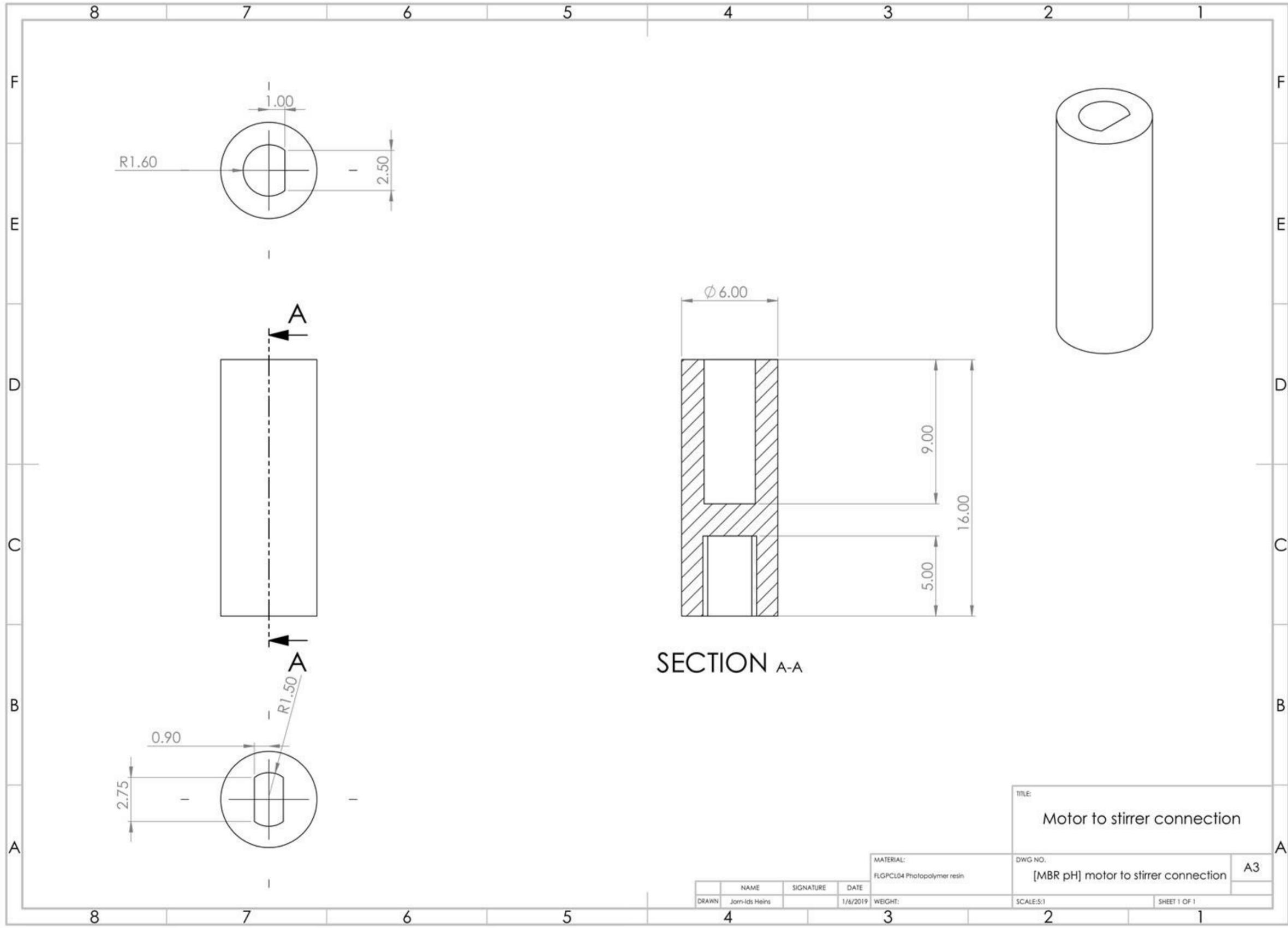




TITLE:		Stirrer nut	
DWG. NO.:		[MBR pH] stirrer nut II	
MATERIAL:		FLGPC104 Photopolymer resin	
DRAWN:		Jorn-ids Heins	
NAME:		SIGNATURE:	
DATE:		1/6/2019	
WEIGHT:		SCALE: 5:1	
SHEET 1 OF 1		A3	

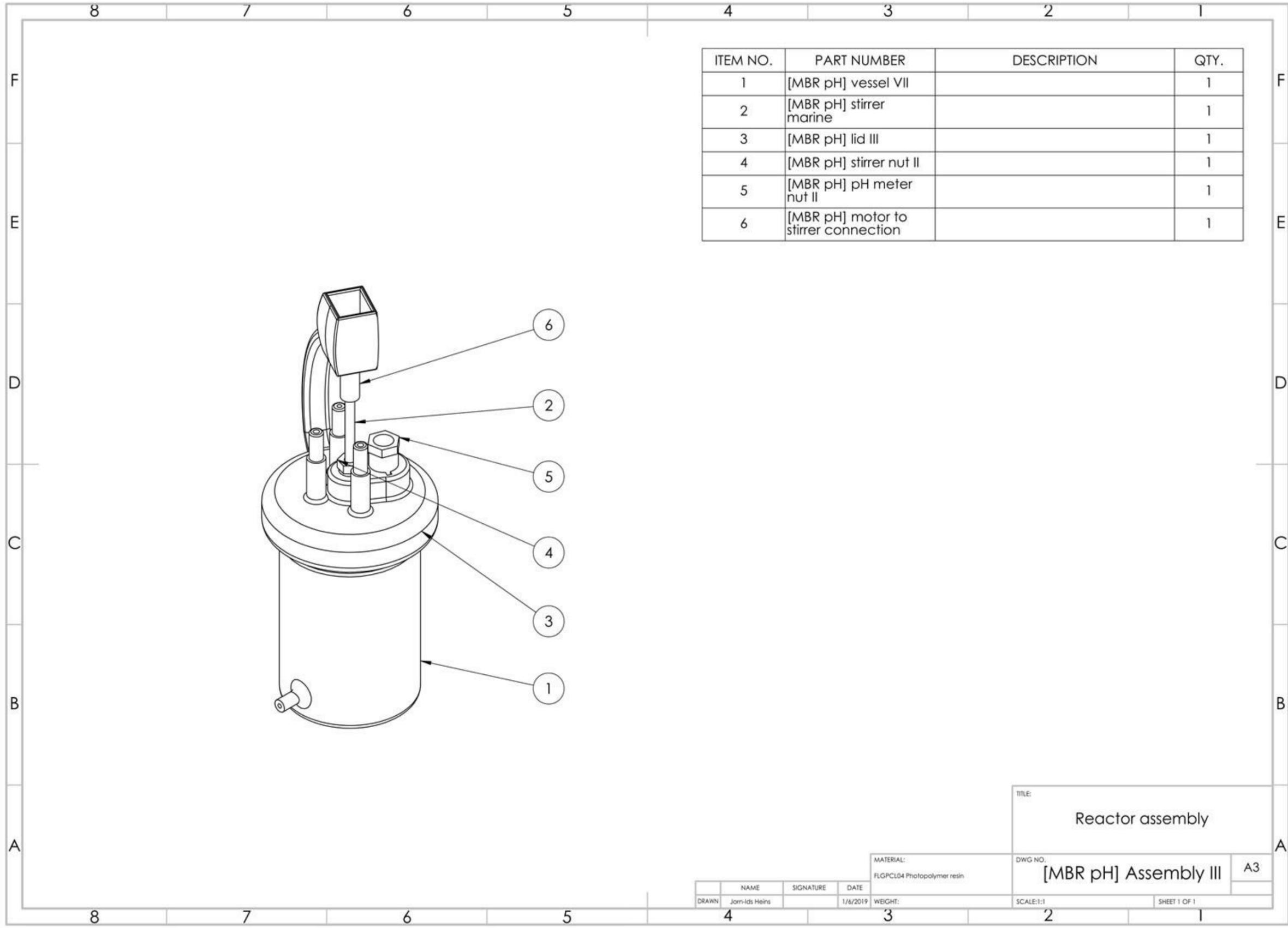


TITLE:		Marine stirrer	
MATERIAL:		FLGPC104 Photopolymer resin	
DWG NO.:		[MBR pH] stirrer marine	
DRAWN:		A3	
NAME:	SIGNATURE:	DATE:	WEIGHT:
Jorn-ids Heins		1/6/2019	
SCALE:1:1		SHEET 1 OF 1	



SECTION A-A

TITLE:		Motor to stirrer connection	
DWG NO.:		[MBR pH] motor to stirrer connection	A3
MATERIAL:	FLGPC104 Photopolymer resin	SCALE: 5:1	SHEET 1 OF 1
DRAWN:	Jorn-ids Heins	SIGNATURE:	DATE: 1/6/2019



ITEM NO.	PART NUMBER	DESCRIPTION	QTY.
1	[MBR pH] vessel VII		1
2	[MBR pH] stirrer marine		1
3	[MBR pH] lid III		1
4	[MBR pH] stirrer nut II		1
5	[MBR pH] pH meter nut II		1
6	[MBR pH] motor to stirrer connection		1

TITLE:
Reactor assembly

DWG NO.:
[MBR pH] Assembly III

MATERIAL:
 FLGPC104 Photopolymer resin

SCALE: 1:1

SHEET 1 OF 1

NAME	SIGNATURE	DATE
Jorn-ids Heins		1/6/2019

A3

