Congreso Nacional de Biotecnología y Bioingeniería
VII Simposio Internacional de Producción de Alcoholes y Levaduras

SUBSTITUTION OF JAR FERMENTOR EXPERIMENTS BY MICRO BIOREACTORS ALLOWING FOR ON-LINE MONITORING AND DIFFERENT OPERATION MODES

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micro bioreactors, *on-line monitoring*, *fed-batch*.

After the “best” strain and media has been screened in small scale, experiments in jar fermentors are currently used to develop a fermentation process. Several on-line signals (as DOT, pH and exhaust gas analysis etc.) are used to monitor and control the process. Usually the DOT is kept above a specific critical level by increasing the stirring speed and aeration rate over time. The pH is usually also controlled and the most suitable value is investigated. Especially the optimization of nutrient feeding is a very important task in process development in jar fermentors. A lot of microbial systems display an overflow metabolism, an osmotic inhibition at elevated concentrations or are characterised by a catabolite repressed product formation. In all these cases, batch operation is not the preferred operation mode and, therefore, these cultures are run in fed-batch in technical scales.

To enhance the speed of process development and the experimental throughput it would be desirable to use automated miniaturized parallel micro bioreactors with full measuring and control options. It is obvious that conventional approaches used on the level of jar fermentors cannot directly be transferred to small scale and new concepts have to be developed.

Micro titre plates (MTP’s) have shown to be a suitable format for small scale fermentations. Techniques will be introduced which allow for on-line measurement of optical density of the culture broth (OD), DOT, pH, NADH and riboflavin fluorescence [Samorski et al. 2005]. If a fluorescent protein as GFP or its derivatives can be fused to a protein of interest, even the product formation can be monitored on-line. A different technique can be used to asses the oxygen and carbon dioxide transfer rate.

Batch operation mode without pH control is still the general state of the art for cultures performed for screening purposes. This is not sufficient for the initial steps of process development. This consideration has resulted in the development of a new technique, which allows to run the screening in fed-batch operation mode [Jeude et al. 2006]. The limiting nutrient (e.g. glucose) is encapsulated in a polymer matrix, which slowly releases the nutrient at an adapted rate during the culture. This technique is applicable in shake flasks as well as in MTP’s. Dramatic increases in product titre (4- up to 400-fold) were observed compared to conventional batch fermentations.

Frequently mineral media with ammonia as nitrogen source are used in production. Therefore, if pH is not controlled, as in a screening reactor, pH will drop significantly due to the consumption of ammonia by the metabolic activity of the cells and by acid production (e.g. as a result of overflow metabolism at elevated concentrations of carbon source inherent for batch cultures). In order to keep the pH in a decent range, high concentrations of buffers are used in conventional screening, which, however, may lead to osmotic inhibition. This results in completely unacceptable different environmental conditions for the investigated microbial systems in small and in technical scale. To solve this problem polymer based slow release systems are currently been developed, which contain pH controlling agents like sodium carbonate or urea, which are compatible with the polymer matrix material. In contrast to fed-batch systems releasing a carbon source, the release of pH controlling agents does not lead to self stabilizing conditions. Therefore, the release kinetics of the pH controlling agents must match as closely as possible the requirement of the microbial culture. A new approach features pH responsive polymer based controlled release systems, which enhance the release of alkaline agents if the pH is decreased. Examples of the successful application of this approach (*E. coli* cultures on glycerol and on glucose) are presented.

Bibliografía.

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