Innovative bioreactors

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Recent papers have described new bioreactor designs. Most innovations addressed either oxygen transfer, shear induced by stirring, control of water activity in organic phase systems or waste biotreatment. Innovations made during the past year were reported in mainly three areas: bioreactor designs for increases in oxygen transfer and decreases in shear stress; bioreactors for two-phase reactions with water activity control; and environmental bioreactors.

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Abbreviations
PED pulsed-electric discharge
TCE trichloroethylene

Introduction

From the simple jar in which the Ancient Greeks fermented their wine to the computer-controlled complex bioreactors commonly used today, great progress has been made in reactor design. Producing more, faster, with higher yields and more reliably have been the driving forces behind this evolution. In past decades, considerations such as shear stress for the cultivation of fragile organisms, establishment of specific conditions for waste biotreatment, or specific means for *in situ* product or byproduct recovery, have stimulated the production of a number of new bioreactor designs. This review reports the authors' perceptions of the new bioreactor designs published during the past year: bioreactor designs for increases in oxygen transfer and decreases in shear stress; bioreactors for two-phase reactions with water activity control; and environmental bioreactors.

Bioreactor designs for better mixing, oxygen transfer and lower shear stress

Mixing, oxygen transfer and shear stress remain the biggest challenges as far as the scale-up to industrial size bioreactors is concerned. These parameters are generally linked, and compromises need to be made, for instance, in aeration to avoid excessive shear stress. The latest developments in bioreactors for better mixing, oxygen transfer and lower shear stress are reported below.

A bioreactor, using a centrifugal pump-type impeller in a conventional fermenter was recently reported [1,2]. As the impeller rotates, circulation is achieved through the draft tube, producing essentially uniform axial flow. The authors claim that their new centrifugal impeller generates very low shear, with favorable oxygen transfer and mixing, as well as low power consumption; however, shear stress was only evaluated using liquid velocity profiles. At this time, direct evaluation with shear sensitive cultures, and comparison of the results with similar commercially available stirrers would be required to fully evaluate the effectiveness of this impeller.

For extremely shear-sensitive cultures, such as mammalian or plant cells, bubble bursting at the surface is sometimes sufficient to generate high stresses that kill the cultures [3]. The development of bubble-free bioreactor systems without conventional aeration–agitation technologies is needed to address this problem. Perfluorocarbons exhibit very high gas-dissolving capacities, and have been applied as a vector to provide oxygen to the culture medium and to remove carbon dioxide in a dissolved form [4,5]. A commercial perfluorocarbon, Foralkyl Br8 (ATOCHEM, Lyon, France), when added to the influent medium in the emulsified form and saturated with oxygen, was able to provide close to the theoretical maximum oxygenation [4]. Similarly, antibiotic production from immobilized *Streptomyces coelicolor* cultures was improved by additional oxygen supplied with perfluorocarbons [5].

The potentially lethal bubble break-up at the gas–liquid interface was minimized by the development of a vortex wave membrane bioreactor [6]. The vortex wave generated very effective mixing under laminar flow conditions by generating, expanding and transporting vortices in an oscillatory flow field [7]. Significant mass transfer enhancement has been achieved under laminar flow conditions, without a major increase in power dissipation. Again, the low shear rate indicates that this vortex wave design may be an effective alternative to conventional bioreactors for shear-sensitive systems.

The mechanical mixing environment of a bioreactor can directly influence the overall productivity by affecting heat and mass transfer. One interesting approach to promote intensive mass transfer was developed, in which the reaction mixture was intensively stirred by ferromagnetic particles [8]. Enhancement of enzymatic cellulose hydrolysis was achieved using such a novel type of bioreactor; however, the relatively high power consumption represents a potential drawback that may hinder the practical application of these ferromagnetic particles.

Alternatively, bioreactors equipped with hydro-ejectors provide a powerful alternative for better aeration and mixing for large-scale bioreactors [9]. Gas–liquid contact occurs not only inside the bioreactor, but also inside the ejector. The jets guarantee a well-mixed reactor, while the power input remains relatively low.

The rheological properties of culture media may change drastically during the course of a fermentation. For high-density cultures, or those producing a viscous product, efficient mixing is sometimes difficult to achieve, resulting in the poor distribution of oxygen and nutrients in the bioreactor. Reciprocating plate bioreactors [10•] provide very good mixing in the vertical direction due to volume exchange caused by the upward and downward motion of the perforated plates. In the horizontal direction, good mixing is produced by the uniform distribution of the perforated plates, and the formation, destruction and reformation of the ring vortex. This resulted in high oxygen-transfer rates as well as spatially homogeneous mixing. The production of pullulan by the yeast *Aureobasidium pullulans* was vastly improved using a reciprocating plate bioreactor.

Bioreactors for enzyme reactions in organic phase

Useful synthetic products can be produced at sufficiently high yield by hydrolytic enzymes if the equilibrium of the reaction is shifted sufficiently towards synthesis. This can be accomplished by carrying out the reaction in an organic solvent. A further increase in the yield can be obtained if the water produced during the hydrolytic reaction can be removed continuously in order to control the water activity [11,12].

Salt hydrates and saturated salt solutions are amongst a number of different techniques that have been tried to remove water generated by the hydrolytic reaction. Although prior works in this field have demonstrated the successful application of these techniques in removing water, there has been no demonstration as yet of a system or reactor design for the control of water activity during such enzymatic reactions.

Recently, a twin-core packed-bed reactor [11] and a packed-bed hollow-fiber reactor [12] incorporating salt hydrate pairs and salt solution, respectively, were reported. A novel twin-core packed-bed reactor consisting of an easily removable inner core of salt hydrate that was separated from an outer core of lipase immobilized on a polypropylene support was constructed and evaluated. The separation of the inner salt hydrate core from the enzyme core, through which the substrate mixture was pumped, allowed for recovery and reuse of the enzyme and salt hydrate. Complete esterification was possible using this design that was not achievable in a reactor without salt hydrate.

In the reactor system using saturated salt solution, the enzyme and salt solution were physically separated by a membrane [12]. The enzyme immobilized on microporous polypropylene matrix was placed on the shell side (outside) whereas the salt solution was circulated on the lumen side (inside). Salt solution diluted by the water formed in the enzymatic reaction was resaturated by passing it through a bed of salt. Complete esterification with controlled water activity was possible with this reactor system that was not achievable in reactors without water activity control.

Other demonstrations of two-phase enzyme reactions utilized hollow-fiber membrane reactors to separate the organic and aqueous phases. Because of low aqueous solubility, the substrates were dissolved in the organic phase. The enzyme was immobilized by entrapment in the hollow fiber on the side in contact with the organic phase, which was maintained at a slightly positive pressure to prevent the aqueous phase from penetrating the membrane into the organic phase. These reactors were used for the inter-esterification of triglycerides and fatty acids [13] and the production of optically active *(2R,3S)*-3-(4-methoxyphenyl)glycidic acid methyl ester [14]. In the latter study, the membrane reactor was integrated to a crystallizer to recover optically pure product. The advantages of these reactor configurations are the reusability of the enzyme, the longer-term stability, and the ease of reloading of the enzyme when the activity declined.

Environmental bioreactors

In recent years, efforts have been directed towards finding cost-effective biotreatments for chlorinated aliphatic hydrocarbon waste. Many of these compounds are readily degradable under aerobic conditions, and some chlorinated aliphatics such as trichloroethylene (TCE) or perchloroethylene (PCE) require either co-metabolism with, for example, methane or toluene, or a combination of aerobic–anaerobic treatment. This stimulated the development of several new reactor configurations, many of them using membranes as a means to separate biocatalyst and waste streams undergoing treatment [15•]. An elegant hollow-fiber membrane reactor configuration was proposed by Parvatiyar *et al.* [16••] to achieve synchronous aerobic–anaerobic treatment. The TCE-contaminated airstream was circulated through the lumen of a hollow-fiber module and an oxygen-free nutrient solution was circulated on the shell side. Diffusion limitation through the biofilm attached to the fibers provided the dual aerobic–anaerobic environment necessary for synchronous treatment. After start-up with toluene and biofilm build-up, TCE vapors were treated; for example, 30% removal efficiency was achieved in 36 s gas residence time for an inlet stream contaminated with 20 parts per million by volume of TCE.

In another study geared towards TCE elimination [17••], a hollow-fiber membrane module was coupled with a fed-batch bioreactor for spatial separation of metabolism and co-metabolism. *Methylosynus trichosporium* OB3b was grown on methane in a fed-batch bioreactor and the suspended culture was continuously circulated through the shell side of a membrane module. TCE-contaminated water was circulated through the lumen of the fibers. In doing so, competition between methane and TCE was avoided and higher biodegradation rates of TCE were obtained.

Furthermore, the use of extractive membrane bioreactors seems to open up new avenues for the treatment of heavily contaminated waste water. Extractive membranes are permeable to organic pollutants, but virtually nonpermeable to water, ionic species or heavy metals. This prevents the pollutant-degrading culture located on the opposite side of the membrane being exposed to extreme pH, high salt concentration or other inhibitory conditions. Waste water containing, for example, chloronitrobenzene (pH~0), 3,4-dichloroaniline (pH>12) or benzene/benzophenone (pH~0) has been successfully treated using such extractive membrane bioreactors [18]; however, the performance can be limited by mass transfer on the polluted water side of the membrane because of the high liquid residence times necessary for the treatment. To overcome this problem, Livingston *et al.* [18] constructed a cascade of three modules, each with a high recycle flow rate, so that mass transfer could be increased independently of the system throughput.

Because of the intrinsic limitations of pollutant-degrading cultures, high interest exists in combining a chemical or physicochemical treatment, such as UV or ozone, with a biological treatment for recalcitrant chemicals. The objective is to optimize the cost of the treatment by first breaking down xenobiotics to more biodegradable entities using a conventional, sometimes expensive, technique, and complete the treatment with a bioreactor. A new combination was recently presented [19•], in which nonthermal electrons were produced by pulsed-electric discharge (PED) and dechlorinated 2,4-dichlorophenol. The innovative part is the use of a nebulizer for the waste water, because aerosols can be more efficiently treated than liquids in PED reactors. The reduced products were then fed to a bioreactor, where they were biodegraded. The combination allowed smaller reactor volumes. Energy costs still need to be optimized.

Other innovative bioreactors

Two new bioreactor designs merit mention. The first is a packed bed where the feed is introduced in a square wave manner, using a new elastic membrane pulsator [20]. Compared to a nonpulsed bioreactor, production of ethanol by *Saccharomyces cerevisiae* increased up to 18%, depending on the frequency of the pulses and the overall hydraulic residence time. Possible explanations for the better performance of the pulsed system are better degassing, less back mixing, and improved mass transfer. The second interesting development is an attempt to establish a continuously aerated plug flow bioreactor [21•]. The reactor is made of a rotating spiral, partially filled with the culture broth. As the spiral rotates, the culture moves along the length of the reactor. Mixing is achieved through aeration. Characterization showed that a plug flow was indeed obtained, and that limited mixing occurred between two adjacent loops. A possible disadvantage of such a system is that it behaves more like a series of batch reactors, so that inoculation is required for each new loop. A demonstration of the feasibility and of the advantages of this reactor setup on a larger scale is still needed.

Conclusions

Recent developments in bioreactor design have attempted to either address some of the limitations of existing bioreactors or open up new avenues in bioprocessing. Clearly, many of the bioreactor designs discussed herein still require improvement, and confirmation of significantly better performance compared to existing designs. The further development of innovative bioreactor designs remains a high priority, because a single bioreactor configuration will never provide a universal solution. In many instances, progresses in reactor design will require similar advances in understanding the fundamentals of bioprocess limitations, so that a more rational, creative and focused approach in bioreactor design can be performed.

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