Ecological forces shape the individual cell proportions in microbial communities

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Abstract

Microbial communities are indispensable for future biotechnology to produce valuable platform chemicals and reduce the exploitation of fossil resources. Yet, the stability of microbial communities in classical continuous reactor set ups is best brief or non-existent. This is due to ecological forces such as stochastic and deterministic properties of communities that contribute to rapid changes in structure and function to varying degrees. The review highlights the differences between these two properties, provides tools for their estimation and gives an outlook on overcoming instabilities of microbial communities in biotechnological reactor systems.

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Introduction

Microbial communities are increasingly used as biocatalysts in biotechnological processes due to the multifunctional properties of their members. In contrast to genetically engineered so-called superbugs, which are engineered to perform the desired steps of a biochemical transformation as a pure culture, microbial communities distribute the necessary transformation steps among different cell types. The involvement of microbial communities in biotechnological production processes have at least two advantages: One is that superbugs require expensive and specifically refined substrates to produce valuable products. Instead, microbial communities can convert cheap complex materials from agriculture and forestry as well as waste materials, which significantly reduces production costs. Such approaches also lead to reduced use of fossil resources for the production of valuable chemicals and support the shift to a circular economy. Second, the functional capacity in communities is vast, mostly redundant or evolving, and thus offers a huge library of possible metabolic transformation pathways [1, 2]. But despite these great benefits, there is a reluctance to use microbial communities more intensively in biotechnology. With the exception of well-known processes such as biogas production, wastewater treatment or the use of microbial communities in the food industry, there are no significant new applications beyond these. In our opinion, the reason for this could be the inability to control complex microbial communities in biotechnological processes. In this statement, we aim to identify the probable causes of process instabilities caused by microbial communities as catalysts and pave the way for solutions to overcome this problem.

The ecology of microbial communities

Ecological paradigms have not yet been considered in the control of biotechnological processes.

However, microbial communities are subject to ecological rules, just like any other population on earth, e.g. the organisms of a forest or of a water body. Following macro-ecology theory, we seek to understand the ecological mechanisms that govern the coexistence of microorganisms in biotechnologically exploited communities.

Mostly, the biocatalysts themselves are not measured and evaluated as segregated values according to their share and function, but rather treated as bulk biomass parameters. In addition, substrate turnover and product synthesis are of interest, as well as abiotic operational parameters such as temperature, pH and off-gas values, which are indispensable for conventional process control. This classical control scheme originates from biotechnological processes, where the biocatalysts are pure populations with well-known physiological properties. But already here the heterogeneity of the population contributed to unstable processes and was therefore an issue in many studies [e.g., 3]. Triggers include cell cycle stages, age distributions, plasmid copy numbers, or gene expression noise [4-8]. Of course, the degree of heterogeneity is much higher in natural and also artificial communities, which affects the stability and efficiency of the processes. To understand what drives assembly of and heterogeneity in microbial communities, ecological theory can be of great help. The main ecological forces affecting the proportions of cell types in communities are stochastic (neutral) and deterministic forces (Figure 1).

Stochastic forces in biotechnological systems

Stochastic behavior is an important ecological property that occurs in communities of any taxonomic level. It assumes that members of a community share the same equivalence and fitness within a community, which implies that they have the same prospects of reproduction and mortality [9]. Under this assumption, deterministic factors like environmental parameters play no role in shaping communities, because all individuals respond in the same way. Although this assumption is clearly counterintuitive to our daily experience, most neutrality-based models provide reliable predictions [10, 11]. In ecology theory, random birth and death of organisms are typical stochastic events. Moreover, systems governed by stochastic forces exhibit intermediate frequencies, i.e., less extreme distributions of community members, which behave therefore largely uniform with respect to each other. Interactions between members of such communities are considered to be small.

We want to understand how these stochastic forces affect microbial communities in bioreactor systems and how they can influence the efficiency of production processes. Typical bioreactors are stand-alone systems operated in continuous cultivation modes. We do not consider batch systems here because community lifetimes are limited in such systems (i.e., only a few generation times of contained organisms) due to rapid onset of nutrient, carbon, and energy scarcity and rapid succession of harvesting steps, and thus ecological forces have less influence. Instead, continuously operated bioreactor systems are constantly fed with carbon and energy sources and contain a community that randomly loses community members due to the dilution rate. This process can be considered as extinction (or cell death). In systems with high stochasticity, there is high functional redundancy in otherwise taxonomically diverse communities. Under these conditions, because cell types with similar functions are present in equal abundance, different cell types with the same function can be easily interchanged. This inevitably leads to structural changes in community composition. Therefore, functional redundancy is a major contributor to changes in the dominance of particular cell types in a bioreactor, which leads to significant structural instability [12-14]. Instability can be such that any given species becomes dominant because there are no interactions among members of the community that would support a lasting dominance of a particular cell type.

We also suspect that bioreactor systems operated at low cell densities are more prone to stochasticity. The relative influence of coincidences such as random birth or death of a cell is greater in less dense communities. Therefore, continuous cultivation systems with low cell densities containing a highly diverse community with random functions are highly susceptible to stochastic events and to structural and consequently functional instability.

Deterministic forces in biotechnological systems

Deterministic behavior is also an important ecological trait that occurs in communities at every taxonomic level. Based on the concept of niche it assumes that members of a community are different from each other, have different characteristics and functions within a community, and are often interdependent. The two aspects of the niche concept relate either to the environmental needs of species ("requirement niche") or to the impacts of species on their environment ("impact niche"), such as the consumption of resources that leads to competition among species [15]. The competitive exclusion principle highlights that a pair of species cannot stably coexist if they feed upon exactly the same resources under the same environmental conditions [16]. Only species with different requirement niches are able to coexist, but whether stable coexistence will be achieved depends on different impact niches. Both aspects indicate that there are trade-offs between species to determine whether stability of a microbial community is reached [17]. In multi-species systems of microbial communities, niche requirements can be highly variable but also highly similar, so niche overlap cannot be avoided.

Considering again at the continuous cultivation mode, we can state that the dilution rate not only promotes cell extinction as a stochastic feature, but also causes selection of cells with a reduced average fitness difference. All cells with a growth rate below the dilution rate are lost, which over time leads to selection of cells that grow equal to or faster than the dilution rate. Thus, the dilution rate contributes to equalizing but not to stabilizing because the niche overlap is not reduced. The dilution rate can also be considered as disturbance that favors faster-growing cells.

In macro-ecology theory, disturbances are called deterministic factors because they shape the environment and communities. Deterministic factors, then, are those that have traditionally been used to steer bioprocesses in desired directions. These are any operating parameters that help select specific cell types through temperature and pH optima, through types of carbon sources and specific nutrient mixtures or agitation rates. Strong deterministic features support interdependencies between cell types of different requirement niches, such as when a substrate is used by one strain and the resulting intermediate serves as a substrate for another strain to produce something. Interdependence lowers the likelihood of monodominant communities with limited function and ensures continuous coexistence among interconnected partners. In highly diverse communities, many such links may exist, but the extinction of one of the partners may also lead to the extinction of the other. Unlike stochastically controlled systems, the system is dominated especially by non-replaceable, abundant microorganisms. Structural change is therefore less likely, while nesting, which describes the persistence of particular cell types, is definitely high in deterministic systems [18, 19]. In high-density systems, only small, albeit continuously provided, resources are available and they are therefore not susceptible to change. Overall, deterministically organized systems appear to be more stable than those under the rule of stochasticity.

Stochasticity vs. determinism

Following the above reasoning, it can be assumed that setting up a bioreactor system in a way where only deterministic forces act can lead to stability and also enable control. However, it is known from many studies that stand-alone systems are almost never stable [12, 20], even though short-term stability is sometimes reported. The rationale for these findings is that in any self-contained system involving living organisms, stochasticity and determinism are simultaneously prevalent, and that in systems involving multiple species, there will always be niche overlaps and uncertain impact niches which can be influenced but never excluded. There are tools that make it possible to determine the proportion of one force or the other, which can give an indication of the chance of setting a system further to the deterministic side for control. However, there is a fundamental recognition that controlling communities in stand-alone bioreactors appears to be impossible.

Determination of stochastic and deterministic shares in microbial communities

To determine the shares of deterministic and stochastic forces the measurement of the individual organisms is necessary. Similar to counting and describing plants in a forest to understand active ecological paradigms also microbial communities needs to be resolved to the individual cell level. This is possible using microscopic technologies among them flow cytometry which allows a fast and cost effective recording of dynamics in microbial community behavior. Microbial communities grown in bioreactors can be routinely analysed according to abundancies of cell types over long periods of time using fingerprinting approaches. Cell types are characterized related to cell size (forward scatter, FSC) and numbers of chromosomes per cell (DNA fluorescence) or nucleic acid contents [21-24]. According to these characteristics, cells are gathering as Gaussian distributions in subcommunities (SC). The changes in numbers of SCs, the position of SCs in a 2D-plot and the numbers of cells per SC inform on community dynamics over time (Figure 2). To determine the proportions of deterministic and stochastic forces in a community, such information can be evaluated using the tool NST (Normalized Stochasticity Ratio; 25). According to theory, the forces of determinism are the stronger the lower is the niche overlap and the higher the niche impact (Figure 2). The niche impact is commonly estimated by correlation or network analyses, with higher determinism indicated by tight correlations or compact networks (26; and only for sequencing data, e.g. 27, 28). Other tools like QPEN and iCAMP use interspecies phylogenetic relationship to estimate their similarity in niches [29, 30], however these methods also rely still only on sequencing data.

Outlook

Multiple-species communities in stand-alone bioreactors would not be able to overcome stochastic forces and create non-overlapping requirement niches and influence impact niches in ways that promote coexistence. As a result, we will not be able to control such systems. And yet, there are system in the environment that are stable over long periods of time, as can be observed in macro-ecology, but also in certain connected basins of wastewater treatment plants and even in the microbiomes of human or animal origin [31, 32]. The fundamental mechanism supporting this stability is dispersal. Recent data indicate that the use of dispersal in loop designed continuous bioreactors greatly contributes to the stability and synchrony of connected complex microbial communities [33]. This research is promising, but more ideas and further consideration of ecological paradigms in biotechnological processes are needed before implementation can be made possible. This may also require that new reactor designs be created for stable and structurally and functionally controlled cultivation of microbial communities.

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This study addressed the stochastic changes and their avoidance in microbial community composition under constant environmental conditions. Inspired by the mass effect paradigm in metacommunity theory, the bioreactors were interconnected to allow different rates of looped mass transfer between communities, which was proven to be effective in stabilization via the rescue effect.

Figures

Figure 1: Ecological forces shape the individual cell proportions in microbial communities. Blue: stochastic forces and Orange: deterministic forces that are common in microbial communities that are cultivated in continuous stand-alone bioreactors. These two forces have different influences on the structure and

function of communities and affect the productivity of biotechnological processes to varying degrees. (SC: subcommunity)

Figure 2: Analyses of the type of ecological forces that shape the properties of microbial communities. Cells are cultivated in continuous bioreactor systems and samples are taken within generation time and analysed on the individual cell level by flow cytometry. Fingerprints per sample are generated and dominant SCs are determined by cell abundance calculation. Networks and co-occurrences are visualized by correlation analyses and proportions of stochastic and deterministic forces are calculated. (SC: subcommunity)