UNIVERSITY OF MARIBOR
FACULTY OF CHEMISTRY AND CHEMICAL ENGINEERING
MARIBOR

Marko Tramšek, Andreja Goršek

Bioreaction engineering

Laboratory exercises manual

Translator: Darja Pečar
Proofreader: Victor Kennedy

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FOREWORD

Bioreaction technique is an integral part of biochemical technique, integrating knowledge of microbiology, biochemistry, genetics and chemical engineering. By definition, a bioreactor system is a system in which biological conversion expels, under the influence of enzymes, microorganisms or animal and enzyme cells. When designing bioreactors, it is essential to take into account biological and technical principles. Living organisms are more sensitive and less stable than conventional chemicals; therefore, a bioreaction system must contain high quality measuring and regulation techniques. Two areas of biochemical reactor design are important. First, it must contain a selection of appropriate reaction parameters for the desired biological, chemical and physical system, with emphasis on microbial growth and conversion. The second area comprises bioreaction parameters: temperature, optimal pH, nutrient content, salts, vitamins, oxygen, optimal mixing, product separation, and prevention of contamination by unwanted organisms.

These are the first instructions for bioreaction engineering laboratory exercises. Biochemical engineering at the Faculty of Chemistry and Chemical Technology in Maribor was introduced in the academic year 2001/2002, when we offered the first generation of this course in the 4th study year. Bioreaction engineering is a very large area, which is impossible to present entirely within the existing program. In addition, in the selection of exercises, we are limited by the existing laboratory equipment. In the coming years we intend to supplement it. In carrying out these exercises, student will come to understand the concepts of fermentation and biological treatment of wastewater. Without understanding these processes, the exploitation of microorganisms for the production of useful products was mastered in ancient cultures. They knew how to make yogurt, cheese, soy sauce, sour cabbage, beer, etc. In the 19^{th} century, the father of modern fermentation technology, Louis Pasteur, scientifically explained the planned conversion of sugar to alcohol and sugar to lactic acid in various products (wine, beer, milk) by using a substrate. Today, fermentation is known to be a biochemical process in which large organic molecules (mostly saccharides) are partially degraded to smaller organic ones. Fermentation is carried out using enzymes produced by yeasts, molds or bacteria. It appears in nature and in industry. The volumetric mass transfer coefficient is of great importance for the design of bioprocesses. In the first exercise, you will determine this coefficient; first in the water

system and later during the fermentation process. Students will find out which process parameters affect its value.

The other two exercises belong to the field of wastewater treatment. Wastewater was known in ancient Rome, but a systematic approach to the problem was not observed until the mid-19th century. At that time, in Europe there was an epidemic of water borne diseases that originated from water pollution. This led to the development of wastewater treatment processes in the beginning of the 20th century, and resulted in setting the first standards for purified water. Today, the problem of sewage is known everywhere in the world. In order to prevent the deaths of fish and other living creatures in the surface waters and sea, wastewater from sewage systems is treated in cleaning devices. During the exercises, students will learn about the anaerobic and aerobic processes of wastewater treatment and their advantages and disadvantages. In both cases, microorganisms, in the presence or absence of oxygen, are fed with organic sewage. You will determine the chemical and biochemical oxygen demand that are measures for organic pollution in waters. Therefore, students will simulate the operation of biological wastewater treatment plants in laboratory reactors.

As future biochemical technologists, students will be able to successfully apply theoretical basics, performance and results of the exercises to industrial practice.

prof. dr. Andreja Goršek

Contents

SYMBOLS

a - area density, $\mathrm{m^2/m^3}$

A_1 - area below the curve $(1-\overline{\gamma}_\mathrm{A})$ regarding time, for medium, s

A_{1E} - area below the curve $(1-\overline{\gamma}_\mathrm{A})$ regarding time, for medium in the absence of yeast, s

c - concentration, mol/L

$\overline{e}$ - coefficient of linear dependence, 1

f - stirrer speed, $\mathrm{min^{-1}}$

f_d - dilution factor, 1

F - temperature correction factor at experimental temperature, 1

$\overline{g}$ - coefficient of linear dependence, 1

H - considered factor (*Re*, f, q_v, V), /

k_L - liquid phase mass transfer coefficient, $\mathrm{m^3/(m^2\ s)}$

$k_\mathrm{L}a$ - oxygen volumetric mass transfer coefficient of, $\mathrm{s^{-1}}$

COD - chemical oxygen demand, /

$\mathrm{BOD_5}$ - five days biochemical oxygen demand, /

q_{10} - mass flow rate of absorbed oxygen, g/h

q_v - volume flow rate, L/h

q_{va} - air volume flow rate, L/h

$Q_{\mathrm{O_2}}$ - rate of oxygen consumption, $\mathrm{m^3/(m^3\ s)}$

m - mass, g

r_biogas - biogas production rate, L/g

r'_COD - mass rate of oxygen chemical consumption, $\mathrm{kg/(kg\ d)}$

r_COD - volume rate of oxygen chemical consumption, $\mathrm{kg/(m^3\ d)}$

$r'_\mathrm{COD,\ dm}$ - mass rate of oxygen chemical consumption to mass of dry matter, $\mathrm{kg/(kg\ d)}$

Re - Reynolds number, 1

T - temperature, K

t - time, s

V - volume of medium, L

V_v - volume of sample that is being filtered, L

γ_A - mass concentration of oxygen at time *t*, g/L

γ_A^* - equilibrium mass concentration of absorbed oxygen, g/L

$\gamma_{A,10}^*$ - equilibrium mass concentration of absorbed oxygen at 10°C, g/L

γ_X - mass concentration of biomass, g/L

$\overline{\gamma_A}$ - dimensional concentration of oxygen, 1

γ_{yeast} - mass concentration of baker's yeast, g/L

γ_{COD} - mass concentration of COD, mg/L

γ_{BOD_5} - mass concentration regarding five days biochemical oxygen demand, g/L

γ_{dm} - mass concentration of suspended dry matter, mg/L

γ_m - mass concentration regarding BOD_5 value of diluted sample on OXI TOP, mg/L

η_{COD} - wastewater treatment effectiveness regarding γ_{COD}, %

η_{BOD_5} - wastewater treatment effectiveness regarding γ_{BOD_5}, %

ϑ - temperature of medium, °C

τ_E - response time due to diffusion resistance of electrode, s

τ_F - response time due to liquid diffusion film, s

ξ - volume ratio, mL/L

1. DETERMINATION OF THE OXYGEN VOLUMETRIC MASS TRANSFER COEFFICIENT IN THE FERMENTATION PROCESS

1.1 INTRODUCTION

Absorption is a process in which one or more components are absorbed from the gas phase (dissolves or reacts) to the liquid phase. In this process, there is a mass transfer from gas to liquid phase. Interphase mass transfer is a process with mass transfer from one phase to another through the interphase surface, which separates them.

Transfer of gases or water has an important influence in the determination of water quality for industrial or home use purposes. It also plays a main factor in recycling wastewater into a clean harmless form. When determining water quality, we come across a term called solubility of oxygen. With aeration (ventilation), contact between the liquid phase and the air is formed and the speed of oxygen transfer is increased. The natural solubility of atmospheric oxygen in rivers and other forms of water has also a key influence on preserving aquatic life, especially fish, bacteria, and other microorganisms that clean water.

Because of their flexibility batch and aeration, fermenters are mostly used in pilot and industrial fermentation processes. Fermentation is a process where organic substances degrade because of aerobic or anaerobic activity of microorganisms, where oxygen is the key element, that assures energy for cell metabolism and is a key element in the synthesis of biomass and other products.

1.2 PURPOSE OF THE EXERCISE

1.2.1 Determination of oxygen volumetric mass transfer coefficient in water.

The purpose of this exercise is to determine the oxygen volumetric mass transfer coefficient, $k_L a$, and its dependence on stirrer speed, volume air flow rate, different liquid phases, different drafts for aeration and different volumes of water in the tank. These values will be determined from experimental data (concentration of dissolved oxygen over time).

1.2.2 Determination of oxygen volumetric mass transfer coefficient in the fermentation process.

The purpose of this exercise is to determine the oxygen volumetric mass transfer coefficient, $k_L a$, during the fermentation digestion of glucose using baking yeast at different stirrer speeds from the experimental data.

1.3 THEORETICAL PART

1.3.1 Determination of oxygen volumetric mass transfer coefficient in water

In the literature, many different mathematical models that describe oxygen transfer in liquid are found. The most often used is the model of two films, which assumes that near the interphase surface two layers of specific thickness are formed from the side of the liquid and gas phase, though which diffusion transfer of oxygen takes place. The result of this model is shown by equation 1.1, which determines the concentration of oxygen in liquid phase regarding time:

$$\frac{d\gamma_A}{dt} = k_L a \cdot \left(\gamma_A^* - \gamma_A\right) \qquad (1.1)$$

where: $k_L a$ – oxygen volumetric mass transfer coefficient , s^{-1}

a - surface density, m^2/m^3

k_L - liquid phase mass transfer coefficient (volume of medium to unit of contact surface and time), $\mathrm{m^3/(m^2\ s)}$

γ_A^* - equilibrium mass concentration of absorbed oxygen, g/L

γ_A - mass concentration of oxygen at time t, g/L

t - time, s

Equilibrium mass concentration of oxygen (in pure water) in dependence on temperature at atmospheric pressure 1.013 bar are shown in the table 1.1.

The capacity of the system, which corresponds to the mass of oxygen that is absorbed in one hour at $\vartheta = 10$ °C, is calculated according to the equation:

$$q_{10} = k_L a \cdot F \cdot \gamma^*_{A,\,10} \cdot V \qquad (1.2)$$

where: q_{10} - mass flow rate of absorbed oxygen, g/h

$k_L a$ - volume coefficient of oxygen transfer, $\mathrm{h^{-1}}$

$\gamma^*_{A,\,10}$ - equilibrium mass concentration of absorbed oxygen at 10 °C, g/L

V - volume of medium, L

F - temperature correction factor at experimental temperature, 1

The temperature correction factor at experimental temperature is determined based on linear interpolation from table 1.1.

Table 1.1: Equilibrium mass concentration of oxygen and temperature correction factor in dependence of temperature

$\frac{\vartheta}{°C}$	$\frac{\gamma_A^*}{g/L}$	$\frac{F}{1}$
0	14.6	1.292
2	13.8	1.221
4	13.1	1.159
6	12.5	1.106
8	11.9	1.053
10	11.3	1.000
12	10.8	0.956
14	10.4	0.921
16	10.0	0.885
18	9.5	0.841
20	9.2	0.814
22	8.8	0.778
24	8.5	0.752
26	8.2	0.726
28	7.9	0.699
30	7.6	0.673

Volume coefficient of oxygen transfer regarding Reynolds number (*Re*), stirrer speed (*f*), air volume flow rate (q_v) and volume of medium in reservoir (*V*) is shown as a linear dependency in the following form:

$$k_L a = \bar{e} + \bar{g} \cdot H \qquad (1.3)$$

where: *H* - considered factor (*Re*, f, q_v, V)

$\bar{e}$, $\bar{g}$ - coefficients of linear dependency

$k_L a$ - oxygen volumetric mass transfer coefficient, s^{-1}

1.3.1.1 Methods of determination of oxygen volumetric mass transfer coefficient in water

In the literature, there are many methods for determining the oxygen volumetric mass transfer coefficient, $k_L a$. With direct methods (method of gases balance, dynamic method, continuous-biological method) the composition is taken into account, while with indirect methods (integral method, method of electrical moment, sulfide-oxide method, CO_2 method, method of glucose oxidation), the composition is not considered.

Integral method

The integral method is based on measuring changes in the concentration of dissolved oxygen over time. With the results acquired, we can determine the value of $k_L a$ from differential equation 1.1, which was previously integrated:

$$\ln\left(1 - \frac{\gamma_A}{\gamma_A^*}\right) = - k_L a \cdot t \qquad (1.4)$$

Equation 1.4 represents a straight line in the form of $y = k \cdot x$, where $-k_L a$ is the slope of the line at the initial part.

1.3.2 Determination of oxygen volumetric mass transfer coefficient in the fermentation process

If we suppose ideal mixing, we can express the mass balance of dissolved oxygen in the liquid phase during the fermentation process as:

$$\frac{d\gamma}{dt} = k_L a \cdot \left(\gamma_A^* - \gamma_A\right) - Q_{O_2} \cdot \gamma_X \qquad (1.5)$$

where: Q_{O_2} - rate of oxygen consumption (volume flow rate on unit of medium volume,

$$q_v / V), \ \mathrm{m^3}/\left(\mathrm{m^3\ s}\right)$$

γ_X - mass concentration of biomass, g/L

In a steady state, at stirrer speed, f_1, there is:

$$k_L a_1 \cdot \left(\gamma_A^* - \gamma_{A_1}\right) = Q_{O_2} \cdot \gamma_X \tag{1.6}$$

An instant change of stirrer speed to f_2 causes a change in the concentration of dissolved oxygen:

$$\frac{d\gamma}{dt} = k_L a_2 \cdot \left(\gamma_A^* - \gamma_A\right) - Q_{O_2} \cdot \gamma_X \tag{1.7}$$

until a new steady state is formed:

$$k_L a_2 \cdot \left(\gamma_A^* - \gamma_{A_2}\right) = Q_{O_2} \cdot \gamma_X \tag{1.8}$$

If we combine equations (1.7) and (1.8):

$$\frac{d\gamma}{dt} = k_L a_2 \cdot \left(\gamma_{A_2} - \gamma_A\right) \tag{1.9}$$

and integrate within borders $t = 0$, $\gamma_A = \gamma_{A_1}$ in $t = t$, $\gamma_A = \gamma_{A_2}$ we get:

$$\gamma_A = \gamma_{A_2} - \left(\gamma_{A_2} - \gamma_{A_1}\right) \cdot e^{-k_L a \cdot t} \tag{1.10}$$

After defining dimensional concentration:

$$\overline{\gamma_A} = \frac{\gamma_A - \gamma_{A_1}}{\gamma_{A_2} - \gamma_{A_1}} \tag{1.11}$$

equation (1.10) is written in dimension form:

$$\overline{\gamma_A} = 1 - e^{-k_L a_2 \cdot t} \tag{1.12}$$

When we aerate the fermentation medium, the concentration of dissolved oxygen increases until it reaches equilibrium. The response time consists of two sources, τ_E and τ_F. The first is the consequence of the film diffusion resistance and the second is due to the diffusion resistance of the electrode. The area below the curve $(1 - \overline{\gamma}_A)$ regarding *t* is written by the equation:

$$A_1 = \int_0^{\infty}\left(1-\overline{\gamma_A}\right)\cdot dt = \frac{1}{k_L a_2} + \tau_E + \tau_F \qquad (1.13)$$

Response time $(\tau_E + \tau_F)$ is determined by the changing oxygen concentration in the medium before inoculation (before the addition of baking yeast). The area below the curve $(1 - \overline{\gamma}_A)$ regarding *t* for the medium without yeast is represented by this equation:

$$A_{1E} = \int_0^{\infty}\left(1-\overline{\gamma_A}\right)\cdot dt = \tau_E + \tau_F \qquad (1.14)$$

Oxygen volumetric mass transfer coefficient in the fermentation process is obtained from the difference of equations (1.13) in (1.14):

$$\frac{1}{k_L a_2} = A_1 + A_{1E} \qquad (1.15)$$

1.4 EXPERIMENTAL PART

Experiments are conducted in the aeration unit shown in Figure 1.1. It consists of a reservoir (3), temperature-oxygen electrode (10), and an appropriate meter (12), a mechanism for aeration (17, 20, 21) and a stirring system (7, 8, 18, 19).

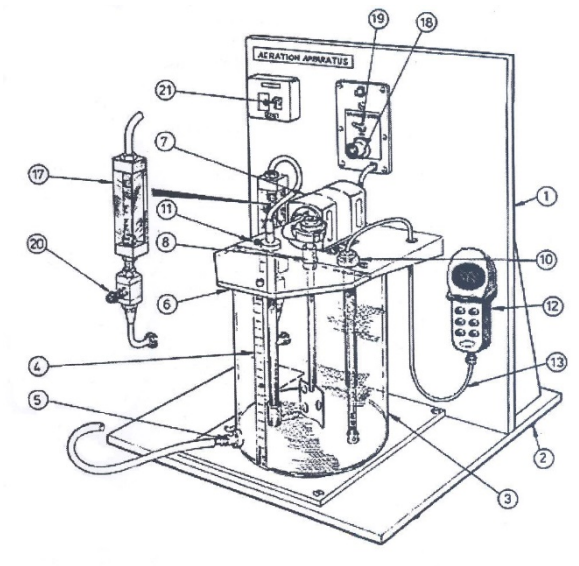

Figure 1.1: Aeration unit.

1.4.1 Chemicals

For the experiment, we need:

- potassium chloride (KCl),
- sodium sulphate (Na_2SO_3),
- cobalt chloride hexahidrate ($CoCl_2 \cdot 6H_2O$),
- synthetic detergent,
- sodium chloride (NaCl),
- distilled water,
- glucose,
- baker's yeast,
- air.

1.4.2 Experimental procedure

1.4.2.1 Calibration of oxygen electrode

The dissolved oxygen meter is made from a combined oxygen – temperature electrode that automatically measures temperature and dissolved oxygen concentration changes. The concentration of dissolved oxygen can be measured in two ways; as a mass fraction of dissolved oxygen in % based on the maximum equilibrium concentration of dissolved oxygen or as a mass concentration in mg/L. Transfer between both modes is made with the button MODE. Before using the electrode, it has to be configured so that the membrane module is filled with the reference fluid and calibrated.

- Filling the membrane with the reference fluid
    1. The electrode is held in a vertical position and the membrane module is carefully screwed off.
    2. The membrane module is filled with reference electrolyte (5 % KCl) up to the edge. Then we check for air bubbles in the fluid.
    3. The electrode is held in the vertical position and carefully screwed back onto the membrane module, where the redundant electrolyte flows off.
    4. Again, we check for air bubbles inside the electrolyte and for any damage to the membrane (wrinkling).
- Calibration
    1. On the meter press CAL, submerge the electrode in the 0.5 % solution of Na_2SO_3, and mix for a few minutes. The value shown on the meter (%) will settle after some time. Then press CAL, which adjusts the meter to 0.
    2. Wash the end of the electrode with distilled water and submerge it in the aeration unit filled with water. Turn on the stirrer and the aeration system. The stirrer speed and aeration are set to maximum. The apparatus should work at these conditions a few minutes (≈ 15 min).
    3. Again, press CAL. After some time, the value on the meter should stabilize at 100 %. If it does not, manually set the value to 100 % with

the +/– buttons. Then press CAL. Calibration is ended and the electrode is ready for measuring.

1.4.2.2 Determination of oxygen volumetric mass transfer coefficient in water

Perform the experiment with the following procedure:

1. Prepare a 10 % solution of Na_2SO_3 by dissolving 10 g Na_2SO_3 in distilled water. Quantitatively transfer the solution to a 100 mL flask and fill it to the mark. Prepare a 1 % solution of $CoCl_2 \cdot 6H_2O$ by dissolving 1g of $CoCl_2 \cdot 6H_2O$ in distilled water, transfer it to a 100 mL flask and dilute it to the mark.
2. Fill the aeration chamber with the appropriate amount of water or other research medium.
3. Insert the appropriate attachment for aeration and a temperature – oxygen electrode.
4. Turn on the stirrer and set the preferred stirrer speed. Add 1.5 mL of Na_2SO_3 solution per 1 L of water and 0.5 mL $CoCl_2 \cdot 6H_2O$ per 1 L of water. The first solution is added with the intention to remove all dissolved oxygen. Cobalt chloride works as a catalyst in the process between sodium sulphate and oxygen. The added amounts are sufficient to remove oxygen from the water. The concentration of dissolved oxygen is measured until it is lower than 10 %. If necessary, add a few drops of each solution, so that the concentration drops to 0 %. When it reaches 0 %, we wait 10 min so that the system settles down.
5. Turn on the aeration system and set the preferred aeration. It is important that air bubbles do not come in contact with the electrode, because that leads to the wrong measurements. Set the stirrer speed to the preferred value.
6. When the conditions, stirring, air flow rate are constant, start measuring concentrations of dissolved oxygen and temperature (the interval is 20 s). Concentration is followed until the point of saturation. The process takes about 10-20 min.

Use a similar procedure for all modifications that are shown in the table. Table 1.2 shows the experiments performed under different conditions.

Table 1.2: Conditions under different modifications of experiment.

No.	Medium	Aeration attachment	Stirrer speed	Medium volume	Air flow
1	dist. water	1	low	10 L	5 L/min
	dist. water	1	medium	10 L	5 L/min
	dist. water	1	high	10 L	5 L/min
2	dist. water	1	optimal	10 L	4 L/min
	dist. water	1	optimal	10 L	8 L/min
	dist. water	1	optimal	10 L	12 L/min
3	dist. water	1	optimal	10 L	5 L/min
	dist. water + detergent	1	optimal	10 L	5 L/min
	dist. water + NaCl	1	optimal	10 L	5 L/min
4	dist. water	1	optimal	10 L	5 L/min
	dist. water	2	optimal	10 L	5 L/min
	dist. water	3	optimal	10 L	5 L/min
5	dist. water	1	optimal	7 L	5 L/min
	dist. water	1	optimal	14 L	5 L/min
	dist. water	1	optimal	20 L	5 L/min

1.4.2.3 Determination of oxygen volumetric mass transfer coefficient in the fermentation process

Perform experiment with the following procedure:

1. Prepare 10 % solution of Na_2SO_3 by dissolving 10 g Na_2SO_3 in distilled water. Quantitatively transfer the solution to a 100 mL flask and fill it to the mark. Prepare 1 % solution of $CoCl_2 \cdot 6H_2O$ by dissolving 1g of

$CoCl_2 \cdot 6H_2O$ in distilled water, transfer to a 100 mL flask and dilute it to the mark.

2. Fill the aeration unit with distilled water (V = 9.5 L).
3. Insert the appropriate attachment for aeration and a temperature – oxygen electrode.
4. Turn on the stirrer and set the preferred stirrer speed. Add 1.5 mL of Na_2SO_3 solution per L of water and 0.5 mL $CoCl_2 \cdot 6H_2O$ per L of water. The first solution is added with the intention to remove all dissolved oxygen. Cobalt chloride works as a catalyst in the process between sodium sulphate and oxygen. The added amounts are sufficient to remove oxygen from the water. The concentration of dissolved oxygen is measured until it is lower than 10 %. If necessary, add a few drops of each solution, so that the concentration drops to 0 %. When it reaches 0 %, wait 10 min so that the system settles down.
5. In a beaker ($V = 1\,\mathrm{L}$) dissolve 150 g of glucose in 500 mL of distilled water.
6. Pour the solution of glucose into the reservoir. Turn on the air pump and set the aeration to 4 L/h and stirrer speed to 8.
7. When the conditions, stirring, and air flow rate are constant, start measuring concentrations of dissolved oxygen and temperature (10 s interval). Concentration is followed until the point of saturation. From the data gathered (concentration of oxygen regarding time), determine the value of the integral in equation (1.14).
8. Set the stirrer speed to 2. Add 40 g of baking yeast (γ_{yeast} = 4 g/L) to the reservoir. Because of the glucose fermentation, the concentration of dissolved oxygen starts to decrease until a new steady state is reached.
9. Set the stirrer speed to 8. Because of the more intense stirring and larger contact surface between fermentation fluid and the air, the concentration of dissolved oxygen increases until a new steady state is reached. Between both steady states, we measure the concentration of dissolved oxygen at 10 s intervals. With this data, determine the value of the integral in equation (1.13).
10. When the experiment is finished, empty the reservoir and clean the aeration unit following the attached instructions.

1.5 Results

1.5.1 Determination of oxygen volumetric mass transfer coefficient in water

The results are given in tabular form:

$\frac{t}{s}$	0	20	40	60	80	100	…	…	…
$\frac{\gamma_A}{g/L}$									
$\frac{\vartheta}{°C}$									

From the temperature data gathered, determine the middle temperature, and then from table 3.1 the appropriate mass concentration of oxygen in water γ_A^*. The experimental equilibrium mass concentration of oxygen, that is, final mass concentration of oxygen, and the value in the table should be the same. Next, draw the graphs:

- $\gamma_A = f(t)$
- $\ln (1 - \gamma_A / \gamma_A^*) = f(t)$

Slope of the graph: $\ln (1 - \gamma_A / \gamma_A^*) = f(t)$ in the linear part represents the value of $-k_L a$.

The capacity of the system is determined using equation 3.2. In the case of making different modifications at different conditions (q_v, V, f), determine the values of $k_L a$ in q_{10} at each condition separately. Next, draw the graphs:

- $k_L a = f(H)$
- $q_{10} = f(H)$

where $H -$ is the condition at which the experiment is performed. From the graph, $k_L a = f(H)$ determine the coefficients of linear dependency (equation (3.3)).

1.5.2 Determination of oxygen volumetric mass transfer coefficient in the fermentation process

The results are given in tabular form:

$\frac{t}{\mathrm{s}}$	0	10	20	30	40	50	…	…	…
$\frac{\gamma_\mathrm{A}}{\mathrm{g/L}}$									
$\frac{\overline{\gamma}_\mathrm{A}}{1}$									
$\frac{\vartheta}{^\circ\mathrm{C}}$									

From the gathered mass concentration data of dissolved oxygen regarding time before the addition of baker's yeast, draw $(1 - \overline{\gamma}_\mathrm{A})$ regarding t, and from the area below the curve determine the sum of response times τ_E and τ_F (equation (1.14)). In the same way, process the mass concentration data of dissolved oxygen regarding time at different stirrer speeds. The value of the integral in equation (1.13) represents the area below the curve. The difference between both areas represents the inverse value of the oxygen volumetric mass transfer coefficient in the fermentation process (equation (1.15)).

2. SYNTHETIC WASTE WATER TREATMENT IN AN AEROBIC REACTOR

2.1 INTRODUCTION

2.1.1 Biological processes of wastewater treatment

Biological wastewater treatment is an alternative to physical/chemical treatment methods. The purpose of biological wastewater treatment is to stabilize organic material in wastewater and to reduce the environmental impact. Different types of fermentation, which can be conducted at aerobic, anaerobic or mixed conditions, comprise biological wastewater treatment. The process is often followed by the release of gases produced by enzyme actions of microorganisms. In anaerobic conditions, in the absence of oxygen, we remove complex molecules. In practice, the anaerobic process is mostly used for stabilizing the renewal of waste sludge from the aerobic process, for processing organic waste from agriculture and sewage from livestock breeding. Benefits of anaerobic procedures include:

- lower production of surplus biological sludge,
- the microbial process needs small amounts of substrate,
- production of methane that can be used as an emergent,
- process can be overloaded if needed.

Weakness of anaerobic procedures:

- sensitive to different toxins ($CHCl_3$, CCl_4, CN and others),
- very long start up time,
- anaerobic processing is preliminary, so we need final processing before releasing it to drainage,
- experience of processing different wastewaters is not so wide compared to aerobic techniques.

2.1.1.1 Anaerobic conversion

Anaerobic fermentation takes place in the absence of oxygen under anaerobic conditions. A source of energy provides incomplete conversion of organic substances, oxidants (sulfates, nitrates and carbonates). About half of the organic substances react to gas or liquid during fermentation. Anaerobic fermentation consists of:

- hydrolysis and fermentation (acid bacteria) and
- the key step of methanogenesis (methanogenic bacteria)

In the last step of the process, biogas is produced with the composition (volume percent):

- 60 % to 80 % CH_4,
- 15 % to 35 % CO_2,
- traces of N, H and SH_2,
- other volatile organic molecules.

In the whole process, there are two different types of bacteria that are essential for anaerobic conversion and act mutually:

- acid bacteria, that produce lower organic acids from organic substances,
- methanogenic bacteria, that convert organic acids to methane (biogas).

In Figure 2.1 the equilibrium established during the process of anaerobic conversion between acid and methanogenic bacteria is shown.

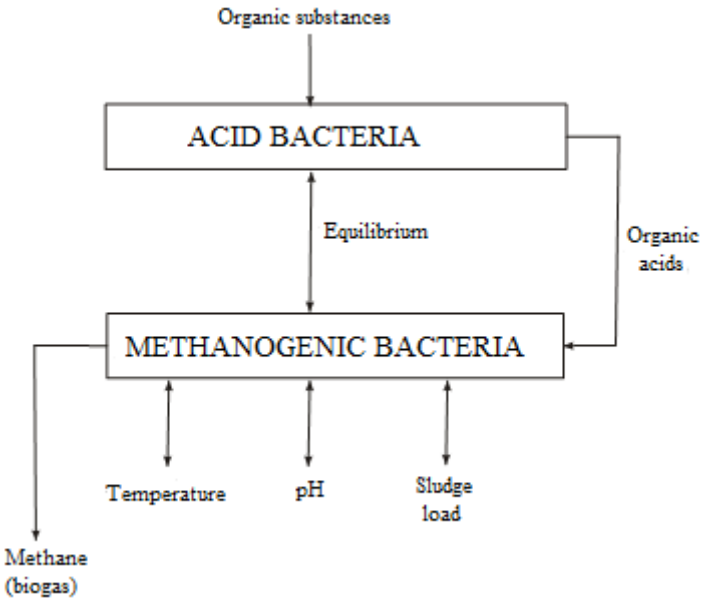

Figure 2.1: Maintaining equilibrium between acid and methanogenic bacteria.

For methanogenic bacteria, slow growth is typical, cell division lasts several more days, and during division they are very sensitive to oxygen and fast changes in conditions. Their activity is decisively influenced by:

- changes in *T* and pH,
- organic load,
- toxicity (sulphur, ammonium, heavy metals and other).

2.2 PURPOSE OF THE EXERCISE

The purpose of this exercise is to show a comparison between acid and methanogenic conversion of anaerobic processes and the influence of different parameters (temperature, pH, flow rate, COD value of inlet, substrate deficiency) on the anaerobic wastewater treatment effect. The experiment is carried out in the Armfield pilot anaerobic reactor. The effect of treatment can be described by daily changing, measuring and determining the parameters:

- value of pH,
- value of COD, γ_{COD},
- production of biogas,
- mass concentration of suspended matter.

2.3 THEORETICAL PART

2.3.1 Description of the anaerobic process parameters

2.3.1.1 Temperature

The anaerobic digestion process (and other fermentation processes) takes place at optimum operating temperature, depending on the properties of the initially selected biomass, it is around 35 °C. At lower temperatures, the activity of microorganisms is reduced, so the effect of wastewater treatment is lower. This property can be demonstrated by the operation of the anaerobic reactor at different temperatures. The temperature is measured with the temperature sensor immersed in the reactor and connected to the temperature control system of the reactor. This automatically maintains the desired reactor temperature by regulating the heater's power.

2.3.1.2 pH value

The most common problem in the transfer of anaerobic technology to industrial practice are the various pH values of the inlet mixtures. Changing these values and measuring their impact on the efficiency of water purification can determine their limit values. These data are important in the planning process. The effect of the inlet pH on the wastewater treatment is demonstrated by the operation of the anaerobic reactor at various pH values at the inlet.

2.3.1.3 Flow rate

The flow rate of wastewater in the anaerobic system directly affects the stability and the effect of the process. The residence time required to reduce contamination depends on the complexity of the inlet substrate (contaminated water); therefore; the residence time as a result of the flow velocity is one of the key factors in the design of anaerobic processes. We can study the influence of the flow rate through the anaerobic reactor on the degree of purification of wastewater.

2.3.1.4 Mass concentration of inlet

The inlet mass concentration, or the degree of contamination of the inlet in the anaerobic purification process, has the greatest influence on the quality or effect of wastewater treatment, especially on the reduction of chemical oxygen demand. This effect is demonstrated by performing experiments in the anaerobic reactor at various inlet concentrations, i.e. at different γ_{COD} (chemical oxygen demand) of the inlet.

2.3.1.5 Substrate deficiency

Macro and micronutrients are indispensable for the successful course of biological systems. Micronutrients can easily establish and maintain biomass in the anaerobic process. The impact of micronutrients can be investigated for introduction to the system. In general, a lack of nutrition in the anaerobic process reduces the degree of purification of the system, despite the low inlet flow rate, because of the accumulation of volatile acetic acid in the system. The effectiveness of the system is also affected by many changes in the properties of biomass, with the final production of an unhealthy and unbalanced microbiological population. This effect can be demonstrated using both reactors in the anaerobic system. The first reactor acts as a control with an adequate nutrient content in the inlet stream and the other as a nutrient-deficient reactor.

2.4 Experimental part

The process of purification of synthetic wastewater is carried out in the Armfield biological anaerobic pilot reactor. Figure 2.2 presents the components of the anaerobic reactor system.

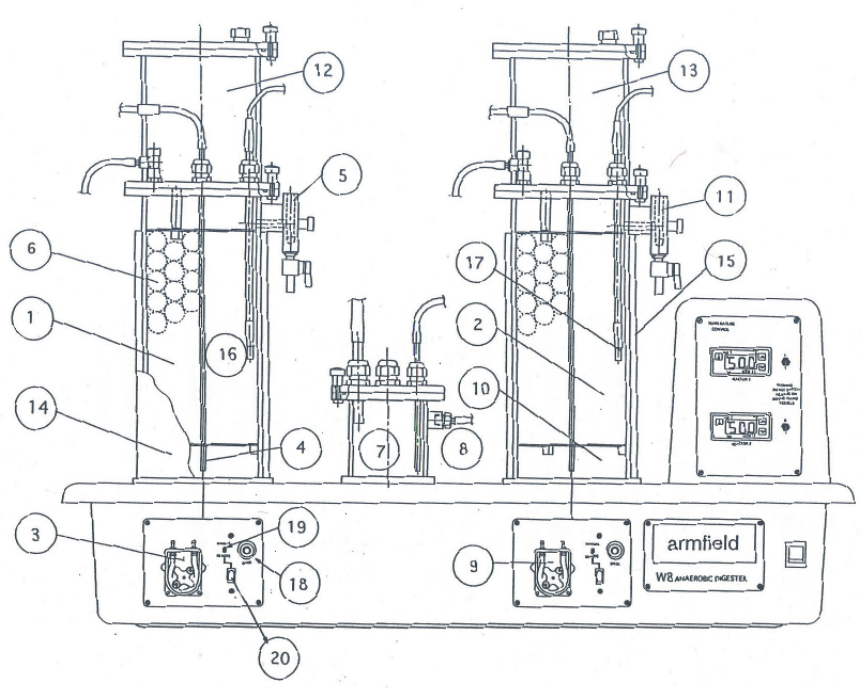

Figure 2.2: Components of the anaerobic reactor system W8.

The anaerobic reactor system consists of two reactor vessels (1, 2) of 5 L. The support material in the reactor (V = 4.3 L) provides a good mixing of the biomass with the inlet. A variable peristaltic pump (3) feeds wastewater from the vessel located along the bottom of the reactor system through the supply pipe (4) into the reactor (1). A 10-step potentiometer (18) allows different speeds of the pump motor, with the maximum speed of the pump 4 (the switch (19) is in the upper position). If the switch (19) is in the lower position, the pump speed can be controlled with a personal computer through connection (20). The effluent from the reactor (1) flows through the gas siphon (5), which prevents losses of the gas produced during bacterial metabolism, into the collection vessel (7). This container allows the reactor (1) to operate at a higher flow rate than the reactor (2), wherein the excess stream leaves the collecting vessel through the tube (8). The variable peristaltic pump (9) allows inlet flow (2) through the inlet pipe (10). The outlet of the reactor passes through the gas siphon (11). The gas produced during the reaction in the reactor (1) or (2) is collected in 5 L gas collection vessels (12) and (13). The gas-collecting vessel (12) is illustrated in Figure 2.3. The gas-collecting vessel (13) is constituted in the same way.

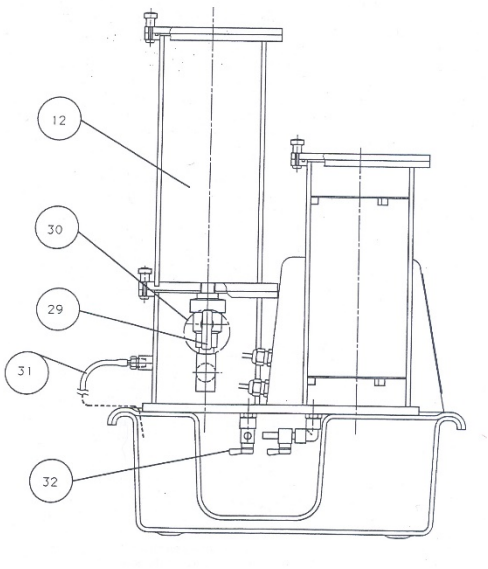

Figure 2.3: Components of the gas collection tank.

The reactor is heated to operating temperature by means of an electric heater in the form of heating jackets (14) and (15). Temperature sensors (16) and (17) transmit the reactor temperature data to the regulators (A) and (F) (Figure 2.4), which, by regulating the power of the heating, provide a constant temperature in the reactors.

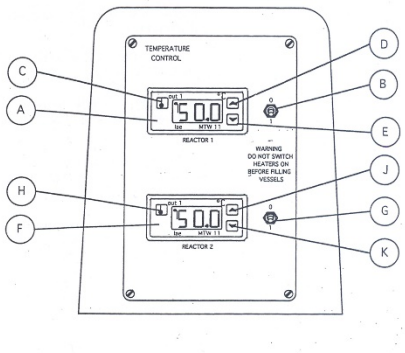

Figure 2.4: Control unit of anaerobic reaction system.

Detailed instructions on the composition, operating requirements and safety measures of the anaerobic reactor system can be found in the instructions given with the appliance.

2.4.1 Determination of process parameters

The effect or the degree of purification of the anaerobic wastewater treatment process is described by monitoring process parameters: pH, biogas production, and mass concentration of suspended solids.

2.4.1.1 pH value

Use a digital pH meter to determine the pH value. The electrode is calibrated with buffer solutions pH = 7 and pH = 4. The calibrated electrode is immersed into the wastewater sample and connected to the digital pH meter. Then read the pH value. The electrode must be calibrated daily.

2.4.1.2 COD value

Preparing the chemicals

- **Sulphuric (VI) acid, *c* (H_2SO_4) = 4 mol/L (1)**

To 500 mL of distilled water, slowly add 220 mL of concentrated H_2SO_4, cool, pour into a 1000 mL volumetric flask and dilute with distilled water to the mark.

- **Silver sulphate – sulphuric (VI) acid (2)**

Dissolve 10 g of silver sulfate (Ag_2SO_4) in 35 ml of distilled water under intensive stirring; add 965 mL of concentrated H_2SO_4.

- **Potassium dichromate, *c* ($K_2Cr_2O_7$) = 0,040 mol/L (3)**

80 g of mercury (II) sulphate (HgSO4) is dissolved in 800 mL of distilled water and 100 ml concentrated H_2SO_4 is carefully added. The solution is cooled to room temperature and then we add 11.768 g $K_2Cr_2O_7$, dried for 2 h at 105 °C, and mix well. Transfer the solution quantitatively to a 1000 mL volumetric flask and dilute with distilled water to the mark.

- **Ammonium iron (II) sulphate, *c* {$(NH_4)_2Fe(SO_4)_2$ $6H_2O$} = 0,12 mol/L (4)**

Dissolve 47 g $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ in distilled water, add 20 mL of concentrated H_2SO_4. Transfer the solution quantitatively to a 1000 mL volumetric flask and dilute to the mark with distilled water. Prepared solution should be standardized daily.

- **Indicator feroin (5)**

Dissolve 1 g $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ in distilled water. Then 1.5 g of 1,10-phenanthroline monohydrate $C_{12}H_8N_2 \cdot H_2O$ is added and stirred until the solid particles dissolve. Transfer the solution quantitatively to a 100 mL volumetric flask and dilute to the mark with distilled water.

Standardization of ammonium iron (II) sulphate

Pipette 10 mL of potassium dichromate solution (3) into a 500 ml round-bottomed flask and dilute with 100 ml of sulfuric acid solution (1). Add 2 to 3 drops of the feroin indicator (5). Titrate the prepared solution with a solution of ammonium iron (II) sulfate (4). The concentration of ammonium iron (II) sulphate in mol/L is calculated by the equation:

$$c = \frac{10 \cdot 0.04 \cdot 6}{V} = \frac{2.4}{V} \qquad (2.1)$$

where: V - volume of ammonium iron (II) sulphate solution, mL,

c - concentration of the ammonium iron (II) sulfate solution, mol/L.

Determination of COD value

The described method for determination of COD value is suitable for the range of oxygen concentration (O_2) between 30 mg/L and 700 mg/L. Samples with higher COD values should be diluted.

Pipette 10 mL of sample (diluted, if necessary) into a 500 mL round-bottomed flask, add 5 mL of potassium dichromate solution (3) and boiling stones. Mix well and slowly add 15 mL of silver sulfate and sulfuric acid solution (2). Mix the contents of the flask and connect it quickly with a return water cooler. Using sandy bath, heat the contents of the flask to boiling in 10 min and continue heating for a further 110 min. After two hours, the flask is rapidly cooled with water to 60 °C; wash the refrigerator with a small amount of distilled water and remove it. Dilute the contents of the flask with 75 mL of distilled water and cool to room temperature. Add 1 to 2 drops of the feroin indicator and titrate with ammonium iron (II) sulfate solution.

10 mL of distilled water should be used in a blank measurement instead of 10 mL of sample. The COD value, expressed in mg/L, is calculated by the equation:

$$\gamma_{COD} = \frac{8000 \cdot c \cdot (V_1 - V_2) \cdot f_d}{V_0} \text{ (mg/L)} \qquad (2.2)$$

where: c - concentration of the ammonium iron (II) sulfate solution, mol/L,
V_1 - volume of ammonium iron (II) sulphate solution used in the blank measurement, mL,
V_2 - volume of ammonium iron (II) sulphate solution used in the sample measurement, mL,
V_0 - sample volume (diluted, if necessary) = 10.0 mL,
f_d - factor of dilution.

The most important parameter describing the effect of anaerobic biological treatment of wastewater is the effect of purification with respect to γ_{COD}, given in percentages regarding wastewater flow determined by the equation:

$$\eta_{COD} = \frac{\gamma_{COD_{inlet}} - \gamma_{COD_{outlet}}}{\gamma_{COD_{inlet}}} \cdot 100\ \% \qquad (2.3)$$

where: η_{COD} - effect of wastewater treatment regarding γ_{COD} (%).

2.4.1.3 Production of biogas

The gas produced during the bacterial conversion in reactors 1 and 2 is collected in the gas collection vessels of both reactors. Collection is based on the water-gas displacement. Gas displaces water so that it leaks out at the base of the collecting vessel as an excess flow.

At the beginning, open the valve at the bottom of the collecting vessel. At the top of the gas-collecting vessel, unscrew the cap and fill the container with distilled water through the inlet opening. When a surplus flow occurs in the bottom of the collecting vessel, close the valve and fill the collection container with distilled water to the top, then close the opening with the cap. Connect the valve with a flexible tube from the reactor and open it to allow the gas to flow from the reactor. On the scale of the collecting vessel, the volume of gas produced can be determined on a daily basis. When the collecting vessel is full of gas, refill it with distilled water. The scale of the gas-collecting vessel is calibrated with a known amount of water, so that

the known volume of water is assigned to the height of the column on the scale. The gas yield expressed in L/g is calculated by the mathematical relation:

$$r_{\text{biogas}} = \frac{V_{\text{biogas}}}{\left(\gamma_{\text{COD}_{\text{inlet}}} - \gamma_{\text{COD}_{\text{outlet}}}\right) \cdot q_v} \tag{2.4}$$

where: V_{biogas} - volume of produced biogas, L/d,

$\gamma_{\text{COD}_{\text{inlet}}}$ - inlet mass concentration of COD, g /L,

$\gamma_{\text{COD}_{\text{outlet}}}$ - outlet mass concentration of COD, g /L,

q_v - volume flow rate of wastewater, L/d,

r_{biogas} - productivity of biogas, L/g.

2.4.1.1 Mass concentration of suspended solids

Mass concentration of the suspended solids is determined gravimetrically. Pre-filter the known volume of sample through a dry weighed filter paper. The filter paper is dried in an oven at 100 °C to a constant weight. The concentration is determined by the equation:

$$\gamma_{\text{dm}} = \frac{m_1 - m_2}{V_{\text{s}}} \tag{2.5}$$

where: γ_{SS} - mass concentration of suspended solids, mg/L,

m_1 - mass of the dry filter paper before filtration, mg,

m_2 - mass of the dry filter paper after filtration, mg,

V_{s} - volume of the sample to be filtered, L.

2.4.2 Experimental procedure

2.4.2.1 Pump calibration

Despite the fact that both pumps have the same technical characteristics, they are not equally effective, so each one needs to be calibrated. The calibration is carried out in a way that, in the

various adjustments of the pump controller, water is drawn from the container beside the reactor and a volume of pumped water is measured with a measuring cylinder. The system works at very low flow rates (2 L/d to 3 L/d), so it is recommended to use a measuring cylinder with an accuracy of 0.1 mL.

The calibration procedure begins with the unwinding of the supply pipe on the reactor, which is connected to the graduated measuring cylinder. Then measure the volume of pumped water at different settings of the pump controller (2.0, 4.0, 6.0, 8.0, 10.0) and draw graph, flow (L/d) regarding pump controller setting. The curve determines the setting of the pump controller at the desired flow rate of water.

2.4.2.2 Preparation of process solutions

Synthetic wastewater

Synthetic wastewater is prepared by dissolving the corresponding quantities of chemicals in distilled water, presented in Table 2.1. Transfer the solution quantitatively to a 1000 mL volumetric flask and dilute with distilled water to the mark. γ_{COD} of prepared solution is about 8000 mg/L.

Table 2.1: Composition of synthetic wastewater

CHEMICAL	MASS CONCENTRATION
Glucose	8.0 g/L
Ammonium hydrogen carbonate NH_4HCO_3	0.4 g/L
Potassium dihydrogen phosphate KH_2PO_4	0.4 g/L
Sodium hydrogen carbonate $NaHCO_3$	0.4g/L
Solution A	1.0 mL/L
Solution B	1.0 mL/L

Solution A is prepared by dissolving magnesium sulphate heptahydrate, $MgSO_4 \cdot 7H_2O$ in distilled water. Dissolve 0.5 g of $MgSO_4 \cdot 7H_2O$, quantitatively transfer it into a 100 mL

volumetric flask and dilute to the mark with distilled water. The prepared solution has a mass concentration of 5 g/L and is stable for several months at room temperature.

Solution B is prepared by dissolving the appropriate amount of chemicals in distilled water (Table 2.2). At room temperature, the solution persists for several months.

Table 2.2: Components of solution B

CHEMICAL	MASS CONCENTRATION
$FeCl_3$	5.0 g/L
$CaCl_2$	5.0 g/L
KCl	5.0 g/L
$CoCl_2$	1.0 g/L
$NiCl_2$	1.0 g/L

2.4.2.3 Starting up the reactor system

The apparatus is assembled and connected in accordance with the instructions provided. Place the RCCB (25) and the four switches (26) on the back of the appliance to the ON position (picture 2.6).

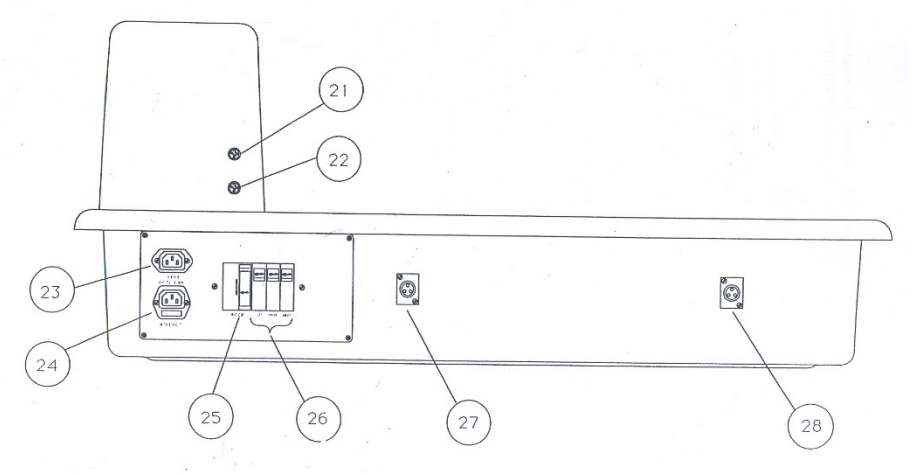

Figure 2.6: Components of the rear part of the anaerobic reactor system.

Distilled water is used as a process fluid. Before turning on the main switch (position ON), set the temperature control switches (A) and (F) to OFF position and switch the pumps

(8) and (9) to the MANUAL position. The pipes are connected in such a way that the main wastewater stream of the vessel (V = 10 L) flows through pump 1, reactor 1 and siphon U into the intermediate vessel (7), and from there through pump 2 of reactor 2 and siphon U into the outlet. Then, remove the feed caps at the top of the two reactors and fill the reactor vessels to the top via the funnel (excess flow appears) and close the supply ports with the feed caps. Fill the intermediate container in the same way. We run the system by turning on both pumps and setting the value on the pump controller to 5.0. Check the flow rate of water in the system. Also, check for possible obstructions (bubbles). Remove all irregularities. Now turn on both temperature controllers and adjust the temperature to 35 °C. The anaerobic reactor is left in this state until a temperature equilibrium is established. The anaerobic process is very long and slow; the experiment can last for several weeks, with the apparatus running constantly 24 hours a day. The reactor heater will heat the reactor content to 35 °C and maintain it at this temperature. When the temperature reaches 35 °C, adjust the temperature of the regulator to 55 °C and observe the temperature rise. At the start up time of the apparatus, both gas collecting containers are also filled.First, remove the caps on the inlet opening at the top of the gas collecting vessels (12) and (13). Check that the discharge valve (32) of the gas-collecting container located below is in the closed position; if not, close it. Then, open the valve (29). Start to fill the gas-collecting container through the funnel. When the excess flow is reached at the base of the gas-collecting vessel, we close the valve (29) and continue with the filling. When the container is fully filled, close the opening with the cap and open the valve (29). Both filled gas containers should keep stationary at least for two hours. Finally, switch off both temperature controllers and discharge all units. The apparatus is now ready for an experiment.

The first stage of the experiment is the preparation, adaptation and cleavage of anaerobic sludge. Take the appropriate sludge from the municipal treatment plant and determine the pH and remove particles (stones, other undesirable particles), adjust the dry matter content (mass concentration of undissolved particles) to 10 g/L and transfer it to reactors (1) and (2). The temperature regulator adjusts the temperature between 35 °C and 37 °C. Set the desired temperature in the reactor by pressing the (C) key simultaneously with the (D) and (E) buttons (plus - minus) increase or decrease the temperature. The heating rate is very slow. When the temperature in the reactor reaches the set value, leave it in that state. After 12 h, continue with the biomass cleavage. Biomass is cleaved with the intention of adapting it to the flow of contaminated water. The cleavage is carried out in such a way that the reactor operates at a low flow rate of about 0.3 L/d on the first day. The flow increases by 0.2 L/d to 0.4 L/d over the

next few days. After reaching 1.5 L/d or operating flow rate, we will continue to operate for a few more days.

2.4.2.4 Continuous flow of the experiment

The biological anaerobic pilot reactor system enables the study of the influence of various factors on the effect of purification. We can perform various experiments:

- comparison of the rate of acid and methanogenic conversion in the anaerobic process,
- the influence of the temperature on the effect of synthetic wastewater treatment,
- the influence of pH values on the effect of purification of synthetic wastewater,
- the influence of the flow rate on the effect of synthetic wastewater treatment,
- the impact of the synthetic wastewater COD rate on the cleaning efficiency,
- demonstration of the influence of substrate deficiency on the wastewater treatment efficiency.

Comparison of the rate of acid and methanogenic metabolism in the anaerobic process (experiment 1)

In this experiment, reactors 1 and 2, pumps 1 and 2 and both gas collecting containers are used. The system is assembled in such a way that the prepared synthetic wastewater flows from the tank to reactor 1, then into the intermediate vessel, into reactor 2, and finally into the discharge vessel. Both reactors are prepared according to the instructions. Adaptation and cleavage of biomass is carried out as described in 2.4.2.3, where the pH of the anaerobic sludge in reactor 1 is adjusted to a value of 5.8 with the use of concentrated hydrochloric acid (HCl). For cleaving the biomass in the reactor 1, synthetic wastewater is used as an inlet flow and for the reactor 2 we use the outlet from reactor 1 as an inlet flow. The experiment is carried out continuously for several days. For the proper operation of both reactors, it is necessary to provide the conditions shown in Table 2.3.

Table 2.3: Conditions of experiment 1.

PARAMETER	REACTOR 1	REACTOR 2
Temperature	35 °C to 37 °C	35 °C to 37 °C
Input flow	synthetic wastewater	outlet from the reactor 1
Input flow rate	2.5 L/d to 3.5 L/d	1 L/d to 1.5 L/d
Input pH	corrected pH with HCl	without pH correction

During the experiment, the effect of both reactors is monitored by daily sampling at the input and output flow and determining the process parameters specified in section 2.4.1.

Temperature impact (experiment 2)

In this experiment, only reactor 2 is used with its pump and a gas-collecting vessel. This is achieved by disconnecting the reactor 2 inlet pipe from the intermediate vessel and the pump. Instead, connect the longer feed pipe that was used in experiment 1 for pump 1 to pump 2. With pump 2, pump the inlet from the tank to reactor 2. From there, the excess flow automatically goes into the drainage tank. The biomass cleavage and adaptation is carried out as described in section 2.4.2.3. If we have already performed experiment 1, we do not have to do this. Synthetically prepared water is used as a process fluid. The experiment takes place in three stages. At the first stage, a temperature of 35 °C is maintained in the reactor from 5 d to 8 d at a flow rate of 1.5 L/d to 2.5 L/d. Perform measurements and analysis of process parameters on a daily basis. In the second stage of the experiment, the temperature is increased to 45 °C, and we continue to operate at an unchanged flow rate of synthetic wastewater for another (5-8) d. During this time, perform the same measurements as in the first stage of the experiment. In the third stage of the experiment, the temperature of the reactor is set to 25 °C, and, at unchanged flow rate, continue to operate for another (5-8) d. Perform the same measurements as in the first and second stages. A comparison of the results of measurements and the analysis of process parameters shows the influence of temperature on wastewater treatment efficiency.

Impact of pH value (experiment 3)

Use only reactor 2 and its associated equipment. Fill it with the fresh anaerobic sludge, which is adapted and cleaved with basic synthetically prepared water. The temperature in the reactor is adjusted to 37 °C, and the pH of the solutions is corrected with HCl to 3.0. In the first stage, the reactor should operate (3-5) d with standard synthetic wastewater. In the next step, the pH of the inlet solution is increased to a value of 10.0 (NaOH is used) and continue to operate until the biogas production is reduced by 50%. At that time, begin to reload the reactor with standard synthetic wastewater until the production of biogas returns to the initial value. In these conditions, the reactor should operate a further (2-3) d. Perform the same procedure when reducing the pH value (HCl) of inlet synthetic wastewater. During the experiment, process parameters are determined and analyzed daily.

Impact of inlet flow (experiment 4)

In this experiment, only reactor 2 and its associated equipment are used. The reactor is filled with fresh anaerobic sludge and the described adaptation and cleavage of the biomass is carried out, using three times diluted, synthetically prepared water with a COD value of about 3000 mg/L. The temperature in the reactor is 37 °C. The experiment is performed by initially adjusting the diluted synthetic wastewater flow to a value of 1.5 L/d and increasing it at intervals of (2-3) d by (0.5-0.75) L/d. We increase the flow rate as long as the wastewater treatment efficiency according to the COD value still drops. During the experiment, perform the daily measurements necessary for the calculation of process parameters. The results of this experiment show the correlation between residence time and the effect of cleaning according to COD value.

- **Impact of the input flow COD value (experiment 5)**

In experiment 5, only reactor 2 and associated equipment are used. The fresh anaerobic sludge is adapted and cleaved as described by the instructions in 2.4.2.3. Use the synthetic wastewater 1 from Table 2.4 as the process fluid.

Table 2.4: Synthetic wastewater of different COD values.

	1	**2**	**3**	**4**	**5**	**6**
≈ γ_{COD} (g/L)	2000	4000	6000	8000	10000	12000
Glucose, γ / (g/L)	2.00	4.00	6.00	8.00	10.00	12.00
NH_4HCO_3, γ / (g/L)	0.15	0.20	0.30	0.40	0.50	0.60
KH_2PO_4, γ / (g/L)	0.15	0.20	030	0.40	0.50	0.60
$NaHCO_3$, γ / (g/L)	0.50	0.50	0.50	0.40	0.30	0.30
$KHCO_3$, γ / (g/L)	0.5	0.50	0.50	0.40	0.30	0.30
Solution A, ξ /(mL/L)	1.00	1.00	1.00	1.00	1.00	1.00
Solution B, ξ /(mL/L)	1.00	1.00	1.00	1.00	100	1.00

During the experiment, prepare several types of synthetic wastewater (Table 2.4). Solutions A and B are prepared in the same manner as mentioned in section 2.4.2.2. The temperature in

the reactor is 37 °C. The experiment should be carried out in such a way that synthetic wastewater of different γ_{COD} values is used as input flow. The reactor should operate with each of the solutions at least (3-4) d. During the experiment, the samples of the input and output flows of both reactors and biogas production are measured and analyzed daily, and then the process parameters are determined. The results of the experiment should also be presented graphically, by plotting the wastewater treatment efficiency regarding the COD value.

Impact of substrate deficiency (experiment 6)

In this experiment we use both reactors, but without interconnection. From vessel 1 (water 1), with pump 1, inlet 1 is introduced into reactor 1. The excess flow automatically flows directly into the outlet and not into the collecting vessel (7). The flow into reactor 2 is driven from container 2 (water 2) by the pump. The excess flow of reactor 2 automatically flows into the outlet. Adaptation and cleavage of the biomass of the two reactors is carried out in the same way as in experiment 5. Before performing the experiment, two types of synthetic wastewater are prepared (section 2.4.2.2), where water 1 contains all chemicals, and water 2 is free of ammonium hydrogen carbonate (nutrient deficiency). The experiment is carried out in such a way that reactor 1 operates with synthetic water 1 and reactor 2 with synthetic water 2. The flow rates of both inlets is adjusted in the range between 1.5 L/d and 2.5 L/d. The experiment should last until a sudden drop in the cleaning efficiency with respect to the COD value at the outlet of reactor 2 occurs as a result of the accumulation of volatile fatty acids. The process parameters are determined daily and at the end of the experiment we compare the results of both reactors.

2.5 RESULTS

The results are presented in tabular and graphical form depending on the experiment. The table below shows the tabulation of the results for each reactor and is useful for all experiments.

Date	$\frac{\vartheta}{°C}$	$\frac{q_v}{L/d}$	$\frac{\gamma_{dm}}{mg/L}$	$\frac{\gamma_{COD\text{-}inlet}}{mg/L}$	$\frac{\gamma_{COD\text{-}outlet}}{mg/L}$	pH - inlet	pH - outlet	η_{KPK}	$\frac{r_{biogas}}{mg/L}$
-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-

In addition to the above mentioned parameters, calculate mass and volume rate of oxygen consumption:

$$r'_{COD} = \frac{\left(\gamma_{COD_{inlet}} - \gamma_{COD_{outlet}}\right) \cdot q_v}{m_b} \quad (2.6)$$

$$r_{COD} = \frac{\left(\gamma_{COD_{inlet}} - \gamma_{COD_{outlet}}\right) \cdot q_v}{V_r} \quad (2.7)$$

where: r'_{COD} - mass rate of chemical oxygen consumption, kg/(kg d),

m_b - mass of biomass, kg,

r_{COD} - volume rate of chemical oxygen consumption, kg/(m^3 d),

V_r - reactor volume, m^3.

Both rates of chemical oxygen consumption, mass and volume, are presented in tabular form.

Date	$\frac{r'_{COD}}{\text{kg/(kg d)}}$	$\frac{r_{COD}}{\text{kg/(m}^3\text{ d)}}$
-	-	-
-	-	-

Graphic presentation of the results depends on the individual experiment and depends on our choice.

The results of experiment 1 make it possible to compare the activity and the wastewater treatment efficiency (with respect to γ_{COD}) of the methanogenic and acid phases of anaerobic processes. In experiment 2, an optimal temperature is determined, which should be approximately 35 °C. In experiment 3, the influence of pH on the wastewater treatment efficiency and on the production of biogas is determined. Experiment 4 provides data on the maximum permissible flow rate of synthetic wastewater on desired wastewater treatment efficiency. In experiment 5, we study the influence of the γ_{COD} of inlet on wastewater treatment efficiency. From the results of this experiment, we can see how at a constant inlet flow rate, γ_{COD} affects the wastewater treatment efficiency and, consequently, the outlet γ_{COD}. In experiment 6, we can observe the effect of substrate deficiency on the growth of microorganisms and consequently the wastewater treatment efficiency.

3. SYNTHETIC WASTEWATER TREATMENT IN AN AEROBIC REACTOR

3.1 INRODUCTION

3.1.1 Biological process of cleaning wastewater

Biological wastewater treatment is an alternative to physicochemical cleaning methods. The primary purpose of biological wastewater treatment is to reduce environmental degradation and to stabilize organic matter in wastewater. The process is based on the action of microorganisms that degrade organic matter into a dissolved and colloidal state. The process is identical to the process of self-purification, which also takes place in nature. Microorganisms in surface waters are present in biological wastewater treatment plants, but their concentration is higher, so cleaning rates are higher than in self-cleaning processes in the natural environment. The process of biological cleaning can be carried out under various conditions: aerobic, anaerobic or mixed (aerobic - anaerobic). Under aerobic conditions, degradable organic compounds are removed, and at the same time, the conversion of nitrogen compounds takes place. Advantages of aerobic cleaning procedures are:

- possibility of cleaning different wastewaters,
- effective removal of organic impurities (waste),
- short start up and life time,
- flexibility and diversity.

Disadvantages of aerobic cleaning are:

- high energy consumption,
- high volume of waste sludge produced,
- sensitivity of microorganisms to sudden change.

With aerobic conversion, energy-rich substances (fats, proteins and carbohydrates) during respiration degrade into energy-poor mineralized products (CO_2 and H_2O). The degradation takes place under the influence of enzymes (biocatalysts) specific to a particular reaction and type of substrate.

3.1.1.1 Process of wastewater treatment with active sludge

The active sludge process is the most commonly used secondary wastewater treatment process. It is used for the removal of dissolved and undissolved substances and colloidal organic impurities from municipal and wastewaters of several types of industries. The wastewater treatment efficiency depends on the following factors:

- the metabolism effectiveness of organic substances and ammonia,
- age of sludge and organic load,
- number and species of microorganisms in the aerator,
- residence time and effectiveness of the reactor,
- environmental factors (concentration of dissolved oxygen, nutrients, alkalinity, pH, temperature),
- effectiveness of mixing of returned sludge, pumping of active sludge and capacity of aeration,
- proper maintenance of cleaning plant,
- proper schooling of the group that works on the cleaning plant, including the test laboratory, maintenance, management and leadership.

3.2 PURPOSE OF THE EXERCISE

The purpose of the exercise is to show the process of aerobic wastewater treatment. The experiment is carried out in the Armfield pilot aerobic reactor. The synthetic wastewater treatment efficiency is monitored by measuring and determining the parameters:

- pH value,
- COD value,
- BOD_5 value,
- mass concentration of dry matter.

3.3 THEORETICAL PART

3.3.1 Describing process parameters

3.3.1.1 Temperature

Biochemical reactions are enzymatically catalyzed and thus dependent on temperature. Increasing the temperature increases the degradation of organic matter and consequently the consumption of oxygen in wastewater. The rate of conversion increases to a certain limiting temperature at which the biological activity is inhibited. The optimal operating temperature of the aerobic reactor is in the range between 25 °C and 30 °C.

3.3.1.2 pH value

A properly selected and maintained pH range is one of the most important factors in the treatment of wastewater, as it directly affects the productivity and activity of enzymes, microorganisms and, consequently, the wastewater treatment efficiency. The optimum pH range is between 6.5 and 8.5. Outside this region, the activity of enzymes is reduced and their structure changes, resulting in interrupted productivity or deactivation of enzymes or microorganisms.

3.3.1.3 Chemical oxygen demand

Chemical oxygen demand γ_{COD} is a mass concentration of oxygen (mg/L) that is required for complete oxidation of the organic substance in the sample to CO_2 and H_2O. It is a criterion for the total quantity of organic substances in the sample (degradable and non-degradable).

3.3.1.4 Five day biochemical oxygen demand

Five day biochemical oxygen demand, γ_{BOD_5}, is mass concentration of oxygen (mg/L) consumed by microorganisms in 5 d for the oxidative decomposition of organic and inorganic substances in the sample. Because of the prolonged degradation process, it is determined every five days at a constant temperature of 20 °C. Two methods are known, a standardized dilution method and a non-standardized manometric method.

3.3.1.5 Mass concentration of dry matter

For the smooth running of the aerobic wastewater treatment process, the amount of dry matter (the sum of non-volatile soluble and undissolved substances) in the reactor should be controlled. Disposal of waste sludge affects:

- outlet flow quality,
- speed of microorganism growth and presence of different microorganisms,
- oxygen consumption,
- sedability of the active sludge suspension,
- appearance of foaming,
- nitrification possibility.

3.4 Experimental part

The Armfield pilot aerobic reactor (Figure 3.1) is intended to simulate the cleaning process of an aerobic biological treatment plant. The components of the biological aerobic pilot reactor are shown in Figures 3.1 and 3.2.

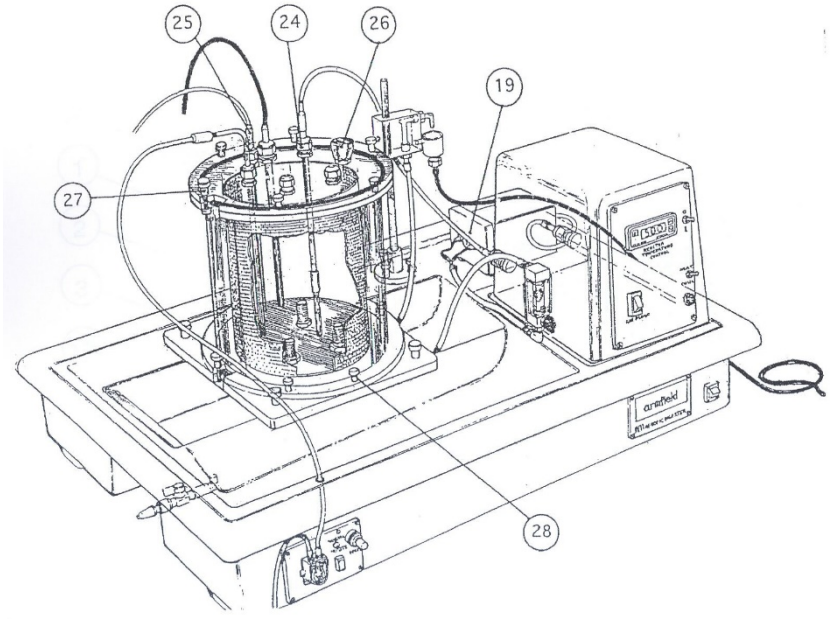

Figure 3.1: Aerobic pilot reactor.

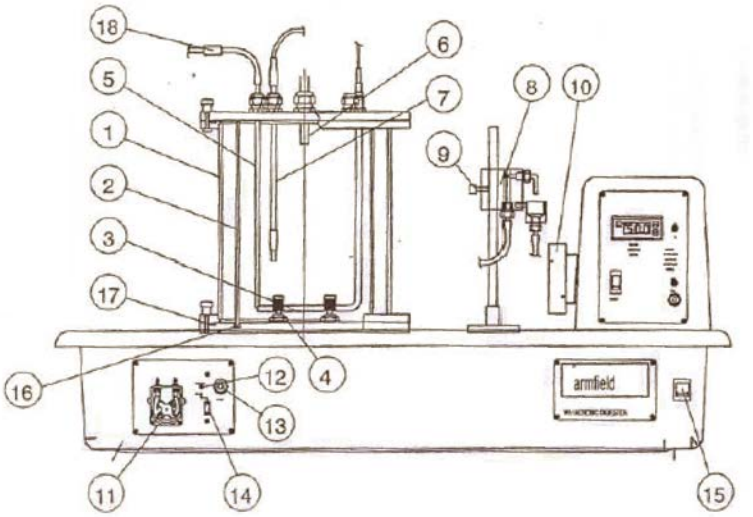

Figure 3.3: Parts of the biological aerobic pilot reactor.

The reactor vessel (1) has a total volume of 12.5 L at the maximum level. The level controller (8) allows the volume to be changed by unscrewing the corresponding screw (9) and moving the top to the desired position. The actual working volume of the reactor is that of a cylindrical permeable cylinder (2) which holds the suspended biomass and leaves the water. The treated wastewater penetrates through the pores of the cylinder, fills the space in the reactor between the permeable cylinder and the reactor wall, and then flows through the outlet at the bottom. The silicone seal on the bottom of the reactor allows the flow of water into the ring. The reactor lid presses the cylinder to the seal and ensures tightness.

The reactor is filled with a peristaltic pump (11) through a submerged tube (5). The 10-way potentiometer (13) allows a maximum speed (pumping) up to 30 min^{-1} with the switch (12) in the "manual" position. When the switch is switched to the "remote" position, the flow rate is controlled by an external device (such as a personal computer), with a connection to the input (14), which simultaneously represents a connection to the data-archiving device.

Two 100 W (3) heaters installed inside the permeate cylinder at the bottom of the reactor maintain the reactor temperature above the ambient temperatures.

The temperature sensor (7) sends information about the reactor temperature to the three-point controller on the control unit. The regulator automatically maintains a constant temperature by regulating the power of the heater.

The air, which suspends and aerates biomass, is introduced into a reactor with a small air pump (19) installed at the rear of the control unit. The air enters at the bottom of the reactor and passes through four nozzles (4) in the form of small bubbles that are evenly distributed

throughout the reactor content. The flow of the air into the reactor is controlled by a flow controller (10).

The permeable cylinder in the reactor eventually becomes impermeable. In this case, it is replaced by a replacement permeable roller. Its design allows easy installation, construction and cleaning of components.

3.4.1 Determination of process parameters

3.4.1.1 Temperature

The temperature is measured with a temperature sensor, which is immersed in the reactor and connected to its temperature control system. This automatically maintains the desired reactor temperature by regulating the heater's power. The operating temperature of 25 °C is set by pressing the key located on the top left corner of the device controller and simultaneously pressing the setting keys (plus / minus).

3.4.1.2 pH value

Use a digital pH meter to determine the pH value. The electrode is calibrated with buffer solutions pH = 7 and pH = 4. The calibrated electrode is immersed through the reactor lid into the suspension and connected to the digital pH meter where pH value is read. The electrode is immersed in the reactor during the process, whereby the coatings are stocked on it. For this reason, we calibrate and clean it daily.

3.4.1.3 COD value

The COD value is determined in the same way as for the anaerobic reactor (section 2.4.1.2.).

3.4.1.4 BOD_5 value

Using the manometric method (the OXI TOP device), the oxygen consumption is determined. The gauge is connected to the incubator bottle. In contrast to the dilution method, the manometric bottle is not full. When the microorganisms consume oxygen dissolved in the sample solution, they start to consume oxygen above the solution. A drop in partial pressure above the solution is a criterion for biochemical oxygen demand.

Preparation of chemicals

Liebman solutions

SOLUTION 1

8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 12H_2O$, 1.7 g NH_4Cl

SOLUTION 2

22.5 g $MgSO_4 \cdot 7H_2O$

SOLUTION 3

27.5 g $CaCl_2$

SOLUTION 4

0.25 g $FeCl_3 \cdot 6H_2O$

When preparing each solution, weigh the appropriate mass of the chemicals and dissolve them in distilled water, place in a 1000 mL volumetric flask and fill up to the mark with distilled water. The prepared solutions can be stored under appropriate conditions (room temperature, etc.) for several months.

Adapted inoculum

Use inoculum from an aerobic reactor.

Selecting the measuring range

Depending on the predicted value of BOD_5, select the corresponding volume of samples from the table 3.1.

Table 3.1: Sample volume for a 5 - day BOD test.

Sample volume, $\frac{V}{\text{mL}}$	Measuring range, $\frac{\gamma(O_2)}{\text{mg /L}}$	Dilution factor, f
432.0	0–40	1
365.0	0–80	2
250.0	0–200	5
164.0	0–400	10
97.0	0–800	20
43.5	0–2000	50
22.7	0–4000	100

Determination of BOD_5 value

Add 1 mL/L to each of the Liebman's solutions and 1 ml/L of the adapted inoculum (only inlet and blank) and, if necessary, a nitrificator (nitrification inhibitor) is added to aerated sample. Pour the precisely measured volume of the sample into a measuring bottle, add a magnetic stirrer, and insert a rubber plug into the neck of the bottle. Two granules of NaOH are placed in the rubber stopper. Care must be taken that the liquid does not come into contact with NaOH. At the end of the bottle, close the OXI TOP devices, mark them, place them in the inductive stirring system, and turn on the stirrer. A description of the OXI TOP operation is found in the instructions accompanying the device. BOD_5 is determined by the equation:

$$\gamma_{BOD_5} = \gamma_m \cdot f_d \qquad (3.1)$$

where: γ_{BOD_5} - BOD_5 value, mg/L

γ_m - measured BOD_5 value of diluted sample on OXI TOP

f_d - dilution factor.

3.4.1.5 Mass concentration of dry matter

The mass concentration of the dry matter is the sum of the mass concentration of the dissolved and undissolved substances, and is determined gravimetrically. Pipette 25 mL of the sample into a dry evaporating dish and dry at 105 °C to constant weight. The dry matter mass concentration in mg/L is determined by the equation:

$$\gamma_{\mathrm{dm}} = \frac{m_1 - m_2}{0.025} \tag{3.2}$$

where: m_1 - mass of evaporating dish with sample before drying, g

m_2 - mass of evaporating dish with sample after drying, g

3.4.2 Experimental procedure

Prepare the device for operation according to the original instructions supplied with the appliance. Before performing the experiment, check the functionality of its components and auxiliary measuring devices. The basic process parameters for the biological aerobic reactor are as follows:

- active volume of reactor, V = 7.5 L,
- mass rate of chemical consumption of oxygen per mass of dry matter, $r'_{\mathrm{COD,\,dm}}$ = 0.35 kg/(kg d),
- mass concentration of dry matter, γ_{dm} = 2.5 g/L,
- COD value of inlet wastewater, γ = 2.3 g/L,
- wastewater flow rate, $q_v = \frac{\left(2.5\,\frac{\mathrm{g}}{\mathrm{L}}\right)\cdot(7.5\ \mathrm{L})\cdot\left(0.35\,\frac{\mathrm{kg}}{\mathrm{kg\ d}}\right)}{\left(2.3\,\frac{\mathrm{g}}{\mathrm{L}}\right)} = 2.8\ \frac{\mathrm{L}}{\mathrm{d}}$,
- residence time, t = 2.7 d,
- inlet pH between 6.5 in 7.5,
- temperature, ϑ = 25 °C.

3.4.2.1 Pump calibration

The calibration of the pump is carried out in the same way as described in chapter 2.4.2.1.

3.4.2.2 Preparation of process solutions

Inoculum

The inoculum is prepared by diluting (if necessary) the sludge from the aerobic treatment plant so that the dry weight mass concentration is 2.5 g/L.

Synthetic wastewater

As an inlet substance, synthetic wastewater is used which, in addition to biodegradable impurities, also contains substrate and buffer solutions necessary for the optimal functioning of microorganisms. Synthetic wastewater is prepared by dissolving the appropriate amounts of chemicals collected in Table 3.2 in distilled water. Transfer the solution quantitatively to a 1000 mL volumetric flask and dilute with distilled water to the mark. The COD value of the prepared solution is γ_{COD} = 11500 mg/L.

Table 3.2: Composition of synthetically prepared wastewater.

CHEMICAL	MASS CONCENTRATION
Glucose	8.0 g/L
Bacterial peptone	2.4 g/L
Meat extract	1.6 g/L
NH_4HCO_3	0.4 g/L
KH_2PO_4	0.4 g/L
$NaHCO_3$	0.4g/L
Solution A	1.0 mL/L
Solution B	1.0 mL/L

Solution A was prepared by dissolving magnesium sulphate heptahydrate, $MgSO_4 \cdot 7H_2O$. Dissolve 0.5 g of $MgSO_4 \cdot 7H_2O$ in distilled water, quantitatively transfer to a 100 mL

volumetric flask and dilute to the mark with distilled water. The prepared solution has a mass concentration of 5 g/L and is stable for several months at room temperature.

Solution B is prepared by dissolving the appropriate amount of chemicals in distilled water, Table 3.3. At room temperature, the solution persists for several months.

Table 3.3: Solution B components.

CHEMICAL	MASS CONCENTRATION
$FeCl_3$	5.0 g/L
$CaCl_2$	5.0 g/L
KCl	5.0 g/L
$CoCl_2$	1.0 g/L

3.4.2.1 Starting up the aerobic reactor

The aerobic reactor is filled with a ready-made inoculum (up to 7.5 L active volume). The contents of the reactor is aerated for 12 h at a constant temperature of 25 °C and at an airflow rate of 1.7 L/min. Then begin with the gradual introduction of synthetic wastewater (γ_{COD} = 2 300 mg/L). Prepared synthetic water γ_{COD} = 11500 mg/L; therefore, it needs to be diluted in a 1:4 ratio (1 L of synthetic wastewater and 4 L of distilled water) prior to introduction into the aerobic reactor. The diluted synthetic wastewater flow is adjusted to 2.8 L/d, corresponding to a residence time of 2.7 d. Continue with aeration and after a few hours, the culture adjusts to the conditions in the reactor. At the same time, the mass concentration of the dry matter is controlled (γ_{dm} = 2.5 g/L). If the concentration of dry matter exceeds the optimal value, sewage sludge is removed through the valve located in the bottom of the reactor.

3.4.2.2 Continuous experiment

The experiment is carried out continuously under constant conditions ($\vartheta = 25°C$, q_{va} = 1.7 L/d, q_v = 2.8 L/d) for a few days. Diluted synthetic wastewater is stored in a vessel (V = 5 L) alongside the reactor and replaced with a new one every day. Immediately after the preparation,

synthetically prepared wastewater has a pH between 6.8 and 7.2, which greatly decreases, even to pH = 3.0, after several hours as a result of its composition and the influence of the environment (temperature, pressure and moisture). This decrease in pH value can lead to the wrong course of the experiment and thus the increase in the COD value and, consequently, to lower wastewater treatment efficiency. During the experiment, monitor the process parameters (temperature, pH, airflow) and observe the changes (color, odor) of wastewater in the reactor and outlet flow. Sample the inlet and outlet flow daily, and determine the values of COD and BOD_5. Control the dry matter content of the reactor daily and, if necessary, control it with the discharge at the bottom of the reactor.

3.5 RESULTS

Results are shown in tabular and graphic form. The table below presents a tabular method of showing data. Wastewater treatment efficiency regarding γ_{COD} is calculated by the equation:

$$\eta_{COD} = \frac{\gamma_{COD_{inlet}} - \gamma_{COD_{outlet}}}{\gamma_{COD_{inlet}}} \cdot 100\ \% \tag{3.3}$$

In a similar way, calculate the wastewater treatment efficiency regarding γ_{BOD_5}:

$$\eta_{BOD_5} = \frac{\gamma_{BOD_5\text{-inlet}} - \gamma_{BOD_5\text{-outlet}}}{\gamma_{BOD_5\text{-inlet}}} \cdot 100\ \% \tag{3.4}$$

Date	$\frac{\vartheta}{°C}$	$\frac{q_{va}}{L/min}$	$\frac{\gamma_{dm}}{mg/L}$	$\frac{\gamma_{COD\text{-inlet}}}{mg/L}$	$\frac{\gamma_{COD\text{-outlet}}}{mg/L}$	*pH* - inlet	*pH* - outlet	η_{COD}
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
Date (period)	$\frac{\vartheta}{°C}$	$\frac{q_{va}}{L/min}$	$\frac{\gamma_{dm}}{mg/L}$	$\frac{\gamma_{BOD_5\text{-inlet}}}{mg/L}$	$\frac{\gamma_{BOD_5\text{-outlet}}}{mg/L}$	*pH* - reactor		η_{BOD_5}
-	-	-	-	-	-	-		-

In graphic form, the results are shown as functions:

- COD value of inlet and outlet flow regarding time, *t*,

- BOD_5 value of inlet and outlet flow regarding time, *t*,
- wastewater treatment efficiency regarding COD and BOD_5 values regarding concentration of dry matter γ_{dm},
- inlet and outlet flow pH regarding time, *t*.