Material Balance and Metabolic Flux Analysis for Styrene Epoxidation Reaction by a *styC* Knockout Mutant of *Pseudomonas Putida* SN1

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Abstract

The reaction of styrene into the enantio-pure (*S*)-styrene oxide by a whole-cell styrenemonooxygenase (SMO) biocatalyst of *Pseudomonas putida* SN1 (*styC*-) was studied quantitatively. And (*S*)-styrene oxide production by *Pseudomonas putida* SN1 (*styC*-) was studied and experiment results were compared with *in silico* metabolic pathway flux analysis. Styrene epoxidation requires one molecule of oxygen (O2) and NADH for the reaction of each styrene, and a carbon source is added to regenerate cellular NADH. The reaction carried out in a side-arm flask in a two-liquid phase system consisted of a phosphate buffer and Di-octyl phthalate (DOP), under the conditions that neither styrene nor O2 was limiting the reaction rate. The metabolic pathway analysis model, consisting of 67 reactions under aerobic condition, was developed based on annotated genome sequence, experimental data and metabolic pathway analysis. The metabolic pathway flux analysis was performed using the model developed and the linear optimization program, MetaFluxNet. This case of objective function of the model was analyzed, maximal (*S*)-styrene oxide production rate. O2 consumption rate and CO2 evolution rate were compared experiment results with *in silico*metabolic pathway flux analysis. The reaction rate decreased in the order; citrate > glucose > formate > no carbon added. For each mole of styrene, an equimolar (*S*)-styrene oxide was produced regardless of the carbon source used, but the oxygen consumption for the conversion of each styrene was significantly varied as follows; 3.36 without added carbon source, 2.13 with glucose, 1.69 with citrate and 3.49 with formate. This result indicates that citrate is the most efficient carbon source for the whole-cell epoxidation reaction in terms of reaction rate and O2consumption. Central metabolic pathway of

host is very different for using carbon source. However, for all the exogenous carbon sources, it was speculated that decoupling between cellular NADH production and styrene epoxidation was serious and most reducing equivalents along with oxygen were wasted without contribution to the SMO reaction. Since oxygen transfer is the most important limiting factor for improving volumetric productivity, metabolic engineering to reduce the decoupling will be crucial for further development of the whole-cell SMO biocatalyst.

References

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