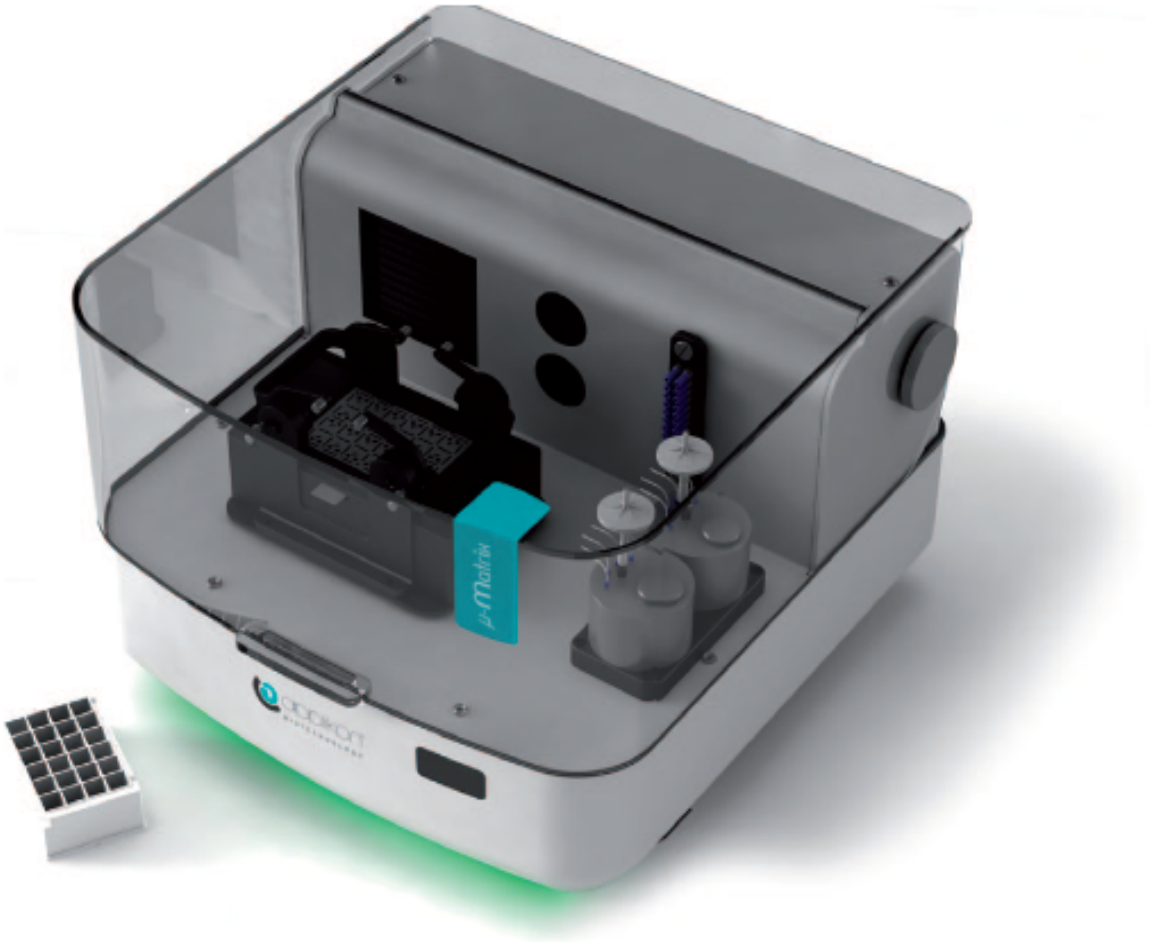
Cell Concentration Sensor for micro-bioreactors

Optical sensor system

E. Lemmens and M. Jansen

Bachelor of Science Thesis



TU Delft Delft University of Technology

Electronic Instrumentation Group

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Optical sensor system

Bachelor of Science Thesis

For the degree of Bachelor of Science in Electrical Engineering at Delft University of Technology

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Abstract

This bachelor thesis describes the development of a sensor system for measuring the cell concentration in small bio-reactors. The study was conducted at Delft University of Technology on behalf of Applikon Biotechnology B.V. The MicroMatrix of Applikon is used for experimental determination of the optimal conditions for bioprocesses in bioreactors. Measuring the cell concentration is important because it is a direct parameter to determine the efficiency of a bioprocess. This thesis will focus only on one of the two chosen sensor techniques of the system, that is, an optical sensor using the optical density technique. Among other things, temperature compensation and an amplifier circuit have been designed for the sensor. The results show that the optical sensor is capable of measuring the cell concentration of yeast within the range of 0 - 120 g/L. This is done with an accuracy between $\pm 10\%$ in the range of 10 - 120 g/L and 10 - 40% between 0 - 10 g/L.

Preface

This thesis describes the development of an optical sensor for cell concentration measurements. This work is done in the context of the course EE3842 "Bachelor afstudeerproject" of the bachelor Electrical Engineering at Delft University of Technology. During the project we have been working at the Electronic Instrumentation Group located at the 15th floor of the Faculty Electrical Engineering, Mathematics and Computer Science. They have been excellent hosts with their support of measurement equipment and knowledge. Especially our supervisors André Bossche and Jeroen Bastemeijer have been a great support during the project with their feedback on our results and progress. We also want to thank our contact person at Applikon Biotechnology, Timo Walvoort, Project Manager Development. He was a great help by giving us a better idea about, for example, the biotechnology market, MicroMatrix and the company itself. We also would like to thank the other group members, Jornel van den Hoorn, Joost van der Kemp, Jeroen Keijsers and Ashvant Mahabir, who have been part of the main project. Together we have accomplished to build a prototype to measure the cell concentration.

Erik Lemmens and Michel Jansen
Delft
July 3, 2014

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Chapter 1

Introduction

The field of bioreactors is developing rapidly. An increasing number of companies are using reactors in their production process, mostly to grow cultures of bacteria or animal cells that can produce useful substances, like insulin. Examples are chemical enterprises, pharmaceutical industries and food producers. Following this trend is the demand for accurate measurement instruments to be used in these reactors. Physical quantities like pH, oxygen and temperature need to be monitored to make sure growing conditions are optimal.

The latest development is the usage of multiple small reactors for experimental determination of the optical conditions for bio-reactions. The reason is that increasing the efficiency of big bioreactors will reduce operational costs. Applikon Biotechnology is responding with the launch of their MicroMatrix bioreactor. This device is capable of growing 24 simultaneous cultures with a volume of 5 mL each, where the conditions of every culture can be individually controlled and monitored. This device allows a customer to choose between different growing conditions, to determine which conditions are best suited to be used in a large scale production facility.

In this project a sensor system is developed that is capable of measuring the concentration of cells in a single reactor. This is a very useful quantity to know, since the factor of success of a bioprocess largely depends on the number of cells available in a culture. Therefore, it is an essential parameter in determining the optimal conditions for a culture.

Current methods to determine the cell concentration in a liquid are mostly offline, meaning a sample has to be taken and analyzed outside of the reactor. This is not ideal because of multiple reasons. First, taking a sample involves the risk of contamination of the entire culture. Since much effort is put into keeping the reactors sterile, taking a sample with an external device is not desirable.
Second, taking a sample to analyse takes time. Probably the sample has to be sent to a laboratory so a technician can analyze it. Depending on the technique used, it may take up to several hours to get results. Ideally, a sensor can monitor a culture at all times and give results directly.

Third, taking a sample is almost impossible because the working volume of the MicroMatrix is only 5 mL. The removed volume of a sample would have a big impact on the total volume. This is why Applikon Biotechnology presented the problem to Delft University of Technology. The problem is presented as bachelor graduation project for six electrical engineering students, with the goal of developing a sensor system that solves these, and more, issues. One of the requirements was that the developed system could be potentially used in their product.

The team that has worked on this sensor consists of six people. These six people were divided into subgroups of two. Each subgroup had a specific task in the project. Later in this thesis, the precise division of tasks between the groups will become clear. For now, it suffices to notice that this thesis specifically describes the design and development of an optical sensor system to measure cell concentration.

Chapter 2

Project

2-1 Project goals

As stated in the introduction, the aim of the project is to develop a cell concentration sensor that solves the issues currently encountered in bioreactor sensors. Before starting the design of the sensor, the requirements that are applicable had to be clear. Therefore, in cooperation with Applikon, a list of requirements was created. This list is stated in the next section. The ultimate goal is to design a sensor that meets all the requirements.

2-1-1 List of requirements

This section contains a list of requirements. The items in this list are not prioritized.

- The sensor has to be able to measure the concentration of cells in a suspension.
- The sensor has to be able to measure cells in the range of 0 - 200 g/L (dry weight)
- The sensor should have an accuracy within $\pm 10\%$.
- The sensor has to be able to measure at least once every hour.
- The sensor has to be scalable so it could be implemented into the MicroMatrix, i.e. a reactor with a work volume of 5 mL.
- The sensor has to be sensitive for a large number of cell types (diameter of 3μm - 30μm).
- The sensor should not influence the bioprocess in the reactor.
- The sensor has to work without taking any samples.
- The sensor has to work within a closed system.
- The sensor has to be sterile and be able to be easily sterilized.

- The sensor has to generate reproducible measurements.
- The sensor should not influence other measurements in the reactor.
- The sensor has to be able to operate safely.

In addition to the list of requirements, a list with desirable features was drafted. These features are not necessary for a correct operation of the sensor, but will generate increased value if they can be implemented. These features are:

- The sensor can differentiate between living and dead cells.
- The sensor can measure in real-time.
- The sensor can communicate with current software systems in the MicroMatrix.
- The sensor is (partly) disposable.

2-2 Considered techniques

In order to be able to design a sensor system that meets all the requirements in the previous section, a literature study was performed that resulted in several techniques that could be used to implement the sensor. The next sections briefly discuss each of the techniques.

2-2-1 Optical

This technology uses light to give an estimation of the concentration of cells in suspension. Several designs are possible, each with their pros and cons [1]. Because this technology will be discussed in dept in the next chapter, the designs are not specified here. Optical sensor systems have been reported with a range between 0 - 16 g/L and an accuracy of 3% [2], but ranges of 0.01 - 12 g/L are also reported as maximum [3]. For determining the proper sensor technique it is assumed that a range of 12 g/L is possible. The optical technique in general is not capable of determining viability of cells [4]. A fiber optic particle concentration sensor has been developed that has a range of 0 - 15 g/L [5].

2-2-2 Impedance

A cell membrane keeps the cell content together and makes sure that the cell is protected against its surroundings. This membrane can be seen (in an electrical point of view) as a capacitor [6]. The impedance technique is based on the change in dielectric constant of a suspension. When a suspension with cells is between two electrodes of a capacitor, the growth of cells will be responsible for a change in the dielectric. [7]. A higher cell concentration will result in a higher dielectric constant, which in turn causes the capacitance to rise [8]. This rise in capacitance is measured and converted in an estimation for the cell concentration. The impedance can be measured at different frequencies [9]. A system has been reported that uses frequencies of 10 kHz and 10 MHz to do a differential measurement to remove the background

offset [10]. This system of [10] has an threshold of 0,5 g/L for yeast. A different measurement system uses the frequency of 60-80 KHz and 1-2 MHz to measure the cell concentration. This system reports an accuracy of 1 g/L in the range of 0-9 g/L but this can be further improved by changing the sensor dimensions [11]. According to [12], there is no theoretically upper boundary for the range of an impedance sensor.

2-2-3 Microwave

This method makes use of the fact that the bioreactor suspension has a specific resonance frequency, which can be measured using microwaves [13],[14]. When the number of cells inside the suspension increases, the resonance frequency will shift due to the change in the dielectric permittivity. A paper has shown that the variation of the resonance frequency and the 3-dB bandwidth exhibits a linear behavior to respectively real and complex dielectric permittivity [15]. By using this technique, very small amount of cells can be measured at gigahertz frequencies [16]. The main disadvantages of this technique is the complexity and the required time to develop such a system. The technique also requires a lot of advanced knowledge about electromagnetic waves. Since the required working frequencies are high, this adds even more complexity.

2-2-4 Acoustic

The last technology that was considered is called acoustic resonance densitometry (ARD). The principle of operation is the same as with microwave sensing, only acoustic waves are used instead of electromagnetic waves [17]. This technique is reported to be a replacement for optical density sensors to detect substances in a suspension [18]. Also, at concentrations above 10^6 cells per ml, ARD is able to replace cell counts [19]. However, research has shown that this technique is capable of destroying living cells if not properly used [20].

2-3 Project choices

To choose the proper technique to make a sensor that meets the requirements, a table was constructed that rates the techniques according to the requirements. In this table the optical technique has been chosen as reference, because it is widely used and is the current standard in cell concentration sensors. The other sensor techniques were given a '+' or '++' for better performance and a '-' or '- -' for worse performance. The ratings are based on the results of the literature study. The requirements in this list are given a priority, because in this bachelor project an important time limitation is present. Our highest priority is feasibility, which includes the limited time, required facilities and cost. Furthermore, range and accuracy were considered important requirements. Non-invasive measurements are in general important in the biotechnology and scalability is also a strong requirement for the sensor to be used in the MicroMatrix. The sensor should be widely applicable for many different cell types to increase the potential of the sensor system for future customers. Disposability and viability are wishes that were given the lowest priority. The other requirements from the list in section 2-1-1 that are not listed, are assumed to be fulfilled by all techniques in the literature study.

Priority		Optical	Impedance	Microwave	Acoustic
1	Feasibility	0	+	- -	-
2	Range	0	+	+	0
3	Accuracy	0	+	++	0
4	Non-invasive	0	0	0	- -
5	Scalability	0	+	-	0
6	Wide sensitivity	0	0	0	0
7	Disposable	0	+	+	-
8	Viability	0	++	++	0

Table 2-1: Ratings for the different techniques

Given the ratings in table 2-1, it is clear that an impedance sensor system meets the most requirements with the least drawbacks. When looking at the most important requirement feasibility, microwave and acoustic do not seem suitable choices. For the remaining options, optical and impedance, there are almost no papers which give any clear information about the maximum range of the different technologies. For an optical system, 0-16 g/L was accomplished. Since this is by far not the same range as the requirement of 0-200 g/L, the decision was made to combine the optical and impedance techniques into a single sensor system to increase the expected range. The optical sensor will cover at least the range of 0 - 12 g/L and the impedance sensor will cover the range of 10 - 200 g/L. In figure 2-1 the proposed system is schematically shown.

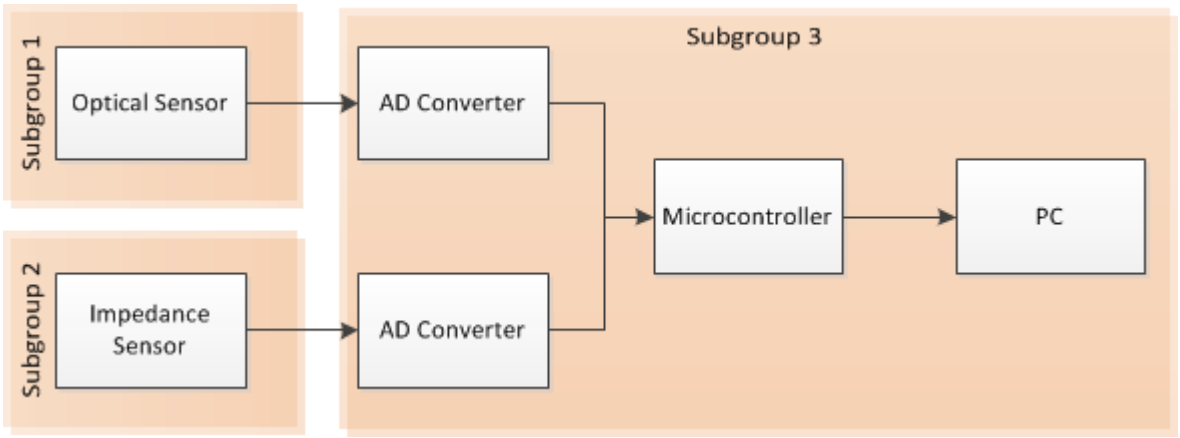

Figure 2-1: Schematic overview of system components.

The two analog sensor signals will be linked to a microcontroller using AD converters. The microcontroller will perform the necessary operations on the signals and computes the cell concentration. Finally, the microcontroller sends a single output to the PC of the lab assistent where the cell concentration will be visible as a single number as well as in graphs.

Figure 2-1 also shows the division of tasks between the three subgroups as mentioned in the introduction. Subgroup 1, as will be described further in this thesis, will design and develop an optical sensor system. Subgroup 2 is responsible for the development of the impedance sensor system and subgroup 3 will develop the software and interface system. These latter subjects will not be discussed further in this thesis.

Chapter 3

Optical sensors

This chapter will start focusing on optical sensors. The impedance sensor and the software and interface system will be developed by other sub-groups as mentioned in the previous chapter and will be outside the scope of this thesis. There are a few different techniques for optical sensors to determine the cell concentration. This chapter will explain how the different techniques work with their advantages and disadvantages. Based on these results and the requirements of the project, one technique will be chosen and further developed.

3-1 Optical techniques

There are currently four different techniques considered to measure the cell concentration [4],[21]. Almost all of them are based on the fact that different materials absorb different wavelengths of light. Every molecule absorbs different wavelengths because the absorption is mainly caused by the chemical structure of molecules. Since cells contain many molecules, many different wavelengths will be absorbed.

3-1-1 Optical Density

Optical density (OD) sensors are based on the turbidity of a substance. A beam of light with certain intensity is transmitted to a receiver with the material in between. The difference in intensity between the transmitted and received signal is absorbed by the material. With more cells in between the transmitter (i.e. a LED) and the receiver (i.e. a photodiode), the intensity will decrease because more cells will start to interfere with the light. Since light is an electromagnetic phenomena, a small fraction of light will also scatter in other directions. With higher cell concentrations, the fraction of scattered light will increase. This means that the cell concentration can also be determined by measuring the intensity of the reflected light. An OD sensor would give the opportunity to do both absorption and reflection measurement at the same time. A disadvantage is that OD sensor can not distinguish nutrients or other

materials from cells, the measured concentration will therefore always be offset by the amount of nutrients.

A formula related to this topic is the 'Beer-Lambert Law'[22] shown in equation 3-1. This law relates attenuation of light with some properties of the material, among others the cell concentration, which the light is traveling through. The 'Beer-Lambert Law' is usually stated as

$$T = \frac{I}{I_0} = \mathrm{e}^{-\epsilon l c} \tag{3-1}$$

Where T is the transmission of light through the medium, I is the intensity of transmitted radiation, I_0 is the intensity of incident radiation. The constant ϵ is called the absorptivity of the medium, l is the pathlength of light transmitted and c is the concentration of attenuating substance. This law makes it possible to calculate the concentration by measuring the incident and transmitted light intensity. However, there are at least six conditions that need to be fulfilled in order to make the Beer-Lambert law valid [23]. These are:

- The attenuating substances must act independently
- The medium under investigation must be homogeneous
- The attenuating medium must not scatter the radiation
- The incident light must consist of parallel rays, each traversing an equal length in the medium
- The incident light should be monochromatic
- The incident light must not influence the substances under investigation

The medium under investigation in this project is likely to be inhomogeneous and will scatter the radiation. Furthermore, the law is only valid for substances in solutions. Since living cells in water form a suspension, the 'Beer-Lambert Law' is not directly applicable in this project. The concepts of transmission and intensity are, however, important in research regarding OD and this project. Therefrom some attention has been given to this topic.

3-1-2 Near infrared spectroscopy

The near infrared (NIR) spectroscopy is quite similar to the OD technique [24]. Light is transmitted through a material to the receiver on the other side. Contrary to OD, the receiver needs to measure the amplitude of the whole spectrum (750 - 1400 nm). As mentioned earlier, every molecule absorbs different (bands of) wavelengths of light, so by analyzing the spectrum it is possible to separate different molecules. Since most absorption bands of different molecules will overlap, advanced multivariate mathematical methods are required to separate all the different molecules. The major drawback of this method is that a lot of calibration is needed in order to separate different materials.

3-1-3 Fluorescence

With fluorescence sensors, high energetic light (i.e. ultraviolet or visible) is send through the medium. Electrons will absorb the energy and will get a higher excitation level. After a while, the electrons will go back to the ground state and transmit a photon with lower energy. It has been found that there is a good correlation between cell concentration and the material NAD(P)H [4],[21], which are organic molecules. However, the correlation is only valid if the concentration NAD(P)H is constant. NAD(P)H is also used in cell metabolism so this will create extra uncertainty. Another drawback is that none of the compounds of the medium should absorb or emit at 360 or 450 nm. Therefore this method is limited to processes with well-defined medium composition.

3-1-4 Microscopy

By using microscopy the cell concentration can be determined by counting the cells which are in the scope of the microscope. Microscopy pictures can be taken of the cells and then send them to a computer. The computer needs to do some image analysis with among other things pattern recognition. The counted cells will be extrapolated to get a estimation of the total cell concentration. This method also makes it possible to study the cell morphology of individual cells. However, image analysis is not easy and costs a lot of processing power, which is not appropriate for the microcontroller. The hardware required for this technique is also very expensive.

3-2 Choices

To be able to choose a suitable technique, the main project requirements need to be taken into account. All techniques satisfy the criteria of non-invasive measurement and the measurement frequency. The concentration of cells in a suspension can be measured by all techniques, however the fluorescence sensor is restricted to processes with a well-defined media. This means the fluorescence sensor is not suitable for a large number of cell types and therefore it does not meet one of the requirements. Another issue of the fluorescence sensor is safety, because it might (only) be working with ultraviolet light. Working with ultraviolet light can cause damage to human body and also to cells, which is clearly not desirable. Other techniques do not have these issues so the fluorescence sensor does not seem like a good option. Microscopy is a very accurate way of measuring the concentration, but it requires expensive hardware and advanced analysis. Since time for the project is limited, developing a sensor using pattern recognition to determine the cell concentration is probably too much work. Therefore microscopy is not feasible within the time and cost limitations. NIR spectroscopy is very useful to determine both the cell concentration and the concentration of other substances (i.e. glucose), but this method requires a lot of time for calibration. For this reason we have chosen for an OD sensor, because it seems the most convenient to implement and the expected costs are low.

3-3 List of requirements

Most of the requirements stated in chapter 2 for the complete sensor also apply to the optical subsystem. However, there are requirements specifically applicable to this subsystem. Therefore, a separate list of requirements has been made to verify whether the optical subsystem will meet the specifications at the end of the project.

- The sensor has to be able to measure cells in the range of 0 - 12 g/L (dry weight).
- The sensor should have an accuracy within $\pm 10\%$.
- The sensor has to generate a DC output voltage.
- The output voltage of the sensor must not be smaller than 0V and must not exceed 3.3V.
- The sensor has to operate with a supply voltage of $\pm$ 5V DC.
- The sensor has to be insensitive for environmental influences like temperature, pH or erosion.
- The parts of the sensor that have direct contact with the suspension have to be disposable.

Requirements stated in chapter 2 are still applicable to the optical subsystem, unless stated otherwise in the list in this section. The output voltage requirements follow from the fact that the software subgroup uses the Arduino onboard AD converter (ADC). This ADC has an input range from 0 to 3.3V [25]. The requirement with respect to the supply voltage is a practical one, this way all the subgroups use the same voltage.

Chapter 4

Design

This chapter will describe how the complete system for the optical sensor is designed. An overview will be given of different blocks in the design and they will be discussed in detail later in this chapter.

4-1 Overview

The previous chapter explained the choice to implement an OD sensor to measure the cell concentration. Since OD sensors can be used in both reflection and absorption way, multiple implementations are available. The choice was made to measure both reflection and absorption, in order to achieve maximal range and accuracy. The consequence of measuring the absorption is that a probe is required. One of the project requirements is that the system should be sterilized. By using a probe, the sensor should be disposable or easy to sterilize.

LEDs and photodiodes will be used as transmitters and receivers. The configuration can be found in figure 4-1. The two LEDs are placed in series so that they have the same current. Furthermore, they are also thermally coupled to each other with an aluminium case for temperature compensation. A transmittance and reflection photodiode receive the radiation of LED1. These signals will be amplified, filtered and sent to the AD converter. LED2 and the reference photodiode will make sure that the intensity of LED1 will be the same for different temperatures.

The design will be verified by using yeast cells. The choice was made to use yeast as a model cell because of its availability, it is sold in every supermarket. Also, yeast is easy to handle and store. But, most importantly, yeast cells have a size that varies between 5 and 10 μm [26]. Therefore they are within the range defined in the list of requirements, so the sensor should be able to measure them. Furthermore, Applikon uses yeast to test their bioreactors.

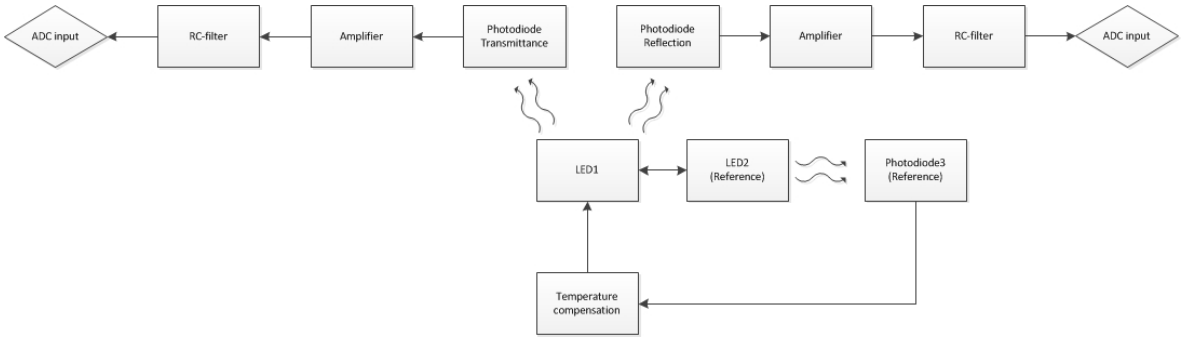

Figure 4-1: Optical System Overview

4-2 Photodiodes & LEDs

The choice for wavelength of the transmitted light is an important one. The previous chapter mentioned that every molecule absorbs different wavelengths because of their different chemical structure. Since all the measurements and calibration of the sensor are conducted with yeast, it is important to know which wavelength is most absorbed by yeast. In figure 4-4 the measurement setup has been drawn. The measurements have only been done with the absorption photodiode for practical reasons. The photodiode is coupled to the transimpedance amplifier of which the feedback resistance has been calibrated at an output of 4,4V, because this is the maximum value in the Op-Amps linear range. The calibration is done with only tap-water, corresponding to a concentration of 0 g/L.

Due to the time limitation only 3 wavelengths have been tested, namely 890 nm, 940 nm and 1300 nm. All these wavelengths are in the NIR spectrum (750 - 1400 nm). This choice has been made because this will prevent interference with visible light, but still has the advantage stated in chapter 3. Another reason is that UV and visible light might damage or influence the cells since they are more energetic. They might raise the energy level of an electron, which is undesirable. Some tests with different wavelengths have been performed from 0 to 30 g/l with a yeast suspension, because this range covers the range specified in the list of requirements. The results are shown in figure 4-2. Based on these results, 940 nm is chosen as wavelength for the optical sensor system because of its linearity and dynamic range. Also, 1300 nm shows a great decay at lower concentrations, probably due to the absorption of this wavelength by water. The 890 nm graph shows a peak around 5 g/L, which makes it impossible to do an unambiguous measurement in the range of 0 - 10 g/L. The measurements are listed in Appendix A

Given the choice of wavelength, the LED TSAL5100 [27] and photodiode BPV22NF [28]. were selected. These LED and photodiode are cheap, EUR 0.37 and EUR 0.69 respectively, which is good from a disposability point of view. Also, the TSAL5100 is capable of handling 100 mA of forward current, enabling it to emit 130 mW/sr. Such a high intensity could be useful when measuring high cell concentrations. This LED has also been chosen because of its relatively small angular displacement of only 10 °, meaning the emitted light is very focused. The photodiode on the other hand has been selected because of its wide angular half sensitivity of 60 °, so it is able to detect small changes in scattered light. Also, this photodiode has a relative small wavelength sensitivity, limiting the effects of ambient light.

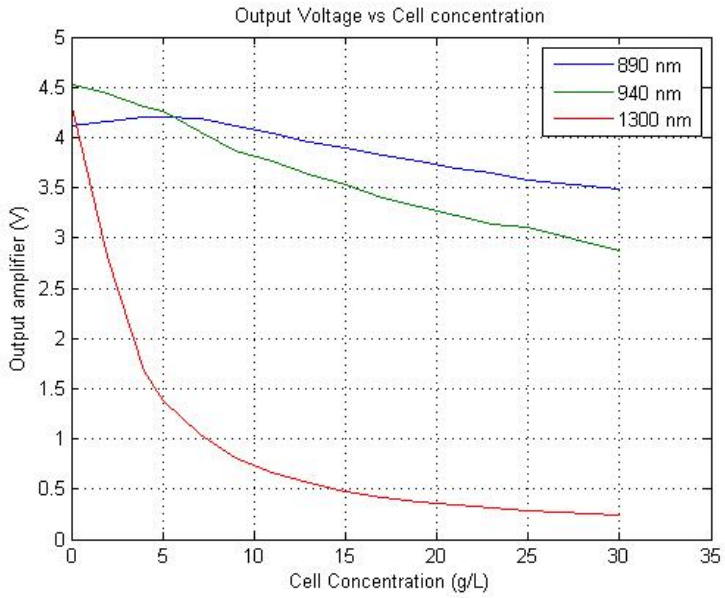

Figure 4-2: Sensor output at different wavelengths

As stated in the previous section, the choice was made to measure both reflection and absorption. This decision results in a design that uses a single LED and two photodiodes. The photodiode that measures absorption will be placed in a probe that is easily disposable. For the development of a prototype, the probe was made of a single glass test-tube because it had about the right size and the photodiode was easy to mount inside it. The tube was necessary to protect the photodiode and circuit against water in the suspension. The reflection photodiode is placed right next to the LED, below the suspension to be measured. A schematic overview of the setup is shown in figure 4-4.

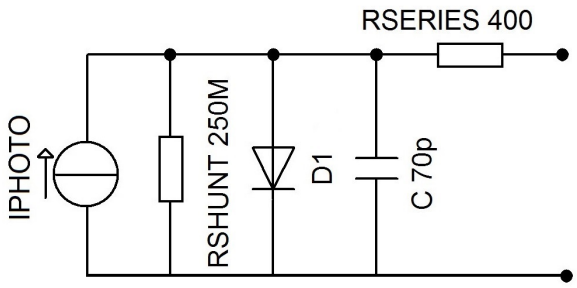

Figure 4-3: Photodiode model of the BPV22NF

To be able to design a circuit that converts the signal from the photodiode into a useful quantity, the photodiodes are considered current sources. The model used for the photodiode is depicted in figure 4-3 [29]. The current source IPHOTO represents the current generated by the incident light. Because a photodiode is a semiconductor device, ideal diode D1 was added to the model to represent the PN-junction. Resistance RSHUNT is the slope of the current-voltage curve of the photodiode at the origin. Ideally, its resistance is infinite, but

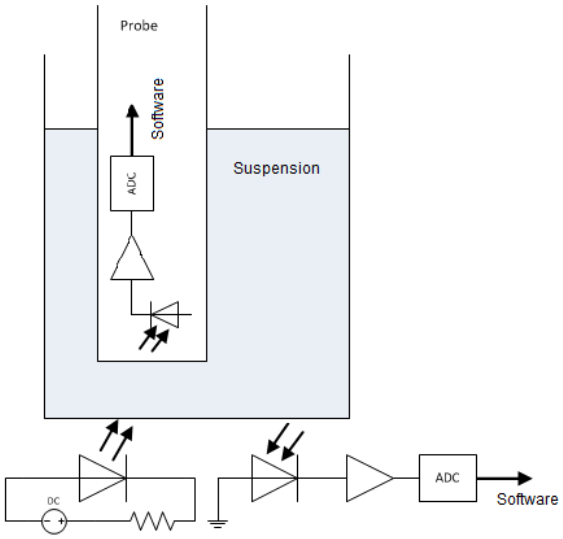

Figure 4-4: Optical sensor system setup

practical photodiodes have values of around 100's of Mega ohms. Resistance RSERIES arises from the wire contacts and the undepleted silicon. Capacitance C is caused by the boundaries of the depletion region. For the BPV22NF, this capacitance is 70 pF [28].

4-3 Amplifiers

To be able to process the information from the photodiodes, the current they generate must be converted to a suitable voltage. This is done by two transimpedance amplifiers. Each photodiode uses its own amplifier circuit. The circuits are build around the OPA381 Operational Amplifier. This Op-Amp is chosen because of its dedicated design for transimpedance topologies, especially for connection to a photodiode [30]. The OPA381 uses a supply voltage of 5V, meeting the supply voltage requirement. The complete amplifier circuit for the absorption sensor is displayed in figure 4-5. The reflection circuit has the same topology and components. Capacitor C1 is used to reduce noise on the Op-Amp supply voltage. The final components in the circuit form a voltage divider, which ensures that the output voltage will not exceed 3.3V since the ADC can only handle input signals below this value. The output of the Op-Amp is limited to 4.5V due to its supply voltage of 5V, and a voltage output swing of typically 0.5V from the positive rail. To set the maximum voltage of the amplifier to 3.3V, a division of 1.36 is required. In order to prevent spikes in the output signal from damaging the ADC, a division of 1.5 is implemented using the resistor values 33Ω and 66Ω.

4-3-1 Feedback resistors

The feedback resistors used in the absorption and reflection circuits set the gain of the amplifiers, since the amplifiers transferfunction is

$$\frac{V_{out}}{I_{in}} = -R_f \tag{4-1}$$

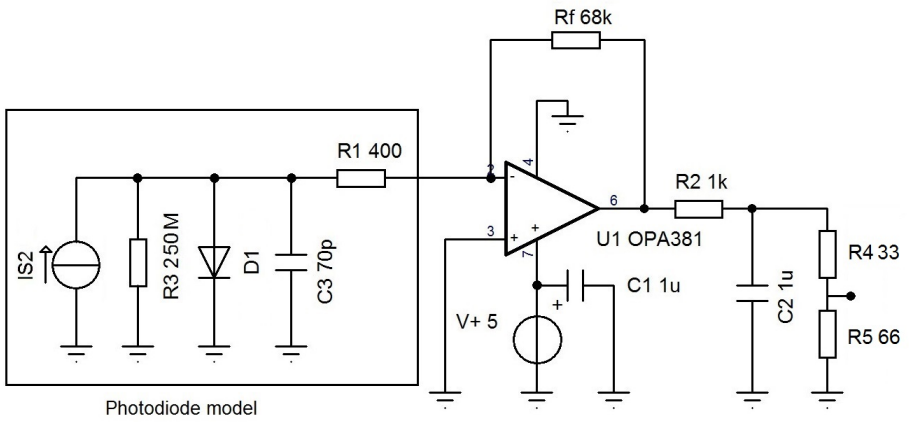

Figure 4-5: Amplifier circuit for absorbance sensor

This is an important parameter because it determines whether the full output voltage range of the OPA381 is being used. If the feedback resistor is chosen too low, the output will only use part of the voltage range of the Op-Amp before optical saturation occurs. This is the point where the sensor output stops changing when the concentration is further increased. In this case, to measure the full concentration range, a high resolution ADC is necessary. If the feedback is chosen too high, the output voltage will be limited by the Op-Amp, even if the photodiode is still sensitive to changes in concentration. This is illustrated in figure 4-6.

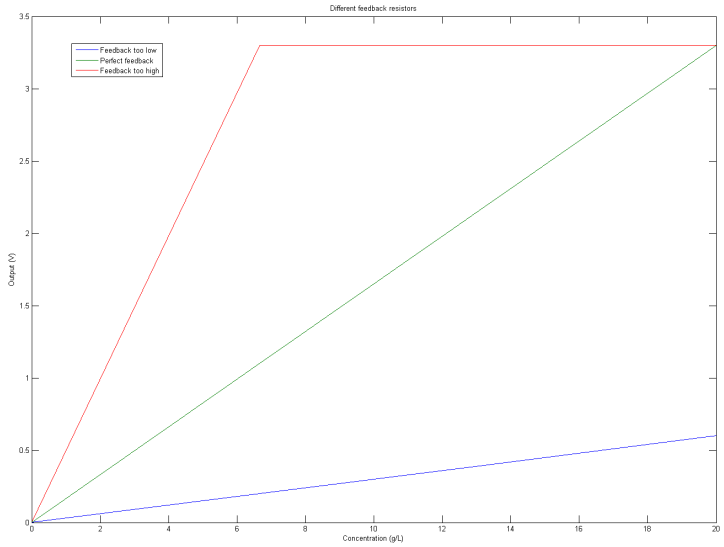

Figure 4-6: Simulation of different feedback resistors

So to find the correct feedback values, it is necessary to determine the photocurrent at the optical point of saturation of the photodiode. This point should then correspond to the maximum output voltage of 4.4V. Because the saturation point also depends on the pathlength and the intensity of the LED, these parameters should be kept constant while performing the

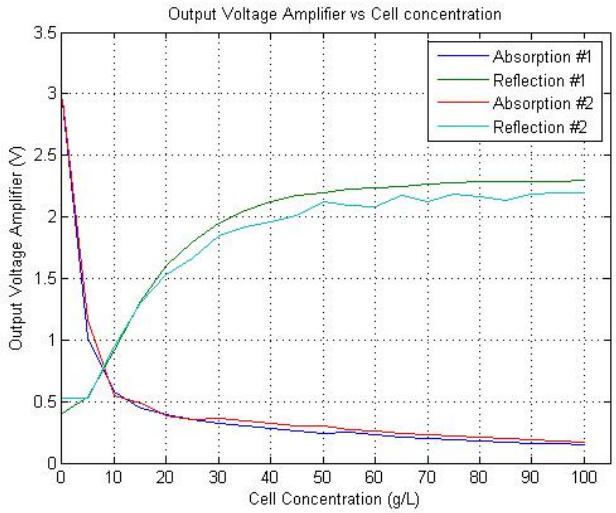

Figure 4-7: Measurements with different cell concentrations

measurements. During the design process, measurements showed that the absorption sensor output dropped tremendously after adding yeast corresponding to 15 g/L as can be seen in figure 4-7. However, when reaching higher concentrations, the sensor the voltage step size is very small. Since the reflection sensor saturates at higher concentrations and has a linear trend at the lower concentrations, the absorption sensor can be used to extend the total range. To increase the voltage step size at higher concentrations, the gain needs to be increased. As a result of this, the amplifier will clip at lower concentrations.

To determine the feedback resistors, two potentiometers are used in order to easily adjust the gain. For absorption, a substance of yeast and water has been made with concentration of 15 g/L. The potentiometers are set at the point where the amplifier is just barely clipping. While the feedback resistor of reflection is determined with a suspension with a concentration of 140 g/L to make sure that the sensor is saturated. The potentiometers were measured with a multimeter so that the feedback resistor for the absorption circuit could be chosen at

$$R_f = 73.2k\Omega \tag{4-2}$$

For the reflection circuit, the feedback resistor is

$$R_f = 68k\Omega \tag{4-3}$$

Because the $73.2k\Omega$ resistor wasn't available, the R_f for the absorption circuit was chosen $68k\Omega$.

4-3-2 RC-filters

After the amplifier stage, a first order RC-filter is placed. This filter has to suppress the output noise before reaching the ADC, and acts as an anti-aliasing filter. The sample frequency of

the ADC is 75 kHz, therefore the signal components above 37.5kHz have to be suppressed to prevent aliasing. Since the Least Significant Bit of the ADC represents 0.8mV, the signal components have to be attenuated below this value in order to be invisible in the digital signal [25]. The RC-filter has a cut-off frequency of

$$f = \frac{1}{2\pi RC} \tag{4-4}$$

Because the oscilloscope only showed (very) high frequency components in the signal, the cut-off frequency has been chosen at 159 Hz. This way the filter is effective but still requires components that are small, generally available and cheap. This is according to our scalability requirement. The component values required for this frequency are $R = 1k\Omega$ and $C = 1\mu F$.

4-4 Temperature compensation

Temperature conditions in the MicroMatrix system can be adjusted according to the customers needs. The available range is approximately 10 °C to 45 °C [31]. The LED used for optical detection has a dependency on temperature that is shown in figure 4-8. Here, the photodiode to measure the curve is placed at a fixed temperature and distance from the LED and its photocurrent is measured using a reverse biased configuration. The temperature of the LED is then varied over the temperature range, while keeping the current constant. The resulting photocurrent is plotted against temperature. It follows that the photodiode output drops 40% due to the change in temperature from 10 °C to 45 °C, which is in accordance with the LED datasheet. Therefore, a dynamic temperature compensation circuit has been designed to make sure the intensity of the LED is constant over the desired temperature range [32]. The choice was specifically made to design a dynamic compensation circuit instead of a static one, because of its better robustness against external influences and lack of calibration. The compensation circuit is shown in figure 4-10.

Its principle of operation is based on a second LED, called the reference LED, that is placed in series with the main LED. The LED's are thermally coupled using an aluminium case, see figure 4-9, to make sure their temperatures are identical. Assuming the two LED's are from the same batch, their temperature dependence and intensity are more or less the same. The reference LED is placed across a photodiode at a fixed distance, L in figure 4-9, which measures its intensity. As the temperature increases, the intensity of the LED will decrease. This decreases the amount of light falling on the photodiode, therefore decreasing its photocurrent according to figure 4-8. This effect will increase the amount of current I_{REF} flowing through the feedback resistor. The base current of the PNP transistor will rise, increasing the LED current. The circuit acts as an optical feedback loop, resulting in a constant LED intensity.

The current source I_{REF} is used to set the circuits point of operation. It is chosen so that the maximum current through the LED flows at 50 °C. At this temperature, the current through the photodiode is $3.3mA$. To induce maximum current through the LED's, the base of the PNP transistor has to drop to zero. This occurs when the OPA381 input is zero. Therefore, the current source I_{REF} is set at the same absolute amplitude but with opposed sign, so the two currents cancel out. The current through the LED's is limited by R_{LIM} at $80mA$, so

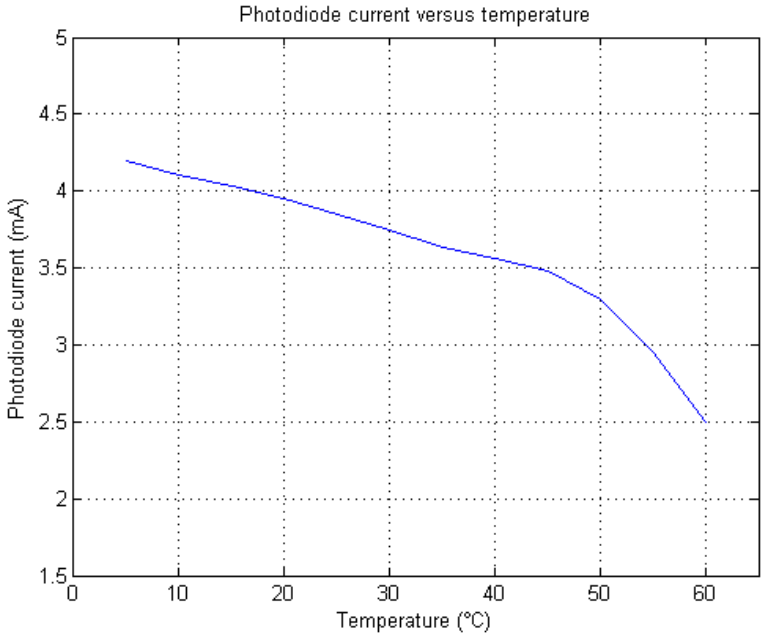

Figure 4-8: LED temperature dependency

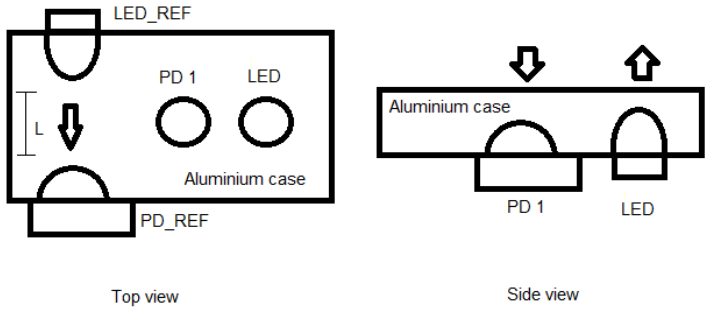

Figure 4-9: Aluminium case with reference photodiode and LED

the current can't damage the LED's if the compensation circuit tries to increase the intensity above this limit. The LED absolute maximum current is $100mA$ [27]. R_{LIM} is calculated as

$$R_{LIM} = \frac{5 - 0.2 - 3.2}{0.08} = 20\Omega \tag{4-5}$$

Here, the supply voltage of 5V is reduced by the voltage drops across the transistor and the two LEDs in series, 0.2V and 3.2V respectively. The resulting value, the voltage drop across the resistor, is then divided by the maximum current allowed to flow. This results in a resistor of 20Ω. The closest available value is 19Ω, so this value is selected to be in the circuit.

Rf is chosen so that the compensation speed matches the slope in the temperature characteristic in figure 4-8. The value is determined by testing the optimal performance of the circuit and is chosen to be $1M\Omega$. For the Operational Amplifier in the circuit, the $OPA381$ was chosen.

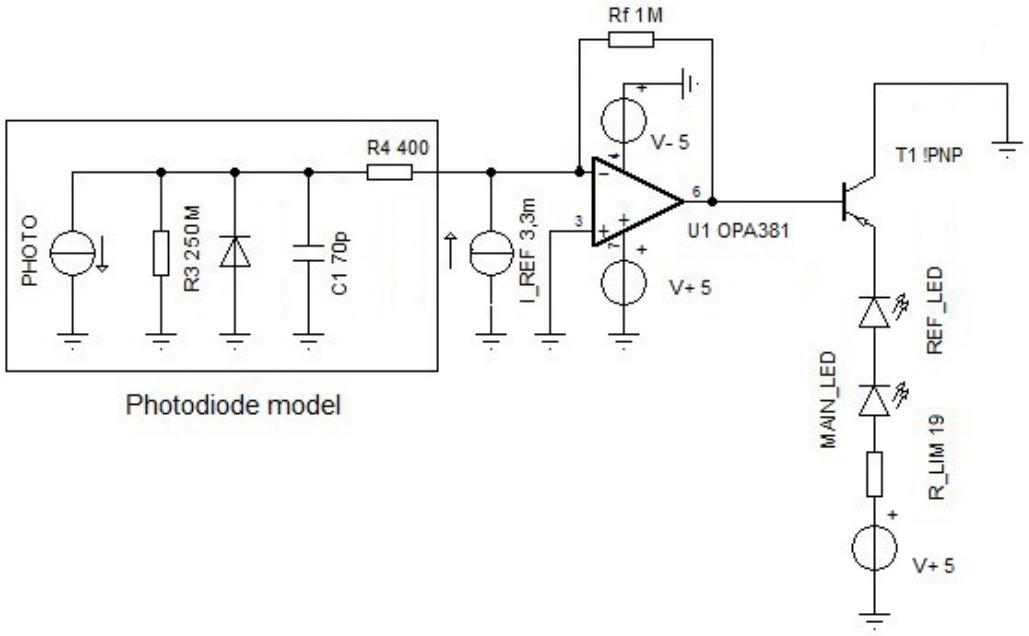

Figure 4-10: Temperature compensation circuit

4-5 Integration

The circuits discussed above are combined onto a development board to serve as prototype. A photograph of the final circuit is shown in figure 4-11. The black line distinguishes between the different subcircuits. The subcircuit most left is the temperature compensation circuit, secondly there is the reflection circuit and the last circuit is the absorption circuit. The board contains a single 12 pin header which connects all the inputs and outputs of the circuit. Appendix B shows the pinout. The output pins 5 and 6 represent the output value of the scattering sensor and the absorbance sensor respectively. They are connected to the ADC and processed by the software subgroup.

For integration within the MicroMatrix several adjustments to the prototype have to be made. First of all, the MicroMatrix requires the use of 48 photodiodes and 24 LEDs, in order to measure the 24 reactors at the same time. Also, the electronics is needed 24 times. The MicroMatrix reactor cassette is depicted in figure 4-12. This is the part of the MicroMatrix where the suspensions will be kept during the culture grow. The cassette is a disposable item with a transparent bottom. The dimensions of a single reactor are 16 x 16 x 40 mm, although only 5 ml of suspension will be kept at each reactor. A probe is supposed to be placed inside each of the 24 cubic reactors. Beneath each of the cubic reactor a LED and photodiode will be placed. At this moment, the width of the probe is 11 mm. Since the suspension inside the reactor has to be stirred constantly, the probe is too large to fit into the reactor. Due to the time limitation and because this is only a prototype, no action has been taken to reduce the size of the probe at this moment.

Inside the MicroMatrix, pH and O_2 measurements are performed using LEDs and photodiodes

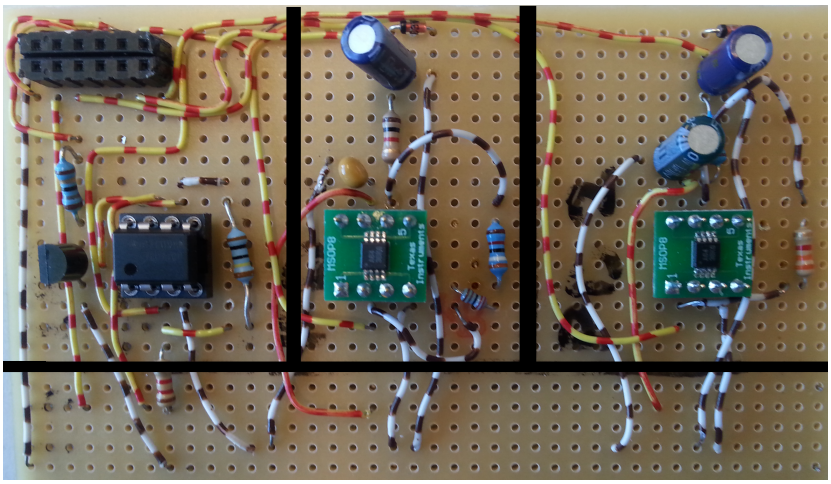

Figure 4-11: Final sensor circuit

that measure color change of pH dots at the bottom of the cassette. See figure 4-13. To make sure the optical sensor does not interfere with these measurements, a multiplexing system is proposed where only a single LED is active at a given moment. For instance, the pH LED could be active once every 2 seconds, while the optical cell concentration LED is active the rest of the time. This way mutual interference is prevented.

Figure 4-12: MicroMatrix reactor cassette

Figure 4-13: pH dots at the bottom of the cassette

Chapter 5

Measurements and Calibration

In order to verify whether our design meets the specifications stated in chapter 2, several measurements where performed. Also, to be able to convert the output of the sensor to an accurate cell concentration value, calibration had to be done. This chapter describes the setup used for both measurements.

5-1 Measurement setup

The optical measurements were performed using an optical rail. This setup is displayed in the photograph in figure 5-1. The setup consist of two vertical stands that hold the absorption probe and a plastic container with a volume of 100 ml. The plastic container is used to hold the yeast suspension. The distance between the probe and the container is variable with a minimum of 5 mm, because of the thickness of the test-tube that the probe is made of. The distance can be kept constant, even with vibrations present. The exact distance can be changed with the screws on the side of the vertical stand. The aluminium case with the LED and photodiode is mounted below the plastic container with tape. It is placed so that the surface of the aluminium case touches the bottom of the container.

5-2 Temperature compensation

To verify whether the temperature compensation circuit is working properly, tests were performed with and without the temperature compensation circuit enabled. Without the compensation circuit, the current through the LEDs is fixed at 80 mA. With the compensation enabled, the current is monitored with a current meter. The intensity of the LEDs is measured with the absorption photodiode at a fixed distance, while varying the temperature of the aluminium case between 10 °C and 45 °C. This varying is done with two cups of water at temperatures of 7 °C and 95 °C. The temperature of the aluminium is monitored with two thermocouples mounted to the case, to ensure the case is evenly heated. Measurements

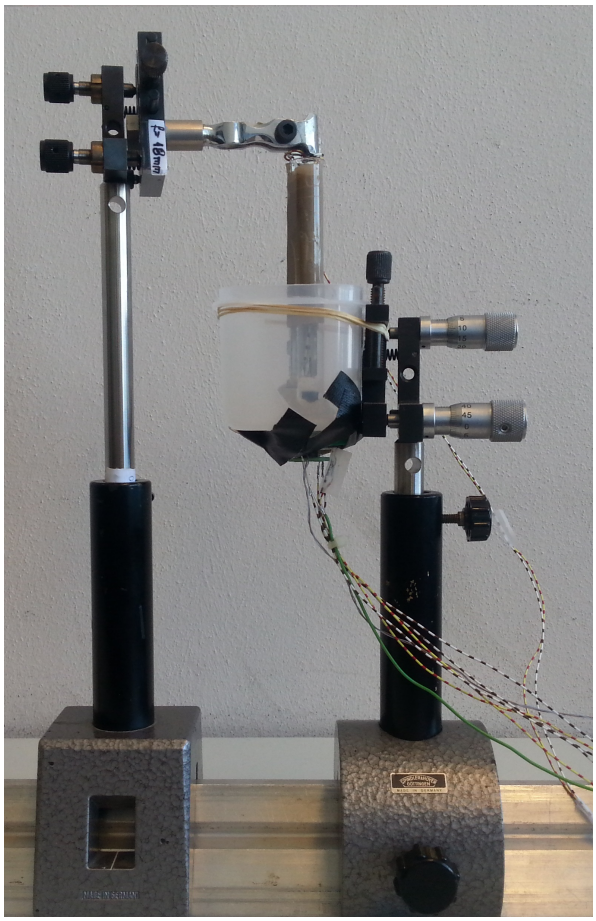

Figure 5-1: Measurement setup

start by dipping the case in hot water, thus increasing the temperature of the aluminium. When the temperature reaches 50 °C, the case is placed in the optical rail to perform the measurement. The photodiode output is plotted against temperature, while the temperature of the case declines. The result is plotted in figure 5-2.

The figure shows the sensor output with temperature compensation and without temperature compensation. Without compensation, the output voltage decline is about 15%. With compensation enabled, the voltage decline is almost 0% over the temperature range 10 °C to 55 °C. From these results it can be concluded that the compensation circuit is working properly.

5-3 Calibration

Because the output voltage of the sensor system is not directly related to the cell concentration by any physical formula, a calibration curve is needed to determine the cell concentration in an unknown suspension. To do this, the plastic container is filled with 100 ml of water. The two outputs, absorption and reflection, are connected to an oscilloscope to measure a DC voltage. Yeast is added to the water with steps of 0.5 g, accurately measured using a scale,

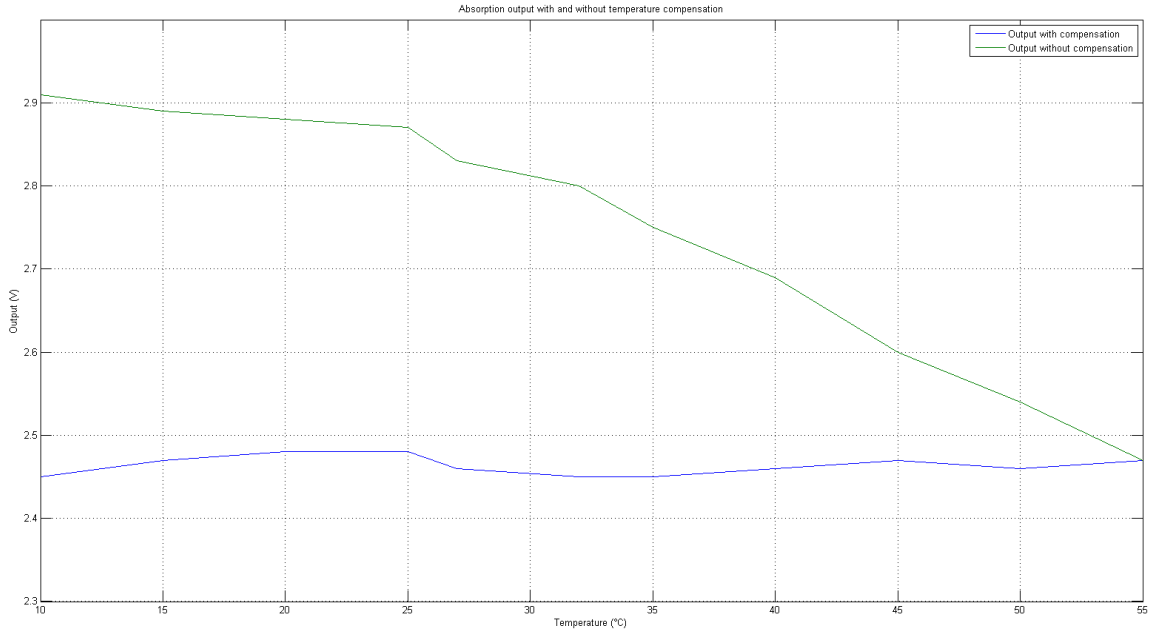

Figure 5-2: Temperature compensation test

corresponding to steps in concentration of 5 g/L. The outputs of the two sensors are plotted against concentration to create the calibration curves, which are shown in figure 5-3. These curves are used to find a correlation between sensor output and cell concentration, with the help of a linear fit model. This correlation is programmed into the Arduino software to be able to convert sensor output to cell concentration.

5-3-1 Linear model

Since the calibration curves have an exponential trend, a linear fit model would not be accurate. However, the reflection sensor seems linear at low concentrations, while the absorption sensor seems to be linear at higher concentrations. Therefore the ranges have been split up, to improve the accuracy of the model. The two calibration curves have been averaged for both sensors. Reflection is modeled within 0 - 30 g/L, while absorption is modeled from 20 until 120 g/L. This way both sensor will fill up each others gaps and even overlap. A model is created by linear regression with a least-squares fit. The MATLABfunction *polyfit* can generate the coefficients for every polynomial based on a least-squares-fit. In this case a polynomial of degree 1 (linear) is used. The generated best fit formulas of reflection and absorption were respectively

$$c = 19,2756 * x - 5,8350 \tag{5-1}$$

and

$$c = -46,8360 * x + 154,6451 \tag{5-2}$$

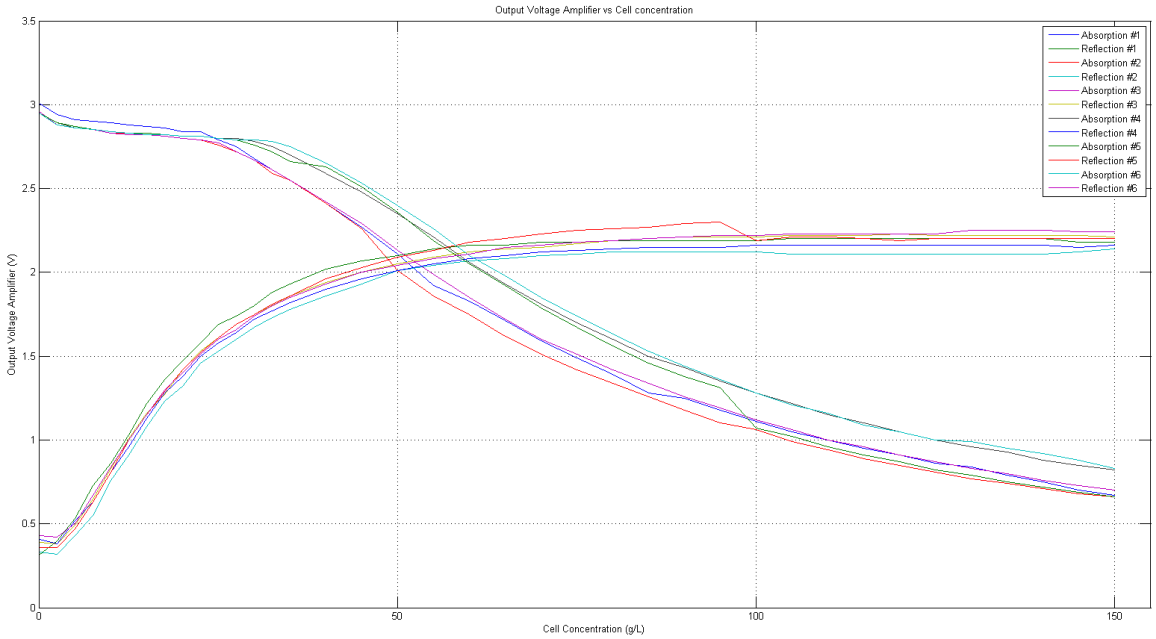

Figure 5-3: Calibration curves for the absorption and reflection sensor

Where x is the output voltage and c is the concentration.

In figure 5-4 and 5-5 it can be seen that both models predict the measured data-points quite accurate.

5-3-2 Error

With the prediction model determined, it is important to know how well the model performs. The coefficient of determination (R^2) is a very useful method to determine the goodness of fit of a model. First the residuals are determined by subtracting the estimated value($\widehat{y}$) from the averaged measured values(y). After that, the residuals are normalized by dividing them by the measured values to get the relative error as shown in equation 5-3.

$$Error_{relative} = \frac{y - \widehat{y}}{y} \tag{5-3}$$

The relative error of both sensors is shown in figure 5-6. The absorption model has only one data-point with a bigger error than $\pm 10\%$ at a voltage of approximately $2.8V$. This voltage is corresponding with a concentration of approximately 20 g/L and therefore it could be corrected by the reflection sensor. The model of the reflection sensor has a relative big error at the concentrations below 10 g/L up to 40%. All other data-points are within the $\pm 10\%$.

Last but not least it is important to know the total goodness of the fit of the model by the coefficient of determination, denoted as R^2. R^2 can be determined by equation 5-4. The

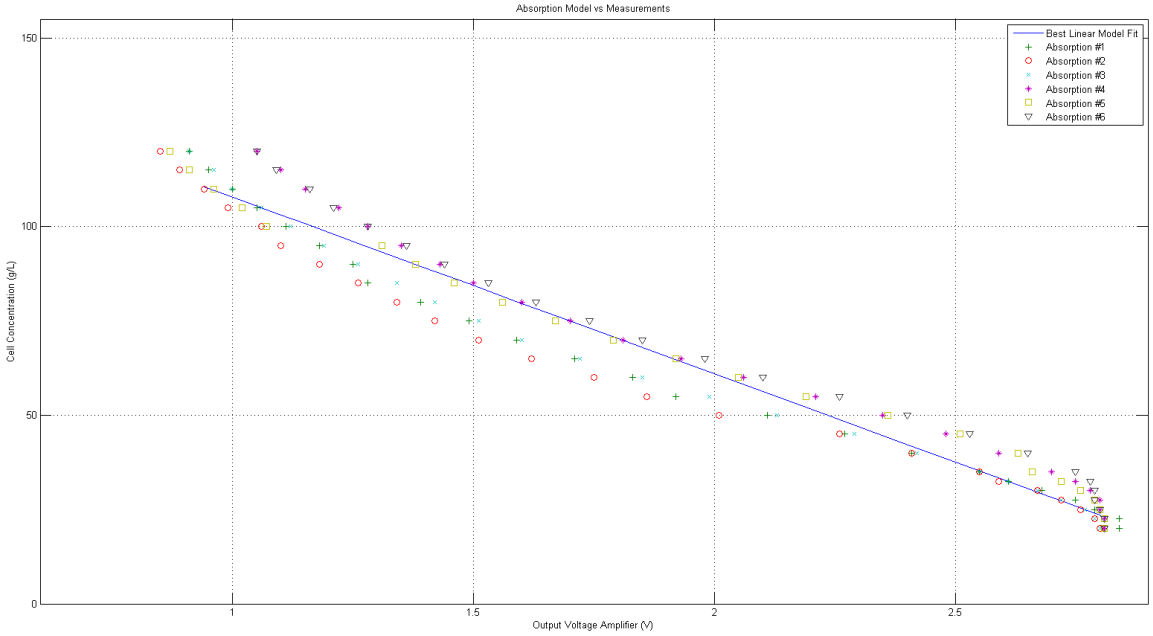

Figure 5-4: Absorption model and measurements

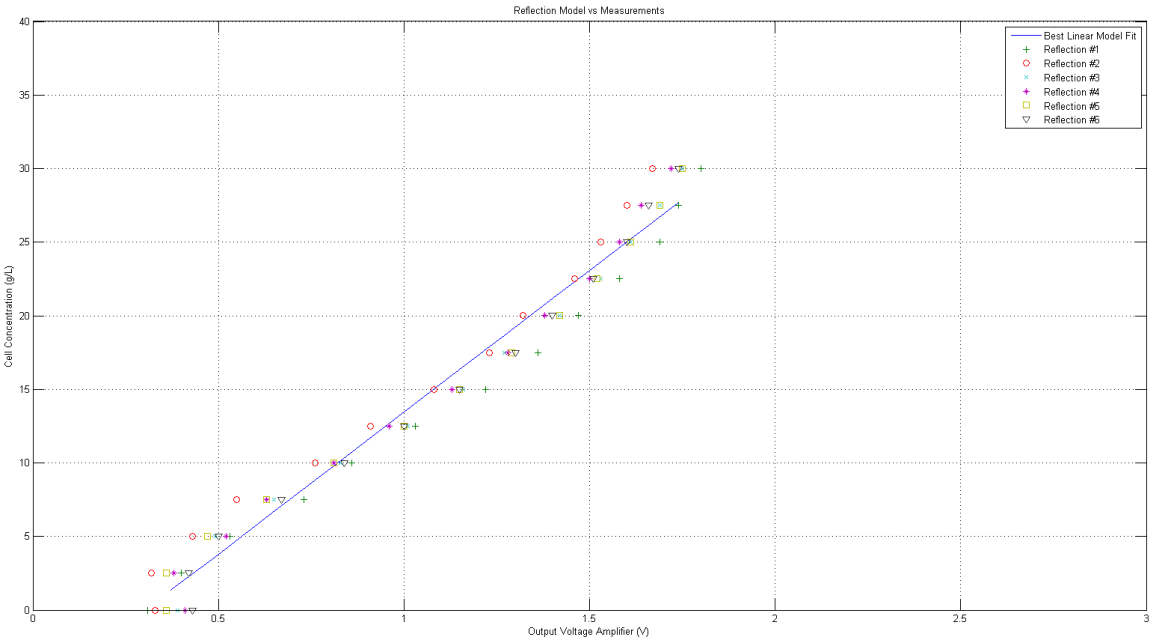

Figure 5-5: Reflection model and measurements

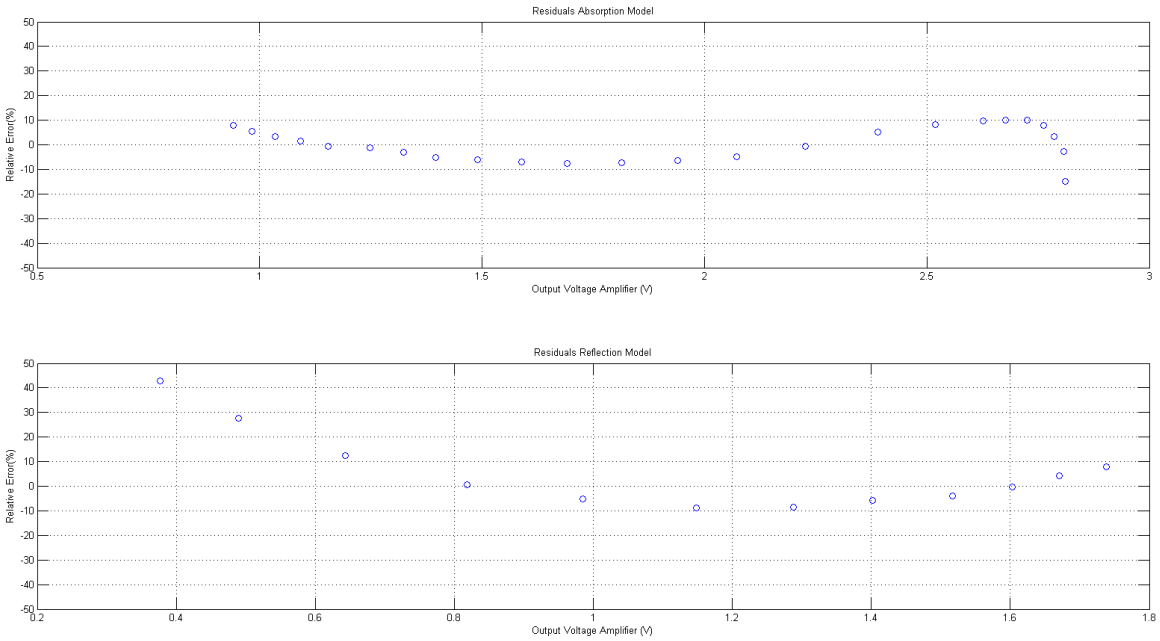

Figure 5-6: Relative error of both sensor systems

residual sum of squares ($SS_{residual}$) is as the name says: the sum of the residuals squared in equation 5-5. The same applies to the total sum of squares (SS_{total}) in equation 5-6.

$$R^2 = 1 - \frac{SS_{resid}}{SS_{total}} \tag{5-4}$$

$$SS_{residual} = \sum y_{residual}^2 \tag{5-5}$$

$$SS_{total} = (length(y) - 1) * var(y) \tag{5-6}$$

By using these equations in MATLAB, a result for R^2 is obtained. For the absorption and reflection models, values of respectively 0,9851 and 0,9833 have been accomplished. These values can be further optimized by performing more measurements because of a better weighted average.

Chapter 6

Discussion and Recommendations

Now that the sensor has been designed and tested, several improvements could be made in order to achieve a better performance. This chapter will compare the requirements stated in sections 2-1-1 and 3-3 with the results reported in chapter 5. Also, several results from the impedance sensor mentioned in chapter 2 will be used to evaluate the total sensor system. For more detailed information regarding the impedance sensor, the reader is referred to [33]. The performance of the total sensor system is also affected by the software used to process the data generated by the sensors. Therefore, references to the software system will be made in this chapter. More information about the software can be found in [34]. The following sections discuss the most important requirements that are not (completely) fulfilled by the sensor system.

6-1 Requirements not fulfilled

6-1-1 Range & Accuracy

The requirements with respect to range and accuracy of the total sensor have not completely been fulfilled by the final prototype. For the optical sensor system, it is possible to measure concentration in the range of 0 - 120 g/L. This range is composed of the ranges of the absorption and reflection sensors, 20 - 120 g/L and 0 - 30 g/L respectively. The accuracy of the optical system varies from $\pm$10% for the range 10 - 120 g/L till 10-40% in the range 0-10 g/L.
The impedance sensor is able to measure cell concentration in the range of 30 - 150 g/L with an accuracy within 15%. Combined, the two sensors give the total system a range of 0 - 150 g/L. The total sensor system thus has a range that is 50 g/L lower then required. The required accuracy of 10% is only met in the range 10-120 g/L. Above and below this range, the accuracy is within 40%. However, the impedance sensor does not work well with the Arduino yet. Further research is required to integrate the impedance sensor with the microcontroller.

6-1-2 Number of cell types

The sensor sensitivity for a large number of different cells is not investigated in dept in this project. All of the measurements were performed using yeast as cell type because of its favorable properties. It is unknown whether the sensor will perform the same when other cells with the same size are used, not to mention the use of bacteria, which are even smaller and have different molecular structures. Further investigation regarding this cell concentration sensor should therefore include research on this topic.

6-1-3 Influence on bioprocess

Another subject that is not completely investigated is the influence of the sensor on the bioprocess in the reactor. Since only measurements with fixed concentrations were performed, it is unknown whether the sensor has influence on growth or production of the cells to be measured. The expectation is that light in the NIR spectrum has no influence on either of these parameters, based on the information found in the literature presented in chapter 2 and 3, but further research is required to be able to confirm this expectation specifically for this sensor.

The impedance sensor has the risk of damaging cells when the voltage applied across the two plates is too high [33]. To be sure this is not the case, further research has to be done with respect to electrical cell properties.

6-2 Requirements fulfilled

These sections discuss the requirements that are completely fulfilled by the sensor system. There is made a distinction between total sensor system requirements and optical sensor system requirements, since the total sensor system consists of the impedance sensor and software components as well.

6-2-1 Total system requirements

Here the fulfilled requirements as stated in section 2-1-1 will be discussed.

Measuring cells in suspension

The total sensor system is able to measure the cell concentration of yeast in suspension. This is done with the combination of the impedance sensor and the optical sensor. The final output of the total system is produced by the software. It is given as absolute concentration value and as graph, where concentration is plotted over time.

Measurement frequency

The requirement of one cell concentration measurement every hour is fulfilled by the total sensor system. The output of the optical sensor is a continuous voltage signal, which theoretically should enable measurements in realtime. The only limitation are the response times of the photodiode, amplifier and RC-filter. A single optical measurement takes about one hundredth of a second to be performed in the software [34]. Since a full measurement of the MicroMatrix requires 24 measurements, the complete matrix could be measured in 0.24 seconds. Optimalisations in software could possibly lower this time, although the requirement with respect to measurement frequency is already met. Also, a configuration where all 24 measurements are performed in parallel will result in significant time benefits, if precautions are taken to reduce interference between different reactors. An impedance measurement takes more time to complete, since more advanced computations have to be performed on the gain and phase information this sensor generates [33], [34].

Scalability

Currently, the prototype of the complete sensor is too big to fit into the MicroMatrix. However, the components used are suitable to scale down in order to fit. Regarding the optical sensor, the biggest challenge is to fit the probe into the reactor. This probe is made of a test-tube and a photodiode. When taken into production, the probe could be reduced in size significantly by designing a different construction that consumes less space but still has the functionality of the current probe. Also a smaller size photodiode could be used. The current dimensions are simply better to handle when building a prototype. The same reasons apply to the LED and photodiode currently placed below the suspension. Possibly surface mounted devices could reduce the sensor size even more.
The impedance sensor uses two plates made from stainless steel to be placed inside the reactor. These plates currently do not fit into the MicroMatrix. There is, however, no reason why these plates couldn't be made smaller so they do fit. The microcontroller is also easily scalable and therefore the requirement of scalability is fulfilled by the total sensor system.

No samples required

The complete sensor system requires no samples to be extracted from the suspension, therefore the requirement is fulfilled. This enables the sensor to be used in reactors with a small working volume, like the MicroMatrix, where taking samples would have a relative high impact on the volume of the suspension.

Work within closed system

The sensor is mounted inside the reactor. It doesn't have to be removed during a culture grow, minimizing the risk of contamination from outside the reactor.

Sterilisability

The entire sensor system is difficult to sterilize. Therefore, the choice was made to make all the components disposable that have direct contact with the suspension. In the case of the impedance sensor, this only includes the two stainless steel plates. The optical case is discussed later in section 6-2-2.

Reproducibility

An important requirement for every sensor is reproducibility. The optical sensor system shows good reproducibility in the range from 0 - 150 g/L, as seen in figure 5-3. This observation could probably use some more foundation by performing more calibration measurements.

No influence on other measurements

Inside the MicroMatrix, pH and O_2 measurements are performed using optical methods. To make sure these measurements are not influenced by our sensor, a multiplexing system is proposed. This enables the sensors to operate independently, reducing mutual influences. Therefore, this requirement is fulfilled by the sensor system.

Operate safely

The sensor requires no operation from outside. It is mounted inside the MicroMatrix and is controlled only by software. Therefore, there is no risk in operating the sensor for lab personnel. Precautions have to be taken when replacing the disposables, as residual suspension could still be present.

6-2-2 Optical requirements

This section discusses the requirements specifically applicable to the optical sensor system, as stated in section 3-3.

Range & Accuracy

The optical sensor meets the requirement with respect to range. Since the required range for the optical sensor is 0 - 12 g/L, an achieved range of 0 - 120 g/L is a respectable result. This is probably due to the fact that two independent measuring techniques, with their own ranges, are used in a single sensor system. Also, the reported sensor systems in literature claim to have accuracies of about 3% [2] or better, where our achieved accuracy is 10%. Presumably the focus of sensor systems in literature tends more towards accuracy than range.

The accuracy of the system is almost meeting the requirements. The accuracy of both sensors is already meeting the specification in the range 10 - 120 g/L with ±10% accuracy, however the reflection sensor only has a ±10-40% accuracy below a concentration of 10 g/L.

A recommendation for the reflection sensor is the position of the photodiode. In our aluminum case, the reflection sensor has a very limited viewing angle and is not directly placed against the bottom of the container. Therefore the current generated by the photodiode is very small. By improving the position of the reflection sensor, the signal-to-noise ratio will most likely increase. This might give better results at higher concentrations since the variation of the incident light is very small in that range.

Another recommendation is to further investigate the effects of LED intensity and pathlength. Due to the limited time available for this project, these parameters were not investigated in depth. It is considered plausible that a higher LED intensity will increase the sensor range because the photodiode captures a higher light intensity, even at higher concentrations. Small variations in intensity will be easier to detect. Therefore, it might be a solution to use different LED intensities for absorption and reflection so both methods can perform optimal within their own range.

The effect of pathlength on the range of the sensor is not exactly clear. A smaller pathlength might increase the absorption sensors range, due to the higher intensity at the photodiode. A pathlength too small could make the suspension less homogenous because cells are clotting between the probe and container bottom, affecting the absorption and reflection performance. For this reason there seems to be a minimal pathlength required for the sensor to operate. This topic requires further investigation.

Supply voltage

The optical sensor system uses a supply voltage of 5V, as required. This supply voltage powers the LEDs and the amplifier circuits.

Output requirements

The output of the sensor is required to be a DC voltage between 0 and 3.3V. This requirement is fulfilled by the optical sensor. As seen in figure 5-3, the sensor output never exceeds 3.3V. This is due to the use of a voltage divider at the output. Also, the output never drops below 0V, because the Op-Amps used in the circuit have no negative supply voltage.

Insensitive for environmental influences

The sensor is insensitive for environmental influences like temperature and pH. The temperature dependance of the LED is compensated by the use of a dedicated temperature circuit that keeps the LED intensity constant over a temperature range of 10 °C to 45 °C. Changes in pH are not considered an issue for the optical sensor, because no electronics or measurement part of the sensor touches the suspension, only the outside of the probe. All the critical measurement components are shielded from direct contact.

Disposability

Disposability is a requirement applicable to parts that have direct contact with the suspension. In the case of the optical sensor, this is only the absorption probe. The basic components

of this probe are a glass test-tube and a photodiode, the total costs of material are less then EUR 1. Therefore this probe could be disposable, eliminating the need to sterilize. A problem with the disposability of the photodiode could be that the sensor has to be re-calibrated every time a new photodiode is installed, since a new photodiode probably has a different response compared to the original one. But since the sensor has to be re-calibrated with new measurement subjects anyway, this is not considered an insurmountable problem.

Chapter 7

Conclusions

The sensor system described in this thesis fulfills most of the requirements stated in the list of requirements in chapter 2-1-1 and 3-3. This conclusion summarizes the results obtained in the previous chapters.

7-1 Optical sensor

The optical sensor system meets most of the requirements stated in section 3-3, as discussed in chapter 6. Accuracy is the only requirement that is not completely fulfilled, since the sensor has an accuracy from 10% to 40% between 0 - 10 g/L and within ±10% between 10 - 120 g/L, where an accuracy of ±10% in the range 0 - 12 g/L is required. The remaining requirements concerning the optical sensor system are completely fulfilled.

7-2 Total sensor system

The most important requirements that are not satisfied by the total sensor system are range and accuracy. The achieved range is 0 - 150 g/L, with an accuracy from ±10% and ±40% between 0 - 10 g/L, ±10% between 10 - 120 g/L and within ±15% between 120 - 150 g/L. These ranges and accuracies are provided by the optical and impedance sensor together. Thus, the accuracy requirement is fulfilled between 10 - 120 g/L, but not in the remaining range. Other requirements that are not completely fulfilled are sensitivity for a large number of cell types and the influence on the bioprocess, simply because these topics have not been investigated due to the time limitation in the project.

Appendix A

Measurement results

A-1 Wavelength measurements

A-1-1 890 nm

Concentration (g/L)	Output (V)
0	4.11
2	4.16
4	4.21
5	4.2
7	4.19
9	4.12
11	4.04
13	3.96
15	3.9
17	3.83
19	3.76
21	3.69
23	3.65
25	3.58
30	3.49

Table A-1: Sensor output as function of concentration for 890 nm

A-1-2 940 nm

Concentration (g/L)	Output (V)
0	4.53
2	4.44
4	4.31
5	4.26
7	4.06
9	3.87
11	3.76
13	3.63
15	3.53
17	3.4
19	3.31
21	3.23
23	3.13
25	3.1
30	2.88

Table A-2: Sensor output as function of concentration for 940 nm

A-1-3 1300 nm

Concentration (g/L)	Output (V)
0	4.33
2	2.82
4	1.68
5	1.38
7	1.05
9	0.81
11	0.67
13	0.56
15	0.48
17	0.42
19	0.37
21	0.34
23	0.31
25	0.29
30	0.24

Table A-3: Sensor output as function of concentration for 1300 nm

A-2 Temperature Compensation

Temperature (°C)	Absorption output (V)	LED current (mA)
10	2.91	80
15	2.89	80
20	2.88	80
25	2.87	80
27	2.83	80
32	2.80	80
35	2.75	80
40	2.69	80
45	2.60	80
50	2.54	80
55	2.47	80

Table A-4: Sensor output as function of temperature without compensation

Temperature (°C)	Absorption output (V)	LED current (mA)
10	2.45	60.0
15	2.47	61.8
20	2.48	63.6
25	2.48	65.5
27	2.48	66.4
32	2.45	68.3
35	2.45	69.6
40	2.46	71.3
45	2.47	73.9
50	2.46	75.8
55	2.47	79.3

Table A-5: Sensor output as function of temperature with compensation

A-3 Calibration

A-3-1 Measurements

1

Concentration (g/L)	Absorption output (V)	Reflection output (V)
0	3,01	0,31
2,5	2,94	0,4
5	2,91	0,53
7,5	2,9	0,73
10	2,89	0,86
12,5	2,88	1,03
15	2,87	1,22
17,5	2,86	1,36
20	2,84	1,47
22,5	2,84	1,58
25	2,79	1,69
27,5	2,75	1,74
30	2,68	1,8
32,5	2,61	1,88
35	2,55	1,93
40	2,41	2,02
45	2,27	2,07
50	2,11	2,1
55	1,92	2,14
60	1,83	2,16
65	1,71	2,16
70	1,59	2,18
75	1,49	2,18
80	1,39	2,19
85	1,28	2,19
90	1,25	2,19
95	1,18	2,19
100	1,11	2,19
105	1,05	2,2
110	1	2,2
115	0,95	2,2
120	0,91	2,2
125	0,86	2,2
130	0,84	2,2
135	0,79	2,2
140	0,75	2,2
145	0,7	2,18
150	0,67	2,18

Table A-6: Sensor outputs as function of concentration

2

Concentration (g/L)	Absorption output (V)	Reflection output (V)
0	2,96	0,33
2,5	2,88	0,32
5	2,86	0,43
7,5	2,85	0,55
10	2,83	0,76
12,5	2,83	0,91
15	2,82	1,08
17,5	2,81	1,23
20	2,8	1,32
22,5	2,79	1,46
25	2,76	1,53
27,5	2,72	1,6
30	2,67	1,67
32,5	2,59	1,73
35	2,55	1,78
40	2,41	1,86
45	2,26	1,93
50	2,01	2,01
55	1,86	2,04
60	1,75	2,07
65	1,62	2,08
70	1,51	2,1
75	1,42	2,11
80	1,34	2,12
85	1,26	2,12
90	1,18	2,12
95	1,1	2,12
100	1,06	2,12
105	0,99	2,11
110	0,94	2,11
115	0,89	2,11
120	0,85	2,11
125	0,81	2,11
130	0,77	2,11
135	0,74	2,11
140	0,71	2,11
145	0,68	2,12
150	0,66	2,14

Table A-7: Sensor outputs as function of concentration

3

Concentration (g/L)	Absorption output (V)	Reflection output (V)
0	2,96	0,39
2,5	2,88	0,38
5	2,86	0,49
7,5	2,85	0,65
10	2,83	0,83
12,5	2,82	1,01
15	2,82	1,16
17,5	2,81	1,27
20	2,8	1,42
22,5	2,79	1,53
25	2,77	1,61
27,5	2,72	1,69
30	2,67	1,75
32,5	2,61	1,8
35	2,55	1,86
40	2,42	1,94
45	2,29	2
50	2,13	2,05
55	1,99	2,09
60	1,85	2,12
65	1,72	2,14
70	1,6	2,15
75	1,51	2,17
80	1,42	2,19
85	1,34	2,2
90	1,26	2,21
95	1,19	2,21
100	1,12	2,21
105	1,06	2,22
110	1	2,22
115	0,96	2,22
120	0,91	2,23
125	0,87	2,22
130	0,83	2,22
135	0,8	2,22
140	0,76	2,22
145	0,73	2,22
150	0,7	2,21

Table A-8: Sensor outputs as function of concentration

4

Concentration (g/L)	Absorption output (V)	Reflection output (V)
0	2,95	0,41
2,5	2,89	0,38
5	2,86	0,52
7,5	2,85	0,63
10	2,84	0,81
12,5	2,83	0,96
15	2,82	1,13
17,5	2,82	1,28
20	2,81	1,38
22,5	2,81	1,5
25	2,8	1,58
27,5	2,8	1,64
30	2,78	1,72
32,5	2,75	1,77
35	2,7	1,82
40	2,59	1,9
45	2,48	1,96
50	2,35	2,01
55	2,21	2,05
60	2,06	2,08
65	1,93	2,1
70	1,81	2,12
75	1,7	2,13
80	1,6	2,14
85	1,5	2,15
90	1,43	2,15
95	1,35	2,15
100	1,28	2,16
105	1,22	2,16
110	1,15	2,16
115	1,1	2,16
120	1,05	2,16
125	1	2,16
130	0,96	2,16
135	0,93	2,16
140	0,88	2,16
145	0,85	2,15
150	0,82	2,16

Table A-9: Sensor outputs as function of concentration

5

Concentration (g/L)	Absorption output (V)	Reflection output (V)
0	2,95	0,36
2,5	2,89	0,36
5	2,87	0,47
7,5	2,85	0,63
10	2,84	0,81
12,5	2,83	1
15	2,83	1,15
17,5	2,82	1,29
20	2,81	1,42
22,5	2,81	1,52
25	2,8	1,61
27,5	2,79	1,69
30	2,76	1,75
32,5	2,72	1,81
35	2,66	1,86
40	2,63	1,96
45	2,51	2,03
50	2,36	2,09
55	2,19	2,13
60	2,05	2,18
65	1,92	2,2
70	1,79	2,23
75	1,67	2,25
80	1,56	2,26
85	1,46	2,27
90	1,38	2,29
95	1,31	2,3
100	1,07	2,19
105	1,02	2,21
110	0,96	2,21
115	0,91	2,2
120	0,87	2,19
125	0,82	2,2
130	0,79	2,2
135	0,75	2,2
140	0,72	2,2
145	0,69	2,2
150	0,66	2,2

Table A-10: Sensor outputs as function of concentration

6

Concentration (g/L)	Absorption output (V)	Reflection output (V)
0	2,95	0,43
2,5	2,88	0,42
5	2,86	0,5
7,5	2,85	0,67
10	2,84	0,84
12,5	2,83	1
15	2,82	1,15
17,5	2,82	1,3
20	2,81	1,4
22,5	2,81	1,51
25	2,8	1,6
27,5	2,79	1,66
30	2,79	1,74
32,5	2,78	1,8
35	2,75	1,85
40	2,65	1,93
45	2,53	2
50	2,4	2,04
55	2,26	2,08
60	2,1	2,11
65	1,98	2,15
70	1,85	2,16
75	1,74	2,18
80	1,63	2,19
85	1,53	2,2
90	1,44	2,21
95	1,36	2,22
100	1,28	2,22
105	1,21	2,23
110	1,16	2,23
115	1,09	2,23
120	1,05	2,23
125	1	2,23
130	0,99	2,25
135	0,95	2,25
140	0,92	2,25
145	0,88	2,24
150	0,83	2,24

Table A-11: Sensor outputs as function of concentration

Appendix B

Pinout

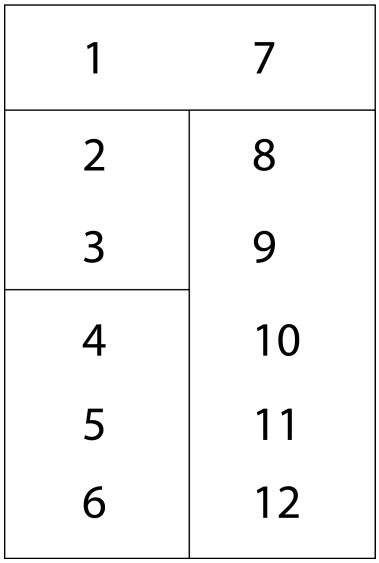

Figure B-1: Pinout of the prototype circuit

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