YILDIZ TECHNICAL UNIVERSITY

FACULTY OF CHEMICAL AND METALLURGICAL ENGINEERING

BIOENGINEERING DEPARTMENT

BIOENGINEERING LABORATORY- II

BYM4242

LABORATORY BOOKLET

Istanbul, 2022/2023

PREFACE

In YTÜ Bioengineering Department, Bioengineering Laboratory I and Bioengineering Laboratory II are required courses in the 7th and 8th semesters. These experiments which prepared within the scope of Bioengineering Laboratory II, are carried out in the 8th semester under the coordination of the department academic staff.

The aim of the course is to make students practice with selected experiments for theoretical vocational courses during Bioengineering education, and evaluate the results obtained by discussing them.

We wish success to all students who took Bioengineering Laboratory II course.

On behalf of Bioengineering Faculty Members,

Prof. Dr. Mehmet Burçin PİŞKİN

LABORATORY RULES

Class ID: 38116848
Class Enrollment Key: BYM4242GR2

- Upload your reports to the Turnitin system by **23:59 on Tuesday** of the next week at the latest!!
- It is sufficient for **one person from each group to register and upload your final report** in the system. Write **ING/Group Number** on the **'Your First Name'** tab to show that you are an English group. On the **'Your Last Name'** tab, write your **Experiment Name**.

  Please do not write your own name and surname!!
- For reports which you are uploaded to Turnitin with a similarity rate of **over 30 percent**, please contact the laboratory coordinator. Points will be deducted from late submitted or uploaded reports to Turnitin system from total grade.
- If the plagiarism score is between 20-30%, 75 points will be given to the plagiarism part of the table.
- If the plagiarism score is below 20%, 100 points will be given to the plagiarism section of the table.
- Upload your reports to the Turnitin system without removing the cover pages from your reports.
- Please deliver the original versions of the reports to the laboratory coordinator via e-mail, and the hard copies of the reports must be hand delivered to the relevant teaching assistants on Wednesday of the next week at the latest.
- If the reports submitted late, each day 10 points will be deducted from reports.

SUCCESS AND EVALUATION STATUS

In-Year Assessment: 30%

Midterm Exam: 30%

Final exam: 40%

- There will be five experiments and one design experiment during the semester. Participation in all experiments is **mandatory**.
- Attend the online class on time.
- Experiment results and calculations will be prepared in a detailed by each group as a single group report, written on the computer until the next laboratory day, and delivered to the responsible research assistant and laboratory coordinator.
- Students are responsible for every step of the experiment and anything that is included in the reports can be asked.

LABORATORY CALENDAR

WEEK	DATE	TOPICS
1	March 1	Meeting with Students, Introduction to course / Presentation of Standards for Engineering Applications
2	March 8	Experiment 1
3	March 15	Experiment 2
		Final Report of Experiment 1
4	March 22	Experiment 3
		Final Report of Experiment 2
5	March 29	Experiment 4
		Final Report of Experiment 3
6	April 5	Experiment 5
		Final Report of Experiment 4
7	April 12	Make-up Experiments
		Final Report of Experiment 5
8	April 19	**MIDTERM WEEK**
9	April 26	Post-Quiz
		Submission of the Design Experiment Booklet
		Final Report of Make-up Experiments
10	May 3	Design Experiments
11	May 10	Design Experiments
12	May 17	Design Experiments
13	May 24	**Deadline for Design Experiment Report Submission**
14	May 31	**Design Experiment Presentations**
15	-	**FINAL EXAM**

EXPERIMENTS AND RESPONSIBLE TEACHING ASSISTANTS

Experiment No	Experiment	Exp. Time	Responsible Teaching Staff	E-mail
1	Plasmid DNA isolation and spectral analysis	09.30	Res. Asst. Başak AKIN	basakakin00@gmail.com
2	Construction and application of urea biosensor	09.30	Res. Asst. Dr. Mustafa NİĞDE	mnigde@yildiz.edu.tr
3	Biofuel production with thermochemical transformation	09.30	Res. Asst. Gülcan Ayşin KARACA	gakaraca@yildiz.edu.tr
4	Size and zeta potential analysis of protein by light scattering method	09.30	Res. Asst. Eda Nur YETİŞKİN MORKAN	yetiskinedaa@gmail.com
5	Baker's yeast production in bioreactor	09.30	Res. Asst. Beyza KARACAOĞLU **(Lab. Coordinator)**	**beyzak@yildiz.edu.tr**
	Design Group 1	-	Res. Asst. Eda Nur YETİŞKİN MORKAN	yetiskinedaa@gmail.com
	Design Group 2	-	Res. Asst. Gülcan Ayşin KARACA	gakaraca@yildiz.edu.tr
	Design Group 3	-	Res. Asst. Dr. Mustafa NİĞDE	mnigde@yildiz.edu.tr
	Design Group 4	-	Res. Asst. Dr. Mustafa NİĞDE	mnigde@yildiz.edu.tr
	Design Group 5	-	Res. Asst. Ayça ASLAN	aslaanayca@gmail.com
	Design Group 6	-	Res. Asst. Ayça ASLAN	aslaanayca@gmail.com
	Design Group 7 (KOOP)	-	Res. Asst. Ayça ASLAN	aslaanayca@gmail.com

DESIGN EXPERIMENT

Week	Work
4	Determination of the problem and subject by the student
5	Approval of the subject by the responsible research assistant
9	Submission of the booklet
10-12	Performing the Design Experiment
13	Submission of the Final Report of Design Experiments
14	Presentations

- Each group must design a Design Experiment which are not including experiments in lab-handbooks. After that you have to write booklet like your laboratory handbook about your experimental design. You have added graphs, data, figures, etc. into Final Report of Design Experiment.
- It is mandatory for the groups to adhere to the determined dates.
- Each group must determine the problem/subject for the design experiment until the 4th week and they has to pay attention to the important dates related to the design experiment and be in contact with the responsible assistant during the design experiment.

EXPERIMENT GROUPS

GROUP NO	STUDENT ID	NAME	SURNAME
1	1905A011	İDİL	AÇAR
	1805A043	YAREN	AĞCA
	1905A022	RUVEYDA	AĞIRMAN
	1905A917	MELIKA	AHMADINASSAB
	1905A020	NİLGÜN EFTALYA	AKGÜL
	1905A911	FURUZA	ALIYEVA
	1905A922	AHMAD	ALTABL
	1805A040	ŞEHRİBAN	ASLANCI
	1905A608	EMİN	AŞUT
	1905A039	YAĞMUR GİZEM	AY
	2005A603	BENSU	AYDOĞMUŞ
	19056912	HODA	CHEIKHKHAMIS
	1905A926	NOUR	CHICKH MOHAMAD

GROUP NO	STUDENT ID	NAME	SURNAME
2	1805A055	HATİCE BEYZA	ÇAY
	2005A604	DERYA	ÇELİK
	1905A018	GİZEM	DEMİRCİ
	1805A015	ŞEVAL	DEMİRTAŞ
	1805A058	ENDERCAN	ERÇETİN
	1905A006	ERGİSU SEZGİN	ESİN
	1805A038	MİNE	EŞBAHOĞLU
	1905A934	HAYTHAM	FASSIH
	1805A046	RAİFE	GENÇER
	1905A051	RÜYA	GİRİŞKEN
	2005A608	BEYZA	GÖZÜKÜÇÜK
	1805A053	EKİN DEREN	GÜRTEKİN
	1805A016	SÜMEYYE	GÜVENÇ

GROUP NO	STUDENT ID	NAME	SURNAME
3	1905A906	HİBE	HARİRİ
	1805A912	ELAF	HOSSREH
	1805A022	FATMA BAHAR	IŞIK
	1905A706	EBRU CEREN	İLHAN
	1905A049	CEYDA	İRİŞEN
	1805A913	FATIMA	JAMOUS
	1905A921	EMİRA	KALİFA
	1805A048	İMRAN ÇAĞRI	KANTARCI
	1805A044	BETÜL	KARADUMAN
	1905A042	DEMİR OLEKSİY	KARASU
	19056904	LOUNA	KARZOUN
	1905A918	NARIN	KASHEKHI
	1805A004	BUSE	KAYGIN

GROUP NO	STUDENT ID	NAME	SURNAME
4	1805A041	SİMGE BİLGE	KEBABCI
	1805A035	BİLGE	KELEŞ
	1805A010	BEYZA	KILIÇ
	2005A612	MUSTAFA VEYSEL	KIRIMİ
	1805A034	BUĞRA	KİREKO
	1805A031	AYŞE NAZ	KOÇ
	1705A038	YUNUS EMRE	KUL
	1905A606	ARYA	LEYLABİ
	1805A917	HALİT	MAIBAR
	1905A903	MOHAMMAD	MOGHADDASI
	1905A003	MELİHCAN	ORAN
	1805A023	BEYZA SILA	ÖÇALAN
	1805A020	İLKNUR	ÖĞÜT

GROUP NO	STUDENT ID	NAME	SURNAME
5	1805A007	HÜSEYİN MURAT	ÖZADENÇ
	1805A051	MUHAMMED MUSTAFA MERT	ÖZDEMİR
	1905A032	SUDE	ÖZHAN
	1905A912	ZEYNEP	RAMAZAN
	1905A913	OLA	RAMAZAN
	1805A018	AHMET	SAFİ
	1905A701	AYŞENUR	SAKOĞLU
	18056065	SHHD	SARAJ
	2005A601	SENANUR BERKEM	SOMAR
	1905A914	SIMAF	SÜLEYMAN
	1905A057	ŞEVVAL	ŞAHİN
	2005A611	BÜŞRA BETÜL	ŞİMŞEK
	19056902	ELMIRA	TAGHIYEVA
	1805A047	BEYDA	TAĞ

GROUP NO	STUDENT ID	NAME	SURNAME
6	2005A705	EMİNE	TAYRAN
	1905A025	CEYDA	TOPAL
	1905A916	ALI	TORABKHANI NOSHAHR
	1805A045	BURAKHAN	TOY
	1805A054	FATMA BERİN	TÜFEKCİOĞLU
	1605A026	AYŞE ŞULE	UÇAR
	1905A902	ZEINA MAZIN	WALI
	1905A607	BORA	YAMAN
	1805A021	BİNNAZ SELDA	YARDIMCI
	1805A014	GİZEM	YAŞAR
	1805A059	BEYDA NUR	YATIKÇI
	1805A028	SELİN	YAVUZ
	1805A025	BURCU	YUNATCI

GROUP NO	STUDENT ID	NAME	SURNAME
7	1805A005	FEYZA	KANDEMİR
	1805A033	SELİN SUDE	AYHAN
	1805A056	ATAKAN	AKDAĞ
	1805A061	SELİN	ADIGÜZEL
	1905A904	AMIRA	KHALIL
	1805A057	İREM İLAYDA	KILIÇ
	1905A005	İPEK	OKTAY

EXPERIMENT 1: PLASMID DNA ISOLATION AND SPECTRAL ANALYSIS

Theoretical knowledge and aim of the experiment :

The DNA molecule is composed of double stranded nucleotides which include five carbon sugar and nitrogenous bases. There are four different types of nucleotides in DNA. Adenine and guanine are purines with double rings, while cytosine and thymine are pyrimidines with single rings. Nucleotides are specifically inserted in DNA molecule. Sugars are combined to each other via phosphate groups which provide interaction of 3' carbon of one sugar with 5' carbon of another. On the other hand, nitrogenous bases are combined with the chain as a side group. Moreover, double stranded structure of DNA molecule is hold together with hydrogen bonds that are localized between nitrogenous bases. Such type of binding is only occured between cytosine and guanine or thymine and adenine bases. By this way, base sequencing in one strand become complementary of other strand. The polarities of two strands are opposite to each other. Therefore, each strand moves oppositely from the other one. Consequently, stair-like double stranded molecule is helically curved and stabilized by hydrogen bonds (Demirsoy vd., 2003).

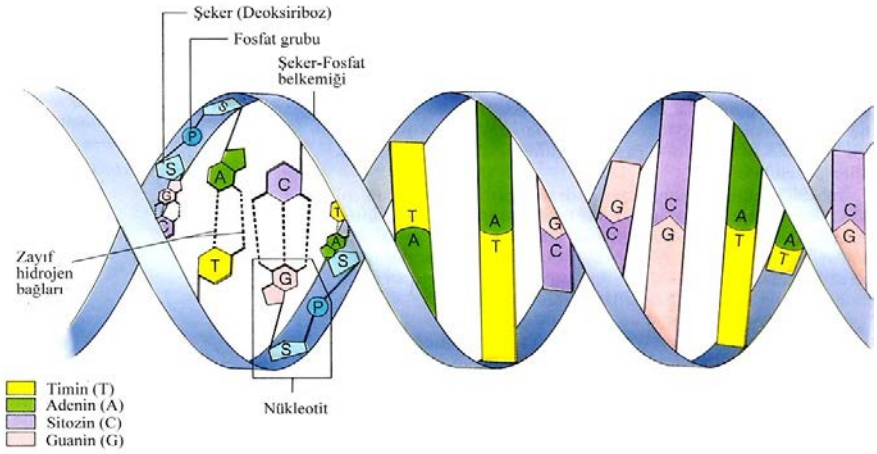


Figure 1. Schematic representation of DNA structure

After discovering that genetic codes are carried by DNA, researches have been mainly focused on enlightening of DNA molecule and expression mechanisms in order to convert this stored knowledge to visible phenotypes. In recent years, the isolation of DNA molecule was achieved and its specific regions were amplified. Therefore, recombinant DNA technology has been started to grow rapidly (Öner vd., 2009).

In researches at the field of genetic engineering, three types of DNA molecules; total cell DNA, phage DNA and plasmid DNA are used. Plasmids are double stranded and self-replicating DNA molecules that are naturally existed in bacterial cytoplasms at out of chromose. The widely use of plasmids as DNA carriers (vectors) in DNA technology studies lead to improvements in plasmid DNA isolation techniques. In general, there are three basic consecutive steps in DNA isolation methods. These steps are; the distruption of cells with lysis

solution in order to obtain high molecular weighted DNA, seperation of DNA-protein complexes by denaturation or proteolysis and purely isolation of DNA from proteins, RNA and the other macromolecules with enzymatic or chemical methods. One of the mostly used technique in plasmid DNA isolation is alkaline-lysis method which was developed by Birnoim and Doly (Birnboim HC, Doly J; 1979). This method provides the distruption of bacterial cells by using sodium hydroxide and sodium dodecyl sulphate (SDS) and selectively precipitation of chromosomal DNA and other cellular components with high molecular weight. Finally, plasmid DNA that is seperated from other cellular constituents is isolated with isopropanol.

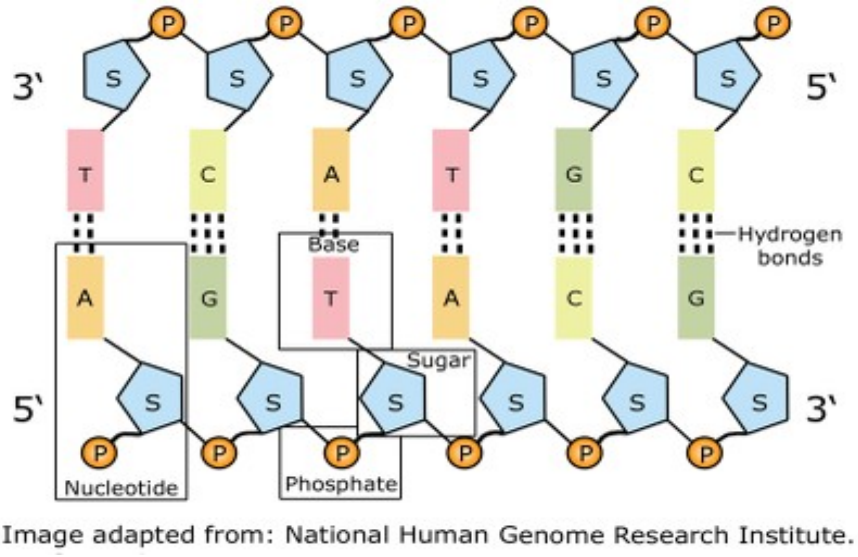


Figure 2. The illustration of DNA molecule (http://www2.le.ac.uk/departments/genetics/vgec/schoolscolleges/topics/dna-genes-chromosomes)

Purpose of Experiment:

Plasmid DNA isolation from bacterial culture by alkaline lysis method and DNA quantification by spectrophotometric method.

Questions that sould be investigated before the experiment:

- What are the common stages of all DNA isolation techniques in general?
- What are the main differences between plasmid and genomic DNA?
- Explain other DNA isolation techniques except plasmid DNA isolation method.
- Which methods are used in order to determine the amount of DNA after isolation?

Experiment Steps:

Buffers, solutions and media that are used in the experiment

Solution I:
0.3M sucrose
25 mM EDTA
25 mM Tris-Cl (pH: 8.0)

Solution II:
0.3 M NaOH
2% SDS
isopropanol
4 M NaCl
Ethanol

Devices that are used in the experiment

-Microcentrifuge, UV spectrophotometer

Consumables that are used in the experiment

-Micropipets, sterile micropipet tips, 1.5 ml microcentrifuge tubes,

Preliminary Preparation

-The culture of bacteria including plasmid DNA should be inoculated into liquid media and cultivated for one night.

METHOD

1. Plasmid DNA is extracted using an alkaline lysis method (Birnboim and Doly, 1979).
2. The E.coli sample (0.03 g) is resuspended in 100 µL ice-cold resuspension buffer [50 mM glucose, 25 mM TrisCl (pH 8.0), 10 mM EDTA (pH 8.0)].
3. Bacterial cells are lysed with 200 µL lysis solution [0.2 N NaOH, 1% (wt/vol) sodium dodecyl sulfate (SDS)] for 4 min and neutralized with 150 µL of chilled 3 M potassium acetate, pH 4.8.
4. The samples are centrifuged at 14,000 rpm for 10 min at 4°C.
5. The supernatant containing the plasmid is mixed with an equal volume of isopropanol and incubated at −20°C for 15 min.
6. Samples are centrifuged at 14,000 rpm for 30 min at 25°C.

7. The supernatant is removed and 500 µL of 70% ethanol is added to the pellet and centrifuged at 14,000 rpm for 5 min at 25°C.
8. The pellet is resuspended in 50 µLMilliQ water (Ultrapure Water).
9. Quantification of the isolated plasmid DNA is performed.

Quantification of Plasmid DNA

The amount of the isolated plasmid DNA is assessed by using UV spectrophotometer.
It is known that 1 OD value corresponds to 50 µg/ml for double stranded DNA molecules. Therefore, the formula below is used in order to evaluate the amounts of double stranded DNA:
DNA (µg/ml) = A_{260} × dilution factor × 50

In order to comment on the protein and RNA contamination in the solution;
A260nm / A280nm ratio is used.
If this ratio is > 1.8, RNA contamination,
if < 1.8 Protein contamination,
if ≈ 1.8, it can be interpreted that there is no contamination.

The questions that should be answered in the final report:

- Discuss the purity of DNA by using values obtained from UV- spectrophotometer.
- Discuss why absorption of the sample was evaluated at 260 nm in order to assess DNA amount.
- Which method would you use in order to evaluate DNA amount instead of using UV spectrophotometer?
- Discuss how to overcome experimental mistakes for this experiment.
- Explain what you should do if you want to determine accuracy and repeatability of the experimental results.

EXPERIMENT 2: CONSTRUCTION AND APPLICATION OF UREA BIOSENSOR

Aim and Theory

A biosensor is an analytical device based on the direct spatial coupling of an immobilized biologically active compound with a compatible transducer, which converts the biochemical signal into a quantifiable electrical signal. It was proposed by Leland C. Clark in 1962. It has wide applications in medical, pharmaceutical, biotechnology, agricultural and military areas. A biosensor consists of a biocomponent and a sensor: The biocomponent specifically recognizes the analyte and the physiochemical changes caused by the interaction between the biocomponent and the analyte such as change of light absorption or electrical charge or frequency of oscillation, etc. are indicated by the transducer. (Figure 3).

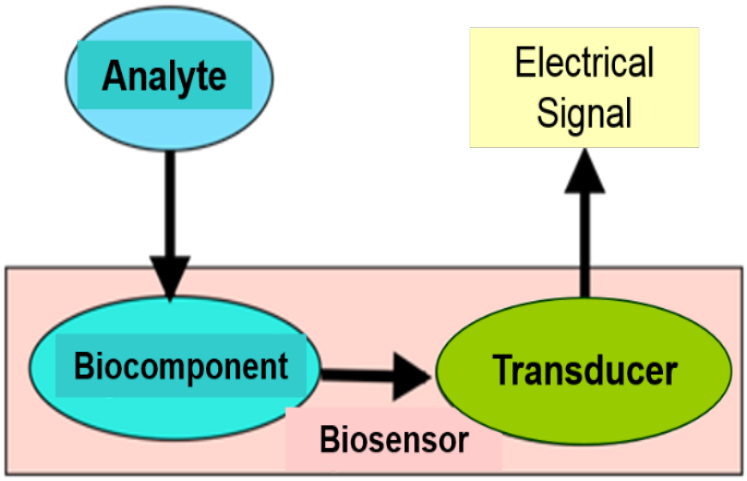


Figure 3. Biosensor.

The key part of the biosensor is the transducer which makes use of the physicochemical change accompanying the reaction. Several types of transducers have been used in the development of biosensors. They are classified according to biological component and type of sensor. It is named according to including biocomponent such as enzyme based, antibody based, nucleic acid based, tissue based, microbial based and polysaccharide based. These biological components are immobilized in chemical or physical way. There are variety of biosensors according to their sensor part such as voltametric (measure electrical potential), amperometric (measure electrical current), conductometric (measure electrical conductivity), measure impedance, optical (measure light intensity), measure mass, thermometric (measure temperature) and measure viscosity.

The substrate in solution is transferred on biosensor surface while biosensors are working. Substrate transfer occurs by diffusion, stirring etc. Substrate is diffused to active site of biocatalyst. Biocatalyst can be impregnated to polymeric porous membrane, linked between sensing element and polymeric membrane, or restrained into gel. Natural polymers such as

polyacrylamide, gelatin, agarose, or synthetic polymers can be used for this confinement process. A reaction occurs between biocatalyst and substrate. Gas molecules (O_2, CO_2, NH_3, etc.) can be released or used, selective ions (H^+, NH_4^+, monovalent anion and cation) can be formed, optic density can be changed, and an electron can be released or used during interaction. The product which is formed by the reaction between biocatalyst and substrate is moved to detector. On the detector surface, these alterations are perceived and transformed into measurable aspect. Measured electrical signal is proportional to analyte concentration.

The aim of this experiment is to construct a solid-state contact PVC-NH_2 membrane pH sensitive urease biosensor and carry out an application of the biosensor.

Pre-Experiment Questions:

- What are the areas of utilization of biosensors nowadays? What are their advantages?
- What is the classification of biosensors? Explain types of biosensors.
- What is potentiometry?
- What is Nernst equation? What is the relationship between the equation and biosensor?
- Supporting material is needed to bind biocomponent to biosensors. What are these materials?
- It is important that biocomponent should remain stable in reaction environment and it should not separate from biofilm. What are the methods to bind biocomponent to the biofilm?
- Why do biosensors have specific response?
- What are the characteristics of an ideal biosensor? Explain briefly.
- Why is an enzyme immobilized in an enzyme-based biosensor? What are the advantages of immobilization?
- Write the reactions that occur in a urea biosensor. Explain working principle of a biosensor.

Experimental Procedure:

Solutions and Chemicals

Solution-1 (% 2.5'lik glutaraldehyde solution):
0.25 g glutaraldehyde
9.25 mL water
Solution-2 (0.05 M phosphate buffer pH 7):
10 mL 0.05 M phosphate buffer
90 mL water
Solution -3 (urease solution):
1 mg urease
20 µL 0.05 M phosphate buffer (pH 7)
Solution -4 (urea solution):
0.1, 0.01, 0.001, 0.0001 M urea solution.

Instruments

-Potentiometer, reference electrode (Ag/AgCl), solid-state contact PVC-NH_2 membrane pH sensitive electrode.

Consumables

-Micropipette, 5- or 10-mL pipette, pendant switch, test tubes, beakers, Eppendorf tubes.

Preliminary preparation

-pH electrode and solutions should be prepared earlier.

Methods:

Urea biosensor is prepared as follows (I. Isildak, 2002):

1. Prepared solid state contact electrode PVC-NH_2 membrane pH sensitive electrode is immersed into urease enzyme solution (Solution-3) and enzyme is applied to membrane surface area.
2. Then the electrode is soaked into 2.5% glutaraldehyde solution (Solution-1) and it is allowed to stay in 15-20 minutes.
3. Electrode is slightly washed with water.
4. Electrode is immersed into enzyme solution (Solution-3) again and, let crosslinking reaction complete for 15 hours at 4 ^oC.
5. After that, the electrode is washed with 0.05 M phosphate buffer and kept in buffer solution at 4 ^oC.
6. If the biosensor is prepared just as this procedure, it can remain stable for weeks.

Calibration curve for urea biosensor:

1. Prepared biosensor and reference electrode are connected to potentiometer and then, electrode points are immersed into solution.
2. The emf values obtained from potentiometer are recorded. This step is repeated for all samples. Sensors should be washed with deionized water before they are immersed into each solution.
3. Each sample is measured for 3 times.
4. All obtained values are listed in a table and calculated average value. Potential value (y-axis) versus –log [urea] (x-axis) graph is plotted.

Determination of concentration of urea solution sample:

1. Electrodes are immersed into solution with an unknown concentration. Then, potential values are measured.

2. Concentration of urea sample solution is determined by using the calibration curve.

Determination of urea concentration by using pH electrode:

1. All procedure is repeated by using pH electrode. pH value (y-axis) versus –log[urea] (x-axis) graph is plotted.

Final Report Should Involve:

- Which principle are measurement and calibration curve of potentiometric biosensors based on?
- Why is calibration curve required?
- How will you measure if concentration of urea solution sample is greater than upper limit of calibration curve?
- How will you measure if concentration of urea solution sample is shorter than lower limit of calibration curve?
- Why do you repeat the measurement of each standard urea solution in the experiment? What is the advantage of that?

EXPERIMENT 3: BIOFUEL PRODUCTION WITH THERMOCHEMICAL TRANSFORMATION

Theoretical Background

Renewable energy, often referred to as clean energy, comes from natural sources or processes that are constantly renew. It has many branches such as solar energy, wind energy, hydroelectric energy, wave energy, biomass energy, etc. If we look at biomass energy among all these. Biomass is renewable organic material that comes from plants and animals, so that it is available from a wide variety of sources. The energy which is derived from these sources are defined as "Biomass Energy". Identifying sources of biofuels such as biodiesel, biogas, biochar can potentially reduce the environmental impact of fossil fuels.

In plants, this energy is coming from Sun, which is stored in form of chemicals, mainly cellulose, after photosynthesis and it can be used in various applications. Storing solar energy in form of biomass is crucial for living organisms. Through photosynthesis, not only organic compounds which can be used as energy source are formed, also oxygen is released to atmosphere which is required for respiration. This transformation of carbon dioxide to organic compounds through photosynthesis results with storage of solar energy in form of biomass. This step is expressed in the equation below.

$$nCO_2 + nH_2O + Sun\ Light + Chlorophyll \longrightarrow (CH_2O)_n + nO_2$$

The formation of carbohydrate monomer which is represented with CH_2O results with 470 kJ of energy and the oxygen is produced due to the water molecule. There are numerous questions unanswered regarding the molecular mechanism conditions to form fresh biomass. These conditions are carbon dioxide, visible region of electromagnetic spectrum, chlorophyll catalyst and a living organism with the ability of photosynthesis. The upper limit of uptake of sunlight by plants is assumed to vary between 8-15%. However, for most plants average value is thought to be 1% or below. Cellulosic biomass contains three main organic constituents such as cellulose, hemicellulose and lignin.

Biomass is produced by some process. For example, by physical process like crushing/grinding, drying, palletization solid fuel is obtained. On the other hand, with bio-chemical processes such as hydrolysis fermentation, anaerobic digestion respectively bioethanol and biogas are produced. As an alternative to fossil fuels, solid, liquid and gas fuels can be produced by thermochemical process of biomass under inert atmosphere with processes such as carbonization pyrolysis, hydrothermal/liquefaction, combustion etc. Years ago, the recovery of the substances/chemicals they contained was a thriving industry in many developed countries.

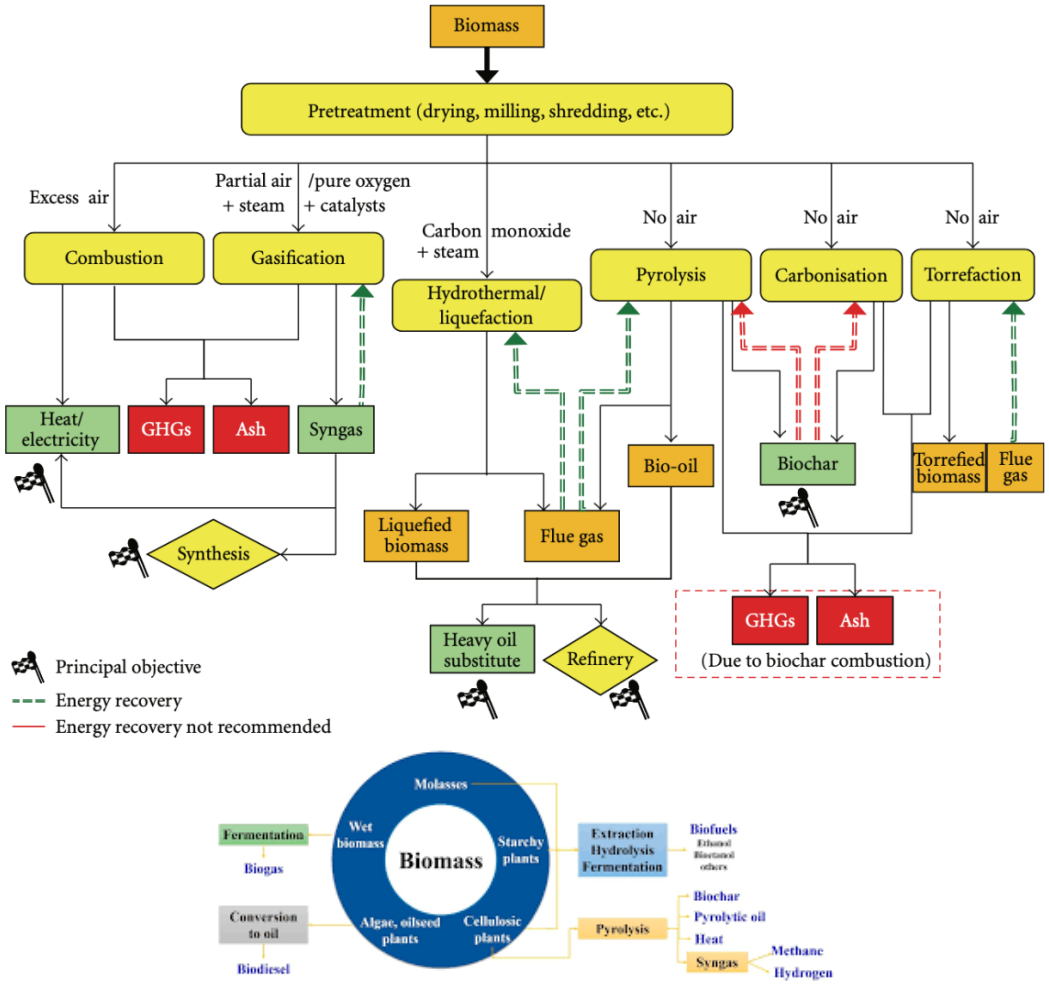


Figure 4. Biomass thermochemical conversion pathways.

Gasification is a process by which carbonaceous materials (biomass) are thermochemically converted into valuable gases, commonly referred to as synthesis gases (syngas) in the presence of a gasifying agent such as air, oxygen, steam, CO_2 or a combination of them at a temperature above 700 °C. Primarily, the produced gas consists of CO, H_2, CO_2 and CH_4.

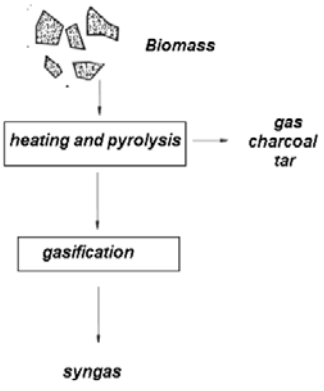


Figure 5. Schematic diagram for biomass gasification.

Pyrolysis is a thermochemical process that can be applied to any organic (carbon-based) product, and through this process, biomass can be converted into other fuels. During this process, the biomass is exposed to high temperature in the absence of oxygen. As a result, biomass is chemically and physically separated into different molecules. Decomposition takes place thanks to the limited thermal stability of the materials' chemical bonds, which allows them to be broken down using heat. This thermal decomposition also leads to the formation of new molecules. As a result of the process, it is ensured that products with a different and generally superior character than the original residue are obtained. That is why today's industry is becoming an important process.

Traditionally, charcoal production is one of the oldest chemical conversion processes known to mankind, used even today. Carbonization is a slow pyrolysis process, a special form, in which biomass is heated and converted into simpler substances with a high percentage of carbon. Carbonization methods are used for the formation of porous carbon from biomass sources. Typically, carbonization consists of heating the biomass in an oxygen-free or oxygen-limited environment, otherwise it will ignite and turn into ash. As the biomass is heated, it goes through certain stages on the way to turn into charcoal, and different structures are formed as a result of different reactions. The reaction conditions are adapted to maximize charcoal production. Mainly charcoal is produced, products other than charcoal are often called by-products. Briefly, after the carbonization or pyrolysis process, high carbon content product which is called biochar is obtained.

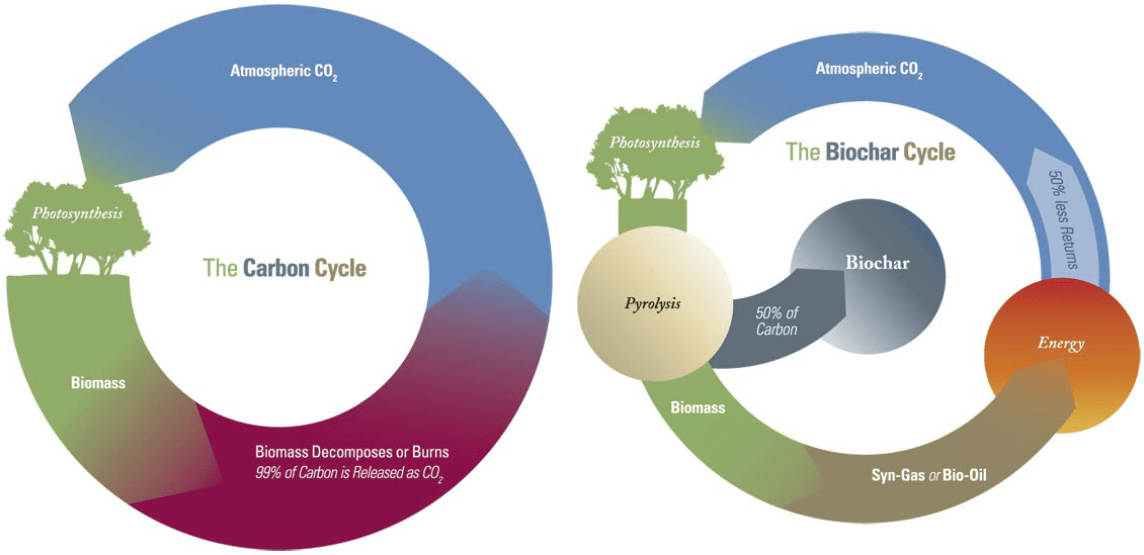


Figure 6. Schematic representation of the carbon and biochar cycle comparison.

Biochar is a charcoal-like substance obtained by burning organic matter from sustainable sources from agricultural and forest waste (biomass) in a controlled process in thermochemical conversion. Materials emit little or no polluting fumes when burning. By this process, organic material is converted into biochar, a stable form of carbon that cannot easily escape into the atmosphere. In addition, the energy or heat generated during this thermochemical conversion can be captured and used as a form of clean energy. Biochar is much more efficient at converting carbon into a stable form and is cleaner than other forms of charcoal. It is used to strengthen soil quality. Biochar can be used as an important tool to increase food security and cultivated land diversity in areas with severely depleted soils, scarce organic resources, insufficient water, and chemical fertilizer resources. In this way, plants reach the necessary nutrients and water more easily while they are growing. In addition, the heat and carriers of renewable energy co-generated during biochar production can be recovered and use as a energy source or this energy gives to local communities who needs them.

Carbonization and pyrolysis of biomass is highly affected from biomass properties and process parameters which are summarized as schematically in figure below.

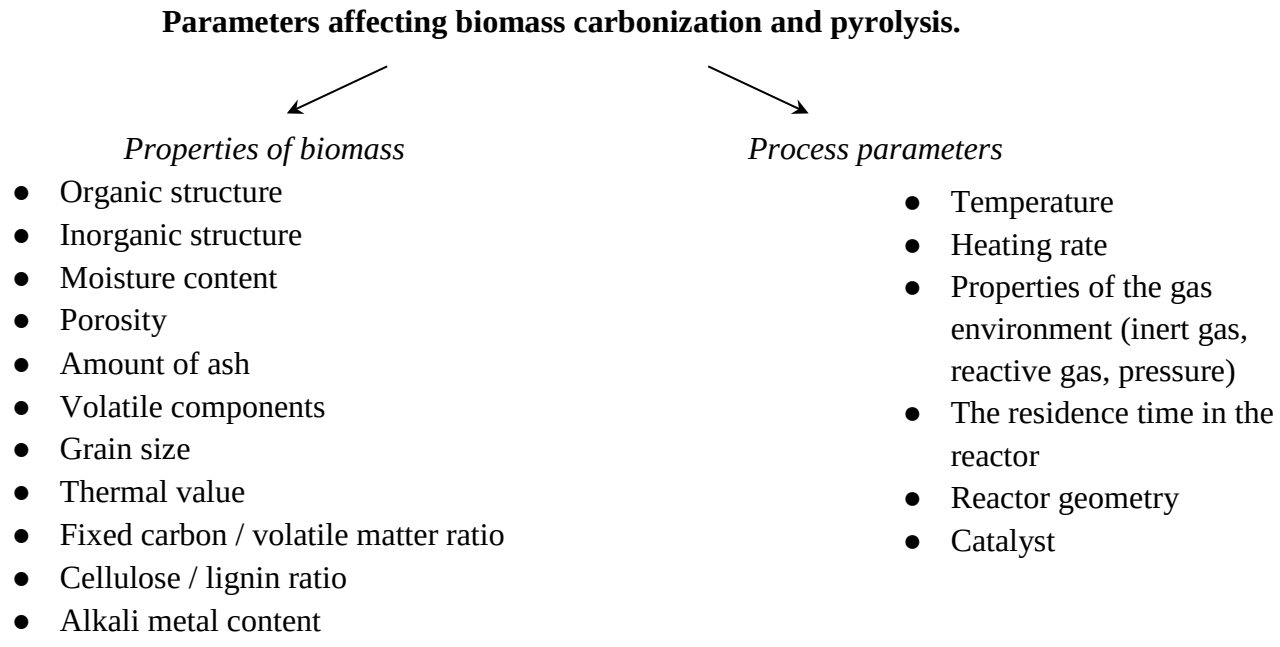


Figure 7. Parameters affecting biomass carbonization and pyrolysis.

Usage Areas

- *Soil healing and use as organic fertilizer*
- *Use in animal farms*
- *Gas retention-Treatment/Filtration*
- *Use in energy storage*
- *Use as a catalyst*
- *Use in structures*
- *Use as adsorbent*
- *Insulation and moisture balancing*

To summarize briefly, biomass is a renewable and sustainable energy source. Biochar is obtained as a result of pyrolysis of biomass. In this way, the carbon cycle is improved. In this experiment, it is aimed to convert the biomass source into biochar by thermochemical degradation process and to calculate the resulting biochar yield.

The aim of the experiment: In this experiment it is aimed to thermochemically degrade biomass source and transform it into biochar and calculate the biochar yield.

Pre-experimental Questions:

- What is biomass?
- What is biochar and its application areas?
- What is pyrolysis?
- What are the parameters that affect biochar yield?

Preliminary study:

- The raw materials are dried at 105 ° C for 24 hours.
- Before starting the experiment, the reactor is purged with nitrogen gas to provide an inert environment condition.

Experimental Procedure:

- Raw materials are weighed on a precision scale.
- The weighed samples are placed in the reactor.
- The temperature and nitrogen flow are adjusted, and the reactor is operated.
- When the desired temperature is reached, the reactor is left at a constant temperature and then the system is cooled.
- After the reactor has cooled down, the nitrogen is shut off and the samples are removed from the reactor.
- Biochar is weighed.

The final report should include:

- The percentage of biochar yield.
- A biochar yield-temperature graph.
- Compare the biochar yields obtained with those in the literature.
- Discuss the the content of biomass according to the obtained results.

EXPERIMENT 4: SIZE AND ZETA POTENTIAL ANALYSIS OF PROTEIN BY LIGHT SCATTERING METHOD

Theoretical Knowledge and Aim of the Experiment

Proteins are macromolecules, formed by covalent attachment of amino acids to each other and fulfill specific functions in biological systems. The most important roles of proteins are the catalysis of chemical reactions as enzymes. Additionally, have different properties such as cell signaling, ligand transportation and be important building materials inside and outside of cell. Therefore, the investigation of proteins is important in bioengineering.

Unlike synthetic and randomly coil natural polymers, proteins have specific and stable 3-dimensional structure in natural state. Proteins, only become functional when the specific 3-dimensional structure. It is important to find the size, surface charge and denaturation point in the investigation of this structure. Light scattering method is widely used to determination of these properties.

Protein sizes are measured with ***Dynamic Light Scattering (DLS)*** methods. In this technique, by using the Brownian motion of the proteins in the solution, first the diffusion coefficient is determined and then the size is determined using diffusion coefficient.

Brownian motion is defined as: “The random movement of particles in a liquid due to the bombardment by the molecules that surround them”. **The presence of Brownian motion is a requirement to use the DLS technique and DLS technique cannot be used in the absence of the Brownian movement!**

In the DLS technique, the laser light is sent onto the sample and scattered light from the particles directed onto a detector. The intensity of the light is a certain value in a certain area on the detector. As a result of Brownian movement of particles, the scattered light also varies continuously. Therefore, the light intensity on the detector also changes constantly because of change of scattering light.

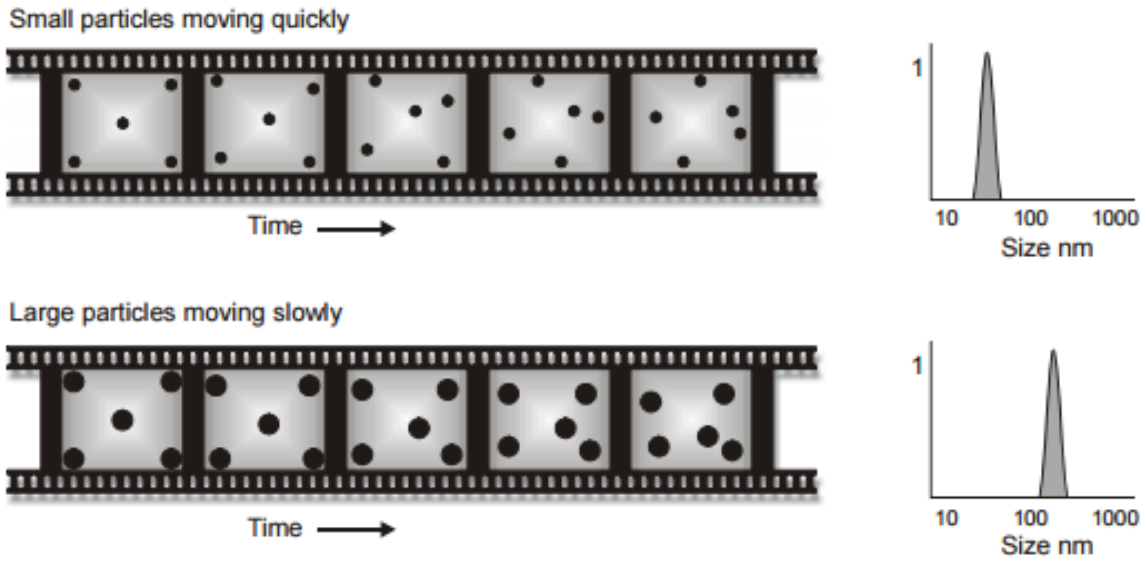


Figure 8. Moving properties of small and large particles

The particles in a liquid move about randomly and their speed of movement is used to determine the size of the particle. It is known that small particles move quickly in liquid and large particles move slowly. If large particles are being measured, then, as they are moving slowly, the intensity of the scattering light will also change slowly. And similarly, if small particles are being measured then, as they are moving quickly, the intensity of scattering light will also change quickly.

In DLS technique, the raw data is obtained by measuring the change of scattering light with time. The size of particle is calculated by using this raw data and Stokes-Einstein equation. Hydrodynamic diameter is calculates using Stokes-Einstein equation. Because hydrodynamic diameter is calculated from the diffusion coefficient, the factors affecting the diffusion, affects the particle size [1,2]. The melting point of proteins can be calculated by temperature-depended size measurement.

Zeta potential of proteins is measured by ***Electrophoretic Light Scattering (ELS)*** technique. In this technique, speed values of proteins are evaluated under an electric field with continuously changing direction. The zeta potential is measured utilizing the effect of Doppler shift and change of speed. All of these features are influenced by the medium pH.

The aim of experiment is to find out how the size and zeta potential of the protein changes at different pH values. You are expected:

- To measure the size and zeta potential of BSA at different pH values.
- To investigate the relation of size and zeta potential with pH considering the isoelectric point of BSA,
- To determine the zeta potential of BSA from zeta potential measurements at different pH values.
- To provide a written report for experimental process.

Pre-Experiment Questions

1. What is the Brownian motion?
2. What is the dynamic light scattering?
3. What are the intensity and volume distributions obtained from dynamic light scattering measurements?
4. What is the Doppler shift?
5. What is the electrophoretic light scattering?
6. What is the zeta potential?
7. What I know about the protein denaturation?
8. What is the isoelectric point of BSA?

Experimental Procedure

Malvern Zetasizer Nano ZS apparatus will be used in the experiment. Instrument equipped with 4.0mW He-Ne laser (632,8nm) and detector at 173° for the measurement of backscattered light. Measurements will be carried out at 25 ± 0.1 °C using viscosity, refractive index and dielectric constant values provided in the software for water as dispersant.

In the experiment, the following operations will be performed step by step for size, zeta potential and melting point measurements.

1. Turn-on the device and allow the laser to be stabilized.
2. The BSA solutions are prepared in phosphate buffer at pH 4.0, 4.5, 5.0 and 8.5.
3. The samples are filtered through 0,45μm filter and put into the capillary cuvette.
4. The cuvette is placed in device and the measurement parameters are entered.
5. The size and zeta potential measurements are performed, respectively.
6. After the measurement's cuvettes are cleaned with distilled water.

Final Report Should Involve

1. Write the size values which measured at different pH as a table.
2. Write the zeta potential values which measured at different pH as a table.
3. Discuss the effect of pH on size and zeta potential considering the isoelectric point of BSA. Determine the isoelectric point of BSA experimentally.
4. Which parameters that can affect the size and the zeta potential of proteins in solution?
5. Compare the experimental isoelectric point with literature values. Discuss the causes of experimental error and how can be fixed.

EXPERIMENT 5: BAKER'S YEAST PRODUCTION IN BIOREACTOR

5.1. Aim of the experiment

The aim of this experiment is to produce *Saccharomyces cerevisiae* in batch mode of a 5 L bioreactor in a closed system, to create a growth curve with the experimental data by taking samples during the process and to observe the growth stages.

5.2. Theoretical Information

A bioreactor is a tank in which organisms (bacteria, fungi, yeasts, etc.) or for the production of products are grown by controlled methods or in some special cases specific reactions are carried out (Figure 9). Bioreactors have an important role in the enzymatic or microbial processing of proteins, organic acids, amino acids, antibiotics. These processes can be either aerobic or anaerobic. The main goal of bioreactors is to provide a suitable environment for the production of the desired product in the most efficient and high quality manner. The bioreactor´s environmental condition like gas (oxygen, nitrogen, carbon dioxide) and liquid flow rates, temperature, pH, concentration of substrate and products, cells number and their composition (proteins and nucleic acids), dissolved oxygen levels, and agitation need to be closely and continuously monitored and controlled.

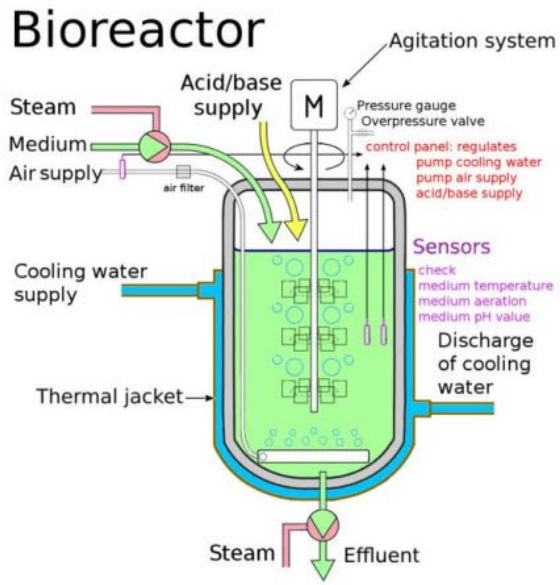


Figure 9. Scheme of the Bioreactor

A biotechnological process can be examined in 3 main parts: preparation of raw materials and upstream processing, fermentation or biological reaction (cultivation) and purification (downstream processing). The general flow diagram of a biotechnological process is given in figure 10. The most important step of the diagram is the production of microorganisms in bioreactors.

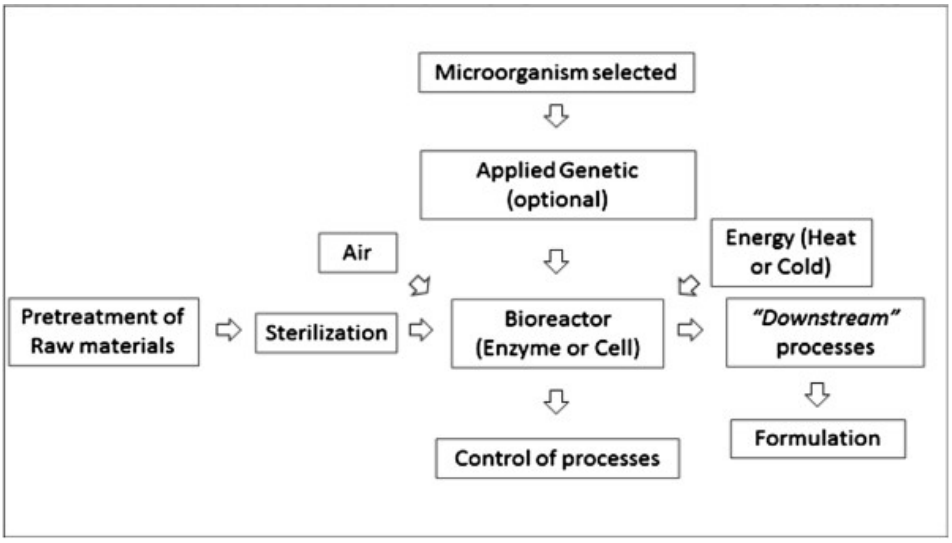


Figure 10. General Flow Chart of the Biotechnological Production Process

5.2.1. Operating Modes of Bioreactors

Biotechnological processes can be classified in various ways depending on whether the medium is fed into the reactor and whether or not oxygen is supplied to the system. Depending on the flow of the medium, bioreactors are divided into 3 groups: **batch, fed-batch and continuous** (Figure 11).

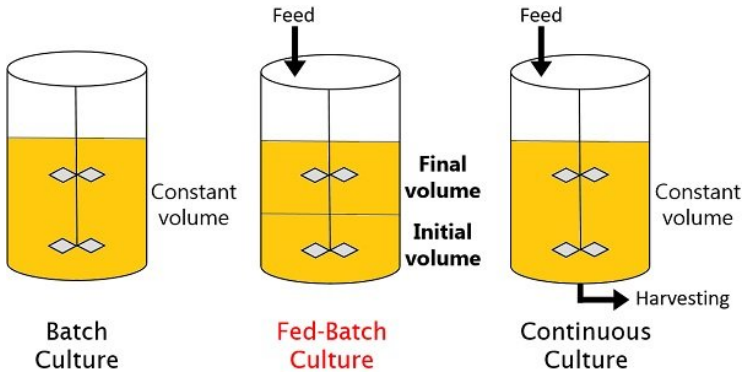


Figure 11: Operation modes of bioreactors

5.2.1.1. Batch Processes

In batch processes, there is no medium that enters the system and leaves the system. All of the substrates are initially put into the reactor. After inoculation with a small amount of biomass, cells continue to multiply until one of the substrates necessary for growth is depleted or until the inhibitory products stop growing. Batch reactors are used in small-scale processes, to test new processes that are not fully developed, to manufacture very expensive products, and in processes that are difficult to transform with continuous processes. In addition, these reactors can be used where high substrate concentrations do not adversely affect the desired biological reaction and the amount of product will be low. The batch reactor has a high conversion advantage, which is achieved by keeping the input in the reactor for a long time, and less effort

is required for process control. However, it also has disadvantages such as high labor costs, product and the difficulty of large-scale production.

5.2.1.2. Fed-Batch Processes

The medium is fed to the reactor continuously, but it is not removed from medium. That is, the volume of liquid in the reactor constantly increases. The fed-batch process is used where the substrate concentration must be low in the medium. The biomass concentration increases continuously and the rate of the biological reaction is controlled by the feed rate as opposed to the batch process.

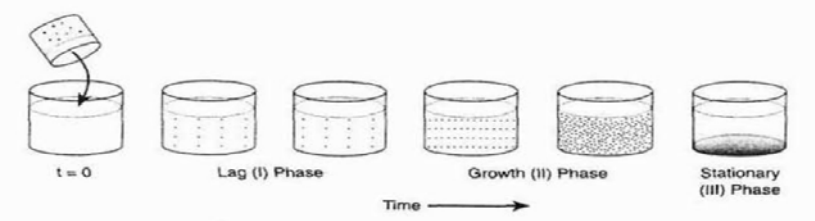
5.2.1.3. Continuous Processes

The medium is fed into the reactor continuously and is usually removed at the same rate. Similar to the fed-batch process, the biological reaction rate is controlled by the feed rate. There are different continuous processes depending on how the feed rate is controlled. Most importantly, the chemostat is operated at a constant input feed rate. On the other hand, the turbidostat is controlled by the inlet feed rate and the outlet cell concentration. pH-auxostat is the control variable pH. The advantage of the continuous process is that the productivity is usually higher than the others. But in practice, batch or fed-batch processes are more preferred. This is because efficiency alone is not a determining factor in process selection and other factors must also be evaluated.

5.2.2. Microbial Growth Kinetics

In bioprocesses, fermentations are carried out by batch, fed-batch or continuous methods.

In **batch cultures**, the fermentation medium is prepared and the microorganism is inoculated. After the pH, temperature and other values of the system are adjusted, no new substrate or microorganism is added to the medium. Fermentation is terminated until the nutrient elements in the medium are exhausted or according to the observed changes in conditions. During the fermentation, no substance is added to the fermenter except for oxygen, antifoam and acids and bases added for pH adjustment. The culture medium, biomass, and metabolite concentration are constantly changing as a result of cell metabolism. After inoculation into sterile medium in batch fermentation, four basic growth phases are observed in the microorganism under appropriate conditions (Figure 12).



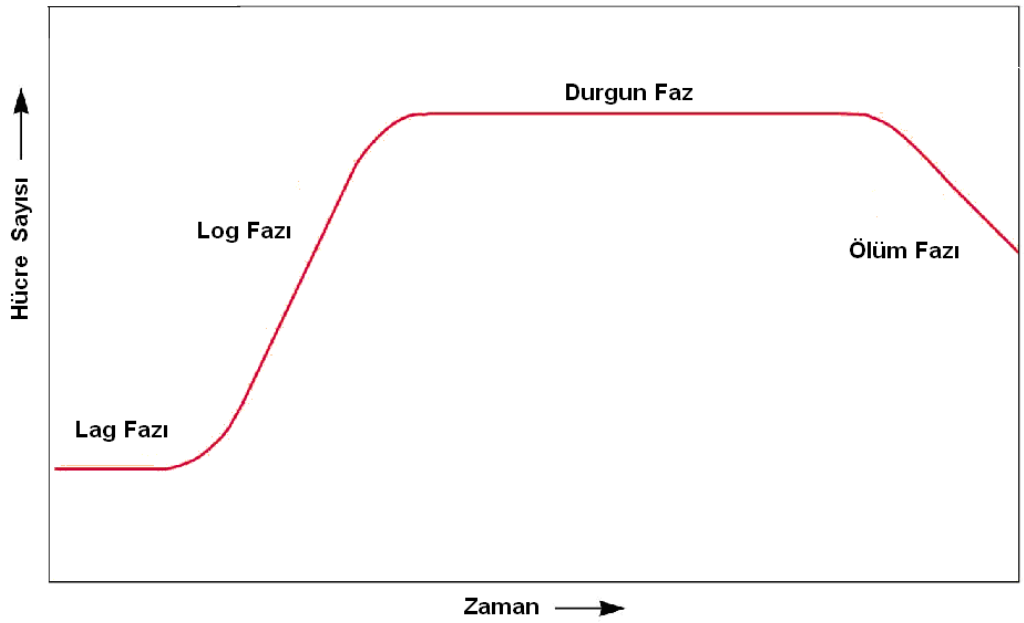


Figure 12. Growth curve in batch culture

1. Lag phase

In the acclimation phase, called the lag phase, there is little increase in cell concentration. During the lag phase, cells adjust themselves to new medium. During this time, cells perform functions such as synthesizing the transport proteins required for the substrate to enter the cell, synthesizing the enzymes needed to use the new substrate, and copying the genetic material of the cells. The length of time of the lag phase depends on which phase the cells were in before the inoculum. Depending on the difference between the inoculum medium of the cells and the fermentation medium, this period may be long or short.

2. Exponential or Logarithmic (Log) phase

At the end of the lag phase, the cell begins to reproduce rapidly as it has adapted to the new culture medium. Cell mass increases rapidly at this stage. While the cell number increases logarithmically, the specific growth rate of the culture remains constant. The log phase is called the proliferation or exponential phase because the cell growth rate is proportional to the cell concentration. In this phase, the cells divide at maximum speed, since the pathway of all enzymes required for metabolism is in the nutrients (as a result of the acclimatization phase) and the cells will use the nutrients in the most efficient way.

3. Stationary phase

The stationary phase is the stable phase. During the stationary phase, the net growth rate is zero as a result of depletion of nutrients or important metabolites. No net growth is observed in this phase, the amount of biomass remains constant. Many important fermentation products, including most antibiotics, are produced in the stationary phase. For example, commercially

produced penicillin using the *Penicillium chryogenurn* is also slowed down by the action of organic acids and toxic substances that occur during cell proliferation.

4. Death phase

It is the period when the energy reserves of the cell are completely depleted and metabolic activity ceases. In business transactions, the death phase has no value. As soon as it reaches this stage, the fermentation is interrupted.

5.2.3. Growth Kinetics

The exponential growth phase (log phase) is indicated as the most important phase of growth kinetics when we want to produce the product and/or the biomass itself. Measuring the exponential growth rate (ie how fast the cells are growing) is the first fundamental step in measuring culture kinetics.

The nutrient ratios of the culture medium and pH, temperature, ionic strength etc. If the physicochemical conditions such as "dt" are suitable, the microorganism (biomass) increase in a certain and small time interval "dx"; It is proportional to the microorganism concentration (x) in the medium. This definition is represented by the following mathematical connections:

$$\mu = \frac{Ln\ X_2 - \mathrm{Ln}\ X_1}{t_2 - t_1}$$

*t_d= ln2/ μ

(μ=specific growth rate; X2=number of cells at the end of log phase; X1=number of cells at the beginning of log phase; t2-t1= log phase time; t_d=double time)

NOTE!! These equations for growth kinetics can only be applied during the log phase. In other words, the graph to be created for the calculation of the specific growth rate and doubling time should be drawn according to the log phase.

*Specific growth rate (μ) defines how fast cells multiply. A higher value of the specific growth rate means that the cells are growing faster. When cells do not grow, their specific growth rate is zero. During the log phase, the specific growth rate is relatively constant.

Saccharomyces cerevisiae is a eukaryotic unicellular microorganism known as baker's yeast that grows by budding. Yeast cells are circular, sized between 2-3 μm and 20-50 μm. 1 gram of wet yeast contains nearly 10 billion cells. There are 600 species of yeast however, only a few of them have commercial value.

Pre-Experiment Questions:

- ❖ What are the features and working principles that a bioreactor should have?
- ❖ What are the main elements that make up the bioreactor?
- ❖ What is inoculation, inoculum, cultivation and fermentation?
- ❖ In what ways do microorganisms reproduce?
- ❖ What method should be followed if microorganisms are to be grown under laboratory conditions?
- ❖ What kind of microorganism is Saccharomyces cerevisiae, what are its characteristics?

Preliminary study:

- *Saccharomyces cerevisiae* is cultured on agar plates and incubated for 48 h at 30˚C.
- It is passed from agar medium to 10 mL liquid medium by sowing a single colony (mini-prep). It is left in a 30°C shaker incubator for 48 hours.
- The volume of broth used in the scale-up process should be 10% of the large scale.
- At the end of the period, 10 mL of culture is transferred to 100 mL sterile medium and left in a 30°C shaker incubator for 48 hours.

The experiment:

- At the end of the period, the entire 100 mL medium is transferred to the large-scale medium (bioreactor).
- When inoculating in the bioreactor (t = 0, control) sampling is started and sampling is continued at regular intervals within 48 hours of growth.
- In the sampling process, the discharge valve of the device is closed, a few mL of liquid medium is transferred to a test tube by using the sampling apparatus.
- The absorbance of the samples will be analyzed spectrophotometrically at 600 nm. Sterile broth will be used as blank. The growth curve of the microorganism will be drawn by using the OD_{600} data.

Final Report Should Involve:

- Determine the time for every sample taken from the beginning of the sampling.
- Record the OD_{600} data and draw a graph of absorbance versus time.
- Indicate growth phases of the microorganism on the graph.
- Determine the log phase.
- Calculate the specific growth rate (μ) and doubling time (t_d).
- Write what needs to be done to accelerate the transition from the lag phase to the logarithmic phase.