PRESSURIZED BIOREACTORS: FUNDAMENTALS AND APPLICATIONS

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ABSTRACT

In this work a review is made on pressurized bioreactors. Several designs are described - batch, chemostat, gas-lift - and the potential applications with examples - studies on oxidative stress, reduction of shear stress, among others, are referred.

There are several reasons for studying the behaviour of biological systems at higher pressures than the atmospheric. Firstly, a large part of the biomass existing in our planet is located in high-pressure environments, like the deep-sea, as it was first demonstrated by ZoBell and Morita (1957). Since then, several microorganisms have been isolated (Erauso et al., 1993) and studies on laboratory conditions were performed to try to understand the hydrostatic and hyperbaric pressure effects on cells physiology (Reysenbach et al., 1991; Nelson et al., 1991). The knowledge of microbial adaptation to high pressures may have many potential applications on enzyme engineering (Kunugi, 1992), on genetic engineering, biochemistry and cell biology.

Pressure effects on microorganisms influences and depends on others environmental parameters affecting microbial growth and metabolism. Increasing pressure can either decrease or increase the temperature upper limits for microbial growth (Bartlett, 1992). Thus, it is important to have laboratory equipment suitable for simultaneous simulate extreme conditions like high temperature and high pressures, for correctly study some thermophilic and barotolerant microorganisms (Nelson et al., 1992).

Cell responses to stress conditions like high temperature have been investigated. It has been observed that the responses are very similar for different kind of stress (Mager and Moradas Ferreira, 1993) and similar to cell response to oxidative stress (Iwahashi et al., 1993). Oxidative stress itself is an important aspect to study, either in a fundamental or applied point of view. Particularly, for aerobic cultures, increasing pressure will increase oxygen partial pressure which could have toxic effects on microorganisms. These effects are not yet completely clear and it seems that cell damage is caused by the reactive oxygen species (H₂O₂ and free radicals, like O₂⁻ and HO⁻) and not by the O₂ molecule. Oxidative stress results from an imbalance between the protective mechanisms of cells and oxygen species production. Some of the protective agents are antioxidant enzymes, such as superoxide dismutase (SOD), catalase and glutathione reductase, among others, and are common to different kinds of cells.
Yeast cells are certainly a good model for studying other eukariotic cells. The antioxidant enzymes present on yeast cells, mainly SOD, have a potential interest in pharmaceutical and in food industries. Westerbeek-Marres et al. (1988) showed that Mn-SOD biosynthesis increases with the increase in oxygen consumption by yeast and that the level of this enzyme is repressed by catabolic repression as well as the respiratory chain components. Also, Clarkson et al. (1991) observed that the specific activity of CuZn-SOD increased with transition from anaerobiosis to aerobiosis in brewing yeast cells. Thus, increasing partial pressure of oxygen could be a way for SOD induction, as was showed by Taniguchi et al. (1992), for Streptococcus lactis cells. It was observed an increase in SOD activity by pressurisation of culture broth with O₂ at 6 atm.

The use of pressure as a way of enhancing oxygen transfer rate to reactors can also be investigated because oxygen is a major growth limiting factor in high cell density aerobic cultures. The traditional way of improving oxygen transfer rates to bioreactors by increasing stirring rate have several limitations like power consumption and cell sensitivity to the high shear stress.

Some works have proved the increasing in cell density by pressurisation for different kinds of cells. For instance, Yang and Wang (1992) successfully used fermentor headspace pressurisation to cultivate E. coli and fragile alga cells.

Pressure effects are not always beneficial (Yabannavar et al., 1992; Onken, 1990) but it should be studied and considered before process scale-up (Trager et al., 1992). In fact, laboratory research of microbial processes are usually performed at atmospheric pressure, but in industrial bioreactors of several tenths of meters high, pressure and consequently gases solubilities are a function of the local position in the reactor, generally increasing by 1 atm for every 10 meters increase in depth (Onken and Liefke, 1990).

Considering the heterogeneity of residence time distribution in large reactors, there will be cells growing at higher pressures (on the bottom) than others (on top) and this could explain some differences in overall bioreactor performances and process productivities found between lab-scale and plant-scale microbial processes.

This is particularly important for processes where dissolved gases are involved. For instance, for the yeast, S. cerevisiae, dissolved oxygen is a factor that causes the switch of the cells from an oxidative to a reductive metabolism, or mixed.

If the unavailability of oxygen constitutes a problem for Baker’s yeast production, the increase on its supply rate can lead to yield decrease on ethanol production (Kuriyama and Kobayashi, 1993). In these processes, there is another gas involved, carbon dioxide. Its partial pressure increase have also metabolic consequences to the cells (Kuriyama et al., 1993). Thus, pressure can not be neglected, as an important parameter of process optimisation for each purpose.

In our lab we have two batch pressurised systems capable of withstanding up to 100 bar of pressure and adapted chemostat able to go up to 150 bar.

We are currently investigating the physiological behaviour of baker’s yeast, analysing in each experiment, the time course evolution of SOD, catalase, glutathione reductase as well as several metabolites (ethanol, glycerol, acetaldehyde, among others), cell density and viability.

At the same time, the chemostat is being used to study the behaviour of E. coli cells in fed-batch operation.
REFERENCES

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