Horizontal Tubular Bioreactors in Biotechnology

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In this review, horizontal tubular bioreactors are discussed regarding their advantages and disadvantages compared to other bioreactor types. In horizontal tubular bioreactors medium flow is characterized by plug flow conditions that can be favorable in the case of inhibition and/or repression bioprocess kinetics. For description of liquid flow simple one-parameter or complex multi-parameters mathematical models have been established. Comparison between these models proved that complex multi-parameters model can describe real situation in the bioreactor more efficiently. Criteria of geometrical similarity are most often used for scale-up of horizontal tubular bioreactors. The successful scale-up procedure should combine the mathematical model of medium flow behavior with bioprocess kinetics in the bioreactor.

Key words:
Horizontal tubular bioreactors, mixing, mathematical modeling, bioprocess kinetics, scale-up

Introduction

In biotechnology different bioreactors are used to conduct different bioprocesses regarding bioprocess kinetics, hydrodynamics and scale of operation. Stirred tank bioreactors have been used most often due to their suitability for conduction of different bioprocesses. However, they have also some disadvantages, such as problems with bearings and sealing, cell damage, high power consumption and high costs of cooling.[1] For these reasons new bioreactor types (e.g. air lift, jet or tubular bioreactors) were developed to solve these problems. In two stage bioprocesses, stirred tank bioreactors can be used in combination with other bioreactor types (e.g. tubular bioreactors). In this situation, biomass cultivation is carried out in a stirred tank bioreactor (first step) and production of metabolites or bioconversion processes take place in tubular bioreactor (second step), respectively. A typical example for such combination is biopesticides production.[2,3] Tubular bioreactors, either vertical or horizontal, have some potential advantages over stirred tank bioreactors.[4,5] They have usually simple construction and possibility to prepare different inner configurations by the use of standard industrial support materials. For construction of tubular bioreactors and their scale-up it is necessary to know less number of parameters than for the stirred tank bioreactors where type and number of impellers, distance between impellers, type and number of impeller blades, type and size of power input has to be known. Mixing in tubular bioreactors is more uniform compared to the stirred tank bioreactors. Thus, it is easier to eliminate "dead" zones that make the scale-up procedure more reliable. Area-to-volume ratio is significantly higher in tubular bioreactors resulting in more efficient mass and heat transfer processes. This is particularly important in bioprocesses with semi-solid or solid substrates, photoreactions (maximum exposure to light) and shear sensitive organisms etc. Due to the plug flow conditions the gradients of concentrations along the bioreactor length are established that is advantage in the case of inhibition and/or repression bioprocess kinetics. In these cases, high productivity and optimal conversion are achieved simultaneously during cultivation. Tubular bioreactors are easy to maintain due to the fact that their basic elements are widely used in bioprocess industry (pipes, pumps, standard fittings). There are some substantial differences between horizontal and vertical tubular bioreactors (tubular and tower bioreactors). In industrial tower bioreactors very often the superficial gas velocity and the power input required for compression of the feed gas to overcome the high static liquid pressure tend to be unallowably high. In tubular bioreactors plug flow is not disturbed by the bioprocess gasses and the hydrostatic pressure can not have inhibiting effect or create practical problems. Although tubular bioreactors have great potentials for use in biotechnology they have also some disadvantages compared to the stirred tank bioreactors. Tubular bioreactors

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are suitable for continuous mode of operation, but in industry most bioprocesses are still run in batch mode. Another disadvantage is a relatively low oxygen supply capacity, what makes them unsuitable for conduction of bioprocesses with high oxygen demand (e.g. biomass and acetic acid production).

During conduction of bioprocess in tubular bioreactors microbial biofilm is very often formed on the inner surface of bioreactors which additionally increases bioprocess efficiency and stability compared to the bioreactors with suspended microbial cells. In tubular bioreactors with microbial biofilm biomass washout can happen very rarely although relatively high inflow rates are used. Major disadvantage of a tubular bioreactor with microbial biofilm is the problem of mass transfer inside the microbial biofilm. This effect usually happens in thicker biofilms where substrate and oxygen limitations are very often present, thus resulting in loss of cell viability. In these conditions, biofilm erosion and sloughing processes[6,7] tend to occur, thus substantially disturbing the bioreactor performance. This problem can be solved by the control of biofilm thickness which can be based on mechanical scraping or abrasion by friction (the self-regulation effect at high hydrodynamic stress). On the other hand, thicker microbial biofilm, in some cases, can be advantageous. In case of substrate inhibition the most favorable substrate concentrations are inside the microbial biofilm where bioconversion rate is optimal for bioprocess conduction. In mixed microbial culture different species grow along the depth of biofilm, for example in wastewater treatment processes the nitrifiers grow near the surface and denitrifiers in the inner biofilm layers which are favorable conditions for the process of simultaneous nitrification and denitrification.[4] Growth of microbial biofilm on the inner surface of tubular photobioreactors is not favorable due to the fact that it can reduce the amount of light available to the phototrophic cells. On the basis of previous consideration it is clear that tubular bioreactors have great potential for conduction of different bioprocesses and therefore they must be used in biotechnology more often.

Bioreactor types and areas of application

In chemical engineering horizontal reactors are well known e.g. rotary drum reactors, rotating cylinders, pipeline contactors and rotary kilns. These reactor constructions have positive effects on the processes inside reactors such as e.g. mixing, heat and mass transfer.[8–10] In our further consideration horizontal tubular bioreactors developed for use in fermentation and wastewater treatment processes, semi-solid or solid state and phototrophic bioprocesses as well as in tissue engineering will be considered.

Horizontal tubular bioreactors in fermentation and wastewater treatment processes

Different constructions of horizontal tubular bioreactors developed for fermentation and wastewater treatment processes are presented in Fig. 1. The simplest construction of horizontal tubular bioreactor (Fig. 1a) is a straight or a spiral tube made to mimic natural flow in the rivers or in the long open channels. This bioreactor construction is very often used in wastewater treatment processes. Another common use of this bioreactor construction is in sterilization technology as a holding section.[4] Biodisc reactor as a horizontal bioreactor has been developed for wastewater treatment[11] (Fig. 1b). It consists of a series of closely spaced discs fixed to a rotating shaft. The discs are usually made of plastic material (polyethylene, PVC, expanded

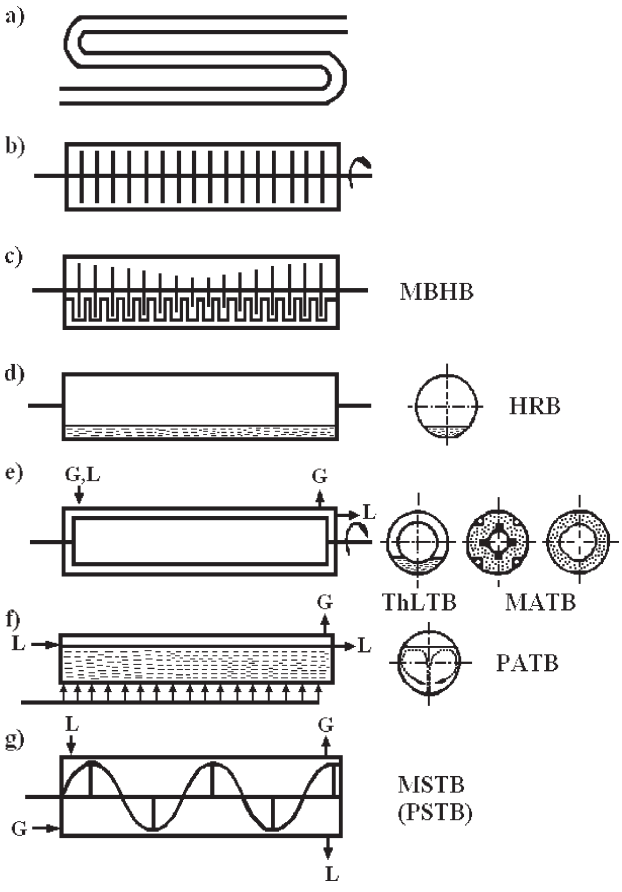
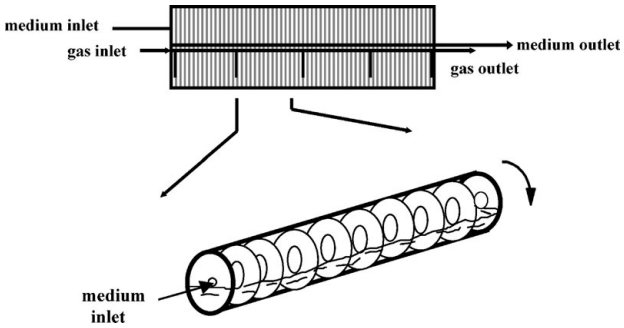


Fig. 1 – *Horizontal tubular bioreactors: a) simple tube, b) biodisc reactor, c) multiple blade tubular bioreactor (MBTB), d) horizontal rotary bioreactor (HRB), e) thin–layer tubular bioreactor (ThLTB) and mechanically agitated and aerated tubular bioreactor (MATB), f) pneumatically aerated and agitated tubular bioreactor (PATB), g) mechanically or pneumatically scraped tubular bioreactor (MSTB or PSTB)*

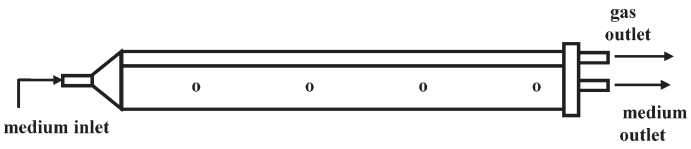
polystyrene) and about 40 % of their area is submersed in liquid phase. During wastewater treatment microbial biofilm is usually formed on the rotating discs that are partially submerged into wastewater to absorb substrate and then raised out of the liquid phase to the air in order to oxidize the absorbed substrate. A similar design is applied in the multiple blade horizontal bioreactor (MBHB) that was constructed for bioprocesses with mycelial microorganisms without the biofilm formation[12] (Fig. 1c). The bioreactor consists of several cylindrical compartments, where each part is sealed off from its neighbouring compartment by a separating plate having an overflow hole in the upper half. The combined splashing of the bioreactor walls and the shearing action between the stirrer blades and baffles installed vertically at the bottom of each compartment in the bioreactor increased mixing and suppressed the formation of biofilm. Similar horizontal cylindrical devices with several rotation discs are used for conduction of different bioprocesses.[13] Horizontal rotary bioreactor (HRB) is characterized by an un-baffled rotating tube[14] (Fig. 1d). This bioreactor type was used for conduction of gluconic acid production by *Pseudomonas ovalis*[15] and cellulase production by *Trichoderma sp*.[16] The thin-layer tubular bioreactor (ThLTB) was designed for verification of Danckwerts renewal theory of mass transfer[17,18] (Fig. 1e). This bioreactor was used for conduction of bioprocesses with relatively low foaming intensity.[19] In case of yeast production this bioreactor construction was modified by incorporation of baffles on the bioreactor wall as well as blades on the central rotating tube in order to increase the oxygen transfer rate. This bioreactor is named the mechanically agitated and aerated tubular bioreactor[19,20] (MATB; Fig.1e). All previously mentioned tubular bioreactors (HRB, ThLTB and MATB) are characterized by a relatively thin liquid layer inside the bioreactor which has positive effect on the mixing intensity and mass transfer phenomena.[21] The pneumatically aerated tubular bioreactor (PATB; Fig. 1f) was developed for aerobic wastewater treatment.[19,22] It is characterized by the absence of any mechanical device and the air (or O_2) is introduced over the entire bioreactor length so that oxygen limitation can be easily avoided. A complex biokinetics with substrate and product inhibition was studied in this bioreactor during continuous ethanol production with *Zymomonas mobilis*. Also, a biomass maturation concept of *Bacillus thuringiensis* was studied in this bioreactor. In both cases, higher substrate conversion coefficients and bioprocess productivities were observed compared to the continuous stirred tank bioreactor.[2,3] The scraped tubular bioreactors can be agitated mechanically (MSTB) or pneumatically with gas jets (PSTB). They were designed for the enzyme production e.g. lipase by yeast and cellulase by fungi[23] (Fig. 1g). In these bioreactors, microbial wall growth was minimized by using rotating internal coils, a moving belt of internal discs or helical ribbons and orifices directly in the tube. These scrapers partially segregate the liquid into moving compartments, where cross-flow aeration, effected by orifices at the bottom is realized in the same way as in PATB. In horizontal tubular bioreactors maintenance of stable suspended biomass concentration gradient along the bioreactor can be a problem due to biomass washout. This problem can be solved by partial recirculation of bioreactor outflow. The effect of recirculation on the plug flow in the mechanically agitated tubular bioreactor was studied and it was observed that the increase of the recycle ratio was related to the decrease of plug flow conditions.[24]

The horizontal rotating tubular bioreactor (HRTB) was designed as a combination of a thin layer[18,20] and a biodisc reactor.[11] Its interior is divided by O-ring shaped partition walls (distance between the partition walls is 0.02 m) that serve as carriers for microbial biofilm. The HRTB was placed on the bearings that enable rotation of the whole bioreactor (Fig. 2). The aeration was done through the central tube fixed in the axis of HRTB. The aeration tube has five extended tubes that were submersed in a liquid phase on five positions along the bioreactor to bring the air into culture.[25,26] HRTB was tested in anaerobic[27,28] and aerobic[29] bioprocesses.



F i g . 2 – *The schematic diagram of horizontal rotating tubular bioreactor*

For anaerobic wastewater treatment a horizontal anaerobic fixed-bed bioreactor was developed. Bioreactor interior was filled with cubic matrices made of polyurethane foam (side of 3–5 mm) containing anaerobic immobilized sludge. This bioreactor is characterized by plug flow conditions (Fig. 3). The axial mixing due to the formation and vertical rise of gases across the horizontal liquid flow, as well as the tubular form of the bioreactor is



F i g . 3 – *The horizontal-flow anaerobic immobilized sludge bioreactor*

expected to promote a plug flow conditions. The gas tube collector along the bioreactor permits minimization of dead volume for gas separation. This bioreactor is also characterized by a relatively short start-up period (8 days) for establishment of stable operating conditions.[30,31]

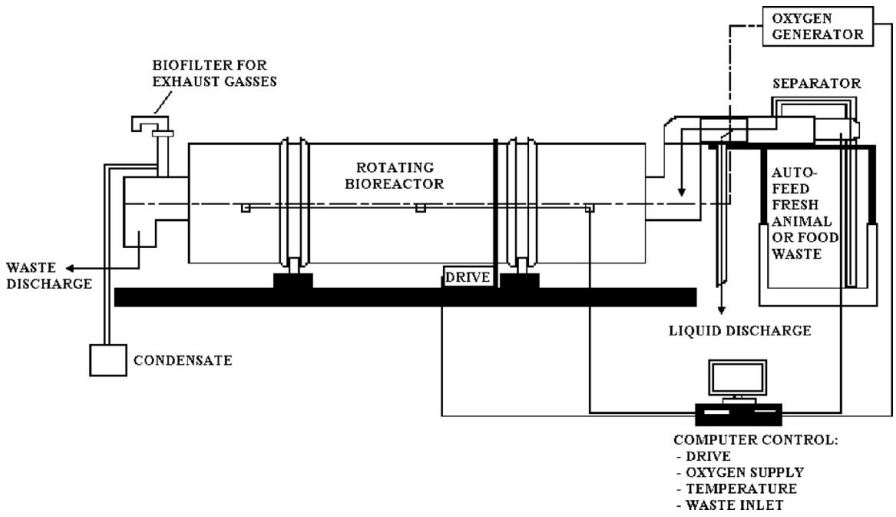
Horizontal tubular bioreactors in bioprocesses with semi-solid or solid substrates

The horizontal tubular bioreactors have also potential for use in semi-solid or solid state bioprocesses. A good example is a bioreactor used for fermentation of sugar cane (Ex-Ferm process) where relatively high sugar consumption and ethanol yield were obtained.[32] In order to increase ethanol production in this bioprocess, equal weights of sugar cane and water are required. Efficient mixing of the high solids loading is achieved by using a rotating bioreactor with a horizontal perforated drum.[33] Horizontal rotating tubular bioreactors are also used for conduction of other bioprocesses with solid substrates.[34,35] For treatment of feed or food solid waste to produce the compost a horizontal rotating tubular bioreactor was developed by EPT Corporation. It is equipped with the systems for monitoring and control of temperature, moisture content, bioreactor rotation speed, oxygen supply and solid waste mass (Fig. 4). During waste treatment in this bioreactor 33 % reduction of solid waste mass was observed and stable compost (odor-free) was obtained in less than five days. This bioreactor can work in batch or continuous mode and its capacity is 20 – 25 tones of solid waste, depending on the material density.[36]

For red wine production, horizontal rotating tubular fermentor with a cap-management device was developed. During red wine fermentation the cap is formed as a consequence of CO_2 trapping in pomace (seeds and skin) and its lifting to the top of the juice. For red wine fermentation it is important that the juice is in contact with the skins to extract color and flavor and therefore the cap has to be periodically submerged. The horizontal fermentor orientation is responsible for a greater contact area between the pomace and the juice than in a vertical tank or vat. In this design, fermentor can rotate itself or devices such as paddles are installed inside the static fermentor. The rotation mixes the juice with the pomace to obtain the similar effect as in the traditional punch-down (pushing the cap into the juice with a large paddle) or pump-over methods. These traditional methods are effective, but labor intensive processes. The major benefit of rotary fermentors is considerable reduction of aging period. These fermentors are manufactured up to 100 m^3 volume.[37]

Horizontal tubular bioreactors in bioprocesses with phototrophic organisms

Horizontal tubular bioreactors can be also applied in photosynthetic bioprocesses where solar energy is used for production of biomass or microbial metabolites. These types of bioreactors are mostly used for cultivation of algae and other phototrophic organisms.[38,39] In these cases, tubular bioreactors are constructed as straight tubes, thin plates with partition walls for regulation of medium flow[40] or solar collector tubes.[41] They are usually made of transparent materials (e.g. plastic or glass)



F i g . 4 – *The horizontal rotating tubular bioreactor for solid waste treatment*

in order to achieve adequate supply of light. The basic principle in all of these constructions is to reduce the light path and thus to increase the amount of light available to cells. These bioreactors are well mixed to ensure optimum light availability to the cells and to enhance gas exchange. They are also characterized by high light utilization efficiency, efficient control of cultivation conditions and ability to operate in continuous mode. Pilot scale units of these bioreactors were used for cultivation of *Spirulina*, *Chlorella* and several marine microalgae.[42,43] The BIOCOIL is a helical tubular photobioreactor consisting of a photostage of small diameter clear plastic tubing (between 2.4 and 5 cm diameter) attached helically around a gas exchange tower. Several parallel bands of tubes are connected to a pumping system.[42,43] Pilot scale of this bioreactor (700 L) was used for cultivation of a wide range of marine microalgae (*Tetraselmis* spp., *Isochrysis galbana*, *Phaeodactylum tricornutum, Chaetoceros* spp. and *Spirulina*) for periods greater than 4 months in semi-continuous mode. This bioreactor design ensures uniform mixing and minimizes adhesion of the algal cells to the inner bioreactor surface. The BIOCOIL can also be scaled up easily and the whole bioprocess can be automated thus minimizing labor costs and improving reliability.[43] The largest industrial horizontal tubular bioreactor for cultivation of algae *Chlorella* was built up in the year 2000 near Wolfsburg (Germany). This bioreactor consists of compact and vertically arranged horizontal running glass tubes of a total length of 500 km and a total volume of 700 m^3. It is placed in a glasshouse of an area of only 10 000 m^2 and the annual production of dry biomass is 130–150 tones.[44]

Horizontal tubular bioreactors in bioprocesses with biological tissues

In tissue engineering the major problem is how to define the environmental conditions where the optimal growth of submersed biological tissues can be achieved. Biological tissues are very sensitive to their environment and, if exposed to harsh conditions, may denature and/or degrade. They must be constantly maintained in a hydrated state at (or near) physiological pH and temperature. For these reasons, very gentle and restricted mixing techniques have been used. In that respect, horizontal tubular bioreactors are the most adequate solution for these bioprocesses. Therefore, for cell culture cultivation an efficient and simple bioreactor design was established that includes a horizontal rotating tube with a coaxial rotating tubular oxygenator[45] (Fig. 5). This oxygenator has micro-porous structure that allows gas exchange without bubbles thus could cause shear stress. The medium is gently mixed by rotation. If the inner and outer cylinders of the bioreactor rotate at the same speed (rpm), then the gradient of medium laminar-flow velocity would be minimized. In this situation, mixing is the result of a secondary flow pattern induced by particle sedimentation through the medium (gravity conditions). When inner and outer cylinders of the bioreactor rotate at different rotation speed (microgravity conditions) the mixing is a consequence of established laminar flows inside cultivation medium. This bioreactor design does not cancel gravity, but ideally maintains continuous freefall conditions for biological tissue growth.[45,46] In literature, many data on the fluid dynamic analyses for this bioreactor design can be found.[47–51]

For animal cell cultivation horizontal rotating cylindrical bioreactor with microcarriers has also been developed. In this bioreactor design the microcarrier motion and its effect on radial medium mass transfer processes inside the bioreactor were studied.[52] In nerve tissue engineering horizontal tubular bioreactors are characterized by microfibers (e.g. hallow fibers) that are used as carriers for development of the neurons.[53,54] A new type of double-mouthed rolling bottle, derived from conventional rolling bottle, has been designed to study the cell growth and the production of monoclonal antibodies of hybridoma cells. It is operated conveniently with a better gas-exchanging efficiency and a lower cost than roller bottles.[55] In case of cardiovascular tissue production horizontal tubular bioreactors are

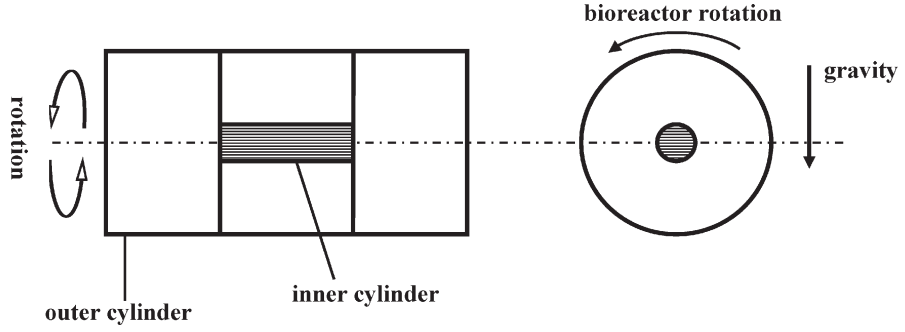


Fig. 5 – *The horizontal tubular bioreactor with inner and outer rotating cylinders*

also used providing reproducible results for specific biomechanical and biochemical parameters that play an important role in tissue engineering. Based on these results, a new bioreactor designs for cardiovascular tissue engineering were made with better mechanical properties and morphological characteristics compared to the static cultivation.[56]

Mathematical modeling and scale-up

For successful construction of horizontal tubular bioreactors, their hydrodynamic characterization and scale-up many variables should be known: mixing (flow) and mass transfer characteristics, profiles of substrate concentration, mixing time as well as distribution of residence and circulation times. Residence and circulation time distributions are obtained by measuring the system response on the pulse or step change in the bioreactor inflow. Mathematical models of mixing coupled with bioprocess kinetic model and computational fluid dynamics are necessary for the successful bioreactor construction.

Mixing and bioprocess kinetics

Characteristics of liquid flow in horizontal tubular bioreactors are possible to describe by one-parameter (dispersion and cascade model) and multi-parameters mathematical models.[57] One-parameter axial dispersion model is the most often used model for mixing (or flow) characterization in horizontal tubular bioreactors and it is based on the mass balance of medium component (c_i) in the liquid phase of the bioreactor. The model is defined by the following equation:

$$\frac{\partial c_i}{\partial t} = D_z \frac{\partial^2 c_i}{\partial z^2} - v_z \frac{\partial ci}{\partial z} \tag{1}$$

The equation 1 is also very often expressed in dimensionless form with Bodenstein (Bo) number as a parameter. This equation could not be solved analytically when the change of liquid flow behavior (ideal mixing flow into plug flow or reverse) occurs in the point of pulse introduction and in the measuring point. In this situation, equation 1 could be only solved numerically. In axial dispersion model radial flow of liquid phase in bioreactor is neglected.

The cascade (tank in series) model can be also used for mixing (or flow) characterization in horizontal tubular bioreactors. The liquid flow in bioreactor can be simulated by the series of ideally mixed cascades (N) and the number of cascades is the variable parameter of this model. The model is defined by the following equation:

$$C_\tau = \frac{N(N\tau)^{N-1}}{(N-1)!} e^{-N\tau} \tag{2}$$

The plug flow conditions are present in horizontal tubular bioreactors when $N \geq 5$ (or $Bo \geq 7$), while ideal mixed flow behavior is realized when $N = 1$. When moving parts are incorporated in horizontal tubular bioreactors (rotating inner tube with or without blades) one-parameter mixing models could not successfully describe flow behavior. Therefore multi-parameters models were established for mixing characterization in these bioreactors. In real horizontal tubular bioreactors axial and radial mixing are present and therefore two-parameters dispersion model was established:[24]

$$\frac{\partial c_i}{\partial t} = -v_z \frac{\partial c_i}{\partial z} + D_z \frac{\partial^2 c_i}{\partial z^2} + D_R \frac{1}{R} \frac{\partial}{\partial R}\left(R \frac{\partial c_i}{\partial R}\right) \tag{3}$$

For thin layer tubular bioreactors mass transfer (e.g., oxygen) can be defined by following multi-parameters dispersion model that defines mixing and oxygen transfer.[18]

$$\frac{\partial c_i}{\partial t} = D_{O_2} \frac{\partial^2 c_i}{\partial R^2} - v_R \frac{\partial c_i}{\partial R} - v_z \frac{\partial c_i}{\partial z} - r_A c_i^{n^*} \tag{4}$$

In real horizontal tubular bioreactor with developed microbial biofilm on the inner surface of the bioreactor the bioprocess kinetics in steady state conditions could be described by the use of Monod model and the mass balance of the system:

$$Q_L d\gamma_S + \frac{\mu_M \gamma_X \gamma_S A_{FX} \delta_F A_F}{Y_{X/S}(K_S + \gamma_S)} dz = 0 \tag{5}$$

When substrate saturation constant (K_S) and biofilm thickness (δ_F) are constant (boundary conditions $z = 0$, $\gamma_S = \gamma_{S_0}$ and $z = z$, $\gamma_S = \gamma_S$) solution of equation 5 is:[24]

$$\ln \frac{\gamma_S}{\gamma_{S_0}} = \frac{\gamma_{S_0} - \gamma_S}{K_S} - \left(\frac{\mu_M \delta_F \gamma_X}{Y_{X/S}}\right) \frac{A_{FX} A_F z}{Q_L K_S} \tag{6}$$

If biofilm thickness is not constant the solution of equation 5 has another form:[24]

$$\ln \frac{\gamma_S}{\gamma_{S_0}} = K_S \left(\frac{1}{\gamma_S} - \frac{1}{\gamma_{S_0}}\right) - \left(\frac{\mu_M \delta_F \gamma_X}{Y_{X/S} \gamma_S}\right) \frac{A_{FX} A_F z}{Q_L} \tag{7}$$

Mass transfer limitation was assumed and incorporated in this model what is in agreement with real performance of bioprocesses in horizontal tubular bioreactors with microbial biofilm. In these bioreactors, ideal biofilm thickness that enables sufficient substrate and oxygen penetration through the whole biofilm layer can be established and it is

mostly effected by the type of microorganism in the biofilm and by hydrodynamic conditions. In the case of biodisc reactor bioprocess model was established on the basis of substrate balance and the use of Monod kinetics.

$$\frac{\gamma_S}{\gamma_{S_0}} = \frac{1}{1 + \frac{(\mu_M \delta_F \gamma_X A_{FX} t_z)}{Y_{X/S}(K_S + \gamma_S)}} \tag{8}$$

The models presented so far are based on pseudo-homogeneous conditions in the bioreactor and consequently they can not explain the bioprocess behavior in details. Therefore, they can not be used in the scale-up procedure. In real bioprocesses, heterogeneity of cultivation medium is often present and therefore this effect has to be incorporated in mathematical model of the bioprocess. The main reasons for development of the model that can describe the heterogeneity of bioprocess are: determination of substrate consumption rate in the case of interaction between bioprocess kinetics and inner or/and outlet transfer processes (inside microbial biofilm or microbial cell) and determination of biofilm growth rate. Complex bioprocess models are usually established so that the heterogeneity of the bioprocess is incorporated in hydrodynamic model which is also coupled with bioprocess kinetic model. As the example of complex bioprocess model for tubular bioreactor with microbial biofilm the following model could be established:[57,58]

$$\begin{aligned} \mathrm{d}V_L \varepsilon_L \frac{\partial \gamma_S}{\partial t} = -v_z \frac{\partial \gamma_S}{\partial z} \mathrm{d}V_L + D_z \frac{\partial^2 \gamma_S}{\partial z^2} dV_L - \\ -(\varepsilon_L \Gamma_S \mathrm{d}V_L + A_{FX} G_S \mathrm{d}V_L) \end{aligned} \tag{9}$$

This model can be used for different bioreactor configurations due to the fact that it was established on the basis of dynamic interaction between kinetic and transport processes. The results obtained by equation 9 show that simple pseudo-homogeneous models can not be used for description of biofilm behavior because of the fact that they neglect interaction between substrate consumption, biofilm growth and mass balance of components in the cultivation medium. However, equation 9 can be solved only by numerical methods.[24]

In horizontal rotating tubular bioreactor (HRTB) a comprehensive mixing study was carried out by different combinations of process parameters [(bioreactor rotation speed (n), dilution rate (D), liquid level in bioreactor (H_M) and distance between partition walls (d_S)]. For mixing description in HRTB three mathematical models were established. Two of them were modified cascade models ("simple" flow model[25] and "spiral" flow model[26,59]) and the third was axial dispersion model with Bodenstein number as a model parameter.[60] Comparison between these models showed that the "spiral" flow model was the most suitable for mixing characterisation in HRTB. In order to incorporate the "spiral" flow model in scale-up procedure it was necessary to make relations between the adjustable model parameters and bioreactor process parameters. The obtained mathematical equations were used for the formation of prediction systems for adjustable model parameters[61,62] and they were in the following form:

$$\begin{aligned} N,\ N_i &= f\,[a_1 - a_N\ \mathrm{f}(H_M,\ d_S),\ \mathrm{Re}_D,\ \mathrm{Re}_N] \\ Q_{CR},\ Q_P &= f\,[a_1 - a_N\ \mathrm{f}(H_M,\ d_S),\ \mathrm{Re}_D,\ \mathrm{Re}_N] \end{aligned} \tag{10}$$

Established prediction system can predict parameters N and N_i with accuracy of ± 1 and parameters Q_{CR} i Q_P with accuracy ± 30 % respectively. After intensive hydrodynamic studies, HRTB was tested in real microbial bioprocesses (aerobic and anaerobic) that were conducted in continuous mode of operation. In both cases, microbial biofilm was developed on the inner surfaces of HRTB. For description of fermentative glucose conversion in HRTB unstructured kinetic model was established that defined biomass growth, products formation and substrate consumption rate by using the modified Monod (Levenspiel) model. This kinetic model defined changes in suspension and in microbial biofilm and it showed relatively good agreement with experimental data. As example, the suspended biomass and substrate concentration along HRTB were defined by following equations[27,28].

$$\begin{aligned} \frac{\mathrm{d}\gamma_X}{\mathrm{d}L} &= \frac{1}{v_z} \cdot \mu \cdot \gamma_X; \\ \mu &= \mu_{\max} \cdot \left(1 - \frac{\gamma_{P_1}}{\gamma_{P_1^*}}\right)^{0.5} \frac{\gamma_S}{\gamma_S + K_S} \end{aligned} \tag{11}$$

$$\begin{aligned} \frac{\mathrm{d}\gamma_S}{\mathrm{d}L} = -\frac{1}{v_z} \cdot \left[\frac{\mu \gamma_X}{Y_{X/S}} + \frac{q_{P_1} \gamma_X}{Y_{P_1/S}} + \frac{q_{P_{1.1}} \gamma_{X_1}}{Y_{P_1/S}} + \right. \\ \left. + \frac{q_{P_{2.1}} \gamma_{X_1}}{Y_{P_2/S}} + m_1 \gamma_{X_1} \right] \end{aligned} \tag{12}$$

In case of tissue engineering, mathematical models have to take into account that biological tissue grows in three dimensions and therefore the problem of boundary conditions for mass balance equations are present very often. These boundary conditions have to be correctly established so that mass balance equations can be numerically solved. The established mathematical models have to determine simultaneously the two effects of tissue

growth: the concentration field of nutrients in the liquid phase and the position of the interface between the tissue and the culture medium. Because of these requests two groups of numerical procedures for solution of mass balance equations were developed: multiple region solutions and single region (continuum) formulations (or phase-field models). Multiple region solutions use independent equations for each phase and couple them with appropriate boundary conditions at the tissue/medium interface. The concentration equations in the bulk medium are coupled to the interfacial conditions and consequently this system of equations and boundary conditions fulfill basic requirements of mathematical models for tissue growth. This approach is based on the Eulerian methods.[63,64] Single region (continuum) formulations (or phase-field models) eliminate the need for separate equations in each phase, by establishing conservation equations that are universally valid. The major advantage of phase-field models is that they do not require the use of quasi-steady approximations and the explicit application of interfacial conditions at the unknown location of a phase boundary. These models are characterized by the phase-field variable which varies in space and time. The transition from biological tissue to cultivation medium is defined by the phase-field variable which varies smoothly but rapidly through the interfacial region. In both numerical approaches medium flow during the cultivation of biological tissue is most often described by Navier – Stokes equation. At a moment, a widely applicable numerical procedure that can be used for the simulation of the growth of biological tissue in the frame of the new field of tissue engineering is still missing.[45,65,66] As example, medium flow behavior and its component concentrations are usually defined in phase-field models by following equations:

$$\frac{\partial v}{\partial t} = -\nabla p - \nabla v^2 + Sc\,\nabla^2 v - Sc\;1/\eta v \qquad (13)$$

$$\nabla\; v = 0$$

$$\frac{\partial c_i}{\partial t} = [-\nabla(v\,c_i) + \nabla^2\,c_i] \qquad (14)$$

These equations (13, 14) can be solved numerically by control volume method after definition of initial and boundary conditions. In this method, tissue surface is discretized with a uniform mesh and the flow-field variables defined over a staggered grid. Forward differences in time and upwind schemes in space (second-order accurate) are used to discretize the partial differential equations, resulting in:

$$v^{t_n+1} = v^{t_n} + \Delta t\,[-\nabla v^2 + Sc\nabla^2 v]^{t_n} - \\ - \Delta t\;Sc\;1/\eta v^{t_n+1} - \Delta t\;\nabla p^{t_n} \qquad (15)$$

$$C_i^{t_n+1} = C_i^{t_n} + \Delta t\;[-\nabla(v\,c_i) + \nabla^2\,c_i]^{t_n} \qquad (16)$$

The orientation of interface medium/tissue is used to determine the medium component fluxes at the tissue surface. This orientation depends also on the direction of the volume fraction gradient of the phase within the cell, and that of the neighbor cell (or cells) sharing the face in question. The established model can successfully describe the process of tissue cultivation.[45]

Basic scale-up rules

It is well known that for scale-up of tubular bioreactors it less number of parameters must be known compared to the stirred tank bioreactors. Simple construction and more uniform flow inside tubular bioreactors are major reasons for simpler and more reliable scale-up. For scale-up of horizontal tubular bioreactors geometrical similarity criteria are most often used. On the basis of these criteria diameter and length of horizontal tubular bioreactors are determined as well as the pump type and the flow rate of medium and air. Besides flow characteristics for successful scale-up of tubular bioreactors it is also important to determine bioprocess kinetics inside the bioreactor. As example, for cultivation of *Candida tropicalis* biomass and substrate balances are defined by following equations:[67]

$$\frac{\mathrm{d}\gamma_X}{\mathrm{d}t_z} = \frac{\mu_M \gamma_S \gamma_X}{K_S + \gamma_S}\,\frac{c_{O_2}}{K_{O_2} + c_{O_2}} \qquad (17)$$

$$\frac{\mathrm{d}\gamma_S}{\mathrm{d}t_z} = -\frac{1}{Y_{X/S}}\,\frac{\mu_M \gamma_S \gamma_X}{K_S + \gamma_S}\,\frac{c_{O_2}}{K_{O_2} + c_{O_2}} \qquad (18)$$

In this case, oxygen balances in liquid (eq. 19) and in gas (eq. 20) phases are defined by following equations:[67]

$$\frac{\mathrm{d}c_{O_2}}{\mathrm{d}t_z} = \frac{k_G a}{\varphi_L}\left(Y_G - \frac{c_{O_2} H}{p_R}\right) - \\ - \frac{1}{Y_{O_2}}\,\frac{\mu_M \gamma_S \gamma_X}{K_S + \gamma_S}\,\frac{c_{O_2}}{K_{O_2} + c_{O_2}} \qquad (19)$$

$$\frac{\mathrm{d}c_{O_2}}{\mathrm{d}t_z} = \frac{k_G a\, R_g T}{\varphi_G}\left(Y_G - \frac{c_{O_2} H}{p_R}\right) \qquad (20)$$

Power required for mixing of aerated broth (P_P/V_L) in this tubular bioreactor was determined on the basis of the pressure gradient ($\mathrm{d}p_R/\mathrm{d}z$) along the axial direction of the bioreactor:

$$\frac{p_P}{V_L} = \frac{v_z}{v_\eta}\frac{dp_R}{dz} \quad (21)$$

On the basis of geometrical similarity criteria combined with bioprocess kinetics a successful scale-up of horizontal tubular bioreactors can be completed.

Conclusions

Horizontal tubular bioreactors are gaining increased interest for conduction of different bioprocesses. They have some potential advantages (e.g., simple construction and maintenance, high surface to volume ratio and flexible inner configuration) over stirred tank bioreactors. However, they have also some disadvantages (e.g., relatively low oxygen supply capacity and unsuitability for batch bioprocesses) compared to the stirred tank bioreactors. In horizontal tubular bioreactors medium flow is characterized by plug flow conditions and this is advantage in case of inhibition and/or repression bioprocess kinetics. Liquid flow behavior in horizontal tubular bioreactors can be described by simple one-parameter (dispersion and cascade model) or complex multi-parameters mathematical models. Comparison between these models pointed out that a complex multi-parameters model can describe bioreactor performance more efficiently. These complex models combine mathematical description of flow pattern with bioprocess kinetics. For scale-up of horizontal tubular bioreactors the criteria of geometrical similarity are most often used. But besides that, flow behavior and bioprocess kinetics should also be incorporated in successful scale-up procedure.

List of symbols

$a_1 - a_N$ experimental coefficients
A_F – biofilm surface, m^2
A_{FX} – specific active biofilm surface, $m^2\ m^{-3}$
c_i – concentration of medium component, mol m^{-3}
c_{O_2} – oxygen concentration in liquid phase, mol m^{-3}
C_τ – distribution of medium component
D – dilution rate, h^{-1}
δ_F – biofilm thickness, m
D_{O_2} – oxygen diffusion coefficient, $m^2\ s^{-1}$
d_S – distance between partition walls in bioreactor, m
D_R – radial dispersion coefficient, $m^2\ s^{-1}$
D_Z – axial dispersion coefficient, $m^2\ s^{-1}$
η – pump efficiency
ε_L – medium porosity
Q_{CR} – circulation flow, $m^3\ s^{-1}$
Q_L – liquid flow rate, $m^3\ s^{-1}$
Q_P – back flow, $m^3\ s^{-1}$
H – Henry constant, mol m^{-3} Pa^{-1}
H_M – liquid level in HRTB, m
k_Ga – oxygen transfer coefficient based on the gas pressure, mol Pa^{-1} m^{-3} s^{-1}
K_{O_2} – saturation constant for oxygen uptake, kg m^{-3}
K_S – substrate saturation constant, kg m^{-3}
L – bioreactor length, m
m_1 – specific maintenance rate of biofilm, kg kg^{-1} h^{-1}
n – rotation speed, s^{-1}
n^* – reaction order
N – cascade number
N_i – number of ideally mixed compartments in cascade
G_S – substrate mass flux in biofilm, kg m^{-2} s^{-1}
γ_{P_1} – ethanol mass concentration, kg m^{-3}
$\gamma_{P_1}^*$ – critical ethanol mass concentration, kg m^{-3}
γ_{P_2} – lactate mass concentration, kg m^{-3}
p – pressure, Pa
P_P – pump power, kW
p_R – system pressure, Pa
q_{P_1} – specific rate of ethanol production in suspension, kg kg^{-1} h^{-1}
$q_{P_{1,1}}$ – specific rate of ethanol production in biofilm, kg kg^{-1} h^{-1}
$q_{P_{2,1}}$ – specific rate of lactate production in biofilm, kg kg^{-1} h^{-1}
R – radial coordinate, m
r_A – reaction rate, mol m^{-3} s^{-1}
Γ_S – volumetric substrate uptake rate in suspension, kg $m^{-3}h^{-1}$
Re_D – Reynolds axial flow number
Re_N – Reynolds rotation number
R_g – general gas constant, J $mol^{-1}K^{-1}$
φ_G – gas fraction in total volume
φ_L – liquid fraction in total volume
γ_S – substrate concentration, kg m^{-3}
Sc – Schmidt number ($Sc = \nu/D_Z$)
γ_{S_0} – initial substrate concentration, kg m^{-3}
T – temperature, °C
t – time, s
t_n – time step, s
t_z – residence time of substrate or biomass in bioreactor, s
t_z^* – mean residence time, s
V_L – liquid volume in bioreactor, m^3
v – medium velocity at the interface tissue/medium, m s^{-1}
v_R – liquid velocity in radial direction, m s^{-1}
v_Z – liquid velocity in axial direction, m s^{-1}
γ_X – biomass concentration in liquid phase, kg m^{-3}

γ_{X_1} – average volumetric density of biofilm, kg m^{-3}
y_G – oxygen mole fraction in gas phase, mol mol^{-1}
Y_{O_2} – oxygen to biomass yield coefficient, kg kg^{-1}
$Y_{P_1/S}$ – ethanol yield, kg kg^{-1}
$Y_{P_2/S}$ – lactate yield, kg kg^{-1}
$Y_{X/S}$ – substrate to biomass yield, kg kg^{-1}
z – axial coordinate
μ – specific growth rate, h^{-1}
μ_M – maximal specific growth rate, h^{-1}
η – permeability, m^3 Pa^{-1} m^{-2} h^{-1}
ν – kinematics viscosity, m^2 s^{-1}
τ – dimensionless time, t/t_z*

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