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Design of a batch stirred fermenter for ethanol production

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Abstract

This paper addresses the batch stirred bioreactor design for ethanol production with yeasts Saccharomyces cerevisiae under anaerobic conditions carried out to improve the performance of the fermentation process. A large, appropriate – sized fermenter is supposed 70 m^3. The operating volume is 52,5 m^3. Batch fermentation was perform with 200 g/l glucose concentration. Fermentation time, is 11,4 hours with ethanol stripping 69,1 $g.l^{-1}$ and 12 hours 75,9 $g.l^{-1}$ without stripping. Computing is stopped when glucose alteration obtain 97 percent. The kinetic constants (K_s, K_p, μ_{max}) of batch fermentation were 2,0 $kg.m^{-3}$, 97,9 $kg.m^{-3}$, 0,476 h^{-1} respectively. Output per a batch is 3 623 kg and a single fermenter can produce 514 batches per year. From it follows that the year vintage is close to the actual 1 862 222 kg. Therefore, the number of 70 m^3 fermenters required 4 bioreactor. Whole heat exchange and heat surface area estimated 338437 J/s and 40 m^2 respectively. The maximum yield of biomass on substrate ($Y_{X/S}$) and the maximum yield of product on substrate ($Y_{P/S}$) in batch fermentation were 82 % and 35,5 % respectively. The present research has shown that high sugar concentration (200 g/l) in the batch stirred bioreactor was successfully converted to ethanol. The achieved results in batch stirred bioreactor with high substrate concentration are promising for scale up operation. The proposed model can be used to design a larger scale batch stirred bioreactor for production of high ethanol concentration.

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Keywords: batch stirred bioreactor design; metabolic processes; bioreactor scale-up

1. Introduction

Biochemical engineering is concerned with conducting biological processes on an industrial scale. This area links biological sciences (genetics, microbiology, animal cell culture, molecular biology, biochemistry, embryology, cell biology, enzyme technology) and engineering sciences such as chemical and reaction engineering. The role of biochemical engineering has become more important in recent years due to the dramatic developments of biotechnology. Biotechnology can be defined as "Commercial techniques that use living organisms, or substances from those organisms, to make or modify a product, including techniques used for the improvement of the characteristics of economically important plants and animals and for the development of microorganisms to act on the environment [1]. The numerous applications of the biotechnology are mainly in areas of pharmaceuticals (antibiotics, antigens, etc.), animal and plant agriculture (higher – yielding food animals, herbicides, insecticides, etc), specialty chemicals (amino acids, enzymes, vitamins, etc.), environmental applications (mineral leaching, toxic waste degradation, etc.), commodity chemicals (acetic acid, ethanol, citric acid, etc.) and bioelectronics (biosensors, biochips). Biological processes have advantages and disadvantages over the traditional chemical processes. The major advantages are mild reaction condition (typically room temperature, atmospheric pressure, fairly neutral medium pH, etc.) specificity (enzyme – highly specific catalyst), effectiveness (enzyme-catalyzed reaction is usually much faster than non-biological catalysts and

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selectively produced the desired product), renewable resources (biomass provides both carbon skeletons and energy required for synthesis), recombinant DNA technology (promises enormous possibilities to improve biological processes). On the other hand disadvantages are as follows: complex product mixtures (cell mass, metabolic products, no conversed original nutrients), dilute aqueous environments (product separation is very expensive), contamination and variability (cells tend to mutate due to the changing environment and may lose some characteristics vital for the success of process). The main biotechnological nodes are pre-treatment processes (e.g. solubilization and hydrolysation of raw materials, sterilization), bio reaction section (bioreactor – the most important and main equipment) and separation section (removal of insoluble, product isolation, purification and polishing). The crown of successful solution of each chemical process is a large scale production at the best economic efficiency.

The submitted diploma work is concentrated on the Design of a batch stirred fermenter for ethanol production and is divided into several parts. Firstly ethanol properties, applications, production and recovery are briefly mentioned. Then the industrial scale batch stirred fermenter design for ethanol production with cells Saccharomyces cerevisiaeis the matter of our concern. Only engineering aspects are taken into account in order to properly model the bioreactor and allow scale-up [2]. Engineering aspects are a combination of metabolic processes that involve stoichiometry, thermodynamics, microbial kinetics and physical processes such as mixing, power consumption, heat transport and mass transport. Finally conclusion summarizes results gained through bioreactor design for ethanol production.

2. Properties, Applications and Microbial Production of Ethanol

2.1. Properties

Ethanol (ethyl alcohol) is a clear colorless liquid with a characteristic agreeable odor. In dilute aqueous solution it has a somewhat sweet flavor but in more concentrated solutions it has a burning taste. Ethanol, CH_3CH_2OH is an alcohol a group of chemical compounds whose molecules contain a hydroxyl group – OH bonded to a carbon atom. Its low freezing point has made it useful as the fluid in thermometers for temperatures below –40°C the freezing point of mercury and for other low-temperature purposes such as for antifreeze in automobile radiators. Ethanol is an alternative energy source. It is an alcohol made by fermenting corn or other similar biomass material. There are three primary ways that ethanol can be used as a transportation fuel [3].

2.2. Ethanol Production Process

There are two basic types of ethanol production plants already alluded to. One is the “wet mill” and the other is the “dry mill”. The wet mill process will soak the grain (corn is the most common so we will use it in our discussion here) until the corn is able to be broken down into its components [4]. At the present time, this product is commercially manufactured via large scale aerobic fed-batch fermentation of selected strains of Saccharomyces cerevisiae [5].

Production of ethanol from biomass requires even more extensive processing to release the polymeric sugars in cellulose and hemicellulose that account for 23 % – 53 % and 20 % – 35 % of plant material, respectively. Cellulose is a beta-linked glucose polymer, whereas hemicellulose is a highly branched chain of xylose and arabinose that also contains glucose, mannose and galactose [6]. Hydrolysis of these carbohydrate polymers is usually accomplished by exposure to acid (contributed either by the biomass or added externally) and by enzymes.

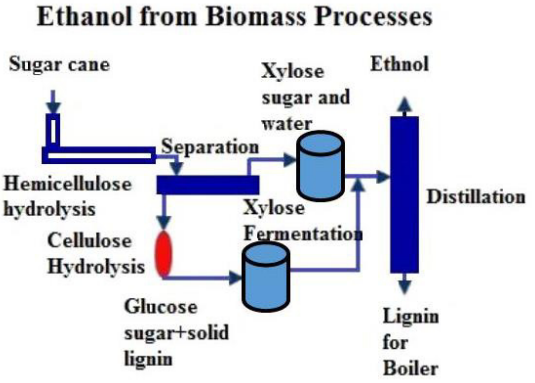


Fig. 1. From biomass process flow sheet for ethanol production [7]

2.3. Bioreactor scale-up

The scale-up of a bioreactor (the step from small scale to a production scale) is a very difficult task because many different aspects of engineering (physical and metabolic processes) and economic considerations need to be taken into account and the final scale-up will necessarily be a delicate compromise between inherently conflicting desirable characteristics. One might regard scale-up as more an art than a science [2]. Multiphase impeller stirred tank reactors enhance mixing of reacting species used in a variety of chemical industries. These reactors have been studied based on Computational Fluid Dynamics (CFD) that can be used in the analysis, design and scale up of these reactors [8].

Production strains are normally first selected in the laboratory, subsequently tested in a number of bioreactor of increasing scale, and the final process verification is carried out in a pilot plant. Unfortunately it is physically impossible to maintain the same process conditions for lab-scale, pilot-scale and industrial scale bioreactors. The reason is that physical processes are directly and metabolic processes indirectly scale dependent. Metabolic processes (microbial phenomena) are theoretically scale independent but practically as a consequence of scale dependent transport phenomena the local environment surrounding the cell will be different in a large scale than in a small scale typically well stirred reactor and may cause metabolic changes. Physical processes (transport phenomena) are scale dependent, they are changed with the reactor scale and described by classical mechanical or chemical engineering. From it follows that likely no process parameter value will be exactly the same in the different reactor scales. Even a completely different reactor design may be preferable. Mathematical models of varying complexity are made up by knowledge combination of metabolic and physical processes. Steps in scale-up are schematically shown in Figure 2 [2].

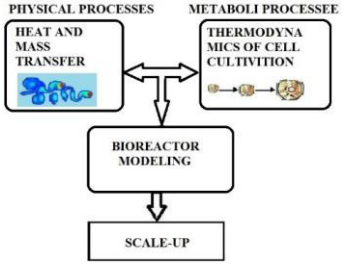


Fig. 2. Schematic representation of analysis steps in scale-up [1 – modified]

The impeller diameter is usually around 1:3 of the reactor diameter for Rushton turbines. The round-bottom facilitates cleaning, sterilization and avoids stagnant zones during operation. Typically there are four equally spaced baffles, equipped near the inner vessel wall, used to break vortexes and so increase the mixing efficiency. Sterile air (or possibly oxygen) for aerobic fermentation is introduced by means of a bubble sparger located below the lowest of the impeller. Cooling (heating) can take place through the reactor wall or by the use of internal coils [2]. Optimal pH in the liquid reaction medium is typically kept by adding acid or base from separate reservoirs and gaseous products such as carbon dioxide leave through the headspace of the reactor as exhaust gas. The scheme of a batch stirred tank fermenter is depicted in Figure 3.

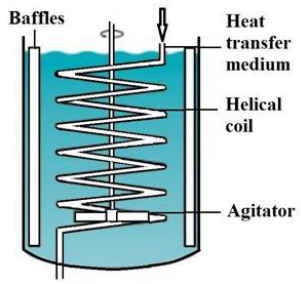


Fig. 3. Helical Coil of a batch type bioreactor [9]

On the basis of the assumed potential market the numbers and the volume of a large scale industrial batch fermenter are chosen. Kinetic data adopted from the literature [2] enables calculation the year and one batch production of ethanol by Saccharomyces cerevisiae under anaerobic conditions via material balance of considered species (see part Mathematical model below). The operating time of the total available year time is 330 days. The potential market equals 7 445 000 kg/year. A large, convenient-sized fermenter is assumed 70 m^3. The operating volume (volume of the liquid phase) is 52,5 m^3. Production per a batch (one cycle time is 11, 4 including 4 hours of turnaround) 3 623 kg and a single fermenter can provide 514 batches per year.

From it follows that the year production equals approximately 1 862 222 kg. Thus, the number of 70 m^3fermenters needed is 4 bioreactor and impeller geometric characteristics and aspect ratios, followed from the scale-up methods [10] are shown in Table 1.

Table 1. Bioreactor geometric characteristics

Nominal volume V_R [m^3]	70
Working tank volume V_L [m^3]	52.5
Agitator speed N [1/s]	2.5
Vessel total height h_R [m]	9.29
Vessel width d_R [m]	3.10
Impeller type	Rushton turbine
Impeller diameter d_s [m]	0.97
Number of impeller n_I [pc]	4
h_R / d_R	3/1
d_s / d_R	1/3.2

2.4. Metabolic processes

A living cell is a complex chemical reactor in which more than one thousand enzyme-catalysed reactions occurred that tend to be organized into sequences called metabolic pathways. The total of all chemical reaction activities is metabolism, material and energy exchange in the cell [11]. Metabolism involves catabolic (energy forming) reactions and anabolic (energy utilizing reactions). It is practically very difficult to identify them qualitatively and even impossible quantitatively [12]. That is the reason that simplified approaches, structured and unstructured models, are used to describe reaction kinetics. Structured models are improvements of unstructured models because of the predictive strength and ability to describe the growth process at different operating conditions. The biomass is divided into a few compartments and cell components with similar function should be placed in the same compartment [2]. Since even that view is often two complex for basic bioengineering analysis unstructured models are very frequently used [12]. Cellular components are pooled into a single biomass component which composition is represented by the total biomass concentration. It is assumed that biomass composition remains constant during fermentation (typical engineering approximation). And just quantitative description of biomass growth (reaction kinetics) during a process, expressed as correlations between reactant/product concentrations, plays the key role for the optimal bioreactor design and secure mode operation because material and energy balances are not possible to form without reaction terms which express conversion of substrates, generation of products and heat in a biochemical process [12]. However before any meaningful investigation of the reaction kinetics the study of the cell stoichiometry and thermodynamics must be successfully done [2].

2.5. Stoichiometry of cell cultivations

2.5.1. Theoretical part

Unstructured model is an attempt to represents all the chemical reactions by a single overall stoichiometric equation that expresses conversion of substrates to biomass and metabolic products (“black box” model). This is clearly an immense simplification of the total biochemical process occurred within a cell. Biomass is considered as unstructured matter with general composition of biogenic elements $CH_xO_yN_zP_mS_n$ and usually written in stoichiometric equations with an average biomass composition ($CH_{1,8}O_{0,5}N_{0,2}$) [2]. Saccharomyces cerevisiae (baker’s yeast) is one of the important industrial microorganisms which are used in the brewing, food manufacturing and genetic engineering industries. Under moderate operating conditions, continuous bioreactors producing Saccharomyces cerevisiae may exhibit sustained oscillations [13].

2.5.2. Practical part

Ethanol production with cells *Saccharomyces cerevisiae* under anaerobic conditions is a typical example of cell cultivation with a primary metabolic product generation directly connected with the biomass growth. From this follows that only one overall equation is necessary for a description of the cell stoichiometry. The key (limiting) substrate is glucose.

$$sC_6H_{12}O_6 + bNH_3 \rightarrow xCH_{1,83}O_{0,56}N_{0,17} + pC_2H_5OH + gC_3H_8O_3 + dCO_2 + eH_2O \quad (1)$$

The basis for calculation of stoichiometric coefficients, *s*, *b*, *x*, *p*, *g*, *d,* and *e* is the Balance of atoms *C*, *H*, *O*, *N*.

$$C: 6s = 1x + 2p + 3g + 1d \quad (2)$$

$$H: 12s + 3b = 1{,}82x + 6p + 2e \quad (3)$$

$$O: 6s = 0{,}5x + 2p + 3g + 2d + 1e \quad (4)$$

$$N: 1b = 0{,}2x \quad (5)$$

For seven stoichiometric coefficients seven independent algebraic equations are in disposal. Balance system is correctly specified.

For ethanol production can be redox balance expressed as follows:

$$e^-: se^-_s = xe^-_x + pe^-_p + ge^-_G \Rightarrow e^- : sC_s\gamma_s = xC_x\,\gamma_x + pC_p\gamma_p + gC_G\gamma_G \quad (6)$$

Where e^-_i is the number a component free electrons, C is the number of carbon atoms of a component, γ_i is the degree of reduction.

$$e^-_s = C_s\,\gamma_s = 4.6 = 24$$

$$e^-_x = C_x\,\gamma_x = 1.4{,}2 = 4{,}2 \quad (7)$$

$$e^-_p = C_p\,\gamma_p = 2.6 = 12$$

$$e^-_G = C_G\,\gamma_G = 3.4{,}67 = 14{,}01$$

The ethanol stoichiometric coefficient, *p* is then:

$$p = 1{,}530 \quad (8)$$

Other unknown stoichiometric coefficients *d*, *b* and *e,* are found from the balance of atoms:

$$b = 0{,}140 \quad (9)$$

$$d = 1{,}649 \quad (10)$$

$$e = 2{,}247 \quad (11)$$

The yield coefficients $(\mathrm{g/g})$, $Y_{s/s}$, $Y_{A/s}$,, $Y_{B/s}$,, $Y_{X/s}$,, $Y_{D/s}$,, $Y_{E/s}$,, of the total stoichiometric equation (1) are summarized in the Table 2.

Table 2 the yield coefficients (g/g), $Y_{i/j}$, for the ethanol fermentation

Component	$Y_{i/j}$ (g/g)
S:	1
B:	0,013
X:	0,126
P:	0,391
G:	0,079
D:	0,403
E:	0,025

Stripping of some ethanol from the liquid phase to the gaseous phase during fermentation process is a common cause of error in the mass balances. If the carbon balance, according to experimental data, shows that some carbon is missing in the products, the redox balance is used to identify so far unknown compound and subsequently satisfied the carbon balance. But in that case there is a good reason to believe that the missing component is ethanol that is stripped off [2].

2.6. Thermodynamics of cell cultivations

2.6.1. Theoretical part

Cells use chemical energy from nutrients quite efficiently but, like any process, some of energy in the substrates is released as heat. Cellular heat production is primary the result of metabolism. Consequently is reasonable to expect an approximately proportional relationship between heat generated and energy substrate utilized [11]. The processed biomass extracted from these species of microorganisms could be utilized as a source of potential protein either for food supplement [14]. Approximate heat balance related to the key substrate consumption is expressed as follows [2]:

$$q_r = (-\Delta_r h) = \sum_{j=1}^{n} {Y_{Sj}}^{mol}(-\Delta_c h_j) - \sum_{i=1}^{n} {Y_{pi}}^{mol}(-\Delta_c h_j) \quad (12)$$

Where q_R is the mole reaction heat and $\Delta_r h$ the mole standard reaction enthalpy ${Y_{Sj}}^{mol}$ are the yield (stoichiometric) coefficients on the left hand (substrate) side and ${Y_{pi}}^{mol}$ the yield (stoichiometric) coefficients on the right hand (product) side of a total stoichiometric equation describing the biochemical process.

Standard combustion heats can be found out either experimentally (ordinary species are listed in Tables) [2] or from Patel-Eriksson empirical relationship [12].

$$(-\Delta_c h_i) = 115\ C_i \gamma_i \quad (13)$$

Where C_i is the number of carbons in a compound and γ_i is its degree of reduction.

2.6.2. Practical part

The reaction heat, q_R (kJ/mol), related to the consumed glucose, is given by:

$$q_R = (-\Delta_r h) = \sum_{j=1}^{n} {Y_{Sj}}^{mol}(-\Delta_c h_j) - \sum_{i=1}^{n} {Y_{pi}}^{mol}(-\Delta_c h_j) \quad (14)$$

Values needed for calculation of reaction heat, q_R , are shown in Table 3.

Table 3 Values for calculation of the mole reaction heat q_R

Component	$(-\Delta_c h_i)$ (kJ/mol)	$Y_{i/j}$ (mol/mol)	$Y_{i/j}(-\Delta_c h_i)$ (kJ/mol)
S: $C_6H_{12}O_6$	2803	1	2803
B: NH_3	383	0.140	53,62
X: $CH_{1.82}O_{0.58}N_{0.16}$	560	0,826	462,56
P: C_2H_6O	1367	1,530	2091,5
G: $C_3H_8O_3$	1661	0,155	257,46
E: H_2O	0	0,247	0
D: CO_2	0	1,649	0

$$q_R = (-\Delta_r h) = 45{,}1\ Kj.mol^{-1} \quad (15)$$

2.7. Microbial kinetics

For successful describing of any fermentation process is necessary to know microbial kinetics that expressed correlations between rates and reactant/product concentrations, temperature and pH, $r = f(c_{,1}, c_{,\dots,} c_{n1}, c_{n?,\dots i1}, c_{i1}, c_{i?,\dots,} T, PH)$. By its inserting in mass balances the substrate conversions and the yield of individual products is possible to predict. This leads to simulations that finally may result in optimal equipment design [2].

Unstructured model is assumed to describe the microbial kinetics of alcohol fermentation yeasts *Saccharomyces cerevisiae* that provides a nice example of products inhibition with specific-growth function of the type [2]:

$$\mu = \mu_{max} \frac{c_s}{K_s + c_s} \left(1 - \frac{c_p}{K_p}\right) \quad (16)$$

Where μ_{max} is the maximum specific biomass growth rate, K_s is the saturation constant of glucose, c_S is the concentration of glucose, c_v is the concentration of biomass, K_p is the inhibition parameter.

Values of kinetics parameters, μ_{max}, K_S, K_p for the description of microbial kinetics and next applied in mathematical modeling were evaluated on the basis of literature information [2]:

$\mu_{max} = 0{,}476\ h^{-1}$

$K_s = 2{,}0\ Kg.m^{-3}$

$K_p = 97{,}9\ Kg.m^{-3}$

2.8. Physical processes

The physical processes (mixing, power consumption, heat transfer, mass transfer, fluid flow pattern, and rheology) are scale dependent and described by mathematical models of varying complexity: the computational fluid dynamics (CFD), the compartment model approach (CMA), formulae and correlations approach (FCA).One of the major phenomena responsible for problems in the scale-up of biochemical process is regarded insufficient mixing. Local concentration and temperature gradients in the reaction medium (mass and heat transfer is negatively influenced) determine microenvironment surrounding the cell and consequently microorganism physiology may be seriously influenced and metabolic changes can occur [10].

2.9. Mixing and power consumption

2.9.1. Theoretical part

The purpose of mixing is achieving concentration and temperature uniformity at each point of the reaction volume as soon as possible. This assumption works reasonable well for small-scale (1–2 l) intensely stirred reactors, with mixing times in the order of 1 s but in large scale systems the time for achieving homogeneity can be in order of minutes [2]. The mixing process requires energy delivered to fermentation medium as the agitator power input transmitted to the fluid. Therefore it is important to understand the interaction between the fluid motion, the agitator speed, and the power input (consumption) and to know how a change of scale effects these relationships [15]. Formulae and correlations approach [10] is used for describing the mixing behavior and the mixing time, pumping capacity and circulation time are the most important quantitative mixing characteristics. In real systems, the mixing device is an important component of the reactor. Good mixing promotes the effective transfer of the substrates and heat to the microorganisms [16].

The pumping capacity, V_P, is that volume of liquid that gets expelled from the impeller per unit time (m^3/s) and is defined by

$$V_p = N_f.N.{d_s}^3 \tag{17}$$

Where N_f is the flow number that depends on impeller type and on the medium viscosity (N_f = 0,72 for Rushton turbine and low viscosity medium), d_s is the stirrer diameter and N is the stirrer rate [2].

The circulation time, t_c, is defined as

$$t_c = \frac{V_L}{V_P} \tag{18}$$

Where V_L is the volume of the liquid phase.

The mixing time, t_m, is the time needed to obtain a certain value of the degree of mixing (homogeneity), m, made a pulse addition of a tracer. As a first approximation, mixing can be assumed to have occurred within 4 t [2]:

$$t_m = 4.t_c \tag{19}$$

Mixing time for stirred tank fermenters above 60 m^3 (the liquid working volume V_L is in liters) may be calculated from the empirical correlation [17] as follows:

$$t_m = 223{,}5\ log(V_L) - 1004{,}6 \tag{20}$$

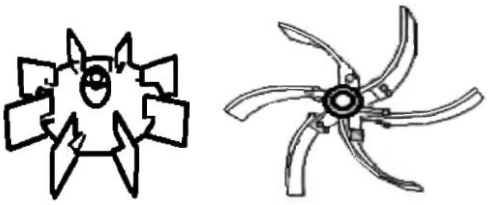
The power consumption, P, for unaerated conditions for multiple impellers put on the same shaft is expressed through the following relation [2]:

$$P = n_I\ N_p \rho_L N^3 {d_s}^5 \tag{21}$$

Where n_J is the number of impellers on the same shaft, P is the power input ($J \cdot s^{-1}$ = W), N_P the power number that is function of Reynolds number and impeller design ($N_P = 5.2$) for Rushton turbine and turbulent flow), ρ the density of the fluid ($kg.m^{-3}$), N the stirrer rate (s^{-1}), d_s the stirrer diameter (m).

The impeller tip speed, v_t should be less than 7,62 m/s [18] because cells can be mechanically damaged and the ratio of power input and volume of liquid, P/V_L, is recommended to be kept in large scale fermenters, from the economical point of view, about $1 - 5\ kW/m^3$ [2].

Some impellers used in stirred tank bioreactors are shown in Figure 4.



a b

Fig. 4. Some impellers used in stirred tank bioreactors [9], [19]

a – rushton impeller with six flat blades, b – axial impeller improve mixing in fermentation

2.9.2. Practical part

The pumping capacity, V_p, the circulation time, t_c, the mixing time, t_m,, the power consumption, P, The impeller tip speed, V_t,, and the ratio of power input and volume of liquid, P /V_L, are:

$$V_p = N_f . N . {d_s}^3 = 0{,}72 \,.2{,}5 \,. 0{,}97^3 = 1{,}643\ m^3 s^{-1} \tag{22}$$

$$t_c = \frac{V_L}{V_P} = \frac{52{,}5}{1{,}643} = 32{,}0\ s \tag{23}$$

$$t_m = 223{,}5\ log(V_L) - 1004{,}6 = 223{,}5\ log(52500) - 1004{,}6 = 50{,}4\ s \tag{24}$$

$$P = n_I\ N_p \rho_L N^3 {d_s}^5 = 4 \,. 5{,}2 \,. 1000 \,. 2{,}5 \,. 0{,}97^5 = 276256\ J \,. s^{-1}\ (W) \tag{25}$$

$$v_t = \pi\ N\ d_s = \pi \,. 2{,}5 \,. 0{,}97 = 7{,}618\ m/s \tag{26}$$

$$\frac{P}{V_L} = \frac{261728}{52{,}5} = 5260\ W . m^{-3} \tag{27}$$

2.10. Heat transfer

2.10.1. Theoretical part

Since biological reactions are very temperature sensitive a careful temperature control (keeping temperature at the optimal value) is therefore absolutely essential [2]. Heat is generated not only by reaction heat, Q_R, which often accompanies reactions but by agitation of the medium (power input), P = Q_R, as well. Removal of heat surplus is done by heat exchange from fermentation medium (hot medium) to water (cool medium).

The heat transfer rate equation is written in the form:

$$Q = K_A A(t_h - t_s) \tag{28}$$

Where A is the heat surface area, t_h the temperature of the hot (fermentation) medium, t_c the average temperature of the cooling medium and k_A the overall heat transfer coefficient relate to the heat surface area.

At industrial scale, heat transfer is often a problem as Q scales with volume of the reactor, that is, for geometrically similar systems with ${d_R}^3$ while cooling surface area scales with ${d_R}^2$ [15]. From it follows that if for small-scale bioreactors (below a few m^3) it is normally sufficient with wall cooling, for large reactors either internal coil is necessary to install above a certain reactor size or fermentation medium may be pumped out of the reactor and cooled in an external heat exchanger [2]. The presence of such internal piping clearly alters mixing pattern and fluid velocities [11] and therefore suitable criterion equation for calculation of the convection heat transfer coefficient on side of fermentation medium should be used.

The overall heat transfer coefficient, K_A, can be calculated from the relation as follows:

$$K_A = \frac{1}{\frac{1}{\propto_h}+\frac{d_{out}}{2\lambda}ln\frac{d_{out}}{d_{in}}+\frac{d_{out}}{\propto_c d_{in}}} \quad (29)$$

Where α_h is the convection heat transfer coefficient on side of fermentation medium, d_{out} is the outer wall diameter, d_{in} is the inner wall diameter, λ is the conductive heat transfer coefficient of the wall and α_c is the convection heat transfer coefficient on side of cooling water

The convection heat transfer coefficient depends on the flow hydrodynamics, physical properties of liquid, the system geometry and is calculated from the following equation:

$$\propto = \frac{Nu\lambda_f}{l} \quad (30)$$

Where $\lambda_f$is the fluid heat conductivity, l is the characteristic system dimension, Nu is the Nusselt number which is calculated from empirical criterion equations which have for each geometry specific form.

Correlations are normally based on dimensionless numbers, which give the Nusselt number, Nu, as a function of the Reynolds number, Re, the Prandtl number, P_r, etc.

Criterion equation in the case of cooling water flowing inside the tube under turbulent conditions is [11,12]:

$$Nu_c = 0{,}023{Re_c}^{0,8}\,{Pr_c}^{0,4} \quad (31)$$

Where Re_c is the Reynolds number, P_{rc} the Prandtl number for cool medium.

$$Re_c = \frac{d_c w_c \rho_c}{\mu_c} \quad (32)$$

Criterion equation in the case that internal coil is added near the inner surface of a bioreactor can be given as [2]:

$$Nu_h = 0{,}17{Re_h}^{0,67}{Pr_h}^{0,37}(\frac{d_m}{d_R})^{0,1}\ (\frac{d_h}{d_R})^{0,5} \quad (33)$$

where P_{rh} is the Reynolds number, P_{rh} the Prandtl number for fermentation medium. d_s the stirrer diameter, $d_h = d_{out}$ the outer diameter of the cooling tube, d_R the inner bioreactor diameter.

2.10.2. Practical part

The total heat exchange can be calculated, from the practical point of view, from the expression:

$$Q = Q_R + Q_P \quad (34)$$

Heat generated by agitation, $Q_P = P$, equals 261 728 J/s. Reaction heat generated during ethanol fermentation is

$$Q_R = {n_s}^R . q_R \quad (35)$$

Where ${d_S}^R$ is the mole amount of consumed glucose during ethanol production (fermentation time equals 11,4 hours when the glucose conversion 97 % is achieved (see part Mathematical model below) and q_R the reaction heat, (kJ/mol).

The mass amount of the consumed glucose is then:

$${m_s}^R = V_L(C_{s0} - C_s) = 52{,}5\,.\,(200 - 6) = 10185\ kg \quad (36)$$

Where c_{S0} is the initial glucose concentration (c_{S0} = 200 g/L) [20]
And c_S is the final concentration of glucose (c_S = 6,0 g/L) and V_L is the volume of the liquid (V_L = 52,5 m^3).

Mole amount of depleted glucose per one production cycle (11,4 hours) equals:

$${n_s}^R = \frac{{m_s}^R}{M_s} = \frac{10185}{180}56{,}583\ Kmol \quad (37)$$

Reaction heat (J/s) is:

$$Q_R = {n_s}^R\,.\,q_R = \frac{56{,}583\,.\,10^3\,.\,45{,}1\,.\,10^3}{11{,}4\,.\,3600} = 62181\quad J/s \quad (38)$$

Mass flow of cooling water, m_c (kg/s), can be calculated from simplified enthalpy balance of a fermenter as:

$$m_c = \frac{Q}{C_{pc}(t_{2c}-t_{1c})} = \frac{Q_R+Q_P}{C_{pc}(t_{2c}-t_{1c})} = \frac{62181+261728}{4190.\ (25-15)} = 7{,}731\ kg.s^{-1} \quad (39)$$

The heat surface area, A, is calculated from the heat transfer rate equation:

$$A = \frac{Q}{K_A.\ (t_h-t_c)} \quad (40)$$

Where t_h the temperature of the hot (fermentation) medium, t_c the medium temperature of the cooling medium and k_A the overall heat transfer coefficient relate to the heat surface area.

The overall heat transfer coefficient can be calculated from the relation as follows:

$$K_{AH} = \frac{1}{\frac{1}{\propto_h}+\frac{d_{vonk}}{2\lambda}ln\frac{d_{vonk}}{d_{vnut}}+\frac{d_{vonk}}{\propto_S d_S}} \quad (41)$$

Calculation of the convection heat transfer coefficient for water α_c. The convection heat transfer coefficient on side of cooling water, α_c, is calculated from the following equation:

$$\alpha_c = \frac{Nu_c\lambda_c}{d_{in}} \quad (42)$$

Where λ_c is the water heat conductivity, d_{in} is the inner pipe diameter, Nu_c is the Nusselt number which is calculated from empirical criterion equations which have for each geometry specific form.

Criterion equation in the case that cooling water flows inside the tube under turbulent conditions is:

$$Nu_c = 0{,}023Re_c^{\,0,8}\ Pr_c^{\,0,4} \quad (43)$$

Reynolds number is given as:

$$Re_c = \frac{d_c\, w_c\, \rho_c}{\mu_c} \quad (44)$$

where $d_c = d_{in}$ is the inner diameter of the pipe (internal coil), w_c is velocity of water flowing within the pipe calculated from the continuity equation, ρ_c is the water density and μ_c is the water viscosity determined for the average temperature.

$$w_c = \frac{V_c}{S_c} = \frac{\frac{m_c}{\rho_c}}{\frac{d_c^{\,2}}{4}} = \frac{\frac{7{,}731}{998{,}2}}{\frac{\pi\ .\ 0{,}127^2}{4}} = 0{,}659\ m/s \quad (45)$$

$$Re_c = \frac{d_c\, w_c\, \rho_c}{\mu_c} = \frac{0{,}127\ .\ 0{,}659\ .\ 998{,}2}{1{,}005\ .\ 10^{-3}} = 83127 \quad (46)$$

Prandtl number is given as:

$$Pr_c = \frac{c_{pc}\ .\ \mu_c}{\lambda_c} = \frac{4190\ .\ 1{,}005\ .\ 10^{-3}}{0{,}599} = 7{,}03 \quad (47)$$

Where λ_c is the water thermal conductivity and c_{pc} the specific heat capacity of water.

The Nusselt number is calculated from the criterion equation:

$$Nu_c = 0{,}023Re_c^{\,0,8}\ Pr_c^{\,0,4} = 0{,}023\ .\ 83127^{0,8}\ .\ 7{,}03^{0,4} = 432{,}8 \quad (48)$$

The convection heat transfer coefficient on side of cooling water is then:

$$\alpha_c = \frac{Nu_c\lambda_c}{d_{in}} = \frac{432{,}8\ .\ 0{,}599}{0{,}127} = 2041\ W\ .\ m^{-2}\ .\ K^{-1} \quad (49)$$

Calculation of the convection heat transfer coefficient for fermentation medium αh
(physical properties of fermentation medium were approximated by the physical properties of water):

The convection heat transfer coefficient on side of fermentation broth, α_h, is given as:

$$\alpha_h = \frac{Nu_h\lambda_h}{d_{out}} \quad (50)$$

where λ_h is the heat conductivity of fermentation medium, d_{out} is the outer pipe diameter, Nu_h is the Nusselt number which is calculated from the empirical criterion equations that for the case that internal coil added near the inner surface of a bioreactor is as follows [2]:

$$Nu_h = 0{,}17 {Re_h}^{0{,}67}\ {Pr_h}^{0{,}37} (\frac{d_s}{d_R})^{0{,}1} (\frac{d_h}{d_R})^{0{,}5} \quad (51)$$

Where Re_h is the Reynolds number, Pr_h the Prandtl number for fermentation medium, d_s the stirrer diameter, $d_h = d_{out}$ the outer diameter of the cooling tube, d_R the inner bioreactor diameter.

Reynolds number is given as:

$$Re_h = \frac{{d^2}_s\, N\ \rho_h}{\mu_h} = \frac{0{,}997^2\ .\ 2{,}5\ .\ 1000}{1{,}2\ .\ 10^{-3}} = 1960208 \quad (52)$$

where d_s is the stirrer diameter, N is the stirrer frequency, ρ_h is the density and μ_h the viscosity of the fermentation medium determined for the temperature 30 °C.

Prandtl number is given as:

$$Pr_h = \frac{c_{p_h}\ .\ \mu_h}{\lambda_h} = \frac{4000\ .\ 1{,}2\ .\ 10^{-3}}{0{,}56} = 8{,}57 \quad (53)$$

Where the thermal conductivity, λ_h, and the specific heat capacity, c_{p_h}, of fermentation medium is approximated as for water.

The Nusselt number is calculated from the criterion equation:

$$Nu_h = 0{,}17 {Re_h}^{0{,}67}\ {Pr_h}^{0{,}37} (\frac{d_s}{d_R})^{0{,}1} (\frac{d_h}{d_R})^{0{,}5} = 0{,}17\ .\ 1960208^{0{,}67}.\ 8{,}57^{0{,}37}\ .\ (\frac{0{,}97}{3{,}1})^{0{,}1}\ .\ (\frac{0{,}139}{3{,}1})^{0{,}5} = 1164 \quad (54)$$

The convection heat transfer coefficient on side of hot (fermentation) medium is then as follows:

$$\alpha_h = \frac{Nu_h \lambda_h}{d_{out}} = \frac{1164\ .\ 0{,}56}{0{,}139} = 4688\ W\ .\ m^{-2}\ .\ K^{-1} \quad (55)$$

The overall heat transfer coefficient, related to the fermentation medium surface area, K_A is given as:

$$K_A = \frac{1}{\frac{1}{\alpha_h} + \frac{d_{out}}{2\lambda} ln \frac{d_{out}}{d_{in}} + \frac{d_{out}}{\alpha_c\, d_{in}}} = \frac{1}{\frac{1}{4688} + \frac{0{,}139}{2\ .\ 15} ln \frac{0{,}139}{0{,}127} + \frac{0{,}139}{2041\ .\ 0{,}127}} = 846{,}6\ W\ .\ m^{-2}\ .\ K^{-1} \quad (56)$$

Where λ_h is the thermal conductivity of the stainless steel wall.

The heat surface area, A, is then as follows:

$$A = \frac{Q}{K_{A_h}\ .\ (t_h - t_s)} = \frac{338437}{846{,}6\ .\ (30-20)} = 40{,}0\ m^2 \quad (57)$$

2.11. Mass transfer

2.11.1. Theoretical part

In bio reactions, the transport of nutrients to the cell surface and removal of metabolites from the cell surface to the bulk of the fermentation are rate processes with times constants not much smaller than those of the cellular reactions. Therefore, mass transfer must be included in an analysis of bio reactions alongside the stoichiometry and cellular reactions [2].transport across the plasma membrane of *S.* cerevisiae, is mediated by several specific plasma membrane transport systems allowing the cell to switch between different, low affinity and high-affinity [21]. A simplified treatment of a flow field in which the overall mass transfer is divided into individual different steps is normally used with good results. An overview of important mass transfer steps in a fermentation process is depicted in Figure [5]. A sparingly soluble gas, usually oxygen, must pass through a series of nine transport resistances from the gas bubble to the cell but in our case only the first two resistances are relevant for the overall mass transfer rate.

By applying the two-film theory the following relation may be derived for the overall flux of the considered component, J_i, from the gaseous to the liquid phase

$$J_i = \frac{n_i}{A_{if}} = K_L\ (c_i^* - c_i) \quad (58)$$

Where n_i is the mole flow rate of the component, A_{if} the interfacial area, K_L the overall mass transfer coefficient (reciprocal value of resistances in the gas and liquid phase), $(c_i^* - c_i)$ the driving force expressed through a liquid phase, C_i the saturation concentration in the liquid phase corresponding to the partial pressure P_i in the gas phase via Henry's law ($P_i = H_i c_i$), c, c_j is the true concentration in the bulk of a liquid phase.

However, the idea of expressing the mass transfer across surfaces by an overall mass transfer coefficient multiplied by a concentration difference is clearly a simplification of a complicated physical reality [2].

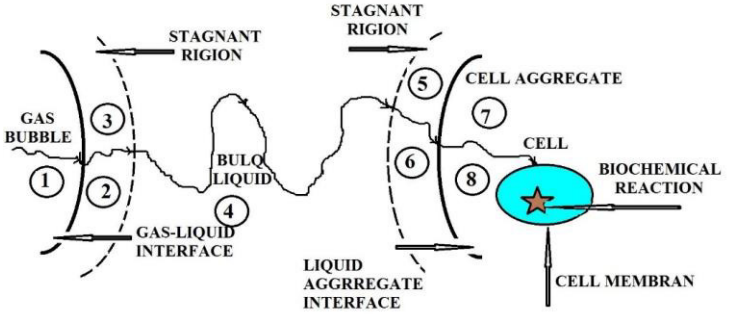


Fig. 5. Overview of steps in the overall mass transfer of oxygen transfer from the gas bubble to the cell [1 – modified]

The overall mass transfer coefficient, K_L, is expressed the following well-known relationship where resistances in the gas and liquid phases are included [2]:

$$\frac{1}{K_L} = \frac{1}{K_L} + \frac{1}{H_i K_g} \tag{59}$$

Where k_L is the liquid-film side mass transfer coefficient, H_i the Henry's constant for the given compound and k_g is the gas-film side mass transfer coefficient.

2.11.2. Practical part

For calculation of $(K_L a_d)_p$ the relation as follows is used [2]:

$$(K_L a_d)_p = \frac{D_i}{D_{O_2}} \ (K_L a_d)_{O_2} \tag{60}$$

Diffusion coefficients of oxygen and ethanol in water (30 °C) [2] is $2.5 \cdot 10^{-9}$ $m^2 \cdot s^{-1}$ and $1.28 \cdot 10^{9}$ $m^2 \cdot s^{-1}$, respectively.

Volumetric mass transfer coefficient of oxygen (for aeration system) is expressed via equation (61).

$$(K_L a_d)_{O_2} = K \ . \ u_s{}^{\alpha} \left(\frac{P_G}{V_L}\right)^{\beta} \tag{61}$$

where $k = 0{,}0018$, $\alpha = 0{,}3$ and $\beta = 0{,}7$ [2].

The superficial gas velocity is defined as:

$$u_s = \frac{V_G}{S_R} \tag{62}$$

Where S_R is the inner bioreactor cross section area.

The aeration rate, V_G, specified in VVM (volume of gas/volume of liquid/minute), equals 0,3.

The aeration rate, V_G, and the inner bioreactor cross section area, S_R, and the superficial gas velocity and the volumetric oxygen mass transfer coefficient $(K_L a_d)$ are then:

$$V_G = \frac{vvm}{60} V_L = \frac{0{,}3}{60} \ 52{,}5 = 0{,}2625 \ m^3/s \tag{63}$$

$$S_R = \frac{\pi \ . \ {d_R}^2}{4} = \frac{\pi \ . \ 3{,}1^2}{4} = 7{,}548 \ m^2 \tag{64}$$

$$u_s = \frac{V_G}{S_R} = 0{,}035\ m/s$$

$$(K_L a_d) = 0{,}0018\,.\,u_s{}^{0,3}(\frac{P_G}{V_L})^{0,7} = 0{,}0018\,.\,0{,}0348^{0,3}\,.(\frac{138128}{52{,}5})^{0,7} = 0{,}163\quad S^{-1} \tag{65}$$

Volumetric ethanol mass transfer coefficient (used in material balance of ethanol) is:

$$(K_L a_d)_i = \frac{D_i}{D_{O_2}}\ (K_L a_d)_{O_2} = \frac{1{,}28\,.\,10^{-9}}{2{,}5\,.\,10^{-9}}\ 0{,}163 = 0{,}083\ S^{-1} \tag{66}$$

2.12. Mathematical model

Material balance of the key species (biomass, *X*, glucose, *S*, ethanol, *P*, glycerol, *G*), expressed as a system of the ordinary differential equations in the liquid phase, is as follows:

$$X\colon\ \frac{dc_X}{dt} = \mu_{max}\frac{C_s}{C_s+K_s}(1-\frac{C_p}{K_p})C_x \tag{67}$$

$$S\colon\ \frac{dc_s}{dt} = -\frac{1}{Y_{x/s}}\mu_{max}\frac{C_s}{C_s+K_s}(1-\frac{C_p}{K_p})C_x \tag{68}$$

$$P\colon\ \frac{dc_p}{dt} = Y_{p/x}\,.\,\mu_{max}\frac{C_s}{C_s+K_s}\left(1-\frac{C_p}{K_p}\right)C_x - (K_L a)_p(C_p - C_p{}^g) \tag{69}$$

$$G\colon\ \frac{dc_G}{dt} = Y_{G/x}\,\mu_{max}\frac{C_s}{C_s+K_s}(1-\frac{C_p}{K_p})C_x \tag{70}$$

and material balance of ethanol stripped into the gaseous phase is

$$P^g\colon\ \frac{dc_p{}^g}{dt} = -(K_L a_d)_p(C_p{}^g - C_p)\frac{V_L}{V^g}\frac{R}{TH_p} \tag{71}$$

where $C_p{}^g$ is the equilibrium ethanol concentration expressed through the liquid phase, V^g the volume of the bioreactor above the liquid phase, R the universal gas constant, *T* temperature in units of Kelvin, H_P is the Henry's constant for ethanol.

System of ordinary differential equations (solved numerically using the Athena Visual Workbench software package – Stewart & Associates Engineering Software Inc., Madison, USA) is used for calculation of concentration time profiles of considered components in the liquid phase and determination of the time fermentation. As an optimization the criterion the high concentration of ethanol in the reaction medium is chosen. Calculation is stopped when glucose conversion achieves 97 %. Time concentration profiles above mentioned species are depicted in Figures 6 and 7. From the Figures 6 and 7 follows that the fermentation time, t_f, equals 11,4 hours with ethanol stripping (C_p = 69,1 g·l^{-1}) and 12,0 hours without stripping (C_p = 75,9 g·l^{-1}), respectively.

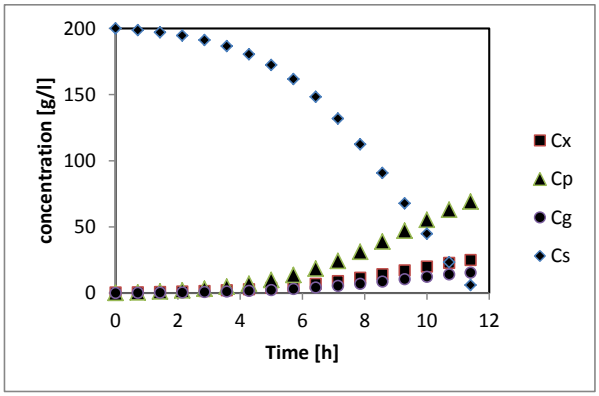


Fig. 6. Concentrations of glucose, biomass, ethanol and glycerol in the liquid phase versus time with ethanol stripping

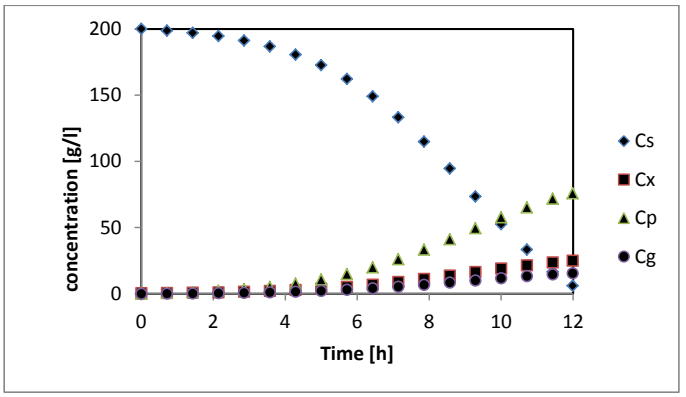


Fig. 7. Concentrations of glucose, biomass, ethanol and glycerol in the liquid phase versus time without ethanol stripping

A mathematical model can be best developed when the process is well understood. To develop such a model for bioprocesses, compositional variable of the organisms must be considered and that model is called a structured model [22].

3. Results and discussion

Following bath stirred industrial scale bioreactor for ethanol production with Saccharomyces cerevisiae is designed:

- the volume of bioreactor, V_R = 70 m^3
- the volume of a liquid phase, V_L = 52.5 m^3
- the bioreactor diameter, d_R = 3,1 m
- the stirrer diameter, d_s = 0,97 m
- the stirring speed, N = 2.5 s^{-1}
- number of impellers, n_I = 4
- the mass flow rate of cooling water, m_c = 7,731 kg.s^{-1}
- heat surface area, A = 40 m^2
- fermentation time, t_f = 11.4 h and operation time, t_{op} = 4 h
- batch production of ethanol, m_P = 3 623 kg
- number of bathes per year equals 514
- year production of ethanol, m_P = 1 862 222 kg

As investigated previously, saccharomyces cerevisiae is utilized extensively in batch fermentations to convert glucose to ethanol for the production of beverages and biofuels. The fermentation of glucose to ethanol demonstrates a series of coordinated enzymatic reactions. This process is interiorly balancing and thermodynamically desirable provided that cellular enzymes use up the net phosphorylated nucleotide produced from substrate level phosphorylation. Mathematical model is set up on the knowledge combination of engineering considerations – microbial phenomena which include stoichiometry, thermodynamics of cell cultivation, microbial kinetics and the physical processes (transport phenomena) such as mixing, power consumption, mass and heat transfer. Values of kinetics parameters, μ_{max}, K_S, K_P for the description of microbial kinetics and next applied in mathematical modeling were evaluated on the basis of literature information. Unfortunately it is physically impossible to maintain the same process conditions for lab-scale, pilot-scale and industrial scale bioreactors. The reason is that physical processes are directly and metabolic processes indirectly scale dependent. Although bioreactor scale-up does not only involve pure engineering considerations but economic considerations as well the next text and discussion will be restricted to the engineering aspects of scale-up. From calculation we obtained batch production of ethanol, 3623 kg/l in the whole media, year production of ethanol 1 806 991 kg/year and for four batch 7 228 ton/year. With stripping 3623 kg/batch, production per year is 1 862 222 kg/year, but without stripping, 3982 Kg/batch, and production per year 1 971 151 kg/year , time for one batch is the sum of production time and turnaround time is 15,4 hours with stripping and without stripping is 16 hours. Investment cost 2105 dolor for major subtotal equipment was estimated and for total capital of fermentation 8946250 dolor calculated. With changing of reactor volume or volume of a liquid phase, proportionally increasing or decreasing production of ethanol for instance, if volume of reactor assumed 60 m^3 proportionally batch production of ethanol become, m_p = 3105 kg/l in the whole media, year production of ethanol 1 548 849 kg/year, and for four batch 6195 ton/year. The aeration rate, V_G = 0,225, Volumetric ethanol mass transfer coefficient (used in material balance of ethanol) (k_La_D) is 0,076 s^{-1} obtained. Decreasing of the initial glucose concentration from to 150 g/L proportionally influence on mass flow of cooling water to 7,70 kg/s, mole amount of

consumed glucose during ethanol production, 42 kmol, reaction heat, 46 635 J/s and other values. Our calculation shoes that was calculated according to the correct scale-up methods. The results from this study could be expected to provide insights into the process performance, optimize the process and aid in the design of processes for large-scale production of ethanol with yeasts Saccharomyces cerevisiae under anaerobic conditions.

4. Conclusion

In conclusion, the present investigation has demonstrated the importance of some fermentation parameters (microbial phenomena) in improving the alcoholic fermentation technology [23] of bath stirred industrial scale bioreactor for ethanol production with Saccharomyces cerevisiae. Bioreactors will be integral to the development of many new high-value products and the replacement of existing chemical-based commodity processes. The proper selection and design of the bioreactor will determine the optimal commercial bioprocess and the corresponding capital investment. The bioreactor should not be regarded as an isolated unit, but as part of an integrated unit operation with both upstream (preparation) and downstream (separations) [24] unit operations.

Simulations can be used for the cost analysis of the optimal design bioreactor [25]. In further research, it is possible to focus on visualization and analysis describes the process using holographic interferometry [26].

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