Increasing the Oxygen Transfer Capacity for High Cell Density Fermentations by Means of a High-Performance Aeration System

Introduction

High cell density fermentation (HCDF) is widely used to provide a high level of productivity to derive proteins or cells. For these cultures the oxygenation capacity of conventional bioreactors like stirred tanks or bubble columns is rapidly exhausted. The dissolved oxygen concentration usually becomes limiting for microbial growth. Besides the reduced productivity caused by limited growth and protein production, the formation of unwanted by-products can also have a strong negative impact. Also the prolonged fermentation time can lead to product losses due to the decomposition by enhanced secretion of proteases.

The usual ways to overcome the limitation of oxygen transfer are the increase of standard process parameters such as agitation speed, gassing rate and head pressure or the introduction of oxygen enriched air. This proceeding is limited by the used equipment especially for head pressure, or by economical aspects. Also special bioreactor types are used.

With the Frings turbine we want to introduce an alternative aeration system which, once mounted to the tank bottom of standard reactors, allows shifting the limit of oxygenation rate at economical specific power input. The turbine works based on a rotor-stator principle as a self aspirating device, which provides the aeration of the entire tank content via highly dispersed gas liquid jets. This technology has already been successfully installed in industrial fermentation systems like acidic acid, bakers yeast or PHB plants up to large scales of 240 m³.

Material and Methods

The oxygenations performance of the Frings turbine was proofed by executing similar fed batch *Saccharomyces cerevisiae* HCDF in a 300 L standard bioreactor, once equipped with a standard 3-stage Rushton-turbine, and once with a Frings turbine 25P. The feeding rate of molasses was controlled with respect for constant respiratory coefficient derived from exhaust air analysis of O_2 and CO_2. Inoculums conditions, pH-profile, temperature and aeration were kept to the same values. The power input was precalculated for both systems and adjusted to similar values.

Fig. 1: Frings turbine P25

The applicability to further expression systems shall be shown by two examples: One chosen system was the production *Thermus aquaticus* (Tag) DNA polymerase in *E-coli* in a fixed profile fed batch process. The process was carried out in a 10 L standard bioreactor equipped with a Frings turbine 5P (courtesy to *Fraunhofer IME).

Furthermore a halophilic *Halomonas elongata* strain secreting extremolytes (hydroxyl-ectoin) was subjected to fed batch fermentation in a 3500 L bioreactor with a Frings 75P turbine controlled by monitoring the dissolved oxygen level (courtesy to **bitop AG).

Results

Fig 2 shows the development of oxygen transfer rate (OTR) and biomass accumulation for the two *Saccharomyces cerevisiae* fermentations over 11.5 h of cultivation. As the Rushton-system reaches its limits of oxygenation after 5 hours at 125 mmol L^{-1} h^{-1} the Frings turbine provides non limited aeration conditions over 10 hours with an oxygen transfer capacity of 215 mmol L^{-1} h^{-1}. As a result of prolonged exponential growth in the Frings system, the final biomass reaches 68 g L^{-1} (dry weight) compared to 46 g L^{-1} after the same time in the Rushton-system.

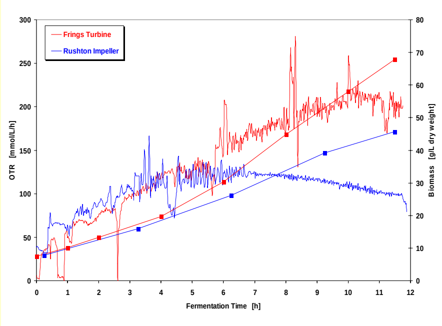

Fig. 2: Comparision of aeration systems for a fed-batch fermentation of *Saccharomyces cerevisiae* up to high cell densities
a) Frings turbine (red) b) Rushton turbine (blue)

The *E-coli* fermentation shown in Fig. 3 reached a maximum OTR of 120 mmol L^{-1} h^{-1} after the initial concentration of glucose was used up. During the fed batch phase and during induction glucose was fed at a limiting rate resulting in a maximum OTR of 0.08 mmol L^{-1} h^{-1}. With moderately high cell densities (OD_{595}<39) cultivated in this fermentation the aeration system reached its limit at no time.

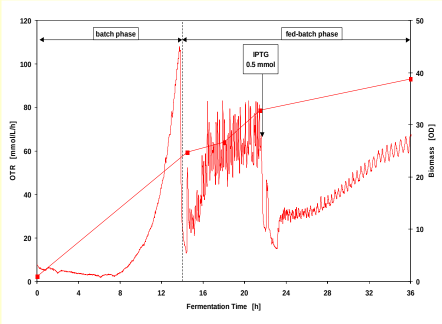

Fig. 3: Batch/Fed-batch fermentation of *E-coli* up to moderately high cell densities aerated by a Frings turbine

The *Halomonas* fermentation at salt concentrations up to 25 % w/v showed expected accumulation of ectoin at cell densities (OD_{595}) up to 45.

All fermentations processed robust and were applicable to the standard measurement and control procedures of the various laboratories.

Results in cell integrity, viability and quality of the said products were comparable with standard stirred bioreactor derived products.

Conclusions

We have demonstrated the superior oxygenation performance of the Frings turbine compared to a standard aeration system. A almost doubling in oxygen transfer capacity at similar power demand opens up all advantages of prolonged growth and production phase without limitation. Consequently the specific reaction rate as well as the cost efficiency of oxygen transfer can be greatly enhanced. Besides the already installed units in industrial large scale fermentations the application on further state of the art bioprocesses with high oxygen demands can surely be recommended.

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