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## **Cross Feeding in Microbial Populations: Mathematical Modelling of a Commensalistic TwoSystem**

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The combination of metabolic activities from different microorganisms is a well known principle in the degradative processes in nature. In this context, the term cross-feeding was coined, denoting the transfer of a metabolite from one organism as substrate to another organism. Cross-feeding is considered an important part of evolution and lately interest has risen in this area. Apart from mathematical modelling based on mass balances, now also approaches using game-theory are applied to quantify these systems and to model interactions between species. An interesting problem for modelling is the development of cross feeding in pure cultures by evolution of a polymorphism within the population [Pfeiffer & Bonhoeffer, 2004]. It exhibits degradation of the primary substrate by one sub-strain into a metabolite which is used by the second sub-strain. The advantage of this phenomenon can be explained by lower cost for the microorganisms regarding enzyme and metabolite concentrations. The cost (synthesis of enzymes, maintenance of metabolite pools) and the pay-off (ATP-yield) are the basis for using the above mentioned game-theoretical approaches to explain the reasons of such behavior. It can be shown that in continuous reaction systems, mixed populations with cross-feeding exhibit stable operating points of coexistence, whereas it is well known, that in mixed cultures competing for a single substrate, only one strain will prevail in a steady state.

In engineering applications, the prediction of process performance is the goal of modelling. The use of mixed cultures in controlled processes beyond food technology and waste treatment is quite rare. This is due to the complex interactions between the different species, which may influence the product quality and reproducibility of the process.

In wastewater treatment, the degradation of xenobiotics usually requires the catabolic activities of more than one strain. As many as 11 strains for the removal of a single compound may be needed for a successful process [Rozgaj & Glancer-Soljan, 1992]. In food biotechnology, industrial scale use of mixed cultures is well established e.g. in milk processing (yoghurt, cheese) or fermentation of vegetables. Another recently developed application is the production of wellness drinks using a combination of yeast and bacteria to produce various organic acids and flavor compounds while degrading ethanol formed by fermentation from a herb extract / sugar mixture (Kombucha).

Since a strict quantitative description of mixed cultures is still not used as a regular tool, we wanted to apply the underlying engineering methods to a model system in order to show their feasibility. The model system was chosen to be simple (2-species) but to have strong interactions between the two strains. Therefore *Lactococcus lactis*, *Candida kefir* and lactose as primary substrate were selected. *Lactococcus lactis* is able to use lactose as substrate and it excretes the metabolite lactate, which causes product inhibition at higher concentrations. *Candida kefir* is not able to metabolize lactose, but it can use the metabolite lactate as a substrate, even though it also has inhibitory effects. This constitutes a commensalistic system in mixed culture, since the presence of *Lactococcus lactis* is necessary for growth of *Candida kefir*.

Model set-up and model verification was conducted on three systems, first on *Lactococcus lactis* and on *Candida kefir* separately and then on the mixed culture of both organisms. The experiments to acquire data for parameter estimation were performed in batch, fed-batch and continuous culture according to the requirements of parameter sensitivity. The components of the mathematical model finally consist of balance equations for substrates (extracellular and intracellular), biomass species and a transport enzyme for lactose transport into *Lactococcus lactis*. In order to accommodate dynamic effects in the mixed culture during changes of the cellular environment, a first order time delay related to the activity of the enzymatic transport of lactate was introduced. This results in a set of eight first order differential equations for the mixed culture.

During the process of model creation and verification, the aforementioned sub-systems

were analyzed separately. Parameter values from these experiments were used to predict the behavior of the mixed culture, which was successful for steady states of the continuous process but required the model expansion for dynamic states of the mixed culture.

Analyzing the operating points predicted by the model, a bifurcation is observed leading to hysteresis effects in continuous culture. This result is similar to the well known problem of ignition and quenching of a continuous adiabatic stirred tank reactor. The ignited state of the chemical reactor corresponds to the mixed culture in the bioreactor, the quenched state to the pure culture of *Lactococcus lactis*.

The creation of a mathematical model, parameter estimation, model verification, analysis of the model and interpretation often results can be done using the reliable tools from chemical engineering. For a well-defined and simple system, process simulation can be successfully implemented and a quantitative prediction is achieved. This is an important step to open possibilities of employing the combined biosynthetic capacities of more than one microorganism in industrial applications.

Pfeiffer T., Bonhoeffer, S.

Evolution of Cross-Feeding in Microbial Populations

The American Naturalist, Vol. 163, No. 6, June 2004, E126- E135

Rozgaj R., Glancer-oljan M.

Total degradation of 6-aminonaphtalene-2-sulphonic acid by a mixed culture consisting of different bacterial genera

FEMS Microbiology Ecology, 86, 1992, S. 229-235