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Process Evaluation for a Trienzymatic System

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Abstract : Multienzymatic catalysis can be used as an alternative to chemical synthesis or hydrolysis of polysaccharides for the production of high value oligosaccharides from cheap resources such as sucrose. However, development of multienzymatic processes is complex, especially with respect to suitable conditions for enzymes originating from different organisms. Furthermore, an optimal configuration of the catalysts in a reaction cascade has to be found. These challenges can be approached by design of experiments. The system investigated in this study is a trienzymatic catalyzed reaction which results in laminaribiose production from sucrose and comprises covalently immobilized sucrose phosphorylase (SP), glucose isomerase (GI) and laminaribiose phosphorylase (LP). Operational windows determined with design of experiments and kinetic data of the enzymes were used to optimize the enzyme ratio for maximum product formation and minimal production of byproducts. After adjustment of the enzyme activity ratio to 1: 1.74: 2.23 (SP: LP: GI), different process options were investigated in silico. The considered options included substrate dependency, the use of glucose as co-substrate and substitution of glucose isomerase by glucose addition. Modeling of batch operation in a stirred tank reactor led to yields of 44.4% whereas operation in a continuous stirred tank reactor resulted in product yields of 22.5%. The maximum yield in a bienzymatic system comprised of sucrose phosphorylase and laminaribiose phosphorylase was 67.7% with sucrose and different amounts of glucose as substrate. The experimental data was in good compliance with the process model for batch operation. The continuous operation will be investigated in further studies. Simulation of operational process possibilities enabled us to compare various operational modes regarding different aspects such as cost efficiency, with the minimum amount of expensive and time-consuming practical experiments. This gives us more flexibility in process implementation and allows us, for example, to change the production goal from laminaribiose to higher oligosaccharides.

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