Fluid mechanical problems in biotechnology

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Summary

The rapid development of biotechnology we are witnessing nowadays is strongly related to the advances in bioprocess engineering. This field of engineering, including both bioreactor engineering and down-stream processing, is an integration of biological, biochemical and engineering principles, leading to the quantitative description and development of biotechnological processes carried out on an industrial scale. A very important part of bioprocess engineering problems involves the fluid mechanics problems occurring in biotechnological equipment. These are both the problems related to bioreactor design and operation, and to down-stream processing operations, such as micro- and ultrafiltration, centrifugation, precipitation, extraction, sorptions, flocculation, mixing, fluid transportation, etc.

This paper is focused mainly on fluid mechanical problems in bioreactor engineering. It begins with a brief reminder of some basic concepts in fluid flow, rheology and turbulence, of relevance to bioreactors. Then the main types of bioreactor design are reviewed, including stirred bioreactors and pneumatically driven bioreactors. The hydrodynamic characteristics of different types of reactors are then outlined, including power consumption, mixing, heat and mass transfer, aeration, and cell demage by hydrodynamic stresses. Finally, some new developments are discussed, including the use of computer fluid dynamics (CFD) to describe hydrodynamics of bioreactors, and the use of the multifractal theory of turbulence to describe some cell damaging effects.

Key words:
bioreactors, hydrodynamic stress, shear.

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biotechnologia

2 (57) 60–77 2002

* Plenary lecture presented at International Conference on "New Horizons in Biotechnology", April 18-21, 2001, Trivandrum (India).

1. Introduction

Almost in all biotechnological processes, fluid mechanics plays an important role. Every time when the suspension of cells in a liquid medium is transported, processed, stirred, heated or cooled, a supply of mechanical power is necessary in order to:

- homegenize the suspension,
- enhance the heat and/or mass transfer,
- transport the suspension to or through the equipment.

This power supply results in fluid flow, which in turn causes different effects on the process conditions. In the case of bioreactors, hydrodynamic stresses may affect microbial morphology, apparent morphology of the broth, broth rheology, microbial growth, product formation, etc. (Fig. 1). It is thus important to understand how

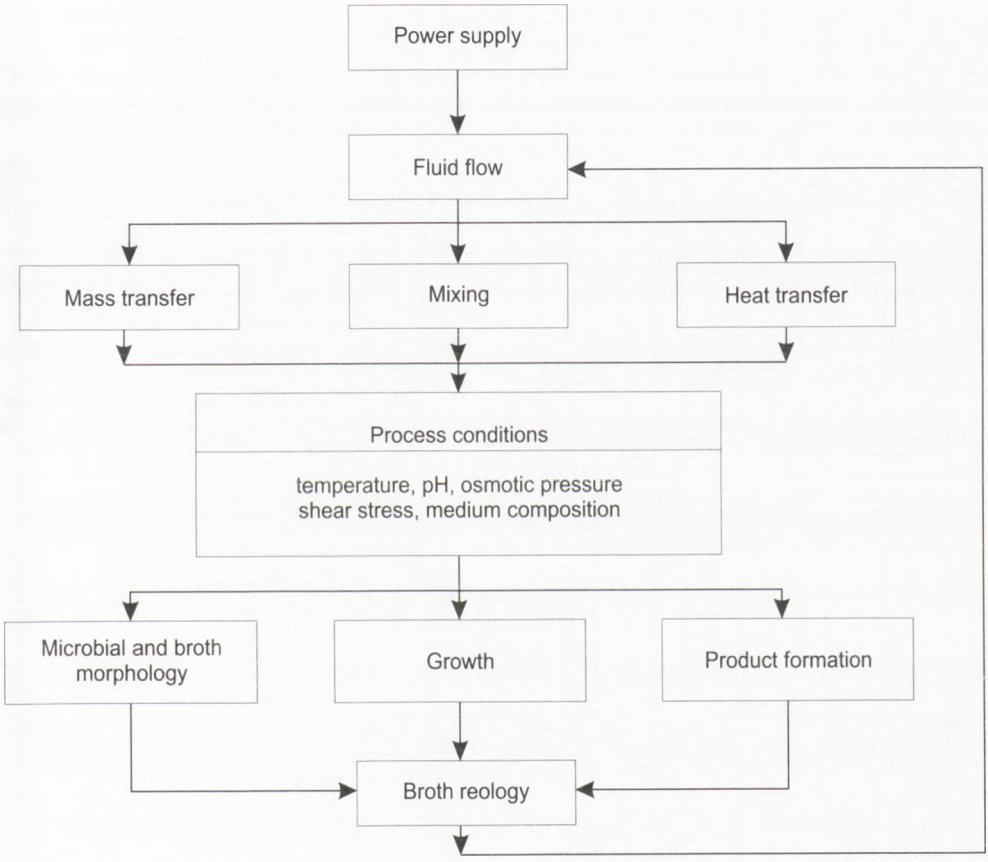

Fig. 1. Relationship between hydrodynamics and process conditions in a bioreactor.

these effects are interrelated and to be able to describe them in a quantitative way in order to properly design process and/or equipment.

2. Basic concepts in fluid flow, rheology and turbulence

At the beginnig, let us recall same basic concepts of fluid flow, rheology and turbulence. In order to bring a real fluid into motion, application of force is necessary. Imagine a layer of fluid confined between two stationary plates (Fig. 2). Upon application of force F to one of the plates, it is set into motion.

After a while, a steady-state velocity profile between the plates is reached with a constant velocity gradient or shear rate

$$\dot{\gamma} = \frac{dv}{dy}. \tag{1}$$

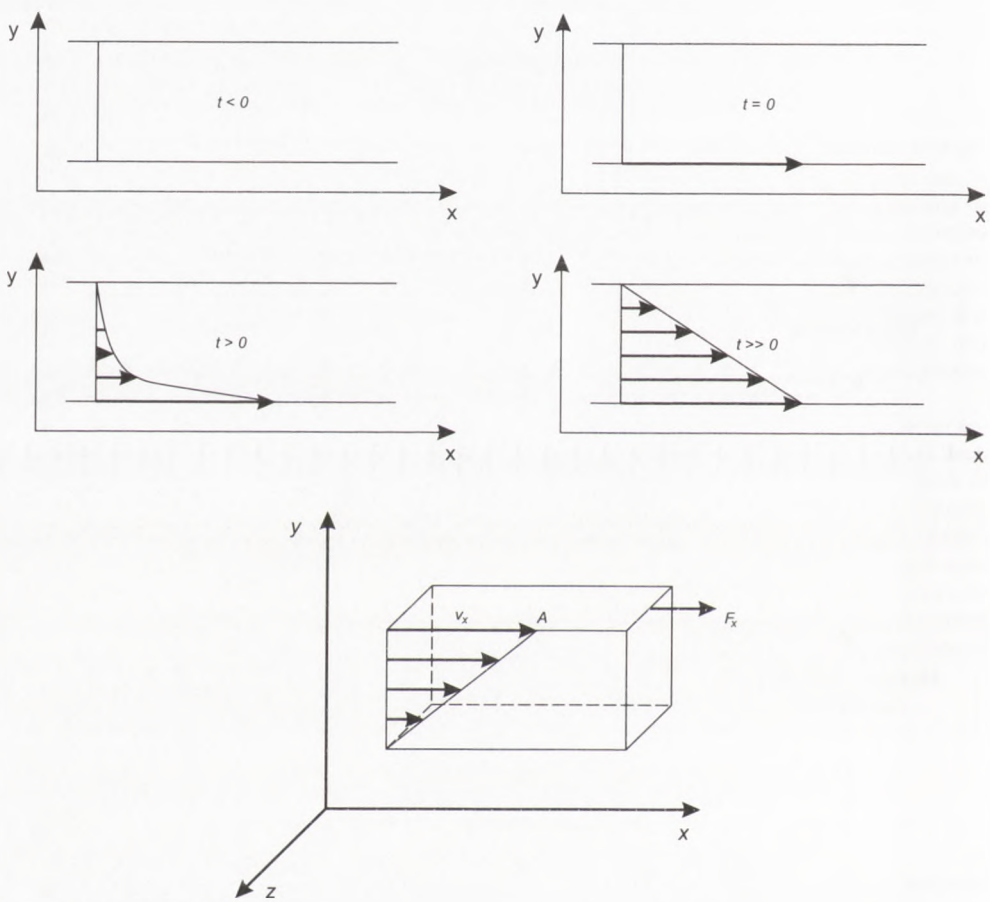

Fig. 2. Shear flow and shear rate.

In order to maintain this gradient, a steady application of the force F is required. The ratio of this force to the plate area is called the shear stress τ

$$\tau = \frac{F}{A}. \tag{2}$$

This shear stress is responsible for most of the damage to cells. The relation between shear rate and shear stress depends on the kind of fluid. For so called Newtonian fluids, the shear rate is simply proportional to the shear stress

$$\tau = \mu\dot{\gamma}, \tag{3}$$

where μ is the viscosity of the fluid. For many biological fluids, however, a non-Newtonian behaviour is observed (Fig. 3). The exponent n is used to characterize the kind of relation between τ and γ for the so-called Ostwald – de-Waele power law.

$$\tau = k\dot{\gamma}^{n} \tag{4}$$

If $n = 1$, $k = \mu$ and the fluid is Newtonian. An analysis of properties of non-Newtonian fluids is the subject of rheology.

When the velocity exceeds certain value, specific for the given fluid and given flow geometry, the fluid flow is no longer stable, and the flow parameters, such as fluid velocity and pressure, undergo random fluctuations. This is called turbulence. Turbulence is characterized by three – dimensional random fluctuations in velocity, as oberved at a single point, caused by the creation of eddies.

According to the classical Kolmogorov`s theory, turbulent motion can be considered as superposition of a spectrum of velocity fluctuations (and eddy sizes) on

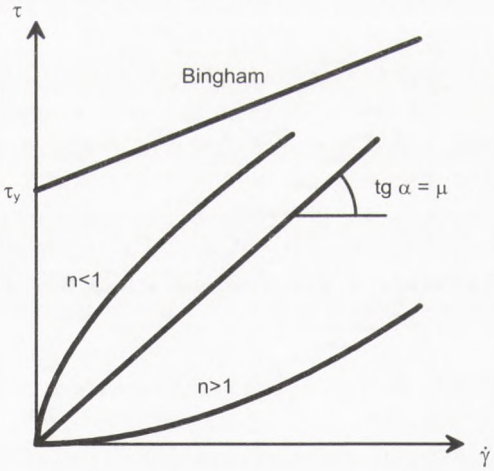

Fig. 3. Relationship between shear rate and shear stress.

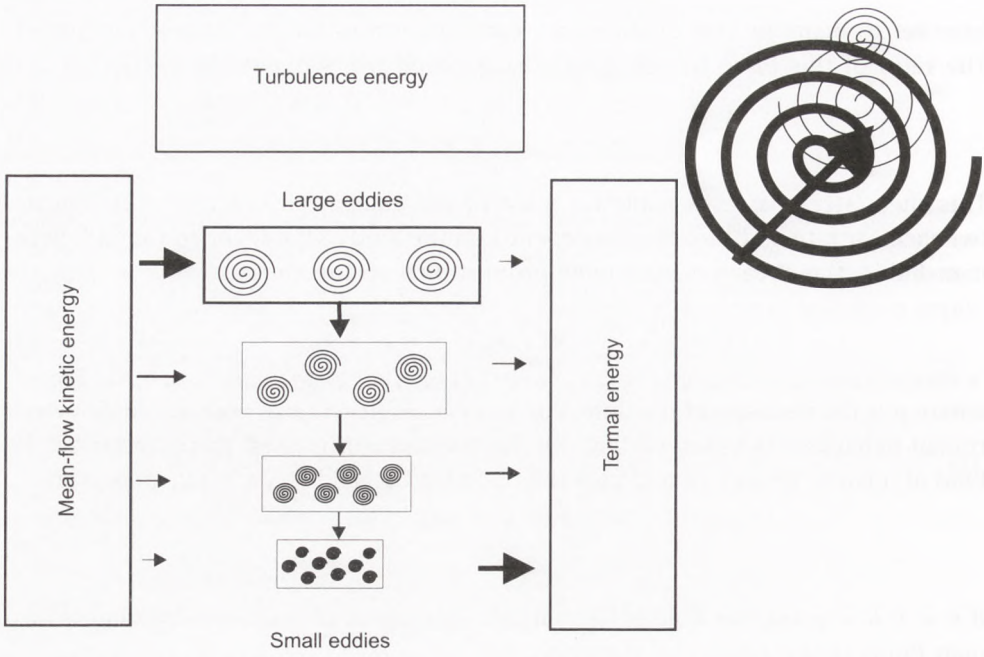

Fig. 4. Energy dissipation cascade [1].

overall mean flow. The power (energy) is supplied to the turbulent flow from the overall mean flow via large eddies that subsequently transfer this energy to a cascade of smaller and smaller eddies. Eventually, very small scale eddies dissipate this energy into heat by viscous forces. This is illustrated in Fig. 4 [1].

In most bioreactors turbulent conditions prevail.

3. Main types of bioreactors

The number of existing bioreactor designs is very large. For animal cell cultures only, more than 70 different bioreactor types are available on the market. The number of those suggested for plant cell cultures, microbial cell cultures, and enzymatic reactions is also significant [2-6,20]. There is no universal design suitable for all culture types, and development of such a universal design is probably impossible. Therefore, it would be impractical to try to discuss all the different designs available.

Instead, I shall describe the main categories of bioreactors and discuss the most important fluid mechanical problems, related to these main groups.

Three main categories of bioreactors, classified according to the means of energy input, can be discerned:

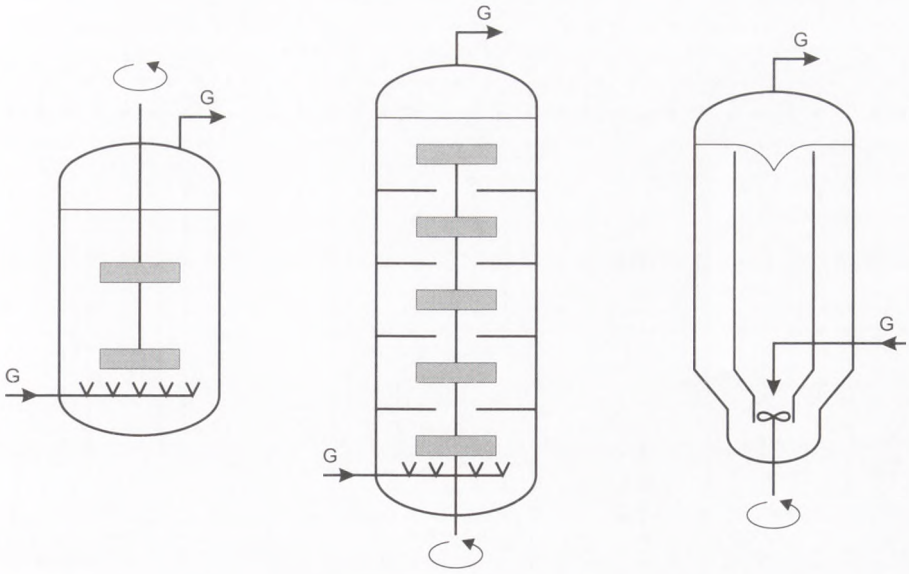

Fig. 5. Mechanically driven bioreactors.

– mechanically driven bioreactors,
– pneumatically driven bioreactors,
– hydraulically driven bioreactors.

The bioreactors with mechanically driven moving parts that enforce the motion of fluid belong to the first category. They may be stirred tank reactors, multistage columns or propeller loop reactors (Fig. 5).

The second category comprises reactors, to which energy is supplied by compressed air, and it includes: bubble columns and air lift reactors (Fig. 6).

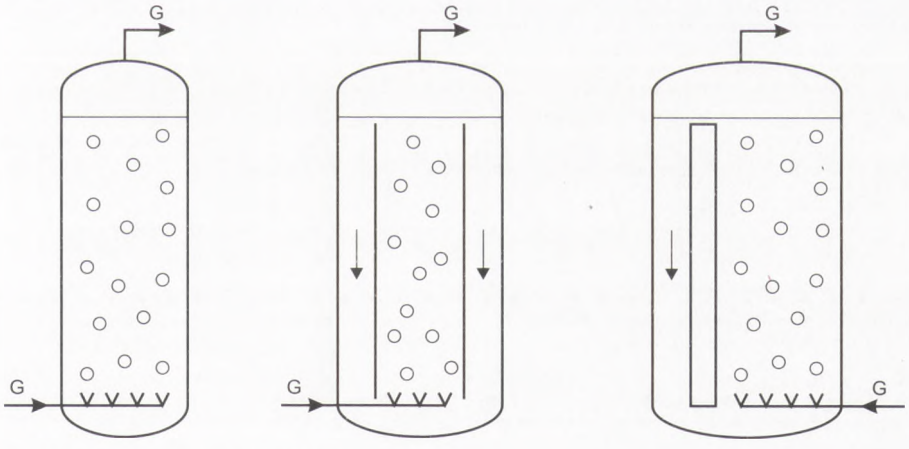

Fig. 6. Pneumatically driven bioreactors.

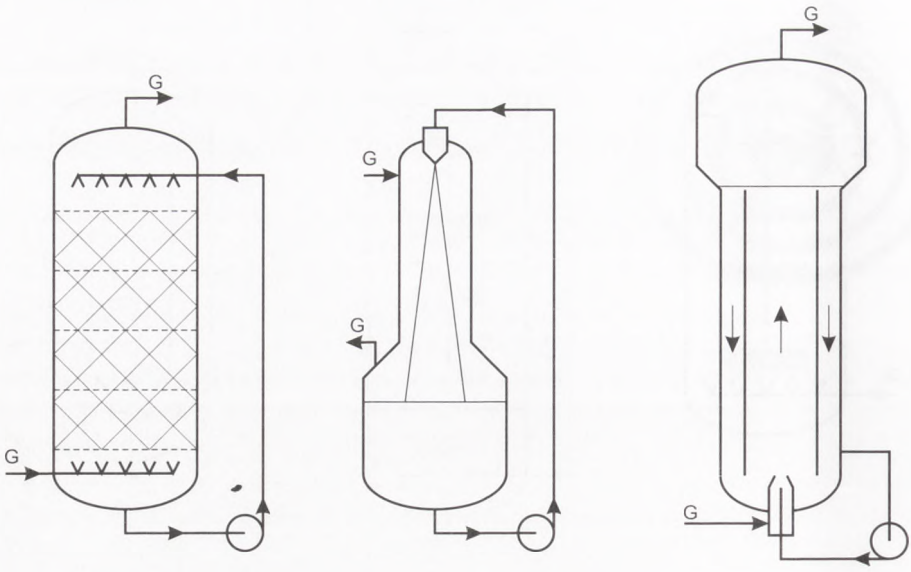

Fig. 7. Hydraulically driven bioreactors.

The third category comprises reactors with external pumps setting the liquid into motion. These are trickle bed columns and jet loop reactors (Fig. 7).

The most common types of bioreactors include stirred tanks, bubble columns and air lift reactors. Their comparison is shown in Table 1 [7].

Table 1

Comparison of the most common bioreactor types [7]

Type	Advantages	Disadvantages
Stirred tank	1. Flexibility and adaptability 2. Wide range of mixing intensity 3. Ability to handle high viscosity media	1. High power consumption 2. High shear rates (possibility of cell damage) 3. High cost
Bubble column	1. Simple construction 2. No moving parts 3. Low cost 4. Ability to handle high cell concentration	1. Poor mixing 2. Limitation to low viscosity media 3. Excessive foaming
Air-lift	1. Simple construction 2. No moving parts 3. High gas absorption efficiency 4. Good heat transfer	1. Poor mixing 2. Limitation to low viscosity media 3. Excessive foaming

Stirred tank reactors may be non-aerated or aerated. A variety of impellers may be used to stir the reactor content, including flat paddles, Rushton turbines, marine propellers, angled blade impellers, profiled impellers, anchor, gate or helical ribbon stirrers, and vaned disc stirrers. Their power requirements, liquid circulation patterns, mixing and gas dispersion properties differ widely.

Stirred tanks are flexible and easily adaptable, they ensure wide range of mixing intensity and may be used for high viscosity media. Their disadvantages include high power consumption, high shear rates in the impeller region, and relatively high cost [2,8,9].

Bubble column is one of the simplest designs, involves no moving parts, is suitable for operation with high cell concentrations, and is relatively cheap. Its disadvantages include poor mixing, excessive foaming, and limitation to low viscosity media [2,3,10].

The same disadvantages exhibit air-lift reactors. Similarly to bubble columns, they are simple in construction and have no moving parts. They also provide for high gas absorption efficiency and good heat transfer [2,10,11].

4. Bioreactor operating conditions

The most important features of a bioreactor include:
- power consumption,
- liquid circulation pattern,
- mixing characteristics,
- solid suspension ability,
- gas dispersion characteristics (gas hold-up, bubble diameter, interfacial area),
- gas – liquid mass transfer,
- solid – liquid mass transfer,
- heat transfer characteristics,
- possible damage mechanism (high shear regions, bubble rupture region).

The above characteristics shall in turn be compared with the most important bioreactor configurations [2-5,11].

a. Power consumption for stirred reactors is usually in the region of 1 – 30 kW/m^3, typically about 10 kW/m^3. For bubble columns it is around 3 kW/m^3, the same is for air-lift reactors (in the two latter cases power is supplied to the system by compressed air).

b. Liquid circulation patterns in stirred reactors may differ, depending on the number of impellers, the aspect ratio (i.e. height – to – diameter ratio), and aeration. In bubble columns, the time-averaged pattern is usually simple: the liquid flows upwards in the center, and downwards near the walls of the column. However, instantaneous patterns are more complicated, with vortices changing their position in the column (Fig. 8). In air-lift reactors liquid circulation pattern is rather simple.

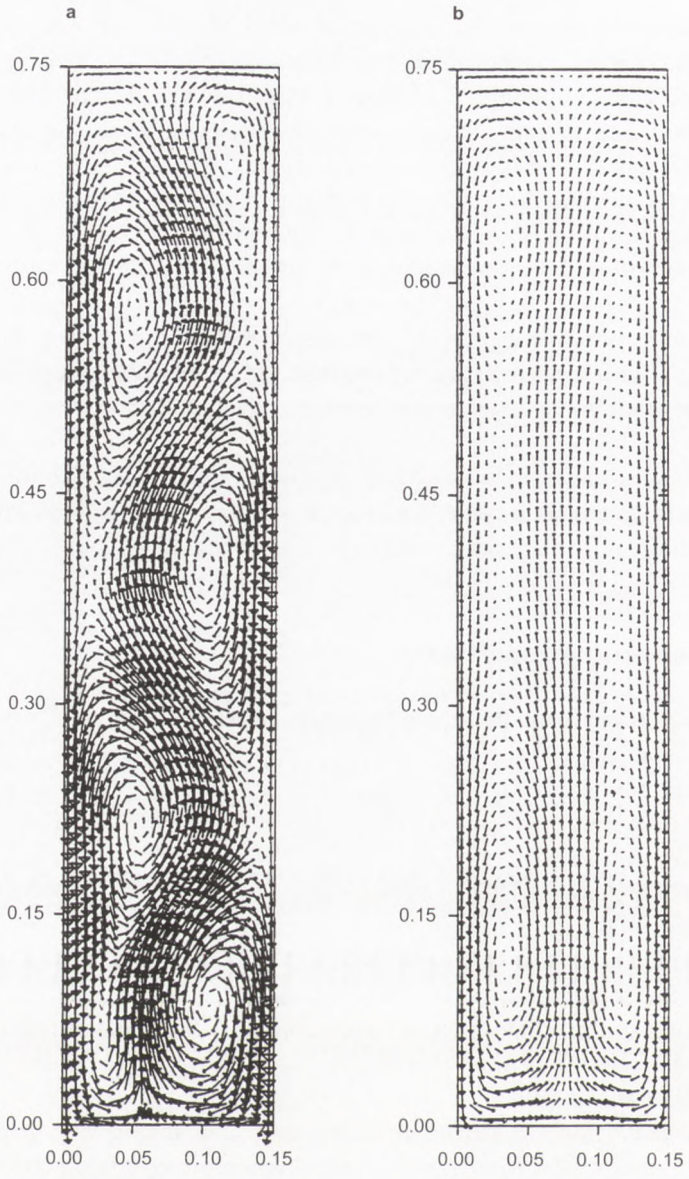

Fig. 8. Time averaged and instantaneous flow patterns in a bubble column [12].

c. Liquid mixing is closely related to the liquid circulation pattern. Stirred reactors ensure relatively good mixing, air-lift reactors being less effective, and bubble columns still less effective then air-lifts.

d. Solid suspension ability is highest for stirred reactors, lower for air-lifts and lowest for bubble columns.

e. Gas dispersion characteristic comprises gas hold-up, bubble diameter and interfacial area between bubbles and the liquid phase.

Gas hold-up is defined as the ratio of the volume occupied by the gas bubbles to the overall volume of the liquid and gas in the liquid medium

$$\varepsilon = \frac{V_G}{V_G + V_L}. \tag{5}$$

It is in the range of 5-20% for stirred reactors, and up to 30% for bubble columns and air lifts. The size of bubbles depends on many factors like the sparger (and/or impeller) geometries, gas flow rate, impeller speed, bubble coalescence and redispersion rates. Interfacial area is an important factor in gas-liquid mass transfer, it is related to gas hold-up and bubble diameter by the relation

$$a = \frac{6\varepsilon}{\bar{d}(1-\varepsilon)}, \tag{6}$$

where $\bar{d}$ is the average bubble diameter (so-called Sauter diameter). A number of empirical correlations for the gas gold-up, bubble diameter and interfacial area are available in the literature.

f. Gas – liquid mass transfer efficiency is slightly lower in stirred tanks than in bubble columns and air-lifts, although in some high – efficiency stirred tanks it may be equally favorable. This, however, often means a necessity to apply excessive shear rates near the impeller.

g. Solid – liquid transfer rates are important for oxygen and nutrients supply to the cells or flocks, as well as for CO_2 removal from the culture. This rate depends to a great extent on the morphology of broth for example size of flocks, and cannot be compared in a simple way for different reactor types.

h. Heat transfer may be effected using external jackets, internal coils, or external heat exchangers. It usually poses no great problems in bioreactor design.

i. Possible cell/flock damage may be caused by excessive shear or by bubble rupture [4,13-15].

The energy dissipation in a stirred vessel is by no means uniform – there are regions near the stirrer, where energy dissipation is many times greater, than the average (Fig. 9). The cell damage, expecially in the regions near the impeller can result from:

- direct interactions between cells and/or microcarriers and turbulent eddies;
- collisions between microcarriers in turbulent flow;
- collisions of microcarriers against the impeller.

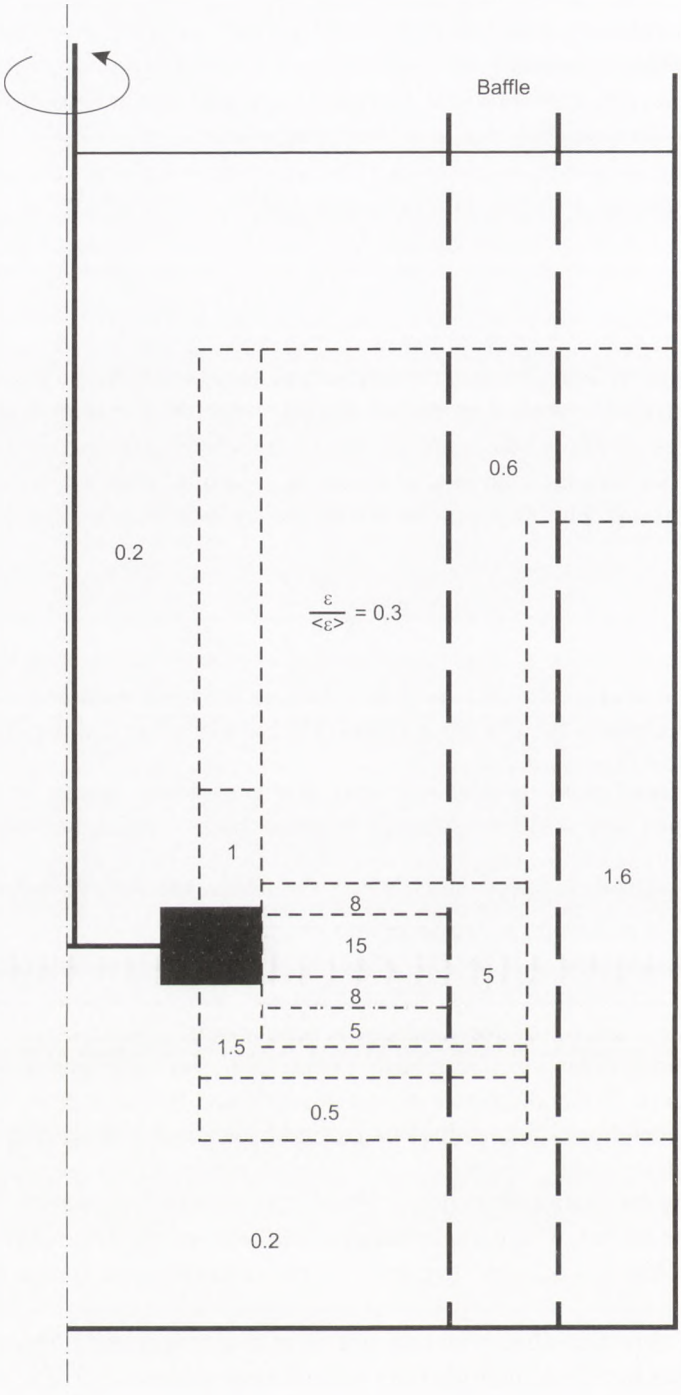

Fig. 9. Energy dissipation rate in a stirred tank [16].

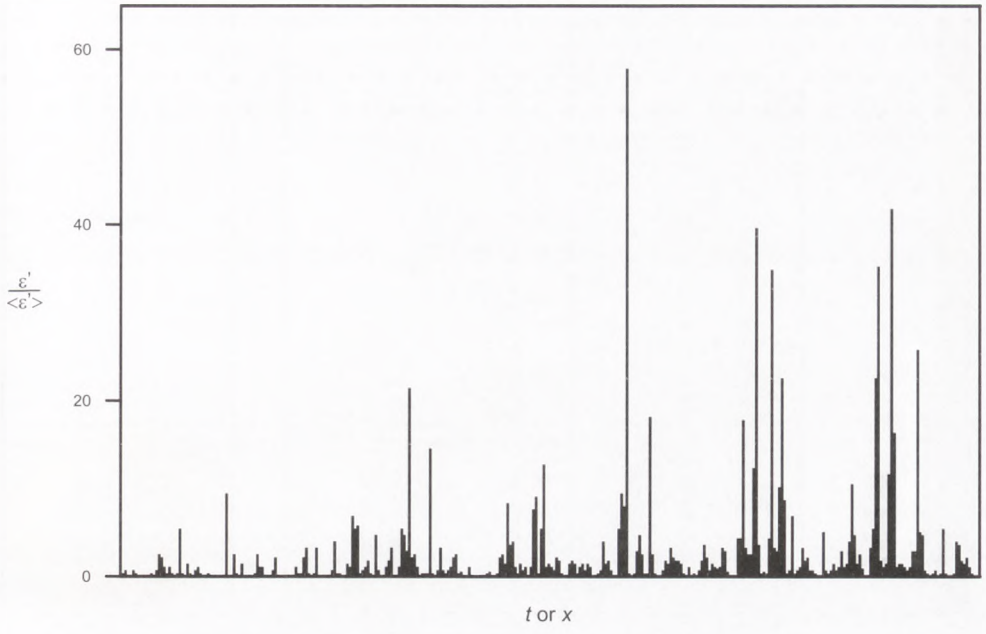

Fig. 10. Local intermittency of turbulence [17].

However, the dissipation field is not uniform not only macroscopically, but also locally (microscopically), with violent outbursts of turbulence occurring in a haphazard way in the fluid. This phenomenon, called local intermittency, complicates the mathematical description of the hydrodynamic situation in the vessel (Fig. 10) [17]. Another important aspect of hydrodynamic stresses action on cells is that they not only influence cells' viability, but also can change their metabolism and secretion.

It was also shown that among important mechanisms of cell damage there is the one associated with bubble bursting. The cells tend to stick to the bubble surface, and very high stresses produced during the bursting process damage them effectively. There is also evidence that cells may be damaged in the regions of bubble formation.

5. New developments

One of the important tools of investigating the hydrodynamic problems is the computer fluid dynamics (CFD). In this technique, the flow field is simulated on a computer, enabling the description of the basic hydrodynamical characteristics of the process. An example of fluid flow field in a bubble column simulated this way (using the FLUENT program) is shown in figure 11. Also figures 12 and 13, showing gas hold-up profiles in a bubble column, and flow field and gas hold – up profile in a stirred vessel, respectively, were obtained in this way (also using the FLUENT pro-

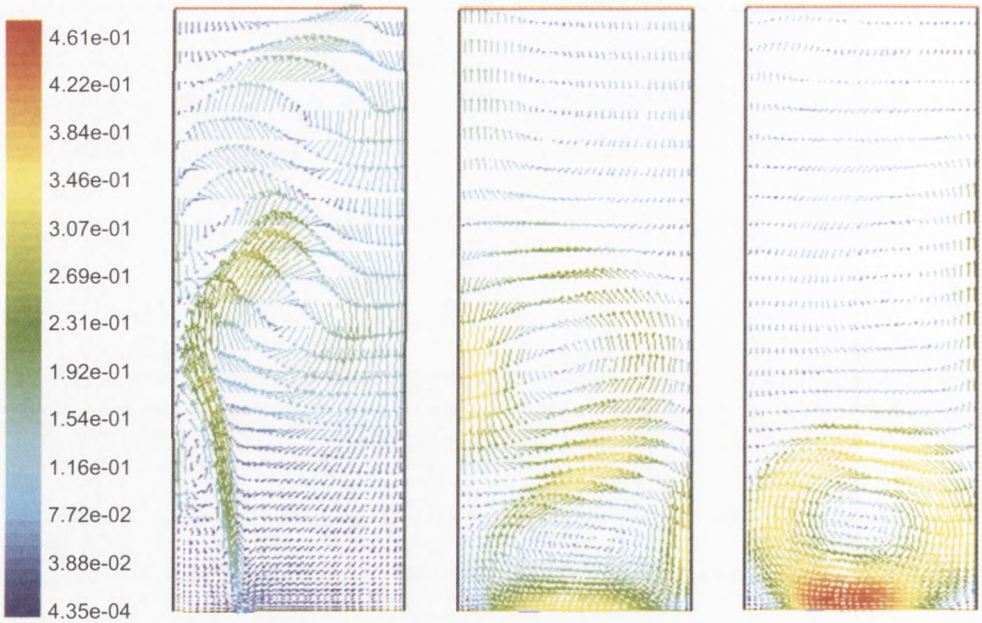

Fig. 11. CFD simulation of the instantaneous flow field in a bubble column (program FLUENT).

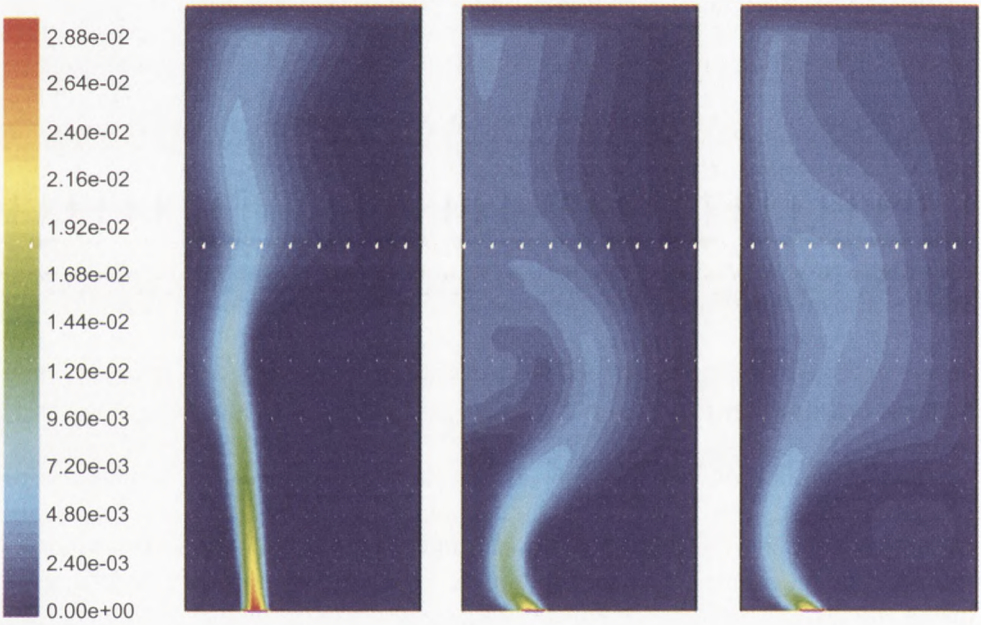

Fig. 12. CFD simulation of the instantaneous gas hold-up distribution in a bubble column (program FLUENT).

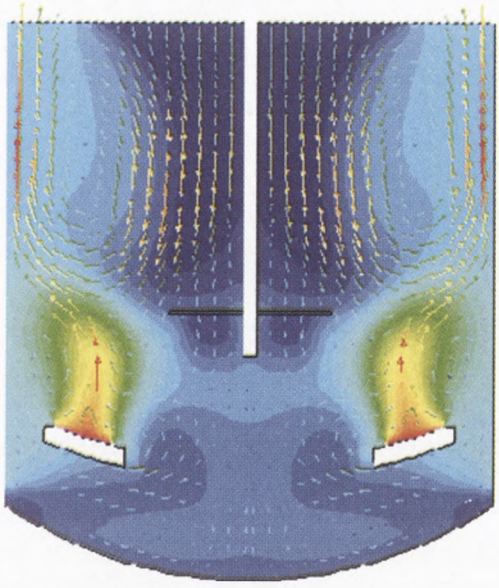

Fig. 13. CFD simulation of the flow field and gas hold-up distribution in a stirred vessels (program FLUENT).

gram). The success of such an approach depends mostly on the kind of assumptions involved in the development of the computer models.

Another important development in the field of turbulence theory is the so called multifractal theory of turbulence. This theory allows the description of the local intermittency of turbulence, described in the previous point. This may be illustrated by the results of the investigation of cell disruption in the viscous subrange of a turbulent field, using erythrocytes as model cells (obtained at Warsaw University of Technology) [18].

Assuming that cell disruption occurs mainly as the result of interactions between cells and turbulent eddies, as it was in the case of our experiments, one can write the kinetic equation for the change in cell concentration in the form

$$\frac{dn}{dt} = \mu n - k_d n, \tag{7}$$

where n is the cell number per unit volume, μ – specific growth rate, k_d – so called death rate constant, and t – time.

The death rate constant can be estimated on the basis of the Kolmogorov theory as

$$k_d = c_v \left(\frac{\langle \varepsilon \rangle}{\nu} \right)^{\frac{1}{2}}, \tag{8}$$

or from the multifractal theory of turbulence as

$$k_d = c'_v \int_{0,12}^{\alpha_{cr}} f(\alpha)P(\alpha)d\alpha = c_v \left(\frac{\langle\varepsilon\rangle}{\nu}\right)^{\frac{1}{2}} \int_{0,12}^{\alpha_{cr}} \left(\frac{\langle\eta\rangle}{L}\right)^{\frac{2\alpha+2-4f(\alpha)}{\alpha+3}} d\alpha, \tag{9}$$

where $\langle\varepsilon\rangle$ represents local average energy dissipation rate, ν is the kinematic viscosity of the medium, $\langle\eta\rangle$ is the so called Kolmogorov scale given by the expression

$$\langle\eta\rangle = \frac{\nu^{\frac{3}{4}}}{\langle\varepsilon\rangle^{\frac{1}{4}}}, \tag{10}$$

L is the scale of the largest turbulent eddies, α is so called scalling exponent, $f(\alpha)$ is eddy frequency, $P(\alpha)$ is the probability density function, α_{cr} is the critical value of α corresponding to the critical stress value, c_v and c'_v – constants (for more details see [13,18,19]).

The experiments were carried out using ovine erythrocytes Merino breed in a Rushton turbine reactor with four baffles and the working volume 0,5 dm^3. The number of unbroken cells was determined using particle counter Coulter Multisizer and in a hemocytometer.

The amount of hemoglobin released from the cells was also measured spectrophotometrically. For erythrocytes $\mu = 0$ and Eq (7) becomes

$$\frac{dn}{dt} = -k_d n, \tag{11}$$

or

$$\ln\left(\frac{n}{n_o}\right) = k_d t. \tag{12}$$

This means that the results should give a straight line in the coordinate system, $\ln\frac{n}{n_o}$, $k_d t$.

As it can be seen from figures 14 and 15, each experiment gives, indeed, a straight line in a semilog plot, which confirms the assumption of the first order mechanism of destruction in a turbulent field. However, different experiments interpreted according to the Kolmogorov theory give different lines, which leaves little chance of extrapolating the results to different conditions (e.g. to scale up the process). The use of the classical theory is therefore seriously limited.

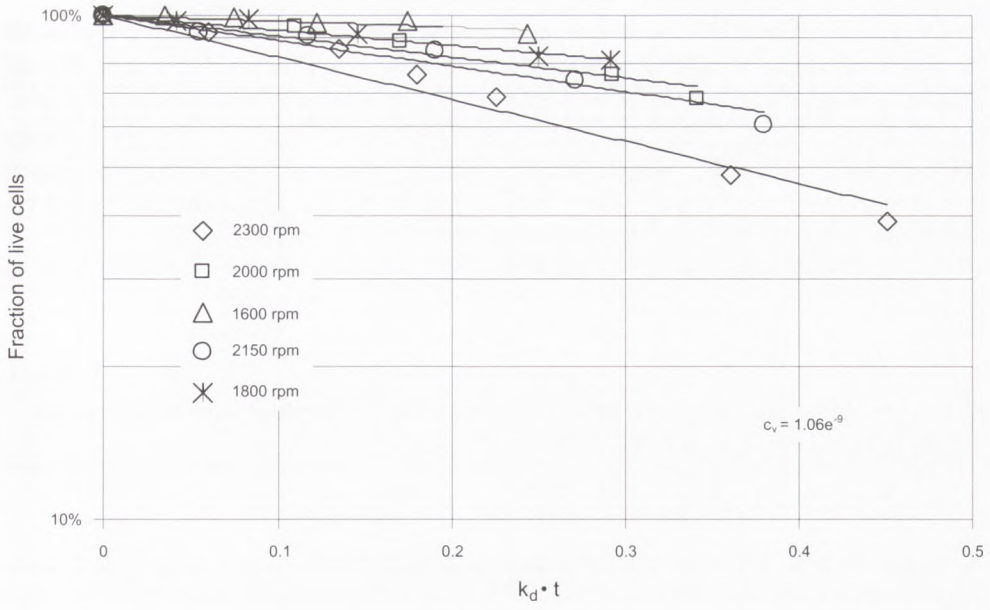

Fig. 14. Number of live cells vs. k_d t according to the Kolmogorov theory.

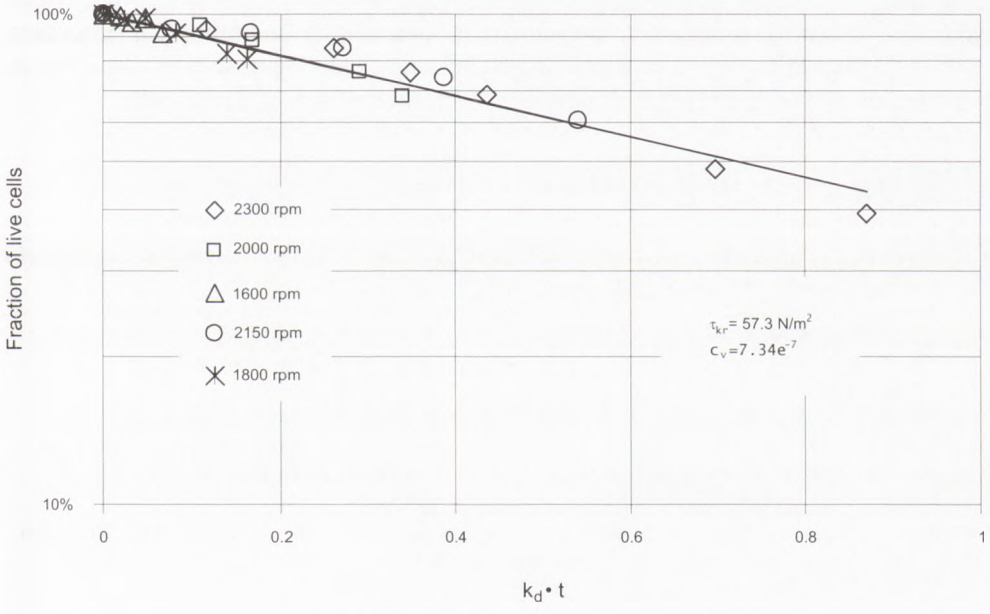

Fig. 15. Number of live cells vs. k_d t according to the multifractal theory.

On the contrary, the same experiments interpreted according to the multifractal theory give a single straight line representing all the experiments. Moreover, the values of k_d calculated from the multifractal theory are in much better agreement with the measured values than those calculated from the classical (Kolmogorov) theory (Tab. 2). This means that the multifractal theory describes cell destruction much better than the classical one, and allows interpolation and extrapolation of the results with much greater degree of certainty.

Table 2

Values of destruction rate constants for different agitation intensities, calculated using Kolmogorov and multifractal theories vs. those measured experimentally

N [rpm]	k_d [s^{-1}] $\times 10^5$ Kolmogorov model	k_d [s^{-1}] $\times 10^5$ multifractal model	k_d [s^{-1}] $\times 10^5$ measured
2300	5.01	9.66	9.65
2150	4.53	6.45	5.27
2000	4.06	4.04	3.88
1800	3.47	1.93	2.38
1600	2.91	7.84×10^{-1}	7.73×10^{-1}

Much more work, however, remains to be done in order to gain better understanding of the hydrodynamic phenomena influencing biotechnological processes. This includes both better understanding of fluid mechanical phenomena, and the interrelation between these phenomena and the behaviour of living cells.

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