Bäthke, Lars (2019): Dynamics of Extracellular Hydrolases Activity in Freshwater Biofilm. Supervisors: Fischer, Bierl (Masterthesis).

ABSTRACT

The designed sampling device proved to be a useful tool, allowing a relation of the measured enzyme activities to the biofilm mass as well as to the occupied area of the biofilm on the glass slides. With properties such as durability and reusability, the exposition in different aquatic environments is possible. The most beneficial properties of the sampling device are the compactness, similarity in surface area and the sampling over a period of time, without disturbance or relocation.

The transfer of the internal protocols for the photometric and fluorometric enzyme activity and kinetic measurements for sewage sludge on biofilm is possible with comparably few adjustments. During the experimental series, signal reduction was increasing over time. This leads to the assumption that quenching properties of the biofilm are related to biofilm age.

In comparing the two different measurement techniques, it is striking that only the respective results of the stream biofilm correlate across the two methods. However, it is important to note that one of the three assessed enzymes also shows a high correlation in the aquarium biofilm. It is expected that the aquarium biofilm possesses properties influencing the correlation manifesting in one of the two measurement techniques.

Regarding the comparison of the two assessed setups of the stream and the aquarium, it can be stated that the first week shows enzyme activity with no significant difference, indicating comparability.

After this first time period, the enzyme activities in the different setups diverge from each other. This shows that if biofilm is transposed into a laboratory setting, there is comparability over one week to the biofilm remaining in the original habitat. It is important to point out that there is no conflict in the results of the Wilcoxon rank sum test over the whole time span and the Tuckey HSD of the ANOVA as well as the t-Test, testing the two parts of spited data sets. The first shows no significant difference between the setups and the others show that the first and the second parts of the spited time frames are significantly different between the setups.

Across the setups, the first and second part do not differ, confirming the Wilcoxon rank sum test that there is no significant difference between the enzyme activities of the stream and the aquarium. It is assumed that these results are highly influenced by the deviations observed in the enzyme activity results.

Here, the comparison to related studies makes clear that there are various reasons for such high deviations. In biofilm the age might be a major factor for differences in enzyme activity. The biofilm in a stream is suspect to much more influences that just the amount off substrate and other compounds present in the water body itself. The overall dynamics of the enzyme activity in biofilm are illustrated best by the change of activities between each measurement. It is usual to observe such changes to be over one hundred percent of the value measured in the measurement before. Variability can be induced by differences in sheer and flow conditions as well as temperature.

In addition to enzyme activity, enzyme kinetics are able to reveal information about the dynamics of the hydrolases activity in the two setups. The moving average model of the acquired $^{app}V_{max}$ and its confident intervals of the stream data is used as a method of determination if the setups are comparable over time. Values falling out of the confident interval indicate differences in enzyme activity. It is notable that the $^{app}V_{max}$ values of the assessed enzymes show a comparable narrow range, especially at the beginning of the series, as compared to the $^{app}K_m$ values.

One of the main assumptions of this work is that the contents of nutrients in the water column which are impacting the enzyme activities of the biofilm are comparable between the setups. This is ensured by the frequent water changes. However, the influence of short-term changes in the respective nutrients or other substances influencing the enzymatic behaviour of the biofilms is not known.

Due to that, the focus is set towards the intercorrelation of the enzyme activities, $^{app}V_{max}$ and $^{app}K_m$ values as well as the correlation to the measured frame parameters. In correlating to the exposition time the importance of the biofilm age toward the enzyme activity is underlined by the example of PDE in both setups. In contrast to that, the picture of Chlorophyll-a representing the algae contents, is not as clear. Here the algae contents are not connected to the enzyme activities to a high degree as it was expected. Only in the aquarium biofilm, significant negative correlation to enzyme activity is present. This indicates that there is a change in biofilm algae contents in the aquarium not retrieved by the stream biofilm.

This is also depicted by the Chlorophyll-a contents slightly decreasing in the aquarium. Overall, the correlations indicate that the biofilm age has a larger influence on the biofilm enzyme activities in the stream and the algae contents in the aquarium. Also, the enzyme activity correlation with the frame parameter are confirming that the diverging enzyme activities after the first week of the experimental series are caused by a change in the composition of the aquarium biofilm.

In the PCA over 50% of the variance is explained by the difference in setups. One important factor among the frame parameter differentiating the setups in the PCA is the temperature. However, the temperature does not show significant correlation to enzyme activities throughout all enzymes assessed.

This illustrates that differences in enzyme activities are not sufficient enough to clearly distinguish between biofilms using the whole time frame of the experimental series.

Only in smaller time intervals there are significant differences between setups. But it is not clear if such differences are influenced by superimposing factors such as biofilm age. The $^{app}V_{max}$ values shows intercorrelation in the stream throughout all enzymes, in the aquarium only between PDE and ß-GLU.

In the PCA, the $^{app}V_{max}$ values do not affect the distribution among the setups defining the first PC. The $^{app}V_{max}$ values represent enzyme activities, at least in the stream where cross method correlation is high. Therefore, the impact of the enzyme activity on the variance of the data set explained by the setup differences of the biofilm is low. The $^{app}V_{max}$ values are not significantly different comparing the stream biofilm with the aquarium biofilm.

In contrast to that, the substrate affinities show a different behaviour. For the substrate affinity only the ß-GLU shows no significant difference, but the phosphatases do. Only the $^{app}K_m$ values of the PDE show significant correlation to frame parameters, in the aquarium to temperature and conductivity and in the stream to the pH.

The fact that the three enzymes assessed show no intercorrelation of $^{app}K_m$ values and no or contradicting correlation to the frame parameter in the two setups underlines that $^{app}K_m$ values might be more independent from superimposing factors. Therefore, $^{app}K_m$ values could be more suitable for characterisation of biofilms.

An additional point is that the biofilms of the second experimental series are clustering around each other with the aquarium data of the first experimental series in the PCA. This leads to the hypothesis that biofilms show specific substrate affinities in different locations. This, being based on the only factors remaining after the frame parameter responsible for separation of the setups in the first PC, are the substrate affinities.

However, it needs to be stressed that events like rain may have an effect on the $^{app}K_m$ values of a biofilm, since the substrate availability is directly affected to a large degree. Consequently, there is, with the short-term changes, a range of substrate affinity specific to a location dependent on the main environmental conditions. The $^{app}K_m$ value quickly reconverts to the observed trend in the stream biofilm after the rain event.

Overall, the substrate affinities might be much more robust as compared to the enzyme activities.