Bioreactor

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I Basic relationships and definitions

Chemical reactors are units, in which chemical transformation of raw materials to products takes place. One common type of them are batch reactors, for which discontinuous operation is the most significant characteristics with periodical inlet and outlet of the reaction mixture. In industry, they are commonly used in production of specialty chemicals, such as pharmaceuticals, paints, pesticides etc.

Reaction rate of a species r_i is defined as rate of change of molar amount of the species in unit volume of the reaction mixture due to chemical reaction

$$r_i = \frac{1}{V}\frac{dn_i}{d\tau} \tag{1}$$

Where τ denotes time. If the species reaction rate is divided by its stoichiometric coefficient υ_i, reaction rate id obtained

$$r = \frac{r_i}{\upsilon_i} = \frac{1}{V\,\upsilon_i}\frac{dn_i}{d\tau} \tag{2}$$

If the reaction mixture volume is constant, the molar amount in equations 1 and 2 can be expressed using concentrations: $n_i = c_{ni} \cdot V$ and the reaction rate can be obtained from relationship

$$r = \frac{1}{\upsilon_i}\frac{dc_{ni}}{d\tau} \tag{3}$$

In many cases, the composition of reaction mixture is expressed using conversion of a specie ζ_i, which is defined as a ratio of reacted amount of the species and the amount entering the reaction.

Common quantity to characterize change of reaction mixture composition is conversion ζ_i, which is defined as a ratio of reacted amount of the species to the amount entering the reaction:

$$\zeta_i = \frac{n_{i0} - n_i}{n_{i0}} \tag{4}$$

Bioreactor is a unit, in which the transformation of raw material onto a product is carried through microorganisms or biochemically active compounds (i.e. enzymes), which were produced by microorganisms.

Reaction kinetics, in which “classical” (biologically inactive) species take part, is usually described by a power function

$$r = k\,(c_A)^{\gamma_A}(c_B)^{\gamma_B} \ldots = k\,\prod_{i=A,B,\ldots}(c_i)^{\gamma_i} \tag{5}$$

i.e. as a product of a kinetic constant k and reaction species concentrations c_i raised to power coefficients γ_i (reaction orders to individual species). To describe the reaction rate of enzymatic reactions, more complicated kinetic models have to be introduced. First important step in this field was offered by Leonor Michaelis and Maud Menten in 1913. They build a theory based on few key assumptions: (i) enzyme (E) and substrate form an enzyme-substrate complex through a reversible reaction, and products are formed during irreversible decomposition of this complex; (ii) total concentrations of enzyme and enzyme-substrate complex are constant through the reaction phase.

$$E + S \rightleftharpoons ES \rightarrow E + P \tag{6}$$

Applying aforementioned assumptions, following equation describing initial enzymatic reaction rate and substrate concentration [S]:

$$v_0 = \frac{V_{\max}[S]}{K_M + [S]} \tag{7}$$

where V_{max} is the maximum (limiting) rate of enzymatic reaction and K_M denotes Michaelis constant. The Michaelis-Menten kinetics has, similarly to e.g. Langmuir isotherm, a saturation character, as is shown in Figure 1.

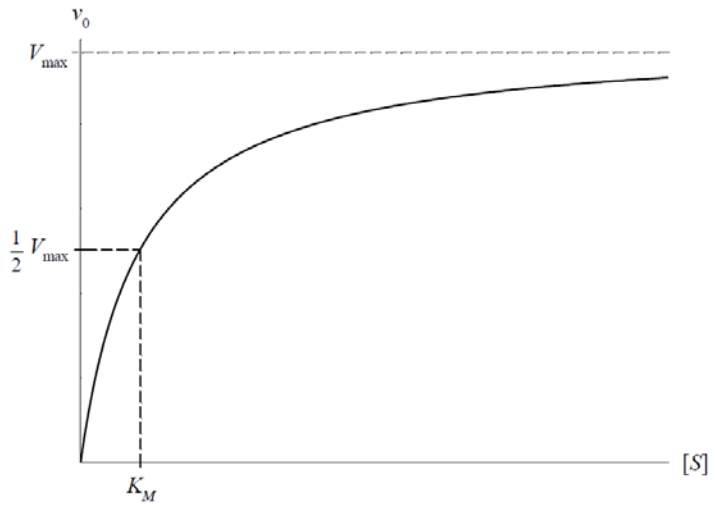


Figure 1, Relationship between initial rate of enzymatic reaction and substrate concentration according to Michaelis-Menten kinetics.

Michaelis and Menten formulated the model based on experimental results of sucrose hydrolysis, which is decomposed on glucose and fructose by enzyme invertase (beta-fructofuranosidase). Equimolar mixture of these two monosaccharides is usually referred to as invert syrup and it is valued by bakers as it is sweeter than sugar and less prone to crystallization. This decomposition is also the aim of this laboratory work. Because the activity of an enzyme is temperature and pH dependent, it is necessary to hold these parameters constant through the reaction. For instance, the invertase enzyme solution used in this work reaches maximum of its activity around pH = 5.

If the information about the (bio)chemical reaction is to be obtained, it is necessary to determine reactants or products concentrations through an experiment. Because saccharides are optically active species, i.e. their molecules incline the plane of polarized light, their concentration is usually determined through polarimetry.

Polarimeter is an instrument which determines the angle, by which the polarized light plane was rotated after the light passed through sample of optically active compounds or their solutions. The angle of rotation α_{20} determined at 20°C depends on the sample length (cuvette length) ℓ and concentration of optically active specie c_i according to an equation

$$\alpha^{20} = [\alpha]_{D,i}^{20}\, c_i\, l \tag{8}$$

where $[\alpha]_{D,i}^{20}$ denotes specific rotation, which is a characteristics for each optically active compound *i*, determined at 20°C using light doublet of wavelength 589 nm from sodium lamp. Important and commonly used rule in polarimetry is Bit's rule of additivity: solution of two optically active compounds A and B with concentrations c_A and c_B shows inclination given by algebraic addition of the contributions of both optically active compounds

$$\alpha^{20} = l([\alpha]_{D,A}^{20}\, c_A + [\alpha]_{D,B}^{20}\, c_B \tag{9}$$

II Aims

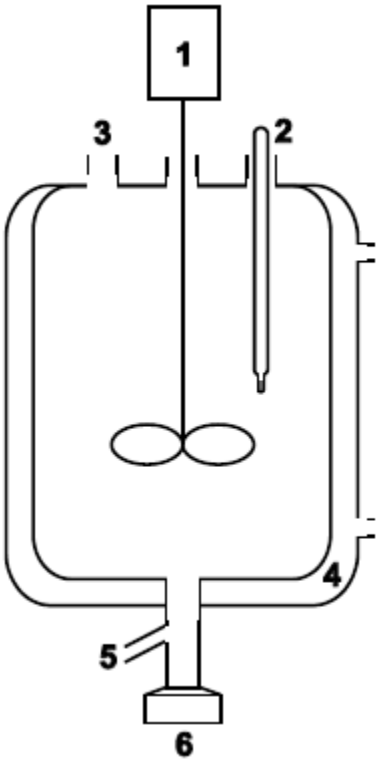
1. Determination of rotation of polarized light plane of reaction mixture through enzymatic reaction.

2. Calculation of sucrose concentration from the measured values of the optical inclination.

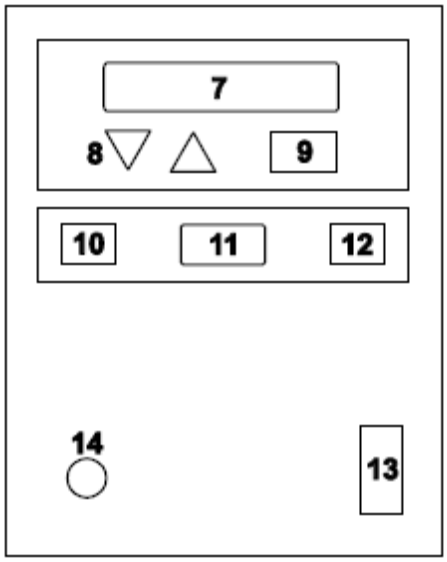
3. Plotting a graph of dependence of sucrose conversion on time.

III Description of apparatus

Experimental apparatus is depicted in figure 2. Glass cylindrical bioreactor with thermostating jacket **4** is equipped with holes in the lid. The left one **3** is used for inserting a glass funnel and filling the bioreactor with individual compounds of the reaction mixture. In the right hole **2** is inserted a digital thermometer to allow temperature verification of the reaction mixture. The temperature is shown on a display placed at the wall to the left from the reactor. The reaction mixture is agitated using turbine impeller **1**. Sampling and flushing of the reactor is made using a valve **6** at the bottom of the reactor vessel. Temperature is maintained by a thermostatic batch circulating water through the reactor jacket. Control panel of the thermostatic bath is depicted on figure 3.



1 – agitator drive with control panel
2 – thermometer
3 – inlet opening for filling
4 – thermostating jacket
5 – outlet
6 – sampling and drain valve
Obr. 2. Batch bioreactor scheme



7 – display
8 – buttons for temperature regulation
9 – confirming botton (enter)
10 – pump switch
11 – display
12 – temperature setting swich
13 – power switch
14 – temperature limmiter controll

Obr. 3. Thermostat controll panel.

Part of the apparatus is also a polarimeter, cuvette of length ℓ = 1 dm, set of bakers (glass and plastic), plastic funnel, measuring cylinders, wash bottle, two 5 l canisters with distilled water, paper tissues, digital pH-meter and necessary chemical species (sucrose, invertase enzyme, acetic acid solution).

IV Work procedure

IV.1 Preparation tasks

At the beginning it is necessary to ensure the agitator, thermostatic batch and polarimetry are connected to power. Also check the vale 6 at the bottom of the reactor vessel is closed. Switch the polarimeter on and let it stabilize for at least 20 minutes. Pressing button 13 is the thermostatic bath switched on and by pressing the button 10, the water circulation through the reactor jacket is started. Display 7 at the control panel shows actual temperature of the water in the thermostatic bath (S is shown on the right of the display 11). Pressing button 12 switches the thermostatic bath into temperature set point setting regime (S is shown on the left of the display 11). In this regime, it is possible to set the required temperature according to task list given using arrows 8 and confirming by button 9.

The mass of sucrose is calculated in a way that after dissolving in given volume of water, solution of required weight fraction will be created in the solution. Calculated amount of sucrose should be verified by laboratory assistant. Sucrose should be weighted in a plastic baker and while stirring continuously, required solution of sucrose is created. Solution is then poured using the funnel in hole 3 into the reactor. At zero stirring speed, the agitator is switched on and impeller frequency is set afterwards. During whole preparation phase, periodically check the thermometer display and adjust set point temperature of the thermostatic bath to reach requested temperature of the sucrose solution.

Further it is necessary to set pH value of the solution to a value close to pH = 5. This step is very sensitive, and thus should be performed with high care. First, take a small sample of the reaction mixture using hole 5. Remove the plastic cover from the pH-meter and wash its tip with distilled water, drying the probe afterwards using a paper tissue. Switch the pH-meter on and immerse the probe tip into the baker with the sample. Stir the sample using the pH meter, slowly moving it the sample and watch the pH value given. Consult the measured value to Your laboratory assistant and discuss the amount of 0.3% acetic acid (if decrease of pH is needed) or NaOH (if increase of pH is needed), which is to be added. After modifying the pH value of the reaction mixture, leave the mixture stirring for few minutes and take a sample again. Perform another adjustment of the pH again, if needed. At the end of the step, pH value should be 5,0 ± 0,1. Switch off the pH-meter, wash its tip with distilled water and replace the plastic cover. Remove the plastic funnel and close the hole 3 by a rubber plug.

After 20 minutes of stabilizing the output of sodium lamp, the polarimeter is ready for measurement. Open the lid of the cuvette space, remove the freely laying cuvette and flush it thoroughly with distilled water to remove impurities.

IV.2 Measurement phase

Measurement is based on determination of optical rotation of the reaction mixture sample. The samples are taken in regular time intervals.

Ask Your laboratory assistant for the enzyme and weight required amount (in miligrams) on analytical balances. Dissolve the enzyme in distilled water in 50ml measuring flask. Stir carefully to dissolve all of the enzyme. Take about 60ml of a sample using valve 6 and pour the enzyme solution into the reactor. Remnants of the enzyme solution from the funnel can be flushed using wash bottle. Read the temperature of the reaction mixture (at the display of the thermometer) and record it into the task list as the measurement no.1, along with time 0.

Flush the cuvette by the sample twice and then fill it up to the top while removing all bubbles from the optical path by inclining the cuvette from side to side. Place the cuvette into the cuvette space of the polarimeter and close the lid. Determination of the optical rotation α_t is made by looking into the ocular and turning the setting ring to minimize contrast between black and orange stripes. If the whole area is orange, the position of the setting ring is very far from the final position and large change is to be made. At the correct setting, the stripped area will take a uniform greyish colour. The value of αt is obtained from reading of the mechanical scale to the left from the ocular. Reading is made in similar way as on typical micro-meter, the scale orientation is from top down. Record the value into the task list. Remove the cuvette and pour all sample along remains from the cuvette flushing back into the reactor. At the end, flush the cuvette with distilled water. Sampling and determination of the optical angle is repeated every 5 minute during first 30 minutes, then take a sample and measure it every 10 minutes for next 70 minutes. Instantaneous values are recorded in the task list along with temperature of the reaction mixture during the sampling.

Never forget to flush the cuvette by distilled water and new sample before each measurement. Also do not forget to flush the sampling baker with distilled water.

At time 30 min verify the pH value in the same way described in the preparation section. This value should be recorded as a remark in the task list. If the value is significantly different from the initial, the pH should be readjusted after a consultation with Your laboratory assistant.

IV.3 Finalizing the measurement

After taking last sample, switch off the agitator, thermostating bath and polarimeter. Verify, that pH-meter is switched off and kept in storage solution. Drain the reaction mixture from the bioreactor and dispose it in the sink. Flush the bioreactor vessel with distilled water. Flush the polarimetric cuvette by distilled water and place empty cuvette in the cuvette space of the polarimeter. Wash all used laboratory equipment in the sink and remove all stains from working place by wet cloth.

V Safety measures

1. Pay special attention when working with acid and do not use it for other purpose ohter than as stated in the work procedure.

2. Handle the p[olarimeter carefully. At all times keep it dry and clean, both around the unit and inside the cuvette space. Also it is necessary to stick to the procedure given here or instructions in users guide, which is kept in the top drawer of the working desk.

3. Incase of polarimeter malfunction, call Your laboratory assistant. It is strictly forbidden to interfere with the polarimeter without supervision.

4. Working with the polarimeter cuvette has to be carefull and any damage must be prevented. It is an expensive material and no spare piece is available.

VI Processing of measured values

First, temperature correction is to be applied onto the rotation angle obtained at temperature of the experiment (α^t) to standard temperature 20°C (α^{20}) using relationship

$$\alpha^{20} = a \cdot \alpha^t + b \qquad (10)$$

Where the values of temperature correction *a, b* are given in table 1.

Tab. 1. Constants of temperature correction

t	30°C	40°C	50°C
a	-	-	1,000
b	-	-	0,876

Initial sucrose concentration (first point at τ = 0 min) is calculated using equation 8.

For succeeding measurements, the concentration of sucrose and invert sugar is obtained using eq. 9. Sucrose concentration c_S is calculated from equation:

$$c_S = c_{S0} - M \cdot c_I \qquad (11)$$

where *M* is the ration of molar weights of sucrose M_S (342 g.mol^{-1}) and invert sugar M_I (360 g.mol^{-1}) and c_I is concentration of invert sugar, which is obtained by substitution of eq. 11 into eq.9:

$$c_I = \frac{[\alpha]^{20}_{D,S}\, c_{S0} - \alpha^{20}}{M \cdot [\alpha]^{20}_{D,S} - [\alpha]^{20}_{D,I}} \qquad (12)$$

Invert sugar is an equimolar mixture of glucose and fructose, the molar weights of which are equal. Their concentrations are then given by

$$c_G = c_F = \frac{c_I}{2} \qquad (13)$$

Values of constants used in the equations 11 – 13 are given in table 2.

Tab. 2 Values of constants.

Constant	Meaning	Value	Unit
M	molar weights ration of sucrose and invert sugar	0,95	1
$[\alpha]_{D,S}^{20}$	specific rotation of sucrose at 20°C	+66,53	angular ° dm^2kg^{-1}
$[\alpha]_{D,I}^{20}$	specific rotation of invert sugar at 20°C	–20,59	angular ° dm^2kg^{-1}
ℓ	cuvette length	1,00	dm

VII Symbols

γ_I	order of reaction to species i	1
a, b	temperature compensation constants	1
α^t	optic rotation angle at temperature *t*	angular °
α^{20}	optic rotation angle at temperature 20°C	angular °
$[\alpha]_{D,S}^{20}$	specific rotation of sucrose at 20°C	angular ° dm^2kg^{-1}
$[\alpha]_{D,I}^{20}$	specific rotation of invert sugar at 20°C	angular ° dm^2kg^{-1}
c_{ni}	molar concentration of i	mol dm^{-3}
c_i	mass concentration of i	kg dm^{-3}
c_I	mass concentration of invert sugar	kg dm^{-3}
c_F	mass concentration of fructose	kg dm^{-3}
c_G	mass concentration of glucose	kg dm^{-3}
c_S	mass concentration of sucrose	kg dm^{-3}
E	enzyme	-
ES	enzyme-substrate complex	-
k	rate constant of chemical reaction	
K_M	Michaelis constant	mol dm^{-3}
ℓ	polarometric cuvette length	dm
n_i	molar amount of species *i*	mol
P	product	-
S	substrate	-
r	reaction rate	mol.$dm^{-3}min^{-1}$
r_i	reaction rate of species *i*	mol.$dm^{-3}min^{-1}$
υ_i	stoichiometric coefficient of species *i*	1

τ	time	min
ζ_i	conversion of species *i*	1
v_0	initial rate of enzymatic reaction	$mol.dm^{-3}min^{-1}$
V	reaction mixture volume	dm^3
V_{max}	maximum (limiting) enzymatic reaction rate	$mol.dm^{-3}min^{-1}$