Microbial Cell Factories

Poster Presentation

Optimisation of substrate feeding in shake flask cultures of *Pichia pastoris* for recombinant protein production

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Background

Pichia pastoris is used as a common host for production of recombinant proteins. Gene expression is mostly controlled by the AOX promoter through methanol addition. The aim of this study was to investigate whether the generally applied methanol addition protocol is optimum and whether the expression in shake flasks can be improved by applying a different feeding scheme.

Therefore we applied the recently described wireless on-line monitoring wireless system (SENBIT® which allows the application of standard sensors such as pH and pO_2 in shake flasks [1]. Moreover, a sensitive sandwich hybridization technology was used for the quantitative analysis of the expression level for process relevant marker genes which to provide data about the physiological state of the cultures and hereby a better understanding of the microbial responses.

Results

The impact of the feeding protocol was studied in shake flask cultures of *Pichia pastoris* for recombinant collagen production with methanol as inducer and carbon source. On-line measurement of pO_2 revealed that the standard methanol feeding protocol is not favourable. The culture is starved for long times for methanol and also oxygen may be depleted shortly after a methanol pulse. A fed-batch like feeding procedure was developed by applying a computer controlled micropump system for semi-continuous addition of methanol. As a result the amount of collagen was improved. Furthermore, also the expression of collagen prolyl 4-hydroxylase, a collagen modifying enzyme was strongly increased which resulted in collagen of higher stability. The improvement of the culture conditions with the new feeding protocol were verified by semi-quantitative analysis of different cellular mRNAs.

Conclusion

Regular feeding of small amounts of methanol in a semi-continuous way improves the behaviour of recombinant cultures of *Pichia pastoris* and increased the amount and quality of collagen in our study. We propose that this method is generally favourable for the optimisation of gene expression in *Pichia pastoris* shake flask cultures.

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References

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