

# Extracellular Enzyme Activity and Polymer Degradation in Microbial Nitrogen Transformations

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## Abstract

Wastewater treatment relies to a large degree on the activity of resident microorganisms and organic matter and the degradation of the polymeric organic matter by extracellular enzymes have been argued to limit the activity of the microbial consortium in such wastewaters. The presence of such extracellular enzymes and their activities was investigated in this thesis.

Several extracellular enzymatic activities, including  $\alpha$ -glucosidase and peptidase activities, are present in activated sludge from the Rya municipal wastewater treatment plant (Rya WWTP), Göteborg. The abundance of extracellular  $\alpha$ -glucosidases indicates the presence and importance of polysaccharides with  $\alpha$ -glucosidic linkages in the influent wastewater. A potentially simple and rapid method, using specific inhibition of  $\alpha$ -glucosidase enzyme activity with castanospermidine, for elucidating the contribution of  $\alpha$ -glucosidases in the overall microbial activity was investigated. It was first demonstrated that castanospermidine indeed inhibited bacterial  $\alpha$ -glucosidase activity in pure cultures of *Chryseobacterium gleum*, *Paracoccus denitrificans*, and *Pseudomonas stutzeri* as well as in activated sludge. In addition, the respiratory activity was reduced significantly by addition of castanospermidine to pure cultures of *C. gleum* and *Ps. Stutzeri* but not *Pa. denitrificans* grown on starch as the sole carbon source. Addition of castanospermidine reduced the overall respiratory activity of activated sludge indicating that  $\alpha$ -glucosidase activity is contributing to the overall metabolic activity of the microbial community in the sludge. It was concluded that the approach using castanospermidine is potentially useful but should be used for qualitative rather than quantitative measurements.

An anoxic bacterial model system used suggested that starch was used as a carbon source by the denitrifying bacteria tested; starch was degraded simultaneously with nitrate utilization. There was a good correlation between starch consumption and  $\alpha$ -glucosidase activity. Further, the  $\alpha$ -glucosidase activity decreased and nitrate utilization increased when glucose was added to the medium containing starch. The influence of monomers on starch utilization was further analyzed using a continuous culture system under anoxic conditions with nitrate as electron acceptor. The bacteria *Ps. stutzeri* was used as a denitrifying model system and this bacterium was grown with a mixture of glucose, acetate, and starch. Acetate was demonstrated to be the preferred carbon source. Starch consumption increased when both glucose and acetate concentrations had reached very low levels in the reactor. All nitrate was consumed and very small amounts of nitrite was detected. Thus, monomers, such as glucose and acetate, may increase the overall activity of denitrifying bacteria in a complex consortium but have negative effects on  $\alpha$ -glucosidase activity and starch utilization.

The effects of polymeric organic matter on the nitrification process were also studied in a nitrifying trickling filter (NTF). The values of  $\alpha$ -glucosidase and peptidase activity in both the biofilm and the bulk water demonstrated that the system contained a high potential of these enzymatic activities. The addition of dissolved organic matters, soluble starch and peptone increased microbial activity but drastically inhibited ammonium oxidation in biofilm samples from the NTF.

**Keywords:** *extracellular enzyme, castanospermine, activated sludge, biofilm, nitrifying trickling filter, denitrification, chemostat*

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