Plasticity: microbial adaptation that surpasses boundaries of the biofilm model

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Introduction. Studies of the adaptability among biofilm communities involved in degradation and nutrient cycling in natural and engineered systems is intriguing from a fundamental perspective, and potentially have considerable value in biotechnological applications. Population size and diversity of these communities are related to the quality and quantity of available resources, as well as their physical-chemical environment. Whether biofilms respond to these factors in a manner that can be predicted is an open question; considering that even replicate pure culture biofilms show notable structural differences (Lewandowski *et al*., 2004, Water Sci. Technol. **49**:359-364; Bester *et al*., 2005, Appl. Environ. Microbiol. **71**:7792-7798), this seems doubtful. We conducted a number of experiments to evaluate the responsiveness of biofilm communities to environmental fluctuations in order to maintain overall community function.

Experimental

Community form-function relationships in wetlands

Experimental wetlands (two 45 x 6 m; three 6 x 3 m) were planted with *Phragmytes* and *Typha* spp and fed with distillery effluent (COD load of ≤12,000 mg/l) to study planktonic microbial communities and associated biofilms forming on geological material (gravel, sand) and plant roots.

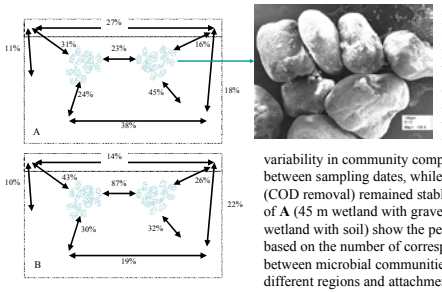
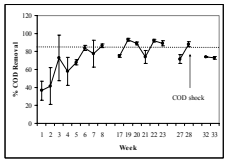
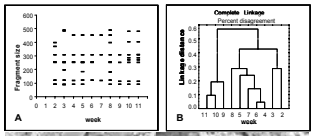


Figure 1. Percent similarity in T-RFLP profiles (*Alu*I) between microbial communities in different regions of the experimental wetlands. Similar high variability in community composition was found between sampling dates, while community function (COD removal) remained stable. Schematic diagrams of **A** (45 m wetland with gravel support) and **B** (45 m wetland with soil) show the percentage similarity, based on the number of corresponding T-RF's, between microbial communities present in the different regions and attachment substrates. Similar results were obtained with *Rsa*I as restriction enzyme.

Figure 2. Mesocosms were used to replicate the wetland experiments to allow variation of environmental conditions – in this case a COD shock load of 24,000 mg/l. In this and similar experiments COD removal efficiency was restored within 3 weeks. The shaded areas indicate periods when fresh water was passed through each system. For comparison, the dotted line represents the average COD removal efficiency of the 45 m gravel wetland after 12 months operation.





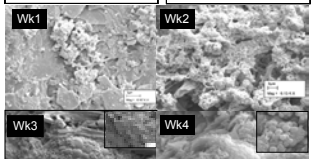
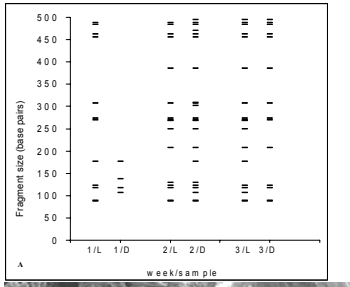


Figure 3. **A**. Dominant restriction fragments (restriction enzyme *Alu*I) from biofilms on the mesocosm gravel during the early stages of development and in response to the COD shock at week 8, showing the sequential nature of community development and the notable change in community structure that persisted beyond 3 weeks after the shock, in contrast to restoring function over this period as shown in Figure 2. **B**. Cluster analysis of the data. **C**. VP-SEM images to illustrate the rapid microbial colonization of the gravel, and morphotype diversity in the resultant biofilms.

Effect of primary producers on form-function relationships

Perspex mesocosms (20cm long X 15cm wide X 15cm high) were filled with the same gravel that were used in experimental constructed wetlands, painted black on the outside or left translucent to evaluate biofilm development in the light and dark.



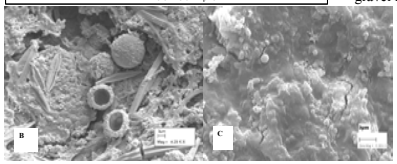
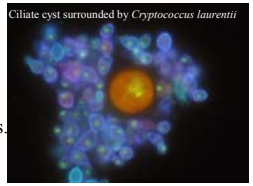


Figure 4. A T-RFLP fingerprint patterns of microbial communities cultivated in the light (L) and dark (D) followed by digestion with *Alu*I, showing shifts in community composition over a three week period, and the effect of light (algae) on community profiles. The SEM micrograph in **B** shows the presence of algae and diatoms in biofilms growing on the support gravel matrix exposed to light. Early biofilm development was slower in the absence of light while the biofilms developing in the deeper layers and shaded sides of the gravel in the system exposed to light formed confluent structures with more EPS production (**C**). The presence of algae and their exudates resulted in higher COD removal efficiency, although the difference was not significant.

Effect of predation on form-function relationships

We have shown earlier (Joubert *et al*., 2006, Microb. Ecol. **52**:178-188) that protistan predation stimulate yeast to form more extensive biofilms and to increase planktonic cell yield from the biofilms. In the present research, we used COD removal rates and respirometry to evaluate the impact of predation on substrate utilization and conversion to CO_2. Yeast were cultivated in polyethylene columns in the presence or absence of protista before measurements. Additional tests included measurement of carbon stored in the non-cellular biofilm fraction and pore clogging as indicator of biofilm biomass accumulation on the attachment substrate (3 mm glass beads).



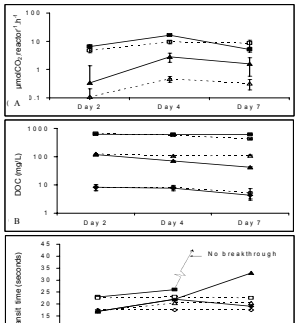


Figure 5. Effect of predation on carbon flow and biomass accumulation. The rates of CO_2 production in the columns receiving different concentrations of YM in the presence and absence of predation are shown in **A**, while **B** shows the DOC concentrations at the outlets of the columns, and **C** shows transit times of 10 ml water through the different columns as an indirect measurement of biofilm accumulation. In general, predation that resulted in higher CO_2 production was accompanied by the highest reduction in DOC values although the impact was not significant at any of the nutrient concentrations ($p>0.05$). It was therefore expected that predation would serve as a mechanism to control biofilm accumulation and we used measurement of the extent of pore clogging to test this assumption. As shown in **C** the opposite was found with

The results in Fig. 5C support the contention that predation stimulate EPS production and more extensive biofilm formation. To test potential implications of this phenomenon on carbon flow through microbial systems, we collected biofilms at the column outlets and used DOC values as an indirect measure of extracellular biofilm biomass (Fig. 6).

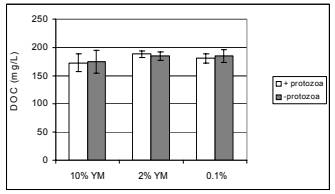


Figure 6. DOC values showing that the amount of carbon stored in the extracellular fraction of biofilms did not differ significantly even though there was large differences in the associated effluent concentrations ranging from 4.4 – 5.3 (0.1% YM influent), 43 – 111 (2% YM), and 425 – 616 mg.l^{-1}. It is clear that a range of environmental conditions dictates the relative amount of energy spent on cell maintenance, cell yield and non-cellular (EPS) yield. Based on the results we propose that labile substrates such as the YM used in our laboratory columns favour cell yield with little investment in storage materials such as EPS while the opposite occurs when biofilm development is dependent on recalcitrant substrates. In fact, it was demonstrated in a related study (Lovis, 2003, Diploma Thesis, Stuttgart University) that biofilms forming on discs of a pilot scale RBC evaluated for treatment of the same distillery effluent used in the wetland experiments consisted of copious amounts of EPS with relatively few bacterial and yeast cells. Nevertheless, we do not have an explanation for the lack of correlation observed in triplicate column experiments between carbon in biofilms and in the aqueous phase.

Carbon channeling in biofilms

To further evaluate the relationship between carbon accumulation in biofilms vs. carbon in the feed, a tube biofilm reactor was developed with the aim to perform an accurate carbon balance on biofilms. This system allows non-destructive, real-time measurement of CO_2 production by biofilms and comparison of these values with cell yield, substrate utilization and carbon investment in the non-cellular fraction of biofilms. A well characterized *Pseudomonas* CT07 strain was grown on sodium citrate.

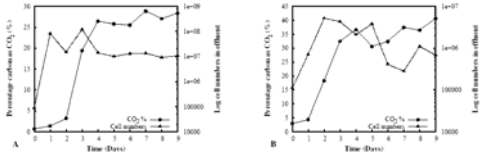


Figure 7. Data demonstrating the relationship between total biofilm respiration rate and the cell numbers released to the planktonic phase by biofilms growing on 1.0 **(A)** and 0.1 mM citrate **(B)**, respectively. These cells represented less than 5% of effluent carbon.

Conclusions

These results illustrate the buffering nature of biofilms – a strategy that is often required to preserve and maintain overall community function in environments with much variation in physical, biological and chemical character. Continuous feedback and response between biofilms and their surroundings, starting with altered levels of gene expression and resulting in changes in architecture, alternating the flow of carbon and energy between cell activity and yield, as well as EPS production appear to be important for this maintenance and serves to remind that biofilms can hardly be constrained to a unified conceptual model.

Acknowledgements

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