DESIGN AND CHARACTERIZATION OF 5-L AUTOCLAVABLE BATCH-CULTURE BIOREACTOR

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY
ŞEYMA ÜNSAL BEYGE

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOTECHNOLOGY

SEPTEMBER 2014

Approval of the Thesis

DESIGN AND CHARACTERIZATION OF 5-L AUTOCLAVABLE BATCH-CULTURE BIOREACTOR

Submitted by **ŞEYMA ÜNSAL BEYGE** in partial fulfilment of the requirements for the degree of **Master of Science in Biotechnology Department, Middle East Technical University** by,

Prof. Dr. Canan Özgen _____
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Filiz Bengü Dilek _____
Head of Department, **Biotechnology**

Assoc. Prof. Dr. Çağdaş Devrim Son _____
Supervisor, **Biology Dept., METU**

Assoc. Prof. Dr. Emren Nalbant Esentürk _____
Co-Supervisor, **Chemistry Dept., METU**

Examining Committee Members:

Assoc. Prof. Dr. Sertaç ÖNDE _____
Biology Dept., METU

Assoc. Prof. Dr. Çağdaş D. SON _____
Biology Dept., METU

Assoc. Prof. Dr. Ayşegül Çetin GÖZEN _____
Biology Dept., METU

Assoc. Prof. Dr. Tülin YANIK _____
Biology Dept., METU

Assoc. Prof. Dr. Emren Nalbant ESENTÜRK _____
Chemistry Dept., METU

Date: 05.09.2014

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last Name: Şeyma Ünsal Beyge

Signature:

ABSTRACT

DESIGN AND CHARACTERIZATION OF 5-L AUTOCLAVABLE BATCH-CULTURE BIOREACTOR

Ünsal Beyge, Şeyma

M.S., Department of Biotechnology

Supervisor: Assoc. Prof. Dr. Çağdaş Devrim Son

Co-Supervisor: Assoc. Prof. Dr. Emren Nalbant Esentürk

September 2014, 101 pages

Bioreactors are systems that use a biological agent to perform a chemical reaction. Developments on the field of biotechnology made production of even previously known non-renewable petro-chemical products to be produced by microorganism by use of bioreactors possible. Also developments on tissue engineering made it possible to produce organs by using bioreactors. Therefore, bioreactors become integral parts of future economy and technology. In this study prototyping of a small scale bioreactor was performed. Heat transfer system which can cause temperature change of minimum 0.2 °C/seconds was produced. Also an agitation system that can operate up to 1500 rpm without any contamination risk was developed and produced. For effective foam/level control a miniature multi-probe foam/level sensor with 2 mm sensitivity and 50 mm working range was developed and tested.

Keywords: Bioreactors, heat transfer, agitation, foam/level sensor

ÖZ

5-L OTOKLAVLANABİLİR KESİKLİ İŞLETİM BİYOREAKTÖR DİZAYNI VE KARAKTERİZASYONU

Ünsal Beyge, Şeyma
Yüksek Lisans, Biyoteknoloji Bölümü
Tez Yöneticisi: Doç. Dr. Çağdaş Devrim Son
Ortak Tez Yöneticisi: Doç. Dr. Emren Nalbant Esentürk

Eylül 2014, 101 sayfa

Biyoreaktörler herhangi bir biyolojik ajan kullanarak kimyasal reaksiyon gerçekleştiren sistemlerdir. Biyoteknoloji alanındaki gelişmelerle birlikte, biyoreaktörler kullanılarak, mikroorganizmalar yardımıyla daha önce yenilenemez kaynaklar olarak bilinen petro-kimya ürünlerinin üretilmesi dahi sağlanabilmiştir. Ayrıca doku mühendisliği alanındaki gelişmeler biyoreaktörler kullanılarak organ üretiminin de önünü açmıştır. Dolayısıyla biyoreaktörler geleceğin ekonomisinin ve teknolojisinin bölünemez bir parçası haline gelmiştir. Bu çalışmada küçük-ölçekli biyoreaktör prototipleme çalışması gerçekleştirilmiştir. Saniyede 0,2 °C ve fazlası sıcaklık değişimine yol açabilen ısı transfer sistemi üretilmiştir. Ayrıca dakikada 1500 devir sayısına çıkabilen ve kontaminasyon problemi olmayan ajitasyon sistemi geliştirilmiş ve üretilmiştir. Etkin köpük/seviye kontrolü için 2 mm hassaslık ve 50 mm ölçüm aralığına sahip minyatür çoklu prob köpük/seviye sensörü geliştirilip test edilmiştir.

Anahtar kelimeler: biyoreaktörler, ısı transferi, ajitasyon, köpük/seviye sensörü

To My Parents and Brother,

ACKNOWLEDGMENTS

I wish to express my deepest gratitude to my supervisor Assoc. Prof. Dr. Çağdaş Devrim Son and to my co-supervisor Assoc. Prof. Dr. Emren Nalbant Esentürk for their guidance, advices, encouragements and insights throughout the research.

I thank to my examining committee members Assoc. Prof. Dr. Sertaç ÖNDE, Assoc. Prof. Dr. Ayşegül Çetin GÖZEN, and Assoc. Prof. Dr. Tülin YANIK for their suggestions and criticism.

I thank my husband Mahmut Beyge for being a wonderful partner both in personal and business life. I am grateful for his technical assistance and moral support.

I am thankful to Bio-Dot Nano ve Biyoteknoloji Ltd. Şti. for financial support of this study.

I also wish to thank The Scientific and Technological Research Council of Turkey (TÜBİTAK) for financial support as BIDEB (2211 - National Scholarship Programme for MSc Students) scholarship.

TABLE OF CONTENT

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

CHEBI	Chemical Entities of Biological Interest
CSTR	Continuously Stirred Tank Reactor
Dint	Double Integer (signed 32-bit integer)
EPS	Exopolysaccharide
HMI	Human Machine Interface
IUPAC	International Union of Pure and Applied Chemistry
INT	Integer (signed 16-bit integer)
Lreal	64-bit Real or floating-point value
Modbus	A serial communications protocol
PID	Proportional-Integral-Derivative
PLC	Programmable Logic Controller
Profibus	Process Field Bus
px	Pixel
REAL	32-bit Real or floating-point value
RJ 45	Registered Jack 45

RPM	Revolutions per Minute
RS232	A standard for serial communication transmission of data
TEFLON	Polytetrafluoroethylene
TFT	Thin-Film Transistor
UDint	Unsigned 32-bit Double Integer
VAC	Volts-Alternating Current
VDC	Volts-Direct Current
VVM	Volume of gas per Volume of bioreactor tank per Minute

CHAPTER 1

INTRODUCTION

1.1. Bioreactors

Reactor is an apparatus or a controlled forcing agent that performs physical or chemical reaction/change into desired direction. Bioreactors are sub-group of reactors that use a biological agent for desired reaction such as production of antibodies, pharmaceuticals, vaccines, organic chemical or organism itself. Bioreactors are a special case of chemical reactors. Developments on synthetic biology, biotechnology and tissue engineering make the bioreactors locomotives for industries such as petrochemical productions, i.e. plastic monomers (Yim, et al., 2011) (Adkins, Pugh, & Nielsen, 2012), jet fuel (Elmoraghy & Farag, 2012) and biodiesel (Patil, Tran, & Giselrod, 2008), and production of organs and tissues (Martin, Wendt, & Heberer, 2004) (Radisic, Marsano, Maidhof, Wang, & Vunjak-Novakovic, 2008)

The classification of bioreactors given below is characterized according to the structure and properties of tank, and bio-active agents.

1.2. Types of Bioreactors

Bioreactors, as reactors, can be classified according to; their thermodynamic properties, numbers of phases and phase types (single-phase, multiphase; solid, liquid, gas), biological agents (anaerobic, aerobic, plant, animal, bacteria, enzyme etc.), size (miniature, micro-scale, large-scale, low-scale), materials used (membrane, column etc.), and their autoclavability (autoclavable, disposable (single-use)).

In this section only classification according to thermodynamic property is explained.

1.2.1. Thermodynamic-Property-Based Classification

According to ideal thermodynamic models at tank level, bioreactors can be classified in three groups. However; bioreactors taken as a whole system, if there is no gas transfer and material transfer they are closed systems, and if there is no gas transfer, but there is material transfer within the system it is also a closed system. In the case of where there is a gas transfer in to/out of the system, the system becomes an open system. Summarized list of bioreactor types based on thermodynamic property are included in ***Table 1.1***.

(a) Batch Reactor: The reactor in ideal model is a closed thermodynamic system. No mass transfer is allowed. The system is generally stirred well. Although most biological reactions performed in batch culture contains solid cell and liquid media, they are classified as single-phase reactors (Caccavale, Samarino, Pierri, & Tufano, 2011).

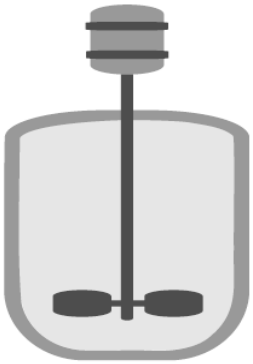


Figure1.1. Batch Reactor (Caccavale, Samarino, Pierri, & Tufano, 2011)

Batch culture bioreactors ***(Figure 1.1)*** are the oldest type of bioreactors and most widely used type (Bellgardt, 2000). Batch culture bioreactors are relatively cheap reactors and need sterilization prior to each use (Bellgardt, 2000). The control is difficult because the system do not follow steady state kinetics; therefore, unexpected results may be observed (Westerterp & Molga, 2006). Batch culture bioreactors are the most flexible bioreactors among others and can be used for experimental purposes (Simon, Weise, & Steinmetz, 2006).

(b) Continuous Reactor: The reactor in ideal model is an open thermodynamic system (in tank level). Both mass and heat transfer are allowed. The system may be stirred (***Figure1.2***) or not ***(Figure1.3)*** (Caccavale, Samarino, Pierri, & Tufano, 2011).

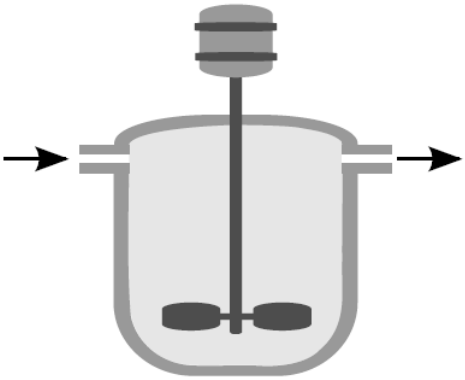


Figure1.2. Continuous Reactor-stirred (Caccavale, Samarino, Pierri, & Tufano, 2011)

Figure1.3. Continuous Reactor-not stirred (Caccavale, Samarino, Pierri, & Tufano, 2011)

Continuous bioreactors are generally used for higher production capacity and have lower run cost than batch systems. Continuous bioreactors need batch start up until steady state kinetics is reached (Williams, 2002). In continuous bioreactors generally biological agent or process substances are recycled to the system which can cause cells to age and mutate (Branyik, Vicente, Dostalek, & Teixeira, 2005) and risk of contamination is high (Williams, 2002).

These types of reactors are non-linear systems, so controlling the process parameters are more difficult. Also most variables are obtained from indirect measurements and first order responses, such as bacterial and substrate concentrations are obtained from pH and temperature data (Bellgardt, 2000) (Ramaswamy, Cutright, & Qammar, 2005). Nonlinear controllers also do not work well and cause excessive variations (Gonzales, Aguilar, Alvarez-Ramirez, & Barren, 1998).

(c) **Fed-Batch (Semi-Batch) Reactor:** Fed Batch reactors are same as batch type reactors except mass transfer is allowed in tank level, the system is converted to open system according to need.

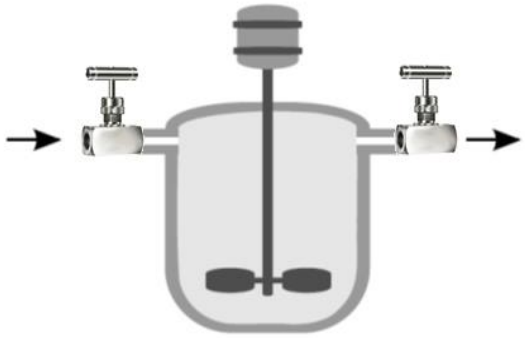


Figure1.4. Fed-Batch Reactor (Caccavale, Samarino, Pierri, & Tufano, 2011)

Fed-Batch systems are nearly same as the batch systems and can be classified under batch reactor system, and they are most widely used variant of batch culture bioreactors ***(Figure 1.4)***. Fed-batch system additionally gives more responsive process and allows introducing additives (Bellgardt, 2000). Since volumetric addition is performed, all process parameter analysis becomes relatively difficult in fed-batch system (Bellgardt, 2000).

Table1.1. Types of Bioreactors According to Thermodynamic Property

Type	**Examples**	**Description**
Batch Reactors	Test tubes, beakers, flasks etc.	When they are closed and chemical reaction is performed, they become batch reactors.
Continuous Reactors	Tubular Reactors/Plug Flow Reactors (Walker, 1961)	Gas/liquid/solid or combinations are passed continuously at very high speed through tubular structure and reactions occur.
	Fixed (packed)-bed reactors (Froment, 1967)	The design is similar to tubular reactor except, there is a bed of catalyst (ex: cell or enzyme) in the tube.
	Fluid-bed (fluidized-bed) reactors (Andrews, 1988)	The design is similar to fixed-bed reactor, except the catalysts are fluidized and stay in equilibrium through flow. When flow is stopped, they are settled. The system is preferred in the use of large, aggregated biological agents.

Table1.1.cont.'d. Types of Bioreactors According to Thermodynamic Property

Type	Examples	Description
Continuous Reactors	Loop reactor (Blenke, 1979)	Loop reactors are special case CSTR with recover and recirculation capabilities of used material with product isolation line, structurally similar to a loop.
	Trickle-Bed Reactors (Satterfield, 1975)	They are a type of fixed-bed reactors in which two phases/materials injected in counter current orientation.
	Bubble Column Reactors (Kantarci, Borak, & Ulgen, 2005)	They have reactor column where mixing is performed by gas distributor at the bottom of the column.
	Rotary Drum Reactors (Sherritt, Chaouki, Mehrotra, & Behie, 2003)	They are a type of solid state reactors in which reactor vessel or drum is rotated for proper mixing. They are generally used for waste water treatment.
	Solid-State Fermenters (Durand & Chereau, 1988)	Cell growth is performed on solid matrix, gas and liquids are fed to the system.

Table1.1.cont.'d. Types of Bioreactors According to Thermodynamic Property

Type	Examples	Description
Continuous Reactors	Mist reactors	Mist reactor is a special purpose bioreactor design for production of hairy roots. The ingredient is given in gas phase (media and oxygen supply in gas phase).
Fed-Batch Reactors	Fed-Batch Reactors (Lim & Shin, 2013)	Unlike batch reactors material injections occur in this type, according to need and not continuously.

1.3. Parts of Batch Culture Bioreactors and Design Considerations

Batch culture bioreactor systems are composed of; reaction vessel, agitator, air delivery system, and control system (Alaghlavi, 2013).

1.3.1 Reaction Vessel

Reaction vessel is the part of bioreactor where the reaction occurs. Before use, the tank must be sterilized. For single-use bioreactors, the tank is sold pre-sterilized/pre-installed (i.e. Sartorius, GE Life Science). For multi-use bioreactors reaction vessel must be sterilized prior to each use. In life sciences sterilization is generally performed by autoclaving at 121 °C, steam

pressure of 1.1 kilograms/square centimetres for 10-15 minutes (Madigan, Michael T.; Martinko, John M., 2006). Therefore, the reaction vessel should withstand 121 °C, 1.1 kilograms/square centimetres pressure.

The vessel should also have high corrosion resistance, and must be easy to clean. In general stainless steel (Zottola & Sasahara, 1994) and borosilicate glass vessels are used. Almost all elements that have contact with reaction side are preferred to be stainless steel and borosilicate glass. Reaction vessels used should be resistant to biofilm formation. Biofilms are composed of cells and exopolysaccharide matrix (EPS) (James, Beaudette, & Costerton, 1995), due to the mass transfer limitations biofilms has an increased resistance to transport of materials (Nichols, 1991). It is also shown that biofilms are formed preferably on scratched areas and crevices of a surface (van Haecke, Remon, Moors, Raes, De Rudder , & van Peteghem, 1990). Borosilicate glass is shown to be more resistant to biofilm formation than stainless steel (Eginton, Gibson, Holah, Handley, & Gilbert, 1995) (Jansen & Kohnen, 1995) (Hyde, Alberg, & Smith, 1997) (Ronner & Wong, 1993).

In a bioreactor vessel active working volume depends on the rate of foam formation (Van't Reit & Tramper, 1991). Accordingly, total bioreactor vessel volume (H_t) can be divided into: headspace volume where no active reactions take place and stands for foam, and working volume (H_l) where all reactions take place ***(Figure1.5).***

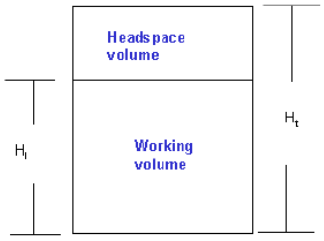


Figure1.5 Bioreactor Vessel Volumes (Singh, Kaushik, & Biswas, 2014)

1.3.2 Agitator

Agitator is the mixing component of a bioreactor. Generally it is composed of external power driver, impeller and baffles (Singh, Kaushik, & Biswas, 2014). Agitators mainly have two functions: near ideal mixing for increased mass transfer and shear effect for breaking air bubbles (Singh, Kaushik, & Biswas, 2014). Agitator makes flow on liquid that causes a shear on the reaction liquid. Shear rate is defined as the velocity of the flow within a given distance. It is a dimensionless unit (1/seconds). Shear stress is the stress on the system as a result of shear/flow. The shear viscosity of a system is the measure of resistance to flow. A Newtonian fluid is a fluid that has same viscous stress arising from flow, as a result is a linear system. A non-Newtonian fluid is a fluid that has variable viscous stress arising from flow; therefore, a non-linear system. Shear rate and shear stress in Newtonian fluids are directly proportional to the flow rate. However, in non-Newtonian fluids flow rate is first order estimation of shear stress and shear rate. Shear rate and shear stress are important parameters in bioreactor design; because, they are approximate measures of power absorption, mixing characteristics and mass transfer phenomena (Coulson & Richardson, 1990) and they give an idea about damage that can be caused to the organism (Chisti, 2001). The reaction media in a bioreactor can behave as Newtonian or non-Newtonian fluid (Sanchez Perez, Porcel, Lopez, Sevilla, & Chisti, 2006). Theoretically in a bioreactor shear stress and shear rate are affected from; gas-liquid interface area, diameter of impeller, diameter of tank, height of fluid, torque, flow index, agitation speed, power input, impellers Reynolds's number, width of impeller blade, volume swept by the impeller, volume of fluid, viscosity, and density of fluid (Sanchez Perez, Porcel, Lopez, Sevilla, & Chisti, 2006). Mixing in a fluid can be

performed in two ways: laminar flow and turbulent flow (Holland & Bragg, 1995) (Sanchez Perez, Porcel, Lopez, Sevilla, & Chisti, 2006). Both flow types can be observed in bioreactors. Thus, agitation analysis for a given bioreactor is affected from many factors and not an easy task to perform.

In a given bioreactor, with assumption of turbulent flow dominates the laminar flow, the effect of shear stress can be estimated by Kolmogorov micro scale of turbulence where viscosity of a solution dominates (Thomas, 1990). In a media which has a viscosity near water, a particle size less than 30 micrometres will not be affected from turbulent flow (Justen, Pauk, Nienow, & Thomas, 1996). However it is also shown that yeast cells which have approximately 5 micrometre size are affected from the turbulent flow (Vrana & Seicher, 1988).

In the field of biotechnology, it can be said that four types of mixing systems dominates; motor based impeller systems (multi or single) (Gogate, Beenackers, & Pandit, 2000), magnetic impeller systems (multi or single) (Lehky), wave-induced agitation systems (Singh V. , 1999), and orbital-shaking systems (Bates, Phillips, & O'Bryan). There are two main design types of impeller systems namely turbine and paddle impeller designs ***(Figure1.6)*** and impeller systems are the most used ones in the field (either motorized or magnetically coupled) (Gogate, Beenackers, & Pandit, 2000). Wave induced agitation systems ***(Figure1.7)*** are specifically designed for single-use bioreactors (Singh V. , 1999). Orbital-shaking systems are also generally used for laboratory scale (small scale) systems (Bates, Phillips, & O'Bryan).

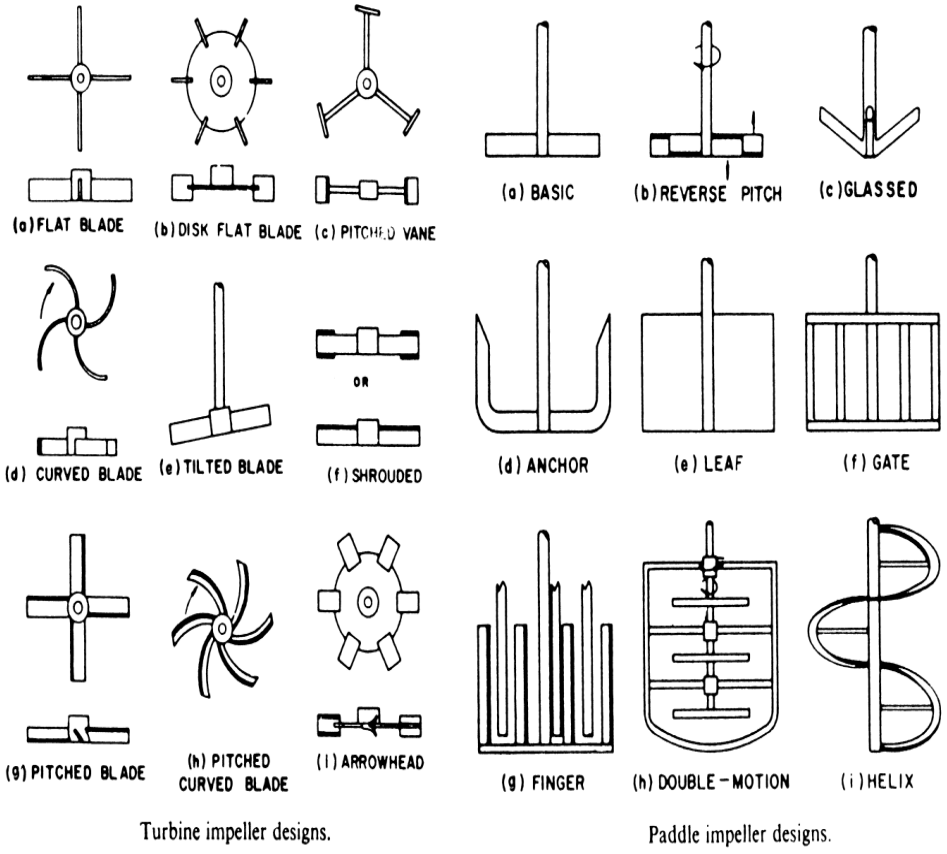


Figure1.6. Design Types of Impeller Systems (Gogate, Beenackers, & Pandit, 2000)

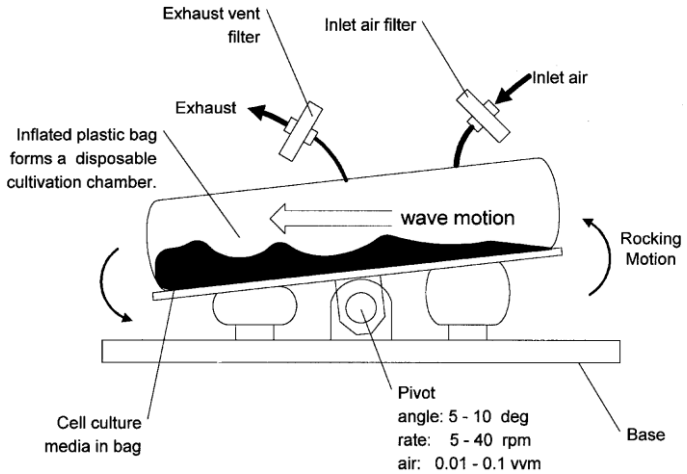


Figure1.7. Wave Induced Agitation Systems (Singh V. , 1999)

As wave-induced agitation and orbital-shaking systems do not have any contact with reaction media they have no impact on sterility. However, impeller systems have contact with reaction media, so they have an impact on sterility. Motorized impeller systems are connected to the tank with at least two O-ring seals. On the other hand magnetic impeller systems are designed to power the impeller magnetically from outside (hermetical seal, no contact with air, air-tight), so they have no impact on sterility (ex: zeta magnetic agitators).

An agitation system must be analysed according to the factors affecting cell integrity, cell damage, and poor mixing that can cause a concentration gradient in the system (ineffective mass transfer), and also according to problems affecting gas-liquid phase (Marks, 2003).

The impeller systems can also be classified as radial flow impellers in which flow occurs in radial side of the rotating shaft and axial flow impellers in which flow occurs along the axis of the rotating shaft. More information on axial shaft design can be obtained at McFarlane *et al.*, 1995, and more information about radial shaft design can be obtained at Nienow and Warmoeskerken (1985). Impellers have mainly two roles: dispersing the air bubbles and increasing the surface area of gas-liquid interface; hence increasing gas transfer rate and providing homogeneity in the system. In two design types a special case of radial impeller system called Rushton-type impellers has the highest gas dispersion ability and dominates the field (Nienow, Warmoeskerken, Smith, & Konno, 1985) (Nienow, A. W.; Wisdom, D. J.; Middleton, J. C., 1977). In any type of impeller, impeller diameter to tank diameter ratio is typically between 1/4 and 2/3 (Harny, Edwards, & Nienow, 1992). An impeller's power consumption linearly correlates with impeller speed to third power and impeller diameter to fifth power. So generally 1/3 impeller diameter to tank diameter is used (Tatterson, G. B., 1991).

And the distance between bottom of tank to impeller is generally between 1/6 tank distance to 1/2 tank distance (Tatterson, G. B., 1994). In multi-impeller systems the distance between the impellers should be at least one impeller distance (Linek, Moucha, & Sinkule, 1996).

1.3.3 Air Delivery System

Air delivery system is where gas mixing, gas transfer to the bioreactor tank, and gas filtering procedures are performed and output gas is disengaged. In general it is composed of inlet air tanks, compressor, sterilization system (both in input and output part), air sparger (where air injection to media occurs) (Singh, Kaushik, & Biswas, 2014). The efficiency of air delivery system is directly affected from the mixing system and also impeller power consumption is affected from sparger (Ni, Gao, Cumming, & Pritchard, 1995). The most important part of the air delivery system is the sparger. The diameter of the sparger is usually smaller than the impeller diameter (Birch & Ahmed, 1996). Sparger has orifices that inject the air to the liquid. The orifices can be plugged from a solid phase, fine particles and high viscosity solutions (Patwardhan & Joshi, 1998). In order to ensure all orifices work on sparger, the Reynolds number of the flow must exceed 2100 (Rewatkar & Joshi, 1993). The sparger must be chosen according to the impeller design. When axial flow impeller is used at high speed there will be nearly no contact between air bubbles and the impeller, which will result in no air bubble dispersion and decreased air bubble surface effective area. The problem can be solved by decreasing the rate of mixing (Murthy, Ghadge, & Joshi, 2007), increasing impeller diameter to create turbulence (Birch & Ahmed, 1996) or positioning the sparger under pumping impeller to increase probability of dispersion and retention time of air bubble (McFarlane & Nienow, 1996a) (Mcflarne & Nienow, 1996b).

In case of radial impeller, specifically the Rushton-type impeller, the choice of sparger is not important because they are very effective on dispersing the air bubbles (Garcia-Ochoa & Gomez, 1998). There are three general types of spargers which are: ring-type (pipe), single orifice, and porous/membrane spargers (***Figure1.8***). The rate of aeration is calculated empirically by volume of gas injected into unit volume of reactor in unit time (VVM, volume of gas per volume of bioreactor tank per minute).

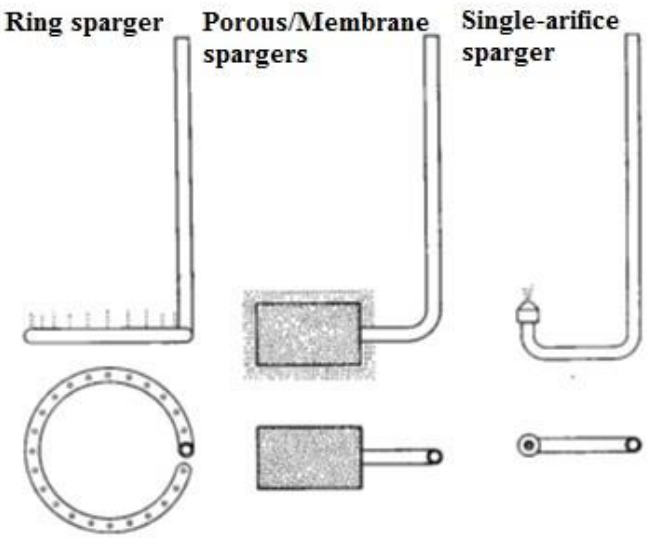


Figure1.8. Spargers (Chain, Paladino, Callow, Ugolini, & van der Sluis, 1952)

Air delivery system (or aeration system) and mixing system are the main parts that have role on mass transfer in bioreactors. Water as a main solvent in biological systems has very low solvation for gases, thus the liquid-gas mass transfer becomes the rate limiting step for reactions (Garcia-Ochoa & Gomez, 1998) (Bouaifi, Hebrard, Bastoul, & Roustan, 2001) (Foger, 2005).

Gas transfer is affected from; bubble velocity (Azbel, 1981) (Moo-Young & Blanch, 1981), size of bubbles (Linek, V.; Kordac, M.; Moucha, T., 2005) (Scargiali, D'Orazio, Grisafi, & Brucato, 2007), presence of surfactant (Linek, V.; Kordac, M.; Moucha, T., 2005), purity of the gas (Worden & Bredwell, 1998), residence time (Poorte & Biesheuvel, 2002).

As mentioned before increased agitation can cause cell damage and cell death. However, studies show that bursting bubbles can cause more stress on cells compared to turbulence (Boulton Stone & Blake, 1993) and can be reversed by using a chemical called Pluronic F-68 (a copolymer of ethylene oxide, propylene oxide and an amphiphilic detergent) which prevents the cell to attach bubbles (Chalmer & Bavarian, 1991). It is also shown that the cell damage is mostly observed on bubble bursting liquid surface (Kunas & Papoutsakis, 1990).

1.3.4 Control System

Control system in a bioreactors is generally composed of 6 elements which are pH control, foam/level control, temperature control, injection and sampling control, motor control, and air delivery system control (generally oxygen sensors are used) (Singh, Kaushik, & Biswas, 2014) (Nisipeanu, Bunciu, & Stanica, 2013). The control is generally made with proportional-integral-derivative (PID) control which basically calculates how far the system is beyond set point, error that is done and error that might be done (Chau, 2001). Model based control for a specific bio-reaction can be implemented, but may not have flexibility and accuracy in terms of both actual mass transfer (Azbel, 1981) (Worden & Bredwell, 1998) and sensors (they are generally not online (de Assis & Filho, 2000) and indirect measurements are performed (Bellgardt, 2000)).

1.3.4.1 pH Control

Adjusting pH according to the biological agents is very important and false regulation can cause irreversible denaturation and instability of proteins (Talley & Alexov, 2010) (Di Russo, Estrin, Marti, & Roitberg, 2012) (Kumar, Tiwari, & Bhat, 2004). False regulation also affects enzyme kinetics (Trivedi & Danforth, 1966) (Voet & Voet, 2004); therefore, making some rules to ensure process performance is important. pH control systems in bioreactors in general contain a pH probe, acid pump and base pump for regulation purpose. Generally pH control is accomplished by on-off mechanism (Chau, 2001). In the control of pH, hold time that is the time needed for fully observation of response is an important parameter (Jagadeesh & Sudhaker, 2010).

1.3.4.2 Foam/Level Control

Foam in bioreactors can block air exit and can add pressure inside the vessel (Singh, Kaushik, & Biswas, 2014). Foam/level control is accomplished by foam/level sensors and antifoamer pumps.

1.3.4.3 Temperature Control

Temperature control system is composed of a temperature probe and heat transfer system (Singh, Kaushik, & Biswas, 2014). Temperature control is also made by an on-off regulation (Jagadeesh & Sudhaker, 2010).

1.3.4.4 Injection and Sampling Control

For regulation of pH, foam level, injection and sampling must be controlled (Singh, Kaushik, & Biswas, 2014) (Nisipeanu, Bunciu, & Stanica, 2013). Injection and sampling system is not a separated system, rather is an integrated constituent. For sterility purposes injection systems use peristaltic pumps (Lyons & Pandit, 2005).

1.3.4.5 Motor Control

In bioreactors stirring and agitation are controlled by the motor (Jagadeesh & Sudhaker, 2010). As agitation is a very important parameter in mass transfer (Sanchez Perez, Porcel, Lopez, Sevilla, & Chisti, 2006), fine control of motor is desired.

1.3.4.6 Air Delivery System Control

In most bioreactors the most important gas is oxygen (Garcia Ochoa & Gomez, 2009). To obtain desired oxygen level with another gas such as nitrogen, carbon dioxide etc., gas property control is needed (Garcia Ochoa & Gomez, 2009). Generally in industry gas mixers are used for this purpose (Garcia Ochoa & Gomez, 2009). Other important parameter is the aeration of bioreactor. The flow rate of the gas is directly related to the aeration speed of a bioreactor and can be controlled by pumps and flow meters (Nisipeanu, Bunciu, & Stanica, 2013) (Jagadeesh & Sudhaker, 2010) (Garcia Ochoa & Gomez, 2009).

1.4 Aim of the Study

The aim of the study was to prototype 5 liters of autoclavable bioreactor by taking into account the design considerations mentioned above in ‘Parts of Batch Culture Bioreactors and Design Considerations’ section. The bioreactor must have had the ability of high speed agitation without performance decrease in sealing efficiency, had fully controlled aeration system into and out of the system, and had the ability of making temperature regulation system based on peltier element which has very fast response time.

The second aim of the study was to produce sensitive and cheap foam/level sensor which has a minimum sensitivity of 2 mm.

The third aim of the study was to verify response time of heating/cooling system, efficiency of sealing system, and verification of the designed foam/level sensor.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

2.1.1. Reaction Vessel

Reaction vessel used in this study was a 5 liter-borosilicate vessel modified from a 5 liters beaker. Firstly, the beakers' upper side was removed and a flange was heat-bended on top of the beaker. The dimensions and properties of the beaker can be obtained from ***Table 2.1***. The initial shape and final shape can be seen in ***Figure 2.1***. After processing the glass it was polished using cerium oxide glass polishing method by polishing set-up refurbisher/grinder (Sabia & Stevens, 2000) (Lee & Lai, 2003).

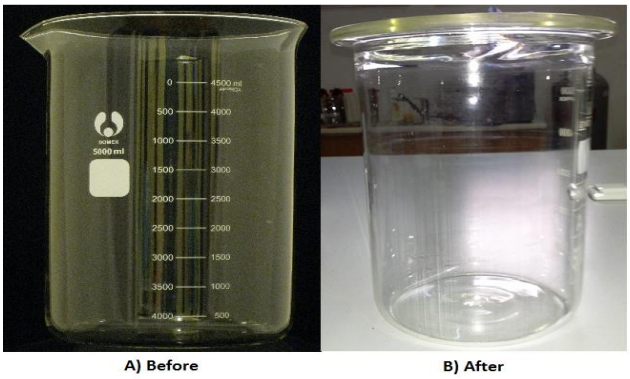


Figure2.1. Initial Shape (A) and Final Shape (B) of Reaction Vessel

Table2.1. The Dimensions and Properties of the Beaker

Specification	**Property**
Vessel total volume	5500 milliliters
Effective volume	4500 milliliters
Height	272 millimeters
Inside diameter	165 millimeters
Outside diameter	176 millimeters
Flange outside diameter	210 millimeters
Flange thickness	10 millimeters
Content	Borosilicate

The lid of the vessel was produced from polytetrafluoroethylene (TEFLON) (CHEBI:53251, CHEBI: the database and ontology of chemical entities of biological interest). According to IUPAC id of poly (1,1,2,3-tetrafluoroethylene), it melts at 326.8°C, has density of 2.20 g/cm^3. Because of its high melting point, high durability and resistance to nearly all chemical compounds rather than 316-stainless steel, Teflon was preferred. The drawings and the final shape of the lid can be seen in ***Figure 2.2.*** The lid's entering side to the vessel was supported with silicone O-ring for sealing.

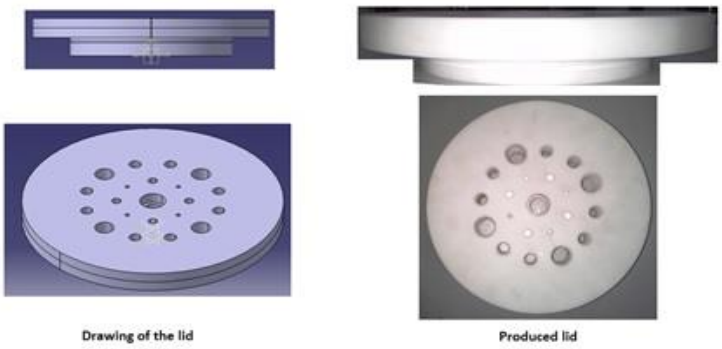


Figure2.2. Drawings and the Final Shape of the Lid

2.1.2. Heating/Cooling System

Heating/Cooling system set up was composed of 2 elements: peltier elements for high-speed heating/cooling and water cooling system as heat sink to take away the excessive heat generated from the other side of peltier element. Also the water cooling system was directed into a radiator heat exchanger for effective cooling and heating. The heating/cooling system support and heat sink parts were made of aluminium. Aluminium has low density, high conductivity, corrosion resistance, non-magnetic, non-sparking, easy to cast properties which make it ideal to be used in heating/cooling system where fast heat transfer and water cooling systems were integrated. The parts of the system are shown in ***Figure2.3.a*** (upper heat sink), ***Figure2.3.b*** (main support), ***Figure2.3.c*** (Peltier element support), ***Figure2.3.d*** (lower heat sink) *and in* ***Figure 2.3.e*** (assembled unit).

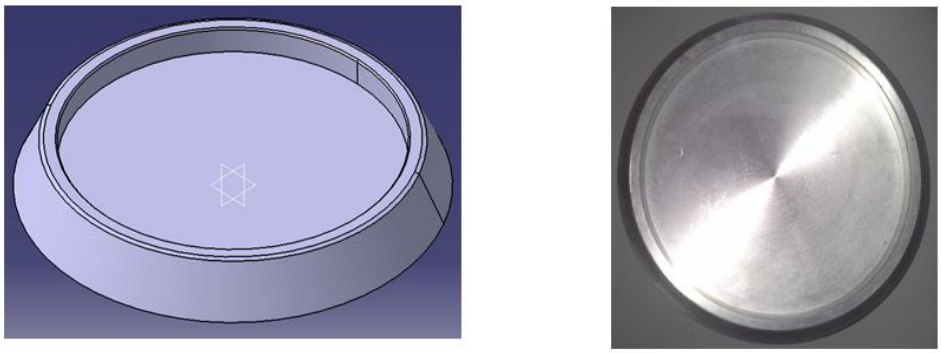
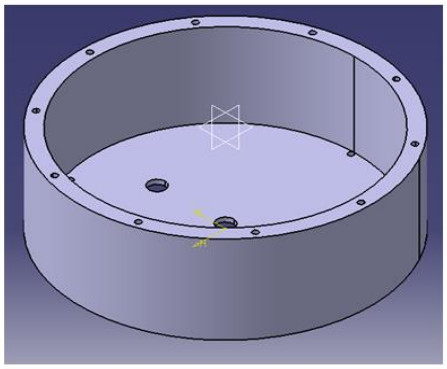


Figure2.3.a. Upper Heat Sink of Heating/Cooling System



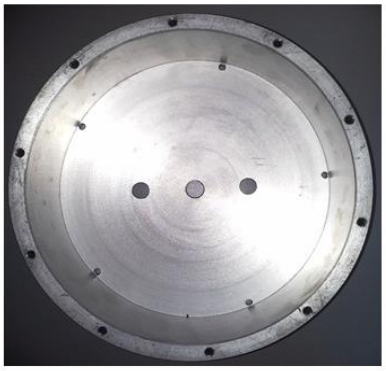


Figure2.3.b. Main Support of Heating/Cooling System

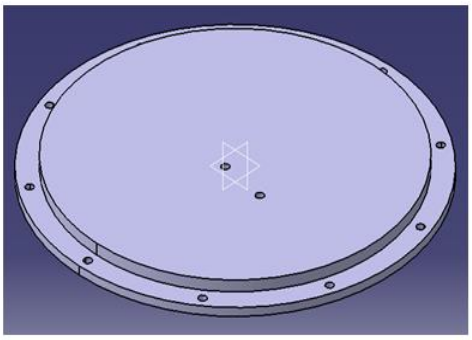


Figure2.3.c. Peltier Element Support of Heating/Cooling System

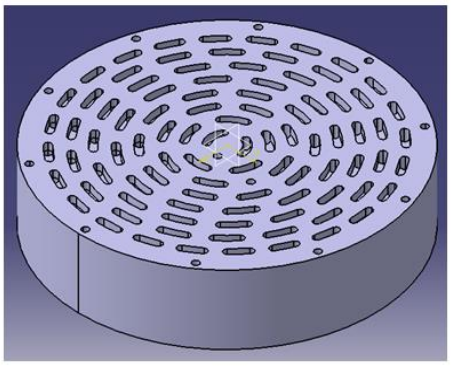
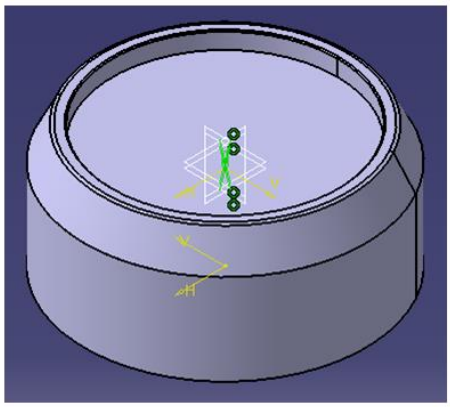


Figure2.3.d. Lower Heat Sink of Heating/Cooling System



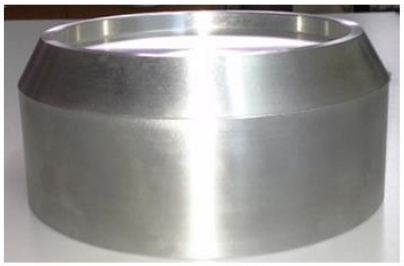
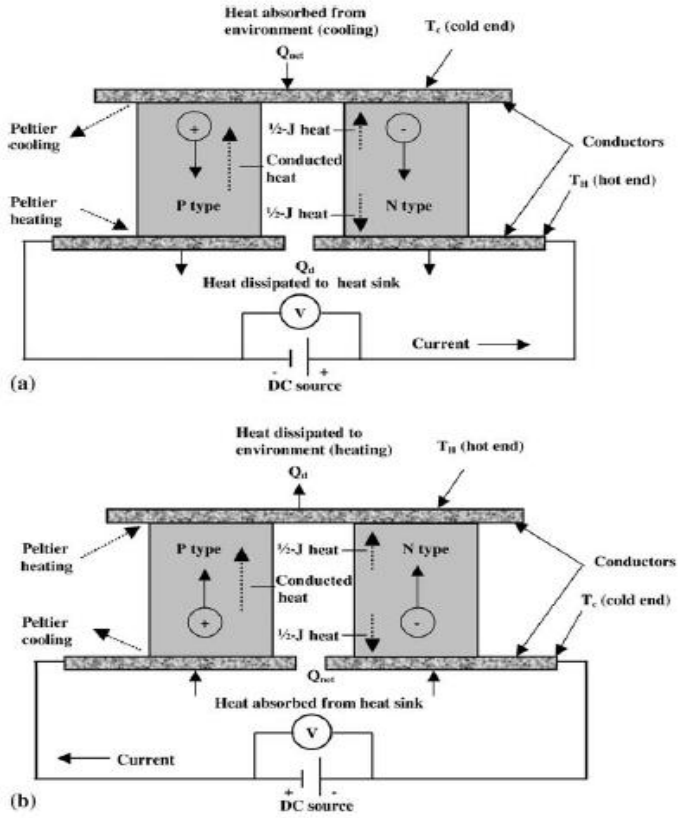


Figure2.3.e. Assembled Unit of Heating/Cooling System

Peltier elements are thermoelectric elements (Bell, 2008) that can work for 100.000 hours, need less maintenance, and also by changing the direction of the direct current, mode of operation can be reversed (from heating to cooling or vice versa ***(Figure2.4)***). A peltier element can function in small environments, is not position dependant, and works with 0.1 °C sensitivity (Riffat & Ma, 2003). The general design of peltier element cooling systems is composed of two heat sinks that have high thermal conductivity ***(Figure2.5)*** (Riffat & Ma, 2003). In general heat sinks are supported by fans or natural convention (Riffat & Ma, 2003). In our designed system, in order to have a very effective and fast cooling/heating, a radiator-based heat sink was used. Peltier elements work with maximum 12 volts direct current (Riffat & Ma, 2003) (Chilingarov, 2013). In order not to give stress on the system, electrical regulation must be done with pulse-width-modulation control and fast-mode-change must be done in case where heat difference between each sides is not exceeding 40 °C (Riffat & Ma, 2003) (Chilingarov, 2013). Total power of heating and cooling is 1600 watts. The peltier element used is shown in ***Figure 2.6.***



Schematic of thermoelectric module operation (a) cooling mode; (b) heating mode.

Figure2.4. Peltier Elements-Modes of Operation (Bell, 2008)

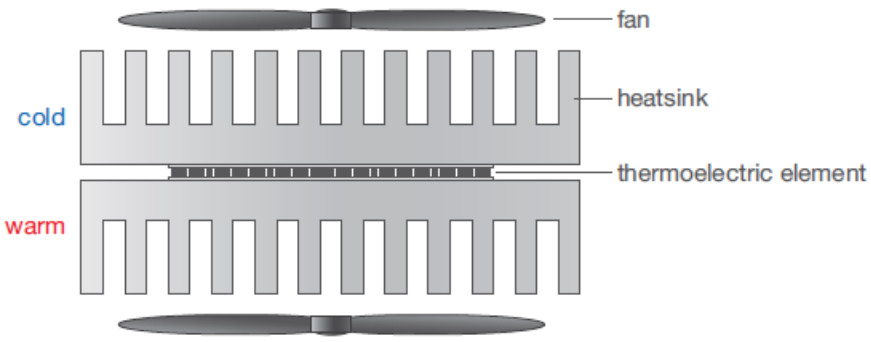


Figure2.5. The General Design of Peltier Element Cooling System (Riffat & Ma, 2003)

Figure2.6. The Peltier Element

The water cooling system was composed of a radiator, water pump, and radiator fan. Properties of the radiator system are shown in ***Table 2.2.*** The radiator is shown in ***Figure 2.7.***

Figure2.7. The Radiator

Table2.2. Properties of the Radiator System

Specification	Property
Supply voltage	220 VAC
Radiator transfer material	Aluminium
Pump flow rate	5 liters/minutes
Signal type	Dry contact (direct 220 VAC)

2.1.3. Agitation System

A motorized impeller system was designed for mixing purpose. The system was composed of motor cage, motor, shaft and impeller system.

2.1.3.1. Motor Cage

Motor cage was produced from 316-grade stainless steel. The lid of the cage was covered with silicone sealing, so motor had no contact with outside. Also the cage's linking part to the vessel lid was sealed with silicone seal. So the motor cage discriminated motor from both outside and inside of the vessel. The drawings and the final product can be seen in ***Figure 2.8***. After production of the cage, the cage was electro-polished (Lee E. S., 2000) in order to prevent biofilm formation (by removing scratches) (Hilbert, Bagge-Ravn, Kold, & Gram, 2003) and to give corrosion resistance (Lee & Lai, 2003).

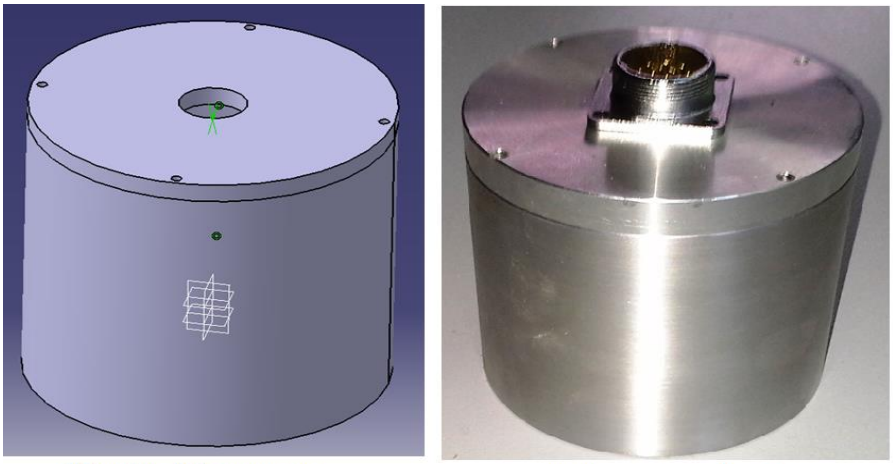


Figure2.8. The Drawings (A) and Final Product (B) of Motor Cage

2.1.3.2 Motor

The motor used was a stepper motor which was driven with a stepper motor driver and the specifications are shown in ***Table2.3***.

Table2.3. Specifications of Motor

Component	Specification	Property
Stepper motor	Size	Nema 34
	Step angle	1.8°
	Steps/revolution	200
	Step accuracy	5%
	Maximum working temperature	80°C
	Insulation class	130°C
	Holding torque	2.75 newton*meter
	Nominal power	18.5 watt
	Type	Hybrid, bipolar
Stepper motor driver	Minimum supply voltage	24 VDC
	Maximum supply voltage	72 VDC
	Maximum current	7.2 A
	Maximum pulse input frequency	300 KHz
	Types of inputs	Optically isolated
	Signals voltage	5 VDC
	Type	Microstepper drive

2.1.3.3. Shaft

The shaft was made of 316-grade stainless steel. After production of shaft, it was polished and electro-polished (Lee E. S., 2000) in order to prevent biofilm formation (by removing scratches) (Hilbert, Bagge-Ravn, Kold, & Gram, 2003) and to give corrosion resistance (Lee & Lai, 2003). The shaft was designed to have metric 10 standard thread in order to change impeller types and impeller sequences easily according to the cell type or experiment used. The drawings of shaft and final product can be seen in ***Figure 2.9***.

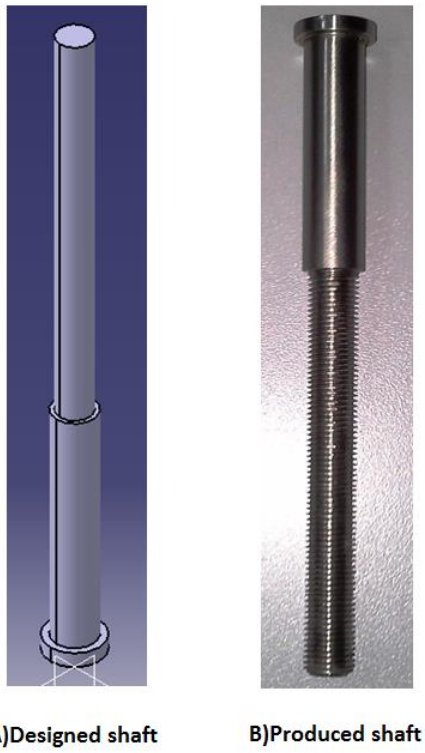


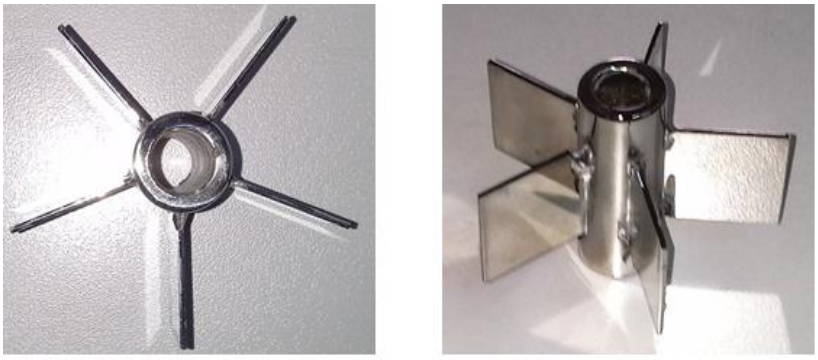
Figure2.9. The Designed (A) and Produced (B) Shaft

2.1.3.4. Impeller System

Three different impellers made of 316-grade stainless steel were designed and produced. The final products can be seen in ***Figure2.10***. All of the propellers were polished but not electro-polished. Electro-polishing was delayed because the systems would be optimized after each test. The system could be configured as multi-impeller system or single impeller system according to the users' choice. The specifications of the impellers are shown in ***Table2.4***.

Table2.4. Specifications of Impellers

Impellers	Specification	Property
Axial impeller-1	Outside diameter	80 mm
	Inside diameter	21 mm
	Effective diameter	49 mm
	Thickness	26.70 mm
	Aspect ratio	22,5:26,70
	Tilt angle	60°
	Number of blades	4
Axial impeller-2	Outside diameter	155 mm
	Inside diameter	21 mm
	Effective diameter	124 mm
	Thickness	16 mm
	Aspect ratio	62:16
	Tilt angle	30°
	Number of blades	6
Radial impeller	Outside diameter	81 mm
	Inside diameter	21 mm
	Effective diameter	60 mm
	Thickness	30 mm
	Aspect ratio	30:30
	Tilt angle	180°
	Number of blades	5



A) Radial Impeller

B) Axial Impeller-1

C) Axial Impeller-2

Figure2.10. Impeller Systems: Radial impeller (A), Axial impeller-1 (B), Axial impeller-2 (C)

2.1.4 Aeration System

The aeration system designed was composed of; gas-mixer, gas pressure/flow rate regulator, pre-gas filter, sparger, outlet gas processing unit and dissolved oxygen probe. The system was designed for mixing four different gases with pre-set ratios, pressure and flow rate.

2.1.4.1. Gas Mixer

Gas mixer system was composed of two helical flow channels connected co-axially. The gas flowed in one direction in the first helical flow channel and then was directed into reverse direction in the second helical channel. Helical channels mixed gases in turbulent flow mixing mode. Each helical flow channel discs were perforated to perform tubular mixing at the same time. The helical channels can be seen in ***Figure 2.11***. Technical specifications of the gas mixer are given in ***Table 2.5.a*** *and* ***Table 2.5.b***.

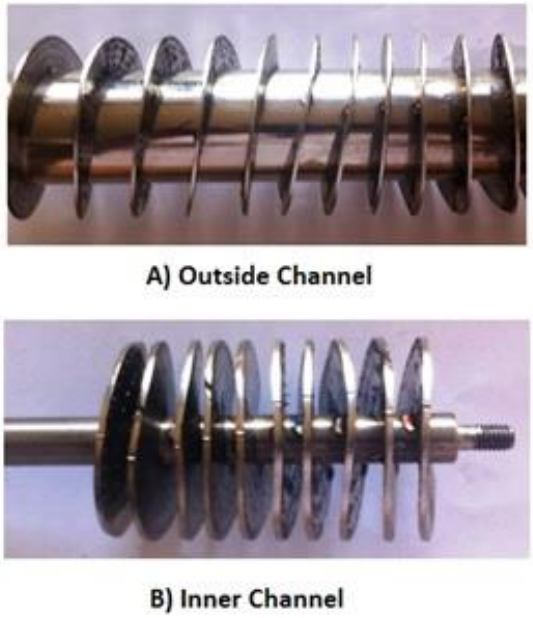


Figure2.11. Gas Mixer: The helical channels

Table 2.5.a. Specifications of Gas Mixer

Flow channels	**Specification**	**Property**
Channel 1	Inside diameter	47 mm
	Outside diameter	51 mm
	Perforation diameter	1 mm
	Number of perforations	80 per disc
	Number of discs	10
	Total height	280 mm
	Material type	316-grade stainless steel
Channel 2	Inside diameter	83.5
	Outside diameter	89
	Perforation diameter	1mm
	Number of perforations	212 per disc
	Number of discs	10
	Total height	300 mm
	Material type	316-grade stainless steel

Table 2.5.b. Specifications of Gas Mixer

Specification	**Property**
Mixing performance at 2 bars	%95
Initial pressure drop inside mixer at 2 bars	0.59 bar
Maximum working pressure	10 bars
Maximum working temperature	300 °C
Pressure drop on exit at 2 bars	0.30 bar

2.1.4.2. Gas Pressure/Flow Rate Regulator

Gas pressure/flow rate regulator was composed of proportional valve, pressure sensor and flow sensor. Each gas inlet to the gas mixer contained proportional valve and the pressure sensors. Gas mixer out had pressure sensor-flow sensor-pressure sensor configuration for regulation of flow and pressure according to the need. Technical specifications of the system constituents are given in ***Table 2.6***.

Table2.6. Specifications of Gas Pressure/Flow Rate Regulator Constituents

Component	**Specification**	**Property**
Proportional valve	Maximum input pressure	16 bar
	Output pressure range	0-12 bar
	Working temperature	-10 °C to +50 °C
	Supply voltage	24 VDC
	Control signal	0-10 VDC
	Linearity	% 0.3
	Resolution	13-bit
Pressure sensor	Working pressure	0-10 bar
	Maximum over pressure	20 bar
	Working temperature	-25 °C to 80 °C
	Maximum temperature	125 °C
	Supply voltage	9VDC to 30 VDC
	Output signal	4-20 mA
	Resolution	13-bit
Flow rate sensor	Flow rate range	0.001 L/min to 2 L/min
	Supply voltage	24 VDC
	Output signal	4-20 mA
	Maximum working pressure	10 bar
	Maximum working temperature	70 °C

2.1.4.3 Pre-gas Filter

Pre-gas filter was used to filter gas for prevention of microorganisms and particular dusts before injection to the reaction vessel. It was composed of two layer U17 Ulpa filter which has $\geq$ 99.999995 overall efficiency of filtration (European standard: **EN1822:2009).** The filter was cut by water-jet (waterjetch). The filters were located into filter cage shown in ***Figure 2.12***. Properties of the filter and filter cage are given in ***Table 2.7.***

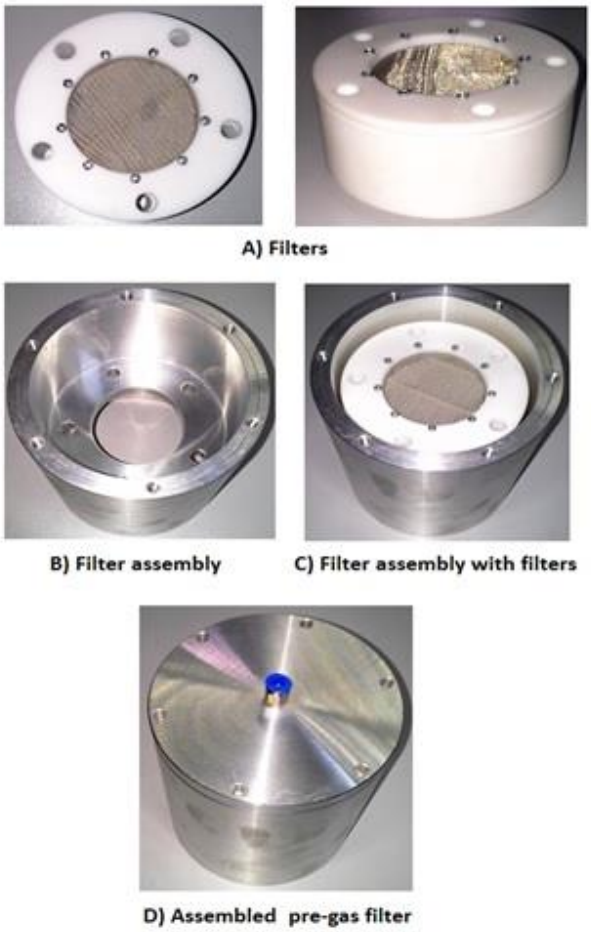


Figure2.12. The Pre-gas Filter System: (A) Filters, (B) Filter assembly, (C) Filter assembly with filters, (D) Assembled pre-gas filter

Table2.7. Properties of the Pre-gas Filter Constituents

Component	**Specification**	**Property**
Filter cage	Material property	Teflon and Aluminium
	Maximum working pressure	10 bar
	Maximum working temperature	200 °C
	Sealing-type	Viton-seal (Dupont)
Ulpa U17 filter	Local collection efficiency	99.9999% (**EN1822:2009)**
	Overall collection efficiency	99.999995% (**EN1822:2009)**

2.1.4.4 Sparger

Sparger is the point where the gas is injected to the reaction vessel. Sparger was produced from Teflon pipe for low cost and easy connections. The structure of the sparger is shown in ***Figure 2.13*** and technical properties are given in ***Table 2.8.***

Figure2.13. The Structure of Sparger

Table2.8. Technical Properties of Sparger

Specification	Property
Material property	Teflon
Pipe inside diameter	6 mm
Pipe outside diameter	10 mm
Sparger type	Ring sparger
Two dimensional radius of pores	1.5 mm
Number of pores	50
Maximum working pressure	10 bar
Maximum working temperature	200 °C

2.1.4.5 Outlet Gas Processing Unit

The system processed the output gas before it was given away to atmosphere. The system was composed of a filter system for sterility and pressure regulator unit that regulates reaction vessel inside pressure. The filter system was same as the pre-filter system. Inside pressure regulator was composed of pressure sensor (same as gas pressure/flow rate regulator), proportional valve and a vacuum pump. Technical specification of vacuum pump is given in ***Table 2.9***.

Table2.9. Technical Specification of Vacuum Pump

Specification	Property
Capacity	1.8 m^3 /minutes
Supply voltage	220 VAC
Control voltage	220 VAC, Dry contact
Minimum pressure range	0.5-2 milibar

2.1.4.6 Dissolved Oxygen Sensor

Dissolved oxygen probe was used to obtain the concentration of dissolved oxygen in vessel. It was composed of dissolved oxygen probe and transmitter. The specifications are given in ***Table 2.10.***

Table2.10. Specifications of Dissolved Oxygen Sensor

Component	Specification	Property
Dissolved oxygen probe	Operation range	6 parts per billion to saturation
	Response time	<90 seconds (to 98% per cent of final measurement)
	Working temperature range	0 °C to 80 °C
	Environmental temperature range	-5°C to 140°C
	Mechanical pressure resistance maximum	12 bar
	Model/Brand	Mettler Toledo/InPro 6800
Transmitter	Model/Brand	Mettler Toledo/M300, Multichannel
	Output signal	0/4-20mA
	Supply voltage	100 VAC to 240 VAC 20 VDC to 30 VDC

2.1.5 Foam Control System

Foam control system was composed of foam sensor and antifoamer injection pump. The foam sensor was produced from one graded electrode composed of 316-grade stainless steel and Teflon, and one singular electrode produced from 316-grade stainless steel. After production the electrodes were electro-polished. Antifoamer pump was a peristaltic pump. The properties of the electrodes and peristaltic pump are given in ***Table 2.11***.

Table2.11. Properties of Foam Control System

Component	**Specification**	**Property**
Foam sensor	Description	Double electrode system
	Minimum sensed distance	2 millimeters
	Maximum working temperature	200°C
	Maximum working distance	100 mm
Peristaltic pump	Minimum flow rate	0.1 milliliters
	Maximum flow rate	50 milliliters
	Working revolution per minute (rpm)	5 to 180
	Supply voltage	24 VDC
	Control type	0-10 VDC Analog control

2.1.6 pH Control System

pH control system was composed of a pH sensor with transmitter, acid peristaltic pump and base peristaltic pump. The specifications of peristaltic pumps were same as antifoamer peristaltic pump. The specifications of pH sensor with transmitter are given in ***Table 2.12***.

Table2.12. The Specifications of pH Sensor with Transmitter

Specification	**Property**
Working pH range	0 to 14
Working temperature range	10 °C to 80 °C
Maximum temperature without work	140 °C
Maximum pressure	Up to 4 bar
Integral temperature probe type	Pt1000
Integral temperature probe range	0 °C to 140 °C
Supply voltage	100 VAC to 240 VAC 20 VDC to 30 VDC
Signal output for pH	4-20mA
Signal output for temperature	4-20mA
Brand/Model	Mettler Toledo InPro3250
Transmitter Brand/Model	Mettler Toledo M300, multi-channel

2.1.7 Main Control System

The main control system was composed of a PLC (Programmable Logic Controller) (Michel, 1990) (Wilhelm, 1985), a touch-screen operator panel/HMI (Human-Machine-Interface), power supplies, solid-state-relay, PLC-relay, optically isolated relay. The specification of each component is given in ***Table 2.13***. Control system can be seen in ***Figure 2.14***.

Table2.13. The Specifications of Main Control System Constituents

Component	**Specification**	**Property**
PLC	User memory	75 kilobytes work, 4 megabytes load, 10 kilobytes retentive memories
	Supply voltage	24 VDC
	High speed counters	Three 100 kilohertz, three 30 kilohertz
	Pulse outputs	Maximum 200 kilohertz four outputs
	Math execution speed	2.3 microseconds/instruction
	Boolean execution speed	0.08 microseconds/instructions
	Analog signal inputs	0-10 V, 4-20mA, 0-20 mA, 0-5 V options are available, 14-bit resolution
	Analog signal output	0-10 V, 4-20mA, 0-20 mA, 0-5 V options are available, 14-bit resolution
	Communication options	RJ45, RS232, Profibus, Modbus
	Web-site control	available

Table 2.13.cont.'d. The Specifications of Main Control System Constituents

Component	**Specification**	**Property**
HMI	General	4.3 inch TFT, 256 colours
	Mode of action	Touch screen
	Resolution	480x272 px
	Communication option	RJ 45
	Configuration software	WinCC
	Maximum number of variables	500
Power supplies	Voltages	12 VDC, 24 VDC, 24 VAC, 220 VAC line
	Amperes	12 Amperes (12 VDC), 4 A (24 VDC), 6 A (24 VAC)
	Main line	220 VAC
Solid state relay	Supply voltage	3-32 VDC
	Load voltage	5-60 VDC
	Response time	> 1 milliseconds
	Maximum load current	10 Amperes
PLC-Relay	Supply voltage	5-24 VDC
	Load voltage	250 V AC/DC, 6 A
	Response time	5 milliseconds
	Release time	8 milliseconds
Optocoupler	Supply voltage	19.2-28.8 VDC
	Load voltage	3-33 VDC
	Load current	3 Amperes
	Typical response time	20 microseconds
	Typical turn of time	300 microseconds
	Operation temperature	-20 °C to 60 °C

Figure2.14. Main Control System

2.1.8 Chemicals

Sodium hydroxide and sodium chloride were obtained from Sigma. Agar, tryptone and yeast extract were obtained from Fluka and used for LB Broth and LB Agar preparations. Antifoam-204 (a mixture of organic non-silicone polypropylene based polyether dispersions) was obtained from Sigma to use as antifoamer.

2.1.9 Encoder

For obtaining the agitation speed an encoder was used to give the exact rpm value of the system. The encoder gave feed-back pulse of 200 per revolution of the motor as the encoder was directly linked to the motor.

2.1.10 High Flow Rate Peristaltic Pump

For foam/level control system, tests were done with a high flow rate peristaltic pump. The specification of the peristaltic pump is given in ***Table 2.14.***

Table2.14. The Specifications of Peristaltic Pump

Specification	**Property**
Minimum flow rate	10 milliliters
Maximum flow rate	1500 milliliters
Working revolution per minute (rpm)	5 to 600
Supply voltage	24 VDC
Control type	0-10 VDC Analog control

2.2 Methods

2.2.1 Ladder Programming

Ladder programming which is also called as relay ladder logic is one of the most popular PLC programming systems (Bryan, 1988). It is a graphical programming which is similar to circuit diagram (Zoubek, Roussel, & Kwiatkowska, 2003). An example of the programme is given in ***Figure 2.15*** (Falcione & Krogh, 1993). The programme execution is started with the first ladder and continued with the second and so on. In the first ladder when 'start' or 'v1' is activated and 'Is2' and 'v3' are not activated, then 'v1' will be activated. 'v1' is linked to 'start' with an 'or' configuration to confer continuous run of 'v1' when the 'start' is inactivated after first activation (Once 'start' is activated 'v1' will run whether 'start' continued to be active or not). 'v1' will be inactivated in case where 'Is2' or 'v3' is activated. The system follows Boolean Logic which is sub-area algebra and the values of the variables are truth values. When the variable is true, then its value is 1(one), and when the variable is false, then its value is 0 (zero). So in case of the first ladder, when 'start' or 'v1' is true and 'Is2' and 'v3' are not true, then 'v1' is true.

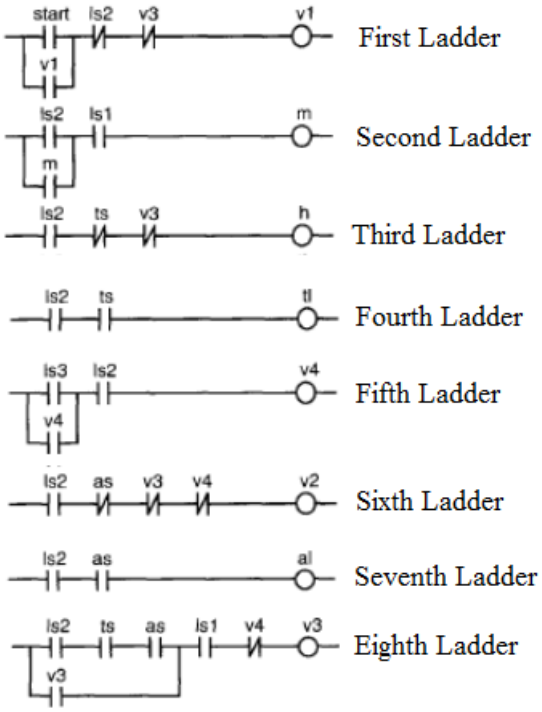


Figure2.15. An Example of Ladder Programme

2.2.1.1 Description of the Chosen Physical Addresses

For all tests the ladder programme addresses are given in the ***Table 2.15***:

Table2.15. The Ladder Programme Addresses

Description	Physical Address
Motor pulse signal-200 kHz pulse output	Q0.0
Motor direction signal	Q0.1
Activation of the stepper drive signal	Q0.2
Drive is activated signal	DI0.1
Encoder signal-100 kHz high speed counter	DI0.0
Radiator pump + Radiator fan activation signal	Q0.3
Peltier element cooling mode signal	Q0.4

Table 2.15.cont.'d. The Ladder Programme Addresses

Description	**Physical Address**
Peltier element heating mode signal	Q0.5
High flow rate peristaltic pump flow rate regulation signal	AQ112
Activation of high flow rate peristaltic pump	Q0.6
180 mm level signal	DI0.2
182 mm level signal	DI0.3
184 mm level signal	DI0.4
186 mm level signal	DI0.5
188 mm level signal	DI0.6
190 mm level signal	DI0.7
192 mm level signal	DI1.0
194 mm level signal	DI1.1
196 mm level signal	DI1.2
198 mm level signal	DI1.3
200 mm level signal	DI1.4
202 mm level signal	DI1.5
204 mm level signal	DI8.0
206 mm level signal	DI8.1
208 mm level signal	DI8.2
210 mm level signal	DI8.3
212 mm level signal	DI8.4
214 mm level signal	DI8.5
216 mm level signal	DI8.6
218 mm level signal	DI8.7
220 mm level signal	DI9.0
222 mm level signal	DI9.1
224 mm level signal	DI9.2

Table 2.15.cont.'d. The Ladder Programme Addresses

Description	Physical Address
226 mm level signal	DI9.3
228 mm level signal	DI9.4
230 mm level signal	DI9.5
Temperature signal	AI96

In this ***Table 2.15,*** DIx.x refers to digital input, AIxxx refers to analog input, AQxxx refers to analog output, DQx.x refers to digital output (where x, is a numerical number). For all tests digital data is obtained directly from the HMI and also from the transmitters used when it is possible.

2.2.1.2 Description of Motor Control

Stepper motor driver was set to 400 pulses/revolution. When set value of rpm was send to the PLC from HMI, the value was converted by the formula given below to give exact pulse rate.

$$Velocity\left(\frac{pulses}{second}\right) = set\ rpm\left(\frac{revolutions}{minute}\right) * \left(\frac{minutes}{60 * seconds}\right) * \left(\frac{400 pulses}{revolutions}\right)$$

So the input rpm value was multiplied by 40/6 to give the correct value to the PLC. After setting the rpm value (32-bit Real Value), if 'activate motor' button was clicked the stepper motor drive would be activated and axis would start to run. Motor's run time was also regulated by entering the run time (Time (32-bit integer value), Dint (32-bit integer value), Int (16-bit

integer value), UDint (32-bit integer value) data types could be used) in minutes to the HMI. After the value was sent to the PLC, the value was converted to milliseconds (the value PLC can understand) by multiplying the entered value with 60000. Motor halt command was written to be controlled by the time value by using timer operation to stop the motor after pre-set operation time was reached.

2.2.1.3 Description of Temperature Read on HMI

Temperature analog input value was firstly normalized (between 0 and 1, into a 64-bit Lreal value) and normalized value was scaled to the range of temperature probe (10 °C to 140 °C). For one point calibration the obtained value was processed by addition or subtraction of the temperature difference between M300 transmitter and HMI screen.

2.2.1.4 Description of Cooling/Heating Test

When heating was activated from HMI**,** Peltier element heating mode signal and Radiator pump + Radiator fan activation signal were activated. When inactivation signal for heating was given the heating was inactivated. When cooling was activated from HMI, Peltier element cooling mode signal and Radiator pump + Radiator fan activation signal were activated. When inactivation signal for cooling was given from HMI the cooling was inactivated. To give time-based operation cooling time and heating time values must have also been given to PLC from HMI. And inactivation was linked to a timer to stop the system in desired time point.

After activation of cooling or heating, elapsed time of the timer was linked to equality function that was activated in 10-500 seconds for each 10 seconds and was linked to move operation for taking temperature value in each 10 seconds.

For mixed cooling/heating test the motor was also activated at the same time for given time interval at the same time.

2.2.1.5 Agitation System Set RPM Stability Test

The motor set rpm and set time values were given for each test. And also the activation signal of the motor was linked to the high speed counter. High speed counter was run in frequency mode with 10 second time interval. Each frequency obtained was linked to a move operation according to the elapsed time linked to the 10-110 seconds for each 10 second comparison operator of equality, when equality was reached the data value of frequency for that given time was multiplied by 6/40 and then moved to the HMI data point value.

2.2.1.6 Level Sensor Test

For level sensor test, setting of the peristaltic pump rate was done by entering the value of desired rate of injection to the HMI. The value was then normalized between 0-1 (with 64-bit Lreal data type conversion). Obtained value was then scaled to 0-10 Volts to give desired signal value to the peristaltic pump. After entering the value of injection rate, 'start injection' button was pressed on HMI that sent activation of high flow rate peristaltic pump signal. The start button was also linked to a timer. When 180 mm signal was activated, elapsed time was moved to 180 mm time data point. For each level same move operations and data points were obtained. Each data points were represented on HMI and taken from HMI as results. The 230 mm level signal was also linked to inactivation of peristaltic pump and timer to stop the process.

2.2.1.7 Foam Control Response Time Test

For obtaining foam level each level signal input was related to the value of level. And move operations were linked to the higher number of digital inputs to send information to HMI. After manually injection 200 microliters of antifoamer, timer operation linked move operators for each elapsed 10 second was activated and the data was observed from HMI with given time tag.

2.2.2 Media Preparation

For test studies Luria Bertani Broth and Luria Bertani agar were used. For Lauri Bertani broth simply 10 g of tryptone, 5 g of yeast extract and 5 g of sodium chloride were dissolved in 1L of distilled water, pH was adjusted to 7.2 (at 37°C) with sodium hydroxide**.**

The solution was autoclaved in the reaction vessel, where all connections were done (except the electrical wiring), at 121°C for 15 minutes by using a 50 L autoclave machine (Lennox, 1955) (Miller, 1987) (Atlas, 1993). For Luria Bertani agar, 10 g of agar was additionally added to 1 L distilled water, and pH was set to 7.2 and then the solution was autoclaved at 121 °C for 15 minutes by using 50 L autoclave machine. Obtained solution was poured into petri-dishes in a Class-2 biosafety cabinet.

2.2.3 Heating/Cooling System Tests

Totally four heating system tests were performed. In each case the temperature was raised approximately 20 °C, and decreased 20 °C under 75% power use. The temperature readings for each 10 seconds were taken from the M300 Mettler Toledo transmitter using the temperature probe

integrated in pH probe. In first test no mixing was performed. In second test the system temperature was tested under 500 rpm agitation. Obtained results logarithmic values were plotted against time and linear curve fitting was performed by using Microsoft Excel (Microsoft, 2010). Logarithmic temperature change was obtained by first order derivative of obtained linear equation.

2.2.3.1 First Order Derivative and Slope of a Curve

The first order derivative of a two-dimensional line is equal to its slope and it was calculated according to the formula given below (Strang, 1991):

$$First\ order\ derivative = Slope = \frac{change\ in\ y}{change\ in\ x} = \frac{\Delta y}{\Delta x}$$

2.2.4 Agitation System Tests

Agitation system was tested for two variable; sealing efficiency under very high speed of rotation and stability of the set velocity. In order to obtain sealing efficiency, autoclaved media containing vessel was agitated at 500 rpm, 1000 rpm, 1500 rpm for 12 hours, 24 hours and 48 hours of operation. In each time interval samples of media were taken from the vessel and plated on LB agar to test growth of organism. Each plate was incubated at 37 °C for 24 hours. After 24 hours each plate was photographed. The test was performed in triplicates.

In the second test the motor was linked to an encoder and velocity of the motor was changed from 50 rpm to 1500 rpm by increasing 50 rpm in each step and the velocity was observed from the encoders' signal linked to the control system for each 10 seconds. For each rpm sets 10 data points were taken. For each group's average value, standard deviation and the relative standard deviation were calculated.

2.2.4.1 Average Value Calculation

Average value, also called as mean value, was calculated according to the formula below by using Microsoft Excel (Microsoft, 2010):

$$Average(Mean)value = \frac{sum\ of\ all\ samples}{number\ of\ samples} = \frac{\sum Samples}{N_{samples}}$$

2.2.4.2 Standard Deviation Calculation

Standard deviation for finite number of the data was calculated by the formula given below by using Microsoft Excel (Microsoft, 2010):

$$\sigma = \sqrt{\frac{1}{N}\sum_{i}^{N}(x_i - \mu)^2}$$

Where;

$\sigma: standart\ deviation$

$N: sample\ size$

$x_i: ith\ sample\ value$

$\mu: average\ (mean)value$

2.2.4.3 Relative Standard Deviation Calculation

Relative standard deviation was calculated according to the formula given below by using Microsoft Excel (Microsoft, 2010).

$$\%\ Relative\ Standart\ Deviation = \frac{\sigma}{\mu} * 100$$

2.2.5 Foam Control System Tests

Foam control system was tested for foam level sensor (level sensor) and response time of foam control system at 1500 rpm agitation. Level/foam sensor level control was tested by addition of media with a rate of 500 mL/min. The height for each time data point was calculated and then plotted against the designed height value.

The response time elapsed after addition of antifoamer for stabilizing the height and for obtaining minimum foam level was obtained with 10 seconds time intervals by plotting the foam level against time after injection of 200 microliter antifoamer manually (Antifoam 204, Sigma Aldrich).

2.2.5.1 Calculation of 'Calculated Height'

Calculated height was obtained by the formula below:

$$Calculated\ height(mm) = \frac{Time\ (miliseconds) * \frac{minutes}{60000(miliseconds)} * Injection\ rate\ of\ pump\ \left(\frac{mL}{minutes}\right) * \frac{1000mm^3}{mL}}{\pi * Radius\ of\ vessel(mm)^2}$$

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Heating/Cooling System Tests

3.1.1 Heating Test with No Mixing

Heating test result with no mixing is shown in ***Figure 3.1***. The data of test is given in **Appendix A.**

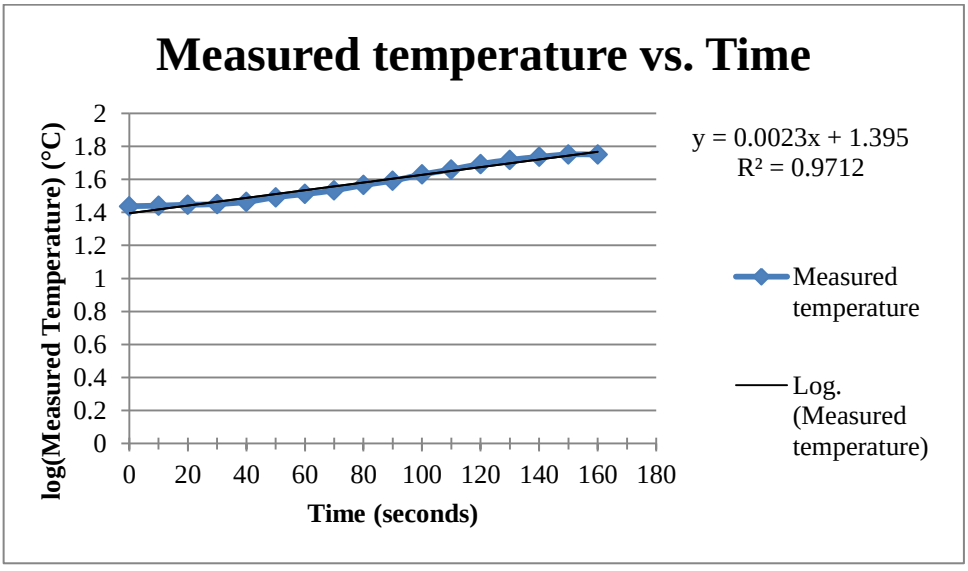


Figure3.1. Heating Test with No Mixing

From first order derivative of linear measured temperature, the average logarithmic temperature change per second was 0.0023. After heating was started, at 30-40 seconds interval higher temperature value change was started to be observed. After 50 °C was seen the heating system was stopped. However, temperature sampling was continued until the temperature was started to decrease. In order to give an idea also about the response time, the upper values and the lower values of temperature were included in analysis. Actually the experiment was set-up for 20 °C change (from 30 °C to 50 °C).

3.1.2 Heating Test Under 500 RPM Agitation

Heating test under 500 rpm agitation results is plotted in ***Figure 3.2.*** The data of test are given in **Appendix A.**

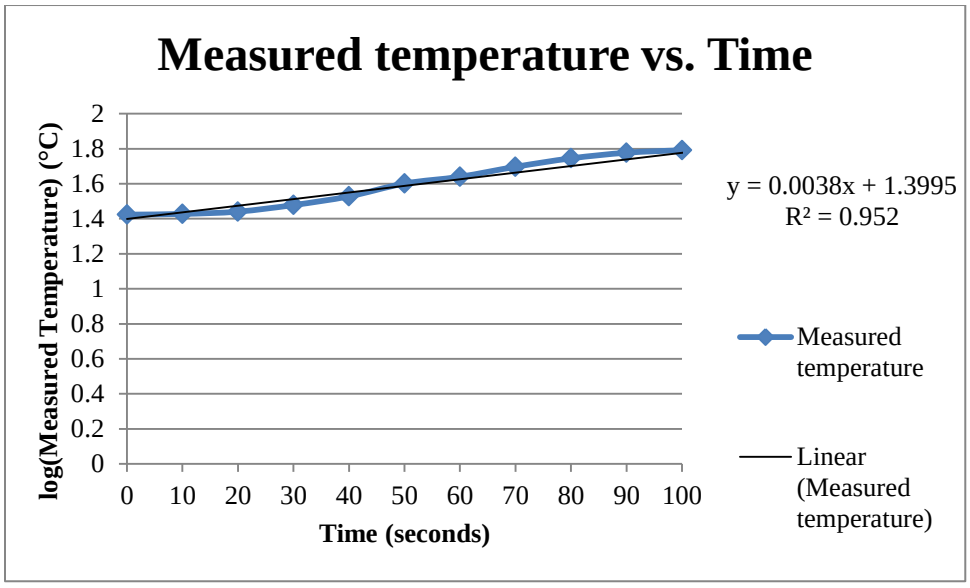


Figure3.2. Heating Test Under 500 rpm Agitation

From first order derivative of linear measured temperature, the average logarithmic temperature change per second was 0.0038. After heating was started at 20 seconds higher temperature value change was started to be observed. After 60 °C was seen, the heating system was stopped. However, the temperature change in each 10 seconds were continued to be taken. In order to observe effective heating, the lower part and the upper part of the heating points were included for analysis. The experiment was actually set-up for 20 °C change (from 30 °C to 50 °C).

3.1.3 Cooling Test with No Mixing

Cooling test under no mixing results is shown in ***Figure 3.3.*** The data of the test are given in **Appendix A.**

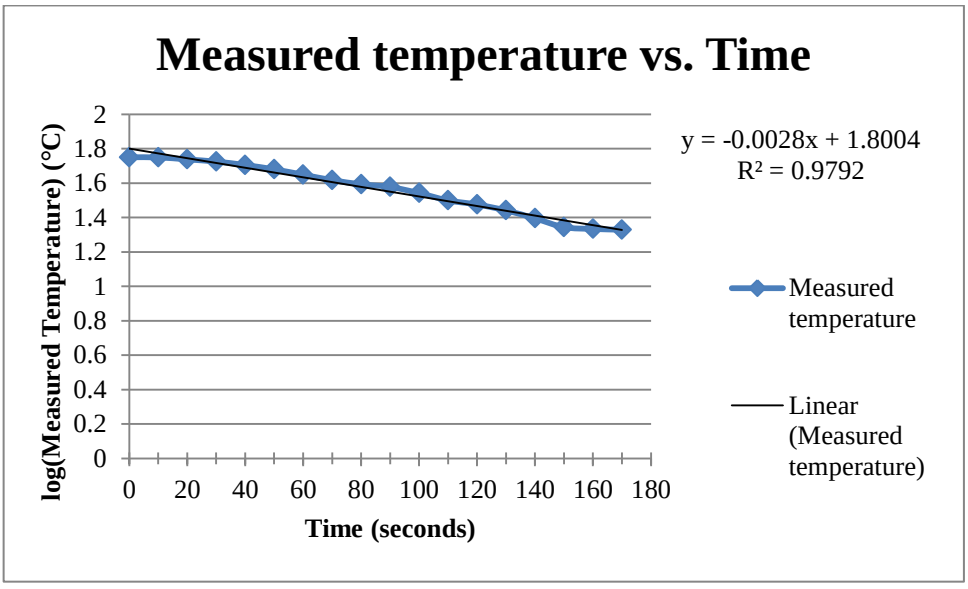


Figure3.3. Cooling Test with No Mixing

From first order derivative of linear measured temperature, the average logarithmic temperature change per second was -0.0028. After cooling was started, at 10 seconds higher absolute temperature value change was started to be observed. After 30 °C was seen the cooling system was stopped. However, the temperature change in each 10 seconds were continued to be taken. In order to observe effective cooling, the lower part and the upper part of the cooling points were included for analysis. The actual experimental procedure was set-up for 20 °C change (from 50 °C to 30 °C).

3.1.4 Cooling Test Under 500 RPM Agitation

Cooling test under 500 rpm mixing results are plotted in ***Figure 3.4.*** The data of the test are given in **Appendix A.**

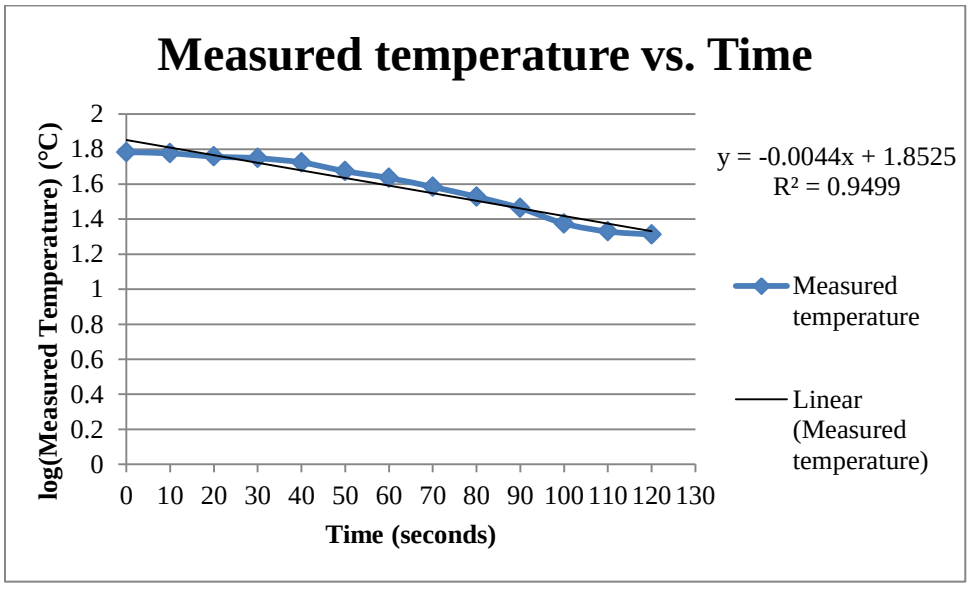


Figure3.4. Cooling Test Under 500 rpm Agitation

From first order derivative of linear measured temperature, the average logarithmic temperature change per second was -0.0044. After cooling was started, between 0-10 seconds higher absolute temperature value change was started to be observed. After 30 °C was seen, the cooling system was stopped. However, the temperature change in each 10 seconds were continued to be taken. In order to observe effective cooling, the lower part and the upper part of the cooling points were included for analysis. Actually the experiment was set-up for 20 °C change (from 50 °C to 30 °C).

In a mixed vessel, heat transfer rate was related to the agitator speed, agitator diameter, density of the fluid, viscosity of the fluid, thermal conductivity of vessel, thermal conductivity of fluid, inside diameter of vessel, temperature gradient, surface area of heat transfer, shear stress, and shear rate (Debab, Chergui, Bekrentchir, & Bertrand, 2010). During the tests all of the parameters were kept constant and heat changes were obtained. Un-mixed logarithmic temperature increase rate average was 0.0023 log(°C)/second, mixed logarithmic temperature increase rate average was 0.0038 log(°C)/second, unmixed logarithmic temperature decrease rate average was -0.0028 log(°C)/second, mixed logarithmic temperature decrease rate average was -0.0044 log(°C)/second.

The response times of the tests were observed to be; 10-20 seconds for unmixed heating, 10-20 seconds for mixed heating, 0-10 seconds for unmixed cooling and 0-10 seconds for mixed cooling.

In both heating and cooling tests, mixed versions have higher absolute heat transfer rates than the unmixed version of the experiment. Heat transfer rate in a mixed vessel is proportional to agitation speed at 2/3 power (Debab, Chergui, Bekrentchir, & Bertrand, 2010). So, obtaining higher heat transfer in mixed vessel was expected.

In peltier elements one side of the element becomes hot and the other side of the element becomes cold. According to the first law of thermodynamics, hot side will transfer heat to the cold side, so in practice cold side is less efficient than the hot side of a peltier element (Riffat & Ma, 2003). Therefore, in each case (mixed/unmixed) cooling must have less average absolute value change in temperature than heating tests. However, according to the results of the cooling experiments, the reverse of the situation was observed. It was most likely due to the radiator heat exchanger used in heating/cooling system. The radiators cooling system pumped cooling solvent at room temperature, the cooling became much more effective compared to the heating. And the response times of the cooling tests were less than those of heating tests. Because heat transfer rate is increased as the temperature gradient is increased. In cooling tests the cooling solvent had room temperature and the vessel had temperature higher than room temperature. However, in heating test the system performed tasks for both to the cooling solvent and the vessels volume. So, cooling solvent and radiator system made pressure on heating and facilitated cooling. Therefore, bioreactor was programmed to low cooling solvent injection rate in heating to decrease the pressure on heating. Also in cooling and heating tests the outside temperature was room temperature as the system will be run in room temperature. Since the room temperature is less than 30 °C, in both heating test and cooling test (50 °C – 30 °C) temperature losses occurred to the environment. Heat lost was helper for cooling but was another pressure for heating.

3.2 Agitation System Tests

3.2.1 Sealing Efficiency Tests

The photographs obtained from the sealing test are given in ***Figure3.5***. Description of each photograph set is given in ***Table3.1***.

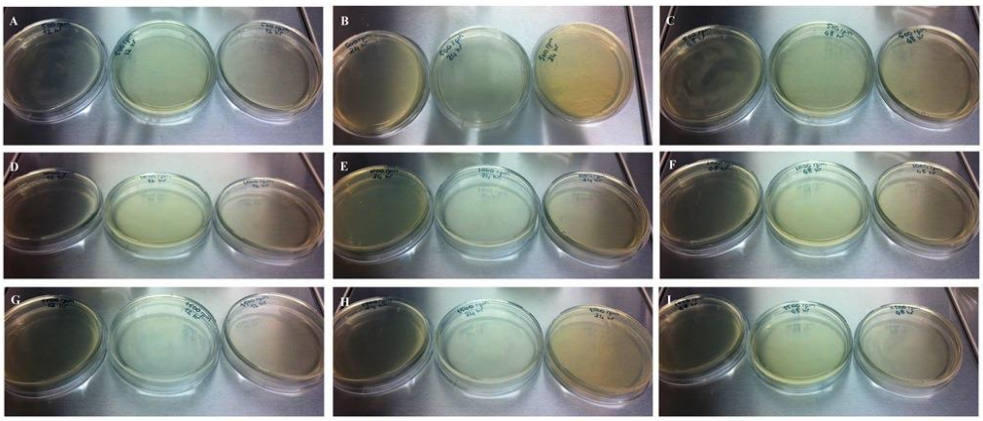


Figure3.5. Photographs of Sealing Efficiency Tests

Table3.1. Description of Photographs of Sealing Efficiency Tests

Photography Set	Description
A	500 rpm, 12 hours
B	500 rpm, 24 hours
C	500 rpm, 48 hours
D	1000 rpm, 12 hours
E	1000 rpm, 24 hours
F	1000 rpm, 48 hours
G	1500 rpm, 12 hours
H	1500 rpm, 24 hours
I	1500 rpm, 48 hours

According to the test results of all rpm and time values there were no microorganism growth on the samples taken from the reaction vessel. Even in very high rpm values (1500 rpm) the system's sealing was not affected. The system had four sealing point; two of them were O-rings and one between the motor cage and the vessel lid and the other between the cage main frame and cage lid. The cage lid, cage main frame and the vessel lid prevent parts to move, because the two seals, between vessel lid and motor cage and between motor cage frame and motor cage lid, were hermetical seals. Even the moving parts' seals were broken these two seals would prevent contact between the vessel system and surroundings, hence would prevent contamination. As a result, if the system was set to the maximum speed and the O-rings were broken the system will still prevent contamination from surroundings. However, for obtaining same performance every time it is used, the half-life of the O-ring seals must be obtained. The half-life tests were not performed in this study.

3.2.2 Velocity Stability Test

Observed average rpm value was plotted against set rpm value in ***Figure 3.6.*** All datasets are given at **Appendix B.**

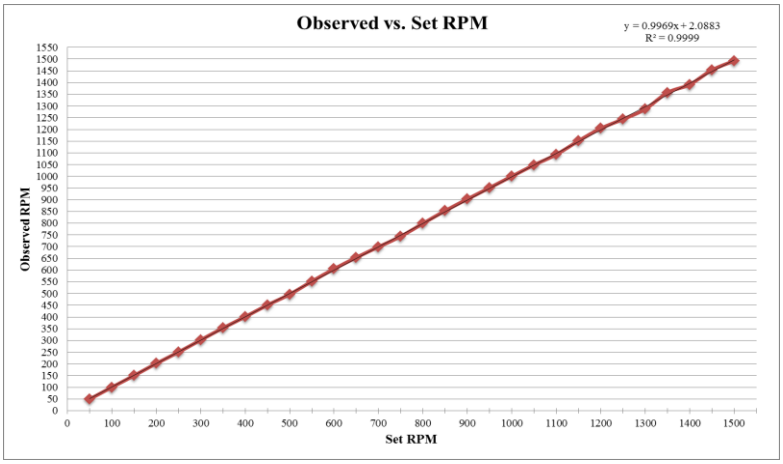


Figure3.6. Velocity Stability Test

The slope of the graph is 0.9969 which is very close to 1. These results showed good-fit with set value and observed value.

Relative standard deviation for each set rpm value is plotted in ***Figure 3.7***.

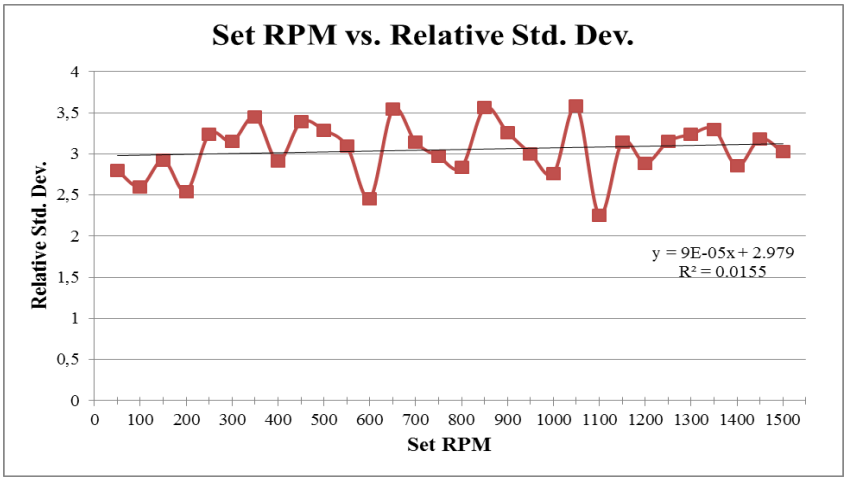


Figure3.7. Relative Standard Deviation of Results of Velocity Stability Test

Relative standard deviation results were between 0% and 5% for each rpm value, which showed at least 95% confidence between observed and set rpm values, and also the results were in acceptable range of hysteria (Åkesson & Hagander, 1999). Observed relative deviations were not following any pattern as the R^2 value observed was near zero. So, there was no factor other than motor's internal dynamics were effective on observed rpm value.

The system was tested as an open loop control although the sensor was incorporated to the system; if the system was used to obtain a closed-loop control better results would be obtained. By using a servo-motor the sensitivity of the system can be increased, since servo motors are closed systems. However, due to the high cost of servo motors, stepper motor was chosen.

3.3 Foam Control System Tests

3.3.1 Foam/Level Sensor Characterization Test

The designed height against calculated height is plotted in ***Figure 3.8.***

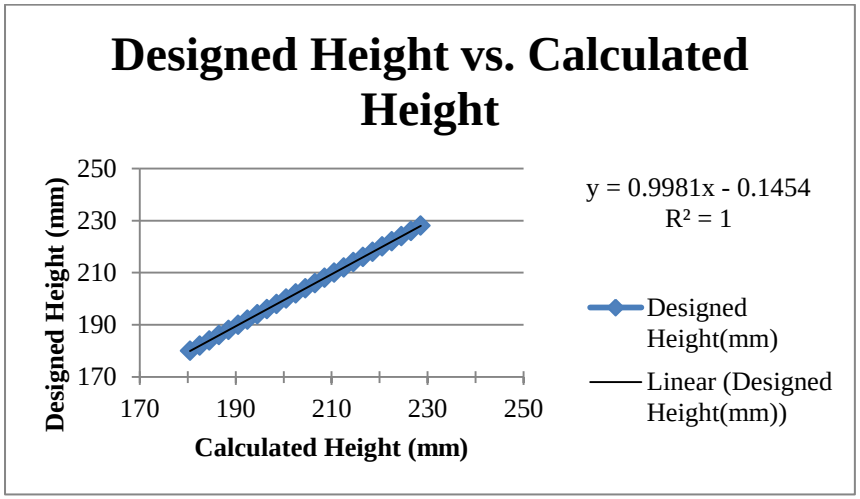


Figure3.8. Foam/Level Sensor Characterization Test

The results were in very good agreement between observed and designed value as plotting performed between calculated value (observed) and designed value, and an R^2 value near 1 was observed. Observed value was calculated with the pump's injection rate and pumps injection rate was set to 500 ml/min. The pump's injection rate was assumed to be same through the test and fluctuations were ignored. The designed probe was a probe which works in discrete pattern. It could be described as multi probe system or apartment probe system. The system designed to have 2 mm sensitivity interval. The system followed probe/isolator/probe configuration that was composed of miniature probes in each probe level. So, as mentioned above the sensor was a discrete (not continuous) sensor. According to the desired

sensitivity its miniature probe distance could be made as low as 0.4 mm which would result in 0.4 mm sensitivity. The size of the bubble in the vessel was related to the agitation rate, gas injection flow rate, viscosity, and pressure. In very low, 160-200 rpm, very little amount of air bubbles would have 1-3 mm diameter (Papoutsakis, 1991) which would be higher than designed sensitivity. In agitation rates higher than 300 rpm, the bubble size ranges between 50-300 micrometres diameter, but bubbles would have very high concentration near to 5000 bubbles/milliliters which would cause very high amount of increase in foam level (Papoutsakis, 1991). In low agitation speed by chance big bubbles could cause hysterias and wrong signals. And in high agitation rate, rate of increase in foam level would be very high and bubbles could stick to the sensing level of the sensor if sensitivity of probe was designed to be high (0.4 mm). Therefore, the sensitivity of the probe was big enough to decrease the hysterias in case of low agitation speed and big enough not to stick very small bubbles.

3.3.2 Foam Control Response Time Test

The foam level after injection of antifoamer is plotted in ***Figure 3.9.***

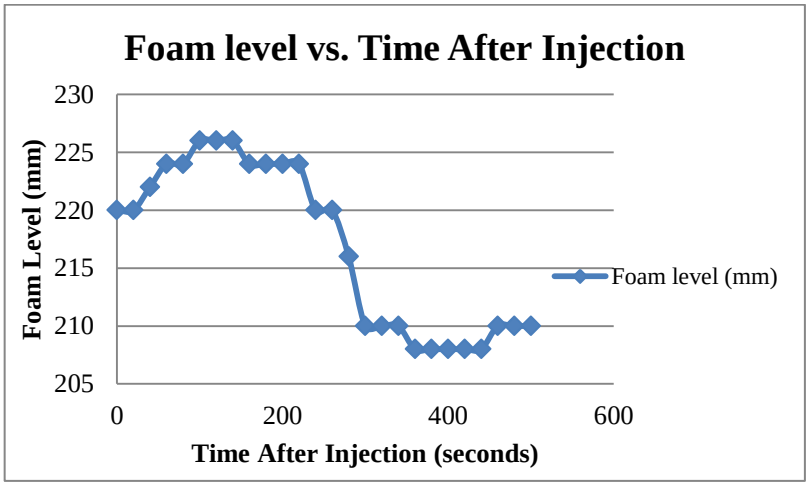


Figure3.9. Foam Control Response Time Test

After injection of antifoamer between 100-140 seconds the foam level was stabilized and started to decrease at 160 seconds. Antifoamer (a mixture of chemical compound which is used to prevent and diminish the foam developed in the reaction vessel) action was observed to be discrete because the sensor used was a discrete/non-continuous sensor. After 400 seconds an increase in the level was observed. That was because of non-homogeneous distribution of the foam at that time because of the action of antifoamer. Since the mixing was continuous, the level of foam stabilized and stayed at 210 mm level. Generally when foam is present in the system 1:20000 volume of antifoamer are added. The system response time was measured under no cell growth condition and 3 VVM flow rate of air was used. As obtained result was the control test, it should not be considered as a real case result. Because obtained duration of the reaction was a very short time for defoaming reaction in presence of bacteria.

CHAPTER 4

CONCLUSION

In this study, prototype of a 5 L autoclavable batch-mode bioreactor was produced. After each part of the bioreactor was produced, some of the most important parameters of the system were tested.

A sterile working condition is also very important parameter to avoid any contamination and unwanted results. The system was tested under 1500 rpm for 48 hours and no contamination was observed. In most small scale bioreactors the maximum working rpm is around 1200 rpm. As our designed system had four layer of sealing and two of them were hermetical, even if the motor was set to 3000 rpm which is the maximum point of the motor, there could not be any contamination. The system's outer two seals were hermetical seals which mean they were air-tight. The seals were not connected to the moving parts and they were independent of the moving shaft so they could not be affected by the velocity of mixing. As a result regardless of the velocity the system was always in air-tight sealing condition. As mentioned above whether the O-ring seals were broken or not, these two hermetical seals would ensure air-tight condition. However, O-rings should be checked and replaced from time to time not to cause any contact between the motor cage air and the vessel air which will cause a dead space in regulation zone (the vessels head volume).

The entire components of our vessel were chosen to withstand at least 130 °C to give the user the opportunity of not moving parts or consider sterility issues relating assembling of the system. The system designed could be fully assembled and autoclaved, and then the process could be started just by adding the electrical connections.

During this study a new miniature multi-probe foam/level sensor was developed and tested on the system. The sensor showed good-fit with designed parameters. Development of a transmitter system with digital output signal is on study. Besides, analog version of the sensor is also under production. As the sensor for foam control had very high sensitivity, and mixing system had very high mixing efficiency, foam control system could respond to foam changes very rapidly.

In small scale bioreactors generally there are no cooling systems and temperature regulation is not tight. In general peltier elements are used in mini-bioreactors (50-200 mL bioreactors) and micro scale bioreactors (microchip based bioreactors). In our heating/cooling systems a very fast temperature regulation could be obtained. In small scale bioreactors most dominated producers (i.e. Sartorius, Applikon, Eppendorf, GE) do not use any cooling systems or they use conventional coolers which is sold separately according to the users' need. Our system had integrated peltier-based cooling system which was the only mini-scale bioreactor that used peltier element for both heating and cooling compared to the products which dominated the bioreactor market (i.e. Sartorius, Applikon, Eppendorf, GE). In order to give the user flexibility on choosing right impeller system, the agitation system was designed to be flexible. However, only the rate control tests were performed for agitation system. An investigation for optimization of agitation system should be performed in future studies.

The aeration system was designed to give the user full control over pressure, flow rate and internal pressure of the vessel. There are many studies showing conflicting results in terms of effect of aeration on cells (Bouaifi, Hebrard, Bastoul, & Roustan, 2001) (Chalmer & Bavarian, 1991) (Kunas & Papoutsakis, 1990) (Thomas, 1990). However, in these studies only the flow rate of the gas is given and there is no viscosity measurements done for proper interpretation. The designed system gave the user flexibility on both pressure and flow rate of gas and the effect of aeration on microorganism will be easier to investigate.

The sparger system was designed from easily replaceable and very cheap materials to give the user the ability to test various design which would best fit the application.

As production of the bioreactor took a lot of time, tests related to aeration, aeration damage, design of aeration system, pH system, gas mixing system could not be investigated and further studies must be done to fully characterize the produced bioreactor. The study was the beginning phase of investigation and production of the bioreactor.

REFERENCES

Adkins, J., Pugh, S., & Nielsen, D. R. (2012). Engineering microbial chemical factories to produce renewable “biomonomers” . *Front. Microbiol.*, 313.

Åkesson, M., & Hagander, P. (1999). Control of Dissolved Oxygen in Stirred Bioreactors. *IFAC.* Lund: Preprints 14th World Congress of IFAC.

Alaghlavi. (2013). Design of fermenter and kinetics. *Bioprocess Engineering*.

Andrews, G. (1988). Fluidized-bed bioreactors. *Biotechnology and Genetic Engineering Reviews*, Vol.6, 151-178.

Astrom, K., & Hagglund, T. (1995). PID Controllers: Theory , Design and Tuning. *Instrument Society of America*.

Atlas, R. M. (1993). *Handbook of Microbiological Media,.* CRC Press.

Azbel, D. (1981). *Two-phase flows in chemical engineering.* New York: Cambridge University Press.

Bates, M. K., Phillips, D. S., & O'Bryan, J. (n.d.). *Shaker agitation rate and orbit affect growth of cultured bacteria.* Thermo Fisher Scientific.

Bell, L. E. (2008). Cooling, Heating, Generating Power, and Recovering Waste Heat with Thermoelectric Systems. *Science*, 1457-1461.

Bellgardt, K. H. (2000). *Bioreaction engineering: Modeling and control.* New York: Springer.

Birch, D., & Ahmed, N. (1996). Gas sparging in vessels agitated by mixed flow impellers. *Powder Technology*, 33-38.

Blenke, H. (1979). Loop Reactors. *Advances in Biochemical Engineering*, 121-214.

Bouaifi, M., Hebrard, G., Bastoul, D., & Roustan, M. (2001). A comparative study of gas hold-up, bubble size, interfacial area, and mass transfer coefficients in stirred gas-liquid reactors and bubble columns. *Chemical Engineering and Processing*, 97-111.

Boulton Stone, J. M., & Blake, J. R. (1993). Gas bubbles bursting at a free surface. *J. Fluid Mech.*, 437-466.

Branyik, T., Vicente, A. A., Dostalek, P., & Teixeira, J. A. (2005). Cpntinous beer fermentation using immobilized yeast cell bioreactor systems. *Biotechnology Progress*, 834-844.

Brich, D., & Ahmed, N. (1997). The influence of sparger design and location on gas dispersion in stirred vessels. *Chemical Engineering Research & Design*, 487-496.

Bryan, L. A. (1988). *Programmable Controller-Theory and implementation.* New York: Industrial text.

Caccavale, F., Samarino, M., Pierri, F., & Tufano, V. (2011). The Chemical Batch Reactor. In F. Caccavale, M. Samarino, F. Pierri, & V. Tufano, *Control and Monitoring of Chemical Batch Reactors* (pp. 10-11). Springer.

Chain, E. B., Paladino, S., Callow, D. S., Ugolini, F., & van der Sluis, J. (1952). Studies on aeration-I. *Bull. World Hlth Org.*, 73-97.

Chalmer, J. J., & Bavarian, F. (1991). Microscopic visualisation of insect cell-bubble interactions. *Biotechnol. Prog.*, 151-158.

Chau, P. C. (2001). *Chemical Process Control: A First Course with MATLAB.*

Chilingarov, A. (2013). Operation of two stacked Peltier elements. *IOP Publishing*.

Chisti, Y. (2001). Hydrodynamic damage to animal cells. *Crit. Rev. Biotechnol.*, 67-110.

Coulson, J. M., & Richardson, J. F. (1990). In J. M. Coulson, & J. F. Richardson, *Coulson & Richardson's chemical engineering* (pp. 227-263). Boston: Butterworth-Heinemann.

de Assis, A. J., & Filho, M. R. (2000). Soft sensors development for on-line bioreactor state estimation. *Computers & Chemical Engineering*, 1099-1103.

Debab, A., Chergui, N., Bekrentchir, K., & Bertrand, J. (2010). An Investigation of Heat Transfer in a Mechanically Agitated Vessel. *Journal of Applied Fluid Mechanics*, 43-50.

Di Russo, N. V., Estrin, D. A., Marti, M. A., & Roitberg, A. E. (2012). pH-Dependent Conformational Changes in Proteins and Their Effect on Experimental pKas: The Case of Nitrophorin 4. *PLOS Computational Biology*.

Durand, A., & Chereau, D. (1988). A new pilot reactor for solid-state fermentation: application to the protein enrichment of sugar beet pulp. *Biotechnology and Bioengineering*, 476-486.

Eginton, P. J., Gibson, H., Holah, J., Handley, P. S., & Gilbert, P. (1995). Quantification of the ease of removal of bacteria from surface. *J. Ind. Microbiol.*, 305-310.

Elmoraghy, M., & Farag, I. H. (2012). Bio-jet Fuel from Microalgae: Reducing Water and Energy Requirements for Algae Growth. *International Journal of Engineering and Science*, 22-30.

Falcione, A., & Krogh, B. H. (1993). Design Recovery for Relay Ladder Logic. *IEEE Control Systems*, 90-98.

Foger, H. S. (2005). *Elements of chemical reaction engineering.* New Jersey: Prentice Hall.

Froment, G. (1967). Fixed Bed Catalytic Reactors — Current Design Status. *Ind. Eng. Chem.*, 59(2), 18-27.

Garcia Ochoa, F., & Gomez, E. (2009). Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview. *Biotechnology Advances*, 153-176.

Garcia-Ochoa, F., & Gomez, E. (1998). Mass transfer coefficient in stirred tank reactors for. *Biochemical Engineering Journal*, 1-10.

Gogate, P. R., Beenackers, A. A., & Pandit, A. B. (2000). Multiple-impeller systems with a special emphesis on bioreactors: a critical review . *Biochem. Eng. J.*, 109-144.

Gonzales, J., Aguilar, R., Alvarez-Ramirez, J., & Barren, M. A. (1998). Nonlineer regulation for a continous bioreactor via a numerical uncertinity observer. *Chemical Engineering Journal*, 105-110.

Harny, N., Edwards, M. F., & Nienow, A. W. (1992). *Mixing in the process industry*. Boston: Butterworth-Heinemann Ltd.

Hilbert, L. R., Bagge-Ravn, D., Kold, J., & Gram, L. (2003). In uence of surface roughness of stainless steel on microbial adhesion and corrosion resistance. *International Biodeterioration & Biodegradation*, 175-185.

Holladay, D., Hancher, C., & Scott, C. (1978). Biodegradation of phenolic waste liquors in stirred-tank, packed-bed, and fluidized-bed bioreactors. *Water Pollution Control Federation*, 2573.

Holland, F. A., & Bragg, R. (1995). Fluids in motion. In F. A. Holland, & R. Bragg, *Fluid Flow for Chemical Engineers* (pp. 4-6). Butterworth Heinemann: Elsevier.

Hyde, F. W., Alberg, M., & Smith, K. (1997). Comparison of fluorinated polymers against stainless steel, glass, and polypropylene in microbial biofilm adherence and removal. *J. Ind. Microbiol.*, 142-149.

Jagadeesh, C. A., & Sudhaker, S. R. (2010). Modeling, Simulation and Control of Bioreactors Process Parameters – Remote Experimentation Approach. *International Journal of Computer Applications*, 81-88.

James, G. A., Beaudette, L., & Costerton, J. W. (1995). Interspecies bacterial interactions in biofilms. *J. Ind. Microbiol.*, 257-262.

Jansen, B., & Kohnen, W. (1995). Prevention of biofilm formation by polymer modification. *J. Ind. Microbiol.*, 391-396.

Justen, P., Pauk, G., Nienow, A. W., & Thomas, C. R. (1996). Dependence of mycelial morphology on impeller type and agitation intensity. *Biotechnol. Bioeng.*, 672-684.

Kantarci, N., Borak, F., & Ulgen, K. O. (2005). Bubble column reactors. *Process Biochemistry*, 2263-2283.

Kumar, P. D., Tiwari, A., & Bhat, R. (2004). Protein Structure and Folding: Effect of pH on the Stability and Structure of Yeast Hexokinase A: Acidic Amino Acid Residues in the Cleft Region are Critical for the Opening and the Closing of the Structure. *J. Biol. Chem*, 32093-32099.

Kunas, K. T., & Papoutsakis, E. T. (1990). Damage Mechanisms of Suspended Animal Cells in Agitated Bioreactors with and Without Bubble Entrainment. *Biotechnology and Bioengineering*, 476-483.

Lee, E. S. (2000). Machining Characteristics of the Electropolishing of Stainless Steel (STS316L). *Int J Adv Manuf Technol*, 591-599.

Lee, S. J., & Lai, J. J. (2003). The effects of electropolishing (EP) process parameters on corrosion resistance of 316L stainless steel. *Journal of Materials Processing Technology*, 206-210.

Lehky, P. (n.d.). Bioreactors-new solutions for old problems. Lozibky, Brno, Czech Republic.

Lennox, E. S. (1955). Transduction of Linked Genetic Characters of the host by bacteriophages P1. *Virology*, 190.

Lim, H. C., & Shin, H. S. (2013). Indroduction to Fed-Batch Cultures. In H. C. Lim, & H. S. Shin, *Fed-Batch Cultures* (pp. 1-10). Cambridge University Press.

Linek, V., Moucha, T., & Sinkule, J. (1996). Gas-liquid mass transfer in vessles stirred with multiple impellers--II. Modelling of gas-liquid mass transfer. *Chemical ENgineering Science*, 3875-3879.

Linek, V.; Kordac, M.; Moucha, T. (2005). Mechanism of mass transfer from bubbles in dispersions: Part II. Mass transfer coefficients in stirred gas-liquid reactor and bubble column. *Chemical Engineering and Processing*, 121-130.

Lyons, E., & Pandit, A. (2005). Design of Bioreactors for Cardiovascular Applications. In N. Ashammakhi, & R. L. Reis, *Topics in Tissue Engineering.*

Madigan, M. t., & Martinko, J. M. (2006). The Autoclaves. In m. t. madigan, & j. m. martinko, *Brock Biology of Microorganisms* (p. 672). New Jersey: Pearson Prentice Hall.

Madigan, M. t., & Martinko, J. M. (2006). The Autoclaves. In M. t. Madigan, & J. M. Martinko, *Brock Biology of Microorganisms* (p. 672). New Jersey: Pearson Prentice Hall.

Marks, D. M. (2003). Equipment design considerations for large scale cell culture. *Cytotechnology*, 21-33.

Martin, I., Wendt, D., & Heberer, M. (2004). The role of bioreactors in tissue engineering. *Trends in Biotechnology*, 80-86.

McFarlane, C. M., & Nienow, A. W. (1996a). Studies of high solidity ratio hydrofoil impellers for aerated bioreactors.3. Fluids of enhanced viscosity and exhibiting coalescence repression. *Biotechnology Progress*, 1-8.

Mcflarne, C. M., & Nienow, A. W. (1996b). Studies of high solidity ratio hydrofoil impellers for aerated bioreactors. 4. Comparison of impeller types. *Biotechnology Progress*, 9-15.

Michel, G. (1990). *Programmable Logic Controllers.* New York: Wiley.

Microsoft. (2010). Microsoft Excell. Redmond, Washington, US.

Miller, H. (1987). Practical aspects of preparing phage and plasmid DNA: growth, maintenance, and storage of bacteria and bacteriophage. *Methods in Enzymology*, 145-170.

Moo-Young, M., & Blanch, H. W. (1981). Design of biochemical reactors mass transfer criteria for simple and complex systems. *Advances in Biochemical Engineering*, 1-69.

Murthy, B. N., Ghadge, R. S., & Joshi, J. B. (2007). CFD simulations of gas-liquid-solid stirred reactor: Prediction of critical impeller speed for solid suspension. *Chemical Engineering Science*, 7184-7195.

Ni, X., Gao, S., Cumming, R. H., & Pritchard, D. W. (1995). A comparative study of mass transfer in yeast for a batch pulsed baffled bioreactor and a stirred tank fermenter. *Chemical Engineering Science*, 2127-2136.

Nichols, W. W. (1991). Biofilms, antibiotics and penetration. *Rev. Med. Microbiol.*, 177-181.

Nienow, A. W., Warmoeskerken, M. M., Smith, J. M., & Konno, M. (1985). On the flooding/loading transition and the complete dispersal condition in aerated vessles agitated by a Rushton-turbine. Wuerzburg, West Germany.

Nienow, A. W.; Wisdom, D. J.; Middleton, J. C. (1977). The effect of scale and geometry on flooding, recirculation, and power in gassed stirred vessels. Cambridge, England.

Nisipeanu, I. A., Bunciu, E., & Stanica, R. (2013). Bioprocesses parameters control in the case of a BIOSTAT A PLUS bioreactor. Slatina, Romania.

Normey-Rico, J. E., & Camacho, E. F. (2007). *Control of dead time process.* London: Springer.

Papoutsakis, T. E. (1991). Fluid-mechanical damage of animal cells in bioreactors . *TIBTEC*, 427-437.

Patil, V., Tran, K. Q., & Giselrod, H. R. (2008). Towards Sustainable Production of Biofuels from Microalgae . *Int. J. Mol. Sci*, 1188-1195.

Patwardhan, A. W., & Joshi, J. B. (1998). Design of stirred vessels with gas entrained from free liquid surface. *Canadian Journal of Chemical Engineering*, 339-364.

Poorte, R. E., & Biesheuvel, A. (2002). Experiments on the motion of gas bubbles in turbulence generated by an active grid. *Journal of Fluid Mechanics*, 127-154.

Radisic, M., Marsano, A., Maidhof, R., Wang, Y., & Vunjak-Novakovic, G. (2008). Cardiac tissue engineering using perfusion bioreactor systems. *Nature Protocols*, 719-738.

Ramaswamy, S., Cutright, T. J., & Qammar, H. K. (2005). Control of continous bioreactor using model predictive control. *Process Biochemistry*, 2763-2770.

Rewatkar, V. B., & Joshi, J. B. (1993). Role of sparger design on gas dispersion in mechanically agitated gas-liquid contactors. *Canadian Journal of Chemical Engineering*, 278-291.

Riffat, S. B., & Ma, X. (2003). Thermoelectrics: a review of present and potential applications. *Applied Thermal Engineering*, 913-935.

Ronner, A. B., & Wong, A. C. (1993). Biofilm development and sanitizer inactivation of Listeria monocryogenes and Salmonella typhimurium on stainless steel and buna-n rubber. *J. Food Prot.*, 750-758.

Sabia, R., & Stevens, H. J. (2000). Performance Characterization of Cerium Oxide Abrasives for Chemical-Mechanical Polishing of Glass. *Machining Science and Technology: An International Journal*, 235-251.

Sanchez Perez, J. A., Porcel, R. E., Lopez, C. J., Sevilla, F. J., & Chisti, Y. (2006). Shear rate in stirred tank and bubble column bioreactors. *Chemical Engineering Jornal*, 1-5.

Satterfield, C. N. (1975). Trickle-Bed Reactors. *AIChE Journal*, 209-228.

Scargiali, F., D'Orazio, A., Grisafi, F., & Brucato, A. (2007). Modelling and simulation of gas-liquid hydrodynamics in mechanically stirred tanks. *Chemical Engineering Research & Design*, 637-646.

Sherritt, R. G., Chaouki, J., Mehrotra, A. K., & Behie, L. A. (2003). Axial dispersion in the three-dimensional mixing of particles in a rotating drum reactor. *Chemical Engineering Science*, 401-415.

Simon, J., Weise, J., & Steinmetz, H. (2006). A comparison of continous flow and squencing batch reactor plants concerning integrated operation of sewer systems and wastewater treatment plants. *Water Science & Technology* , 241-248.

Singh, J., Kaushik, N., & Biswas, S. (2014). Bioreactor-technolgoy & design analysis. *The scitech journal*, 28-36.

Singh, V. (1999). Disposable bioreactor for cell culture using wave-induced agitation. *Cytotechnology*, 149-158.

Strang, G. (1991). Introduction to Calculus. In G. Strang, *Calculus* (p. 2). WEllesley: Wellesley-Cambridge Press.

Talley, K., & Alexov, E. (2010). On the pH-optimum of activity and stability of proteins. *Proteins*, 2699-2706.

Tatterson, G. B. (1991). *Fluid mixing and gas dispersion in agitated tanks.* New York: McGraw-Hill Inc.

Tatterson, G. B. (1994). *Scaleup and Design of Industrial Mixing Processes.* New York: McGraw-Hill.

Tatterson, G. B. (1994). *Scaleup and Design of Industrial Mixing Processes.* New York: McGraw-Hill Inc.

Thomas, J. R. (1990). Problems of shear in biotechnology. In M. A. Winkler, *Chemical engineering problems in biotechnology* (pp. 23-94). London-New York: Elsevier Applied Sciences.

Trivedi, B., & Danforth, W. H. (1966). Effect of pH on the Kinetics of Frog Muscle Phosphofructokinase. *J. Biol. Chem.*, 4110-4114.

van Haecke, E., Remon, J. P., Moors, M., Raes, F., De Rudder , D., & van Peteghem, A. (1990). Kinetics of Pseudomonas aeroginosa adhesion to 304 and 316-L stainless steel: role of cell surface hydrıphobicity. *Appl. Environ. Microbiol.*, 788-795.

Van't Reit, K., & Tramper, J. (1991). *Basic Bioreactor Design.* New York: Marcel Dekker.

Voet, D., & Voet, J. G. (2004). Effects of pH. In D. Voet, & J. G. Voet, *Biochemistry* (pp. 486-487). New Jersey: John Wiley & Sons, Inc.

Vrana, D., & Seicher, L. (1988). Cytomorphological comparison of mechanical and chemical defoaming of a yeast culture. *Folia Microbiologica*, 144-147.

Walker, R. (1961). Chemical Reaction and Diffusion in a Catalytic Tubular Reactor. *Physics of Fluids (V.4)*, 1211.

Westerterp, K. R., & Molga, E. J. (2006). Safety and runaway protection in batch and semibatch reactors: A review. *Chemical Engineering Research & Design*, 543-552.

Wilhelm, R. E. (1985). *Programmable COntroller Handbook.* Indiana Polis: Hayden.

Williams, J. A. (2002). Keys to bioreactor selection. *Chemical Engineering Progress*, 34-41.

Worden, R. M., & Bredwell, M. D. (1998). Mass transfer properties of microbubbles: II. Analysis using a dynamic model. *Biotechnology Progress*, 39-46.

Yim, H., Haselbaeck, R., Niu, W., Pujol-Baxley, K., Burgard, A., Boldt, J., et al. (2011). Metabolic engineering of Escherichia coli for direct production of 1,4-butanediol. *Nature Chemical Biology*, 445-452.

Yong, H., Wu, M., Jİn-Hua, S., & Guoping, L. (2004). Parameter-dependent lyapunov functional for stability of time-delay systems with polytopic-type uncertainties. *IEEE Trans. Autom. Contr.*, 828-832.

Zhong, Q. C. (2006). *Robust control of time delay systems.* Verlag: Springer.

Zottola, E. A., & Sasahara, K. S. (1994). Microbial biofilms in the food processing industry- Should they be a concern? *Int. J. Food Microbiol.*, 125-148.

Zoubek, B., Roussel, J. M., & Kwiatkowska, M. (2003). Towards automatic verification of ladder logic programs. *Computational Engineering in Systems Applications*.

APPENDIX A

DATA of HEATING/COOLING SYSTEM TESTS

***Table A.1*. Heating test with no mixing**

Time (s)	Measured temperature (°C)
0	27.38
10	27.61
20	27.96
30	28.20
40	29.08
50	31.03
60	32.46
70	34.15
80	36.79
90	39.09
100	42.66
110	45.68
120	49.37
130	52.24
140	54.51
150	56.38
160	56.3

***Table A.2*. Heating test under 500 rpm agitation**

Time (s)	Measured temperature (°C)
0	26.53
10	26.76
20	27.59
30	30.08
40	33.71
50	39.92
60	43.66
70	49.74
80	55.59
90	59.84
100	61.82
110	62.9
120	61.26

***Table A.3*. Cooling test with no mixing**

Time (s)	Measured temperature (°C)
0	56.3
10	56.2
20	54.69
30	53.17
40	50.77
50	47.93
60	44.61
70	41.48
80	39.22
90	37.86
100	34.87
110	31.6
120	29.92
130	27.65
140	24.84
150	22.09
160	21.57
170	21.34

***Table A.4*. Cooling test under 500 rpm agitation**

Time (s)	Measured temperature (°C)
0	60.63
10	59.68
20	57.18
30	55.98
40	52.94
50	47.21
60	43.15
70	38.37
80	33.74
90	29.04
100	23.71
110	21.36
120	20.53
0	60.63
10	59.68
20	57.18
30	55.98

APPENDIX B

DATA of VELOCITY STABILITY TESTS

***Table B.1*. Data of Set RPM vs Observed RPM in each 10 seconds**

	setRPM	**observed RPM in each 10 sec.**
DataSet1.1	**50**	48.35
DataSet1.2	**50**	50.12
DataSet1.3	**50**	51.34
DataSet1.4	**50**	49.84
DataSet1.5	**50**	49.17
DataSet1.6	**50**	50.63
DataSet1.7	**50**	47.92
DataSet1.8	**50**	48.13
DataSet1.9	**50**	52.07
DataSet1.10	**50**	49.18
DataSet2.1	**100**	98.42
DataSet2.2	**100**	99.15
DataSet2.3	**100**	96.98
DataSet2.4	**100**	102.36
DataSet2.5	**100**	100.91
DataSet2.6	**100**	104.04
DataSet2.7	**100**	96.85
DataSet2.8	**100**	103.42
DataSet2.9	**100**	101.74
DataSet2.10	**100**	98.57

Table B.1.cont.'d. **Data of Set RPM vs Observed RPM in each 10 seconds**

	setRPM	**observed RPM in each 10 sec.**
DataSet3.1	**150**	144.35
DataSet3.2	**150**	148.43
DataSet3.3	**150**	152.76
DataSet3.4	**150**	150.94
DataSet3.5	**150**	149.98
DataSet3.6	**150**	157.72
DataSet3.7	**150**	146.63
DataSet3.8	**150**	154.46
DataSet3.9	**150**	143.82
DataSet3.10	**150**	151.13
DataSet4.1	**200**	192.36
DataSet4.2	**200**	199.74
DataSet4.3	**200**	208.61
DataSet4.4	**200**	201.52
DataSet4.5	**200**	203.87
DataSet4.6	**200**	207.55
DataSet4.7	**200**	205.97
DataSet4.8	**200**	197.49
DataSet4.9	**200**	197.02
DataSet4.10	**200**	202.63
DataSet5.1	**250**	237.84
DataSet5.2	**250**	246.86
DataSet5.3	**250**	254.67
DataSet5.4	**250**	258.29
DataSet5.5	**250**	252.16
DataSet5.6	**250**	248.75
DataSet5.7	**250**	257.12
DataSet5.8	**250**	240.83
DataSet5.9	**250**	241.63
DataSet5.10	**250**	261.46

Table B.1.cont.'d. **Data of Set RPM vs Observed RPM in each 10 seconds**

	setRPM	**observed RPM in each 10 sec.**
DataSet6.1	**300**	286.83
DataSet6.2	**300**	298.37
DataSet6.3	**300**	310.42
DataSet6.4	**300**	313.86
DataSet6.5	**300**	301.49
DataSet6.6	**300**	307.51
DataSet6.7	**300**	294.76
DataSet6.8	**300**	288.98
DataSet6.9	**300**	306.36
DataSet6.10	**300**	311.38
DataSet7.1	**350**	345.76
DataSet7.2	**350**	357.47
DataSet7.3	**350**	335.97
DataSet7.4	**350**	361.46
DataSet7.5	**350**	367.03
DataSet7.6	**350**	359.81
DataSet7.7	**350**	340.82
DataSet7.8	**350**	335.74
DataSet7.9	**350**	365.43
DataSet7.10	**350**	360.64
DataSet8.1	**400**	382.38
DataSet8.2	**400**	397.45
DataSet8.3	**400**	411.04
DataSet8.4	**400**	415.37
DataSet8.5	**400**	401.15
DataSet8.6	**400**	399.87
DataSet8.7	**400**	385.69
DataSet8.8	**400**	409.27
DataSet8.9	**400**	413.97
DataSet8.10	**400**	391.74

Table B.1.cont.'d. **Data of Set RPM vs Observed RPM in each 10 seconds**

	setRPM	**observed RPM in each 10 sec.**
DataSet9.1	**450**	428.46
DataSet9.2	**450**	430.83
DataSet9.3	**450**	470.82
DataSet9.4	**450**	455.06
DataSet9.5	**450**	437.38
DataSet9.6	**450**	451.28
DataSet9.7	**450**	463.42
DataSet9.8	**450**	467.85
DataSet9.9	**450**	461.23
DataSet9.10	**450**	443.83
DataSet10.1	**500**	477.34
DataSet10.2	**500**	481.37
DataSet10.3	**500**	497.21
DataSet10.4	**500**	480.86
DataSet10.5	**500**	501.68
DataSet10.6	**500**	522.28
DataSet10.7	**500**	483.98
DataSet10.8	**500**	518.64
DataSet10.9	**500**	509.92
DataSet10.10	**500**	490.13
DataSet11.1	**550**	551.42
DataSet11.2	**550**	531.64
DataSet11.3	**550**	572.82
DataSet11.4	**550**	560.71
DataSet11.5	**550**	564.47
DataSet11.6	**550**	534.73
DataSet11.7	**550**	541.61
DataSet11.8	**550**	528.26
DataSet11.9	**550**	562.84
DataSet11.10	**550**	573.43

Table B.1.cont.'d. **Data of Set RPM vs Observed RPM in each 10 seconds**

	setRPM	**observed RPM in each 10 sec.**
DataSet12.1	**600**	610.21
DataSet12.2	**600**	608.06
DataSet12.3	**600**	582.13
DataSet12.4	**600**	590.15
DataSet12.5	**600**	598.97
DataSet12.6	**600**	615.15
DataSet12.7	**600**	623.76
DataSet12.8	**600**	628.47
DataSet12.9	**600**	601.97
DataSet12.10	**600**	592.38
DataSet13.1	**650**	617.82
DataSet13.2	**650**	631.42
DataSet13.3	**650**	681.96
DataSet13.4	**650**	677.76
DataSet13.5	**650**	664.45
DataSet13.6	**650**	669.97
DataSet13.7	**650**	648.74
DataSet13.8	**650**	625.45
DataSet13.9	**650**	646.82
DataSet13.10	**650**	673.93
DataSet14.1	**700**	667.73
DataSet14.2	**700**	679.94
DataSet14.3	**700**	702.26
DataSet14.4	**700**	714.24
DataSet14.5	**700**	720.75
DataSet14.6	**700**	698.35
DataSet14.7	**700**	673.95
DataSet14.8	**700**	719.27
DataSet14.9	**700**	731.34
DataSet14.10	**700**	684.93

Table B.1.cont.'d. **Data of Set RPM vs Observed RPM in each 10 seconds**

	setRPM	**observed RPM in each 10 sec.**
DataSet15.1	**750**	730.78
DataSet15.2	**750**	715.16
DataSet15.3	**750**	741.84
DataSet15.4	**750**	767.68
DataSet15.5	**750**	771.24
DataSet15.6	**750**	780.63
DataSet15.7	**750**	734.72
DataSet15.8	**750**	724.35
DataSet15.9	**750**	728.15
DataSet15.10	**750**	744.82
DataSet16.1	**800**	823.44
DataSet16.2	**800**	765.21
DataSet16.3	**800**	786.48
DataSet16.4	**800**	810.43
DataSet16.5	**800**	833.96
DataSet16.6	**800**	818.38
DataSet16.7	**800**	782.36
DataSet16.8	**800**	794.36
DataSet16.9	**800**	772.82
DataSet16.10	**800**	803.55
DataSet17.1	**850**	808.86
DataSet17.2	**850**	834.74
DataSet17.3	**850**	862.14
DataSet17.4	**850**	885.82
DataSet17.5	**850**	825.68
DataSet17.6	**850**	815.45
DataSet17.7	**850**	854.96
DataSet17.8	**850**	882.32
DataSet17.9	**850**	890.55
DataSet17.10	**850**	873.74

Table B.1.cont'd. **Data of Set RPM vs Observed RPM in each 10 seconds**

	setRPM	**observed RPM in each 10 sec.**
DataSet18.1	**900**	895.92
DataSet18.2	**900**	862.73
DataSet18.3	**900**	910.14
DataSet18.4	**900**	923.16
DataSet18.5	**900**	887.82
DataSet18.6	**900**	931.72
DataSet18.7	**900**	936.84
DataSet18.8	**900**	941.13
DataSet18.9	**900**	873.88
DataSet18.10	**900**	868.68
DataSet19.1	**950**	940.43
DataSet19.2	**950**	910.79
DataSet19.3	**950**	915.62
DataSet19.4	**950**	957.47
DataSet19.5	**950**	983.72
DataSet19.6	**950**	990.75
DataSet19.7	**950**	943.48
DataSet19.8	**950**	926.49
DataSet19.9	**950**	967.25
DataSet19.10	**950**	978.36
DataSet20.1	**1000**	975.47
DataSet20.2	**1000**	1015.42
DataSet20.3	**1000**	1032.42
DataSet20.4	**1000**	1021.84
DataSet20.5	**1000**	987.84
DataSet20.6	**1000**	963.68
DataSet20.7	**1000**	989.47
DataSet20.8	**1000**	968.25
DataSet20.9	**1000**	1004.27
DataSet20.10	**1000**	1043.45

Table B.1.cont.'d. **Data of Set RPM vs Observed RPM in each 10 seconds**

	setRPM	**observed RPM in each 10 sec.**
DataSet21.1	**1050**	1001.14
DataSet21.2	**1050**	1042.76
DataSet21.3	**1050**	1021.87
DataSet21.4	**1050**	1094.48
DataSet21.5	**1050**	1100.04
DataSet21.6	**1050**	1006.67
DataSet21.7	**1050**	1012.46
DataSet21.8	**1050**	1087.82
DataSet21.9	**1050**	1054.86
DataSet21.10	**1050**	1065.23
DataSet22.1	**1100**	1125.24
DataSet22.2	**1100**	1090.72
DataSet22.3	**1100**	1062.83
DataSet22.4	**1100**	1083.55
DataSet22.5	**1100**	1071.31
DataSet22.6	**1100**	1134.46
DataSet22.7	**1100**	1087.98
DataSet22.8	**1100**	1064.76
DataSet22.9	**1100**	1098.78
DataSet22.10	**1100**	1112.32
DataSet23.1	**1150**	1098.72
DataSet23.2	**1150**	1121.25
DataSet23.3	**1150**	1186.79
DataSet23.4	**1150**	1201.06
DataSet23.5	**1150**	1163.65
DataSet23.6	**1150**	1173.78
DataSet23.7	**1150**	1177.92
DataSet23.8	**1150**	1099.15
DataSet23.9	**1150**	1134.76
DataSet23.10	**1150**	1157.47

Table B.1.cont.'d. **Data of Set RPM vs Observed RPM in each 10 seconds**

	setRPM	**observed RPM in each 10 sec.**
DataSet24.1	**1200**	1152.36
DataSet24.2	**1200**	1232.68
DataSet24.3	**1200**	1176.86
DataSet24.4	**1200**	1187.43
DataSet24.5	**1200**	1253.67
DataSet24.6	**1200**	1218.92
DataSet24.7	**1200**	1243.74
DataSet24.8	**1200**	1225.73
DataSet24.9	**1200**	1166.75
DataSet24.10	**1200**	1187.94
DataSet25.1	**1250**	1201.74
DataSet25.2	**1250**	1214.15
DataSet25.3	**1250**	1251.85
DataSet25.4	**1250**	1293.23
DataSet25.5	**1250**	1205.61
DataSet25.6	**1250**	1195.93
DataSet25.7	**1250**	1234.64
DataSet25.8	**1250**	1284.73
DataSet25.9	**1250**	1256.48
DataSet25.10	**1250**	1298.45
DataSet26.1	**1300**	1246.48
DataSet26.2	**1300**	1294.27
DataSet26.3	**1300**	1255.29
DataSet26.4	**1300**	1263.41
DataSet26.5	**1300**	1241.84
DataSet26.6	**1300**	1301.26
DataSet26.7	**1300**	1354.57
DataSet26.8	**1300**	1248.86
DataSet26.9	**1300**	1318.45
DataSet26.10	**1300**	1342.87

Table B.1.cont.'d. **Data of Set RPM vs Observed RPM in each 10 seconds**

	setRPM	**observed RPM in each 10 sec.**
DataSet27.1	**1350**	1292.32
DataSet27.2	**1350**	1298.96
DataSet27.3	**1350**	1315.53
DataSet27.4	**1350**	1398.36
DataSet27.5	**1350**	1326.67
DataSet27.6	**1350**	1367.48
DataSet27.7	**1350**	1372.73
DataSet27.8	**1350**	1402.75
DataSet27.9	**1350**	1386.81
DataSet27.10	**1350**	1409.96
DataSet28.1	**1400**	1398.76
DataSet28.2	**1400**	1423.28
DataSet28.3	**1400**	1356.67
DataSet28.4	**1400**	1378.24
DataSet28.5	**1400**	1342.94
DataSet28.6	**1400**	1445.46
DataSet28.7	**1400**	1436.78
DataSet28.8	**1400**	1427.47
DataSet28.9	**1400**	1364.85
DataSet28.10	**1400**	1342.49
DataSet29.1	**1450**	1395.65
DataSet29.2	**1450**	1423.45
DataSet29.3	**1450**	1476.28
DataSet29.4	**1450**	1512.32
DataSet29.5	**1450**	1513.78
DataSet29.6	**1450**	1492.64
DataSet29.7	**1450**	1465.36
DataSet29.8	**1450**	1389.79
DataSet29.9	**1450**	1412.56
DataSet29.10	**1450**	1451.25

Table B.1.cont.'d. **Data of Set RPM vs Observed RPM in each 10 seconds**

	setRPM	**observed RPM in each 10 sec.**
DataSet30.1	**1500**	1437.84
DataSet30.2	**1500**	1483.38
DataSet30.3	**1500**	1496.64
DataSet30.4	**1500**	1532.54
DataSet30.5	**1500**	1551.52
DataSet30.6	**1500**	1572.04
DataSet30.7	**1500**	1469.36
DataSet30.8	**1500**	1446.45
DataSet30.9	**1500**	1473.72
DataSet30.10	**1500**	1463.62