
**YILDIZ TECHNICAL UNIVERSITY**

**FACULTY OF CHEMICAL AND METALLURGICAL ENGINEERING**

**BIOENGINEERING DEPARTMENT**

# BIOENGINEERING LABORATORY- II

# BYM4242

# LABORATORY BOOKLET

**Course Coordinator**
**Prof. Dr. Mehmet Burçin PİŞKİN**

**Laboratory Coordinator**
**Res. Assist. Başak AKIN**

**Istanbul, 2023/2024**

# 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: 42826343
# Class Enrollment Key: BYM4242GR2

- Upload your reports to the Turnitin system by **23:59 on Thursday** 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 **ENG/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!**
- In the Turnitin plagiarism rate section of the report evaluation form;
  - Students with a plagiarism rate of over 40% will receive 0 points.
  - Students whose plagiarism rate is between 30-40% will receive 50 points.
  - Students whose plagiarism rate is between 20-30% will receive 75 points.
  - Students whose plagiarism rate is below 20% will receive 100 points
- There is no right to revise the Turnitin report. 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 Friday 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: 40%** | **Experiment Reports** | **20%** |
| | **Pre-quiz** | **10%** |
| | **Post-quiz** | **10%** |
| | **Design Experiment Booklet** | **10%** |
| | **Design Experiment Reports** | **10%** |
| | **Presentation** | **20%** |
| | **Team Member and Team Leader Evaluation** | **20%** |

**Midterm Exam: 20%**

**Final exam: 40%**

- There will be two experiments and one design experiment during the semester. Participation in all experiments is **mandatory**.
- Students who arrive to the experiment more than 10 minutes late will not be admitted to the pre-quiz. Students who get less than 50 points in the pre-quiz will not be able to participate in the experiment. As with participation in the experiment, those who do not participate in the pre-quiz for the second time or who do not receive the sufficient grade will be deemed not to have participated in the experiment and will fail the course. Experiment results and calculations will be prepared in detail 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 who do not contribute to the report will not receive a grade from the report, and they will not be allowed to take the post-quiz during the report submission. Therefore, they will be graded zero points from this experiment report and post-quiz.
- Students who did not take the post-quiz will be graded zero regardless of their contribution to the report.
- Students who get <50 points in the post-quiz have only 1 make-up chance for each experiment.
- Each student has the right to participate in one make-up experiment. Students who do not participate in one experiment fail the course.
- Students are responsible for every step of the experiment and anything that is included in the reports can be asked during post-quiz.
- **Students who cannot participate in the experiment with a valid excuse must contact the laboratory coordinator.**

**Team Member - Team Leader Evaluation Surveys**

- For each experiment, two students from the group will act as the group leader. Group leaders will be responsible for tasks such as conducting the experiment, giving and receiving instructions, distribution of tasks in the experiment report, report preparation and assembling the final version of the report during that week. Group members will evaluate these qualifications of the team leaders through the online 'Group Members' Team Leader Evaluation Form', which will be shared accordingly. At the same time, the team leader will evaluate the group members one by one, in terms of giving and receiving instructions, working within the team, and contributing to the report, through the online Team Leader's Group Members Evaluation Form. Group leaders are assigned to following tasks:

| | |
|---|---|
| **Task 1** | Experiment 1 (Report) |
| **Task 2** | Experiment 2 (Report) |
| **Task 3** | Design Experiment (Report) |
| **Task 4** | Design Experiment (Booklet) |
| **Task 5** | Design Experiment (Presentation) |

● Each student will serve as a team leader at least once during the semester.

*** Students who cannot participate in the experiment with a valid excuse must contact the laboratory coordinator.

# LABORATORY CALENDAR

| WEEK | DATE | TOPICS |
|---|---|---|
| 1 | February 23 | Meeting with Students, Introduction to course / Presentation of Standards for Engineering Applications |
| 2 | March 1 | Experiment 1 (Group 1, Group 6)<br>Experiment 2 (Group 2, Group 5) |
| 3 | March 8 | Experiment 1 (Group 3, Group 8)<br>Experiment 2 (Group 4, Group 7) |
| | | Final Report of Experiments/Post-quiz |
| 4 | March 15 | Experiment 1 (Group 5, Group 2)<br>Experiment 2 (Group 6, Group 1) |
| | | Final Report of Experiments/ Post-quiz |
| 5 | March 22 | Experiment 1 (Group 7, Group 4)<br>Experiment 2 (Group 8, Group 3) |
| | | Final Report of Experiments/ Post-quiz |
| 6 | March 29 | Make-Up Experiments |
| | | Final Report of Experiments/ Post-quiz |
| 7 | April 5 | **Submission of Design Experiment Booklet** |
| | | Final Report of Make-Up Experiment/Post-quiz |
| 8 | April 12 | **HOLIDAY WEEK** |
| 9 | April 19 | **MIDTERM WEEK** |
| | | **Standardization Lecture** |
| 10 | April 26 | Design Experiments |
| 11 | May 3 | Design Experiments |
| 12 | May 10 | Design Experiments |
| 13 | May 17 | **Deadline for Design Experiment Report Submission** |
| 14 | May 24 | **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. Assist. Nurseda SÜRGİT | nursedasurgit@gmail.com |
| 2 | Baker's yeast production in bioreactor | 09.30 | Res. Assist. Beyza KARACAOĞLU | beyzak@yildiz.edu.tr |
| - | Design Group 1 | - | Res. Assist. Ayça ASLAN | aslaanayca@gmail.com |
| - | Design Group 2 | - | Res. Assist. Selcan AKAR | seakar@yildiz.edu.tr |
| - | Design Group 3 | - | Res. Assist. Elif Nur YILDIZ | nur.yildiz@yildiz.edu.tr |
| - | Design Group 4 | - | Arş. Gör. Gülcan KARACA | gakaraca@yildiz.edu.tr |
| - | Design Group 5 | - | Res. Assist. Başak AKIN **(Lab Coordinator)** | basak.akin@yildiz.edu.tr |
| - | Design Group 6 | - | Res. Assist. Nurseda SÜRGIT | nursedasurgit@gmail.com |
| - | Design Group 7 | - | Res. Assist. Zişan TOPRAK | zisantoprakytu@gmail.com |
| - | Design Group 8 | - | Res. Assist. Muhammet ÇELİK | mcelik@yildiz.edu.tr |

# DESIGN EXPERIMENT

| Week | Work |
|---|---|
| 4 | Determination of the problem and subject by the student |
| 5 | Approval of the subject by the responsible research assistant |
| 7 | Submission of the booklet |
| 10-12 | Performing the Design Experiment |
| 13 | Submission of the Final Report of Design Experiments |
| 14 | Presentations |

- A and B groups will decide on the subject together. A and B groups must have the same subject; however, **the experiments will be done separately**.
- 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 need to add 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 have 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

| Gr. A1 | | |
|---|---|---|
| Student Number | Name - Surname | Leader |
| Ç1905B045 | EYLÜL YILMAZ | Task 1 |
| 1805A040 | ŞEHRİBAN ASLANCI | Task 2 |
| 1905A001 | EDA ARSLAN | Task 3 |
| 1905A004 | CANSU KATIKSIZ | Task 3 |
| 19056908 | EMEL BOSTANI | Task 4 |
| 19056906 | BELAL ABDULLAH | Task 5 |

| Gr. A2 | | |
|---|---|---|
| Student Number | Name - Surname | Leader |
| 1905A017 | İREM KOÇHAN | Task 1 |
| 1905A021 | AYLİN AKMAN | Task 2 |
| 1905A024 | ONUR AKINCILAR | Task 3 |
| 1905A026 | EKİN BERK KAYA | Task 3 |
| 1905A028 | YAĞIZ KARAKAŞ | Task 4 |
| 1905A029 | SILA ERCAN | Task 5 |

| Gr. A3 | | |
|---|---|---|
| Student Number | Name - Surname | Leader |
| 1905A044 | OZAN AYDIN | Task 1 |
| 1905A045 | HACER SÜLÜN | Task 2 |
| 1905A046 | FATMA ŞAFAK İŞEN | Task 3 |
| 1905A047 | EKİN HAZAL AYANOĞLU | Task 3 |
| 1905A052 | REFİYE İCLAL MERİÇ | Task 4 |
| 1905A053 | DOĞA ZEREN | Task 5 |

| Gr. A4 | | |
|---|---|---|
| Student Number | Name - Surname | Leader |
| 1905A062 | YUSUF CAN SAİN | Task 1 |
| 1905A063 | OZAN ORKUN KABA | Task 2 |
| 1905A702 | OĞUZHAN KARAĞÖZ | Task 3 |
| 1905A909 | CHILANAY RZASOYLU | Task 3 |
| 1905A911 | FURUZA ALIYEVA | Task 4 |
| 1905A915 | SAFİYE AHDAB | Task 5 |

| Gr. A5 | | |
|---|---|---|
| Student Number | Name - Surname | Leader |
| 1905A933 | AYAT AMER | Task 1 |
| 2005A002 | ELİF OCAK | Task 1 |
| 2005A008 | HATİCE ŞEYMA BAŞARAN | Task 2 |
| 2005A013 | ELİFNAZ ERTÜREN | Task 2 |
| 2005A014 | NURGÜL FİLİZ | Task 3 |
| 2005A015 | GÖRKEM ONUR YILDIRIM | Task 4 |
| 2105A046 | SENANUR KİRİŞCİ | Task 5 |

| Gr. A6 | | |
|---|---|---|
| Student Number | Name - Surname | Leader |
| 2005A030 | YAĞMUR ZEYNEP DEMİR | Task 1 |
| 2005A031 | FURKAN CANLI | Task 1 |
| 2005A034 | BEYZA GÜNEY | Task 2 |
| 2005A037 | ELÇİN DÖNGEL | Task 2 |
| 2005A038 | İREM BAYRAK | Task 3 |
| 2005A043 | YAREN YÜCEL | Task 4 |
| 2105A011 | MELİKE DÜNDAR | Task 5 |

| Gr. A7 | | |
|---|---|---|
| Student Number | Name - Surname | Leader |
| 2005A901 | NAYA MOKAYED | Task 1 |
| 2005A903 | TUQA HLOUBI | Task 1 |
| 2005A904 | KARDELEN ÇAVUŞ | Task 2 |
| 2005A906 | RAWAN MOHAMED YASINOUMER | Task 2 |
| 1905A007 | RABİA NUR KAYA | Task 3 |
| 1905A927 | AHMED VELİT ABUDEN | Task 4 |
| 1905A008 | ELİF YILDIZ KÜÇÜKTEPE | Task 5 |

| Gr. A8 | | |
|---|---|---|
| Student Number | Name - Surname | Leader |
| 2005A926 | İREM GÜLBEYAZ TUNÇER | Task 1 |
| 2005A927 | GÖKÇE MELEK YILMAZ | Task 1 |
| 2005A928 | YOUSSEF AALAOU | Task 2 |
| 2005A930 | MARIAM ELADAVY | Task 2 |
| 2005A934 | YOUSSEF KHANTOMANI | Task 3 |
| 2005A935 | BUŞRA İDRİS | Task 4 |
| 2005A936 | HUSSIN ALBAGHDADI | Task 5 |

| Gr. B1 | | |
|---|---|---|
| Student Number | Name - Surname | Leader |
| 1905A009 | ŞİMAL GEDİK | Task 1 |
| 1905A010 | ASLIHAN EK | Task 2 |
| 1905A012 | MERVE AĞAY | Task 3 |
| 1905A013 | CEREN KALENDER | Task 3 |
| 1905A014 | AZRA NUR ERDEN | Task 4 |
| 1905A015 | ŞİMAL USTAOĞLU | Task 5 |

| Gr. B2 | | |
|---|---|---|
| Student Number | Name - Surname | Leader |
| 1905A030 | DOĞA GEZER | Task 1 |
| 1905A031 | SERHAT ZEREN | Task 2 |
| 1905A035 | YAREN FİDAN | Task 3 |
| 1905A037 | AYŞENUR AVCI | Task 3 |
| 1905A041 | YAĞMUR MAZMANOĞLU | Task 4 |
| 1905A043 | AYNUR ÖZ | Task 5 |

| Gr. B3 | | |
|---|---|---|
| Student Number | Name - Surname | Leader |
| 1905A054 | İREM NUR KOÇ | Task 1 |
| 1905A056 | FATMA ECEM BAN | Task 2 |
| 1905A058 | ECE BERFİN GEZGİNCİ | Task 3 |
| 1905A059 | ZEHRANUR BEYAZKAYA | Task 3 |
| 1905A060 | GÖKÇE NUR GÜLAÇ | Task 4 |
| 1905A061 | BEYZA ÇELEBİ | Task 5 |

| Gr. B4 | | |
|---|---|---|
| Student Number | Name - Surname | Leader |
| 20056070 | İREM DEMİRBAŞ | Task 1 |
| 1905A928 | AYAH AASHOUR | Task 2 |
| 1905A929 | HEBA IBRAHIM | Task 3 |
| 1905A930 | LAVA TASH | Task 3 |
| 1905A931 | AYSU YILMAZ | Task 4 |
| 1905A932 | BAYAN ALSHAYEB | Task 5 |

| Gr. B5 | | |
|---|---|---|
| Student Number | Name - Surname | Leader |
| 2005A018 | KEMAL TAHSİN MENTEŞE | Task 1 |
| 2005A019 | KAAN DANIŞ | Task 1 |
| 2005A022 | İSHAK GÜRZ GÜLEÇYÜZ | Task 2 |
| 2005A023 | NUR AĞAOĞLU | Task 2 |
| 2005A024 | BERİL ÖZDEN | Task 3 |
| 2005A025 | ALEYNA KÖSE | Task 4 |
| | EDANUR TAÇKİN | Task 5 |

| Gr. B6 | | |
|---|---|---|
| Student Number | Name - Surname | Leader |
| 2005A049 | FURKAN OĞUZHAN TEKCAN | Task 1 |
| 2005A051 | MELİKE NUR ALPTEKİN | Task 1 |
| 2005A057 | ESRA ÖZDEMİR | Task 2 |
| 2005A059 | ABDULLAH ÖZTÜRK | Task 2 |
| 2005A605 | BESTE DAYIOSMAN | Task 3 |
| 2005A607 | ZEHRA KILIÇ | Task 4 |
| 2005A912 | ABDULRAHMAN SALİHOĞLU | Task 5 |

| Gr. B7 | | |
|---|---|---|
| Student Number | Name - Surname | Leader |
| 2005A915 | HAMZA YILDIZ | Task 1 |
| 2005A916 | NANCY HASSAN FATHY ABDELATTY | Task 1 |
| 2005A919 | LANA ALKASIRI | Task 2 |
| 2005A921 | SEYED YOUSEF SEYED AHMAD LAVASANI | Task 2 |
| 2005A922 | AHMAD SAQIB QAEYM | Task 3 |
| 2005A923 | RAHMAH HAMEED MUHI OBADA | Task 4 |
| 2005A911 | HAGAR YASER AHMED NASSER | Task 5 |

| Gr. B8 | | |
|---|---|---|
| Student Number | Name - Surname | Leader |
| 2005A937 | ALAA ABDELVAHED | Task 1 |
| 2105A031 | ALPTEKİN KAHYA | Task 1 |
| 2105A602 | BARKIN BALCI | Task 2 |
| 2105A603 | AYŞİM ÇİSEN AKYOL | Task 2 |
| 2105A917 | ZAKARYA AHMED MOHAMMED ZABARA | Task 3 |
| 2105A929 | LUJAIN SHABAN | Task 4 |
| 2205A603 | ALEYNA ESMA BİLGİN | Task 5 |

## 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).

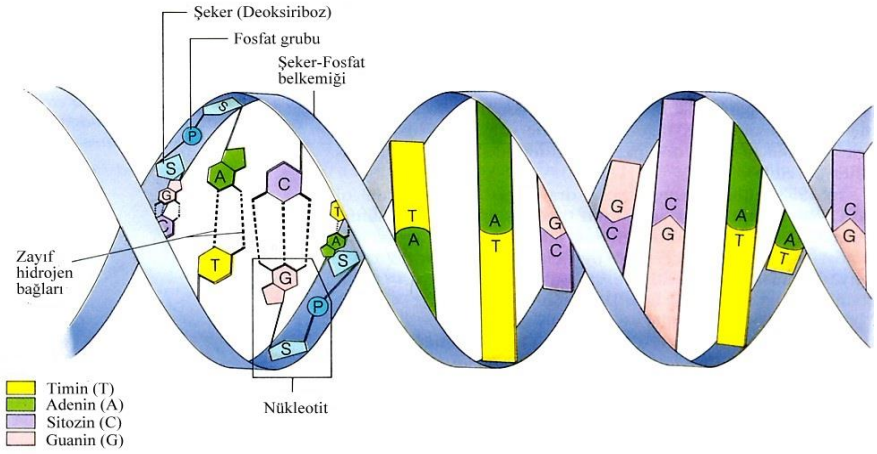



**Figure 1. Schematic representation of DNA structure**

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.

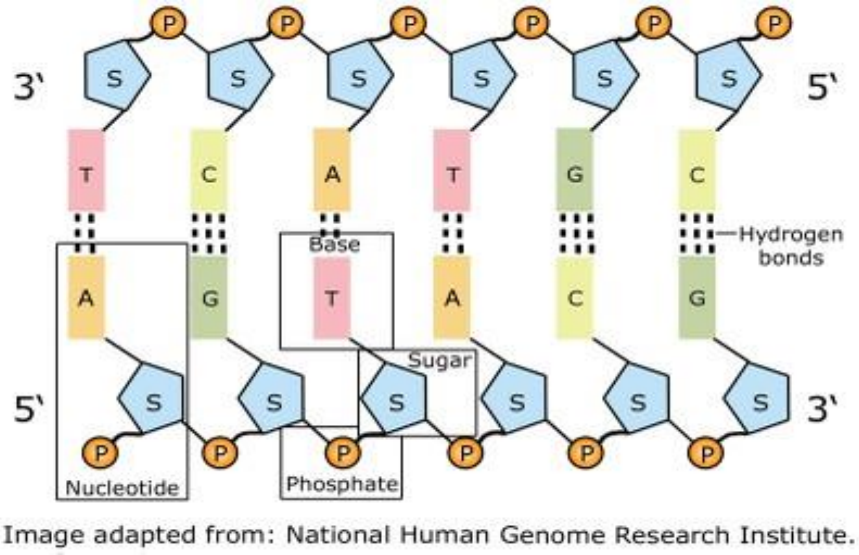



**Figure 2. The illustration of DNA molecule** (http://www2.le.ac.uk/departments/genetics/vgec/schoolscolleges/topics/dna-genes-chromosomes)

## 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 glucose
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: BAKER'S YEAST PRODUCTION IN BIOREACTOR

## 1.1. 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.

## 1.2. 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.

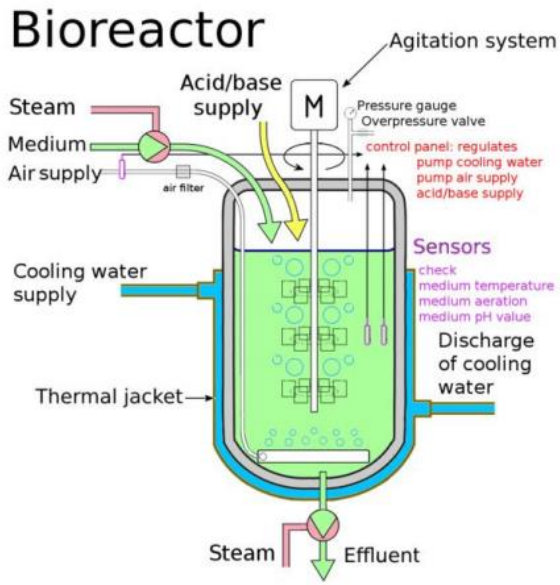



**Figure 9. Scheme of the Bioreactor**

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.

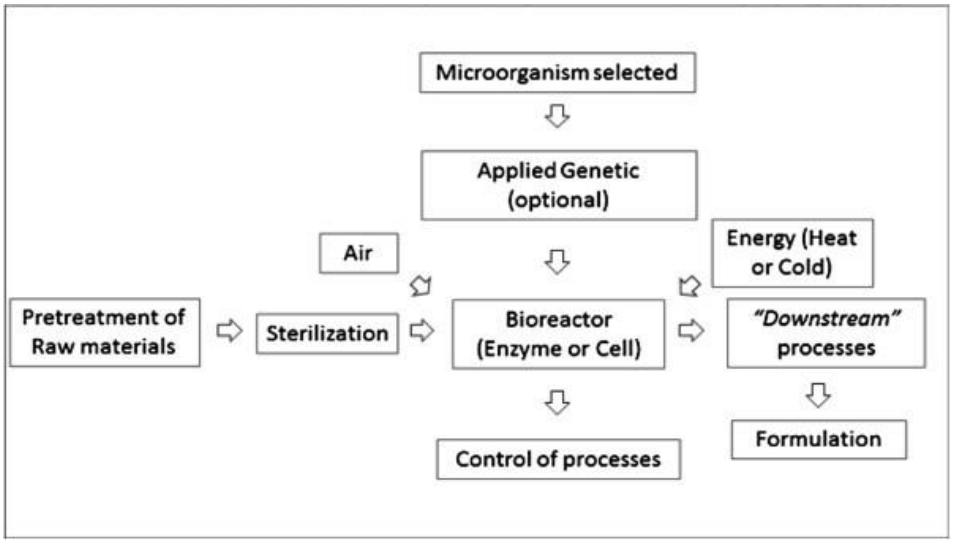



**Figure 10. General Flow Chart of the Biotechnological Production Process**

### 1.2.1. 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).

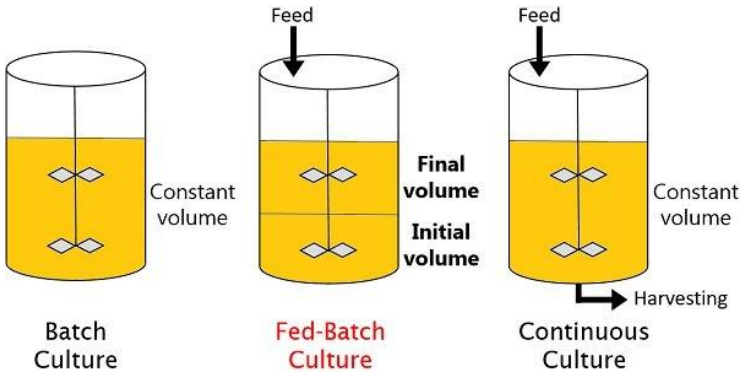



**Figure 11: Operation modes of bioreactors**

#### 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.

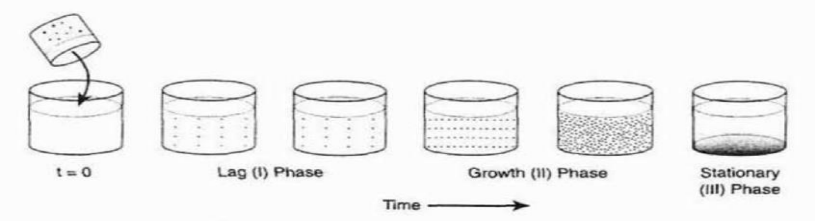
#### 5.2.1.3. Continuous Processes

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.

### 1.2.2. 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).



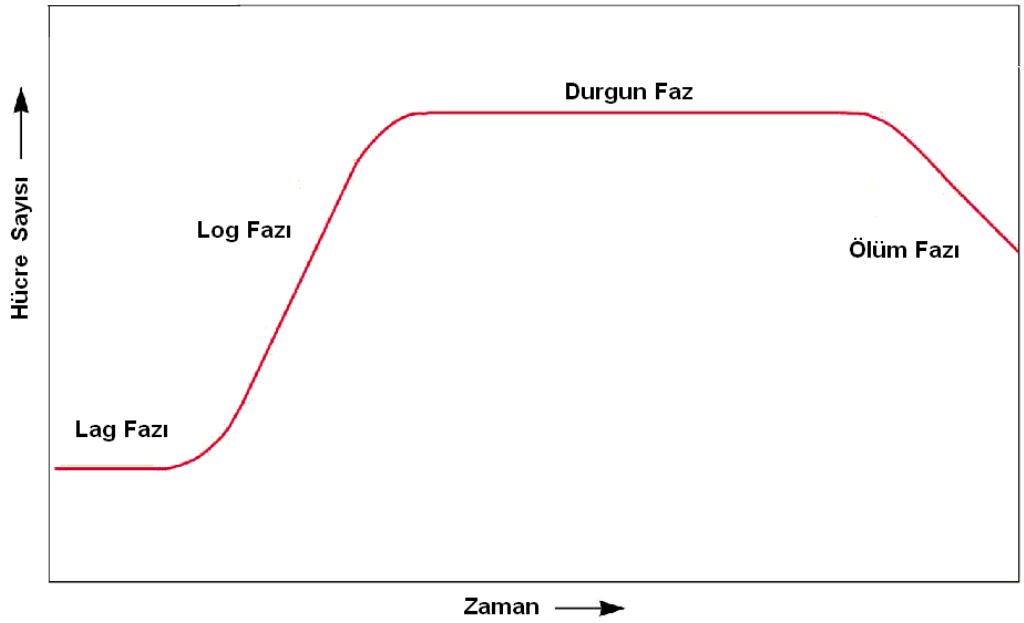



**Figure 12. Growth curve in batch culture**

## 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.

### 1.2.3. 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 ($\mu$) and doubling time ($t_d$).
- Write what needs to be done to accelerate the transition from the lag phase to the logarithmic phase.