ENZYMATIC FINGERPRINTS OF ACTIVATED SEWAGE SLUDGES

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INTRODUCTION

Enzymatic reactions are suspected to play a key role in the degradation of organic wastewater micropollutants by activated sludge micro-organisms. Whereas easily biodegradable organic compounds are almost completely eliminated by properly operated sewage plants usually, tremendous variations of the removal efficiencies for some polar micropollutants are reported. Often attempts to explain such differences are made without reference to the enzymatic process level. To characterize and to compare specific patterns of the activities of extracellular sludge enzymes and to track their time-dependant variations, a broad range of enzyme activities were assayed in vivo in activated sludge samples of five luxembourgish and a german sewage plants. The recorded enzymatic fingerprints might provide a rationale for the observed differences in the (co-)metabolic substrate transformation capacities of the investigated sludges.

PURPOSE

- **Adaptation of enzyme activity tests for activated sludge samples**
- **Linking enzymatic fingerprints of activated sludges to influent characteristics of different WWTPs**
- **Variations of enzymatic activities within the influent, effluent and biological steps**

MATERIALS & METHODS

ENZYMATIC ACTIVITY TESTS

- **Perkin Elmer Spectrophotometers: Lambda 2 or 550 S**
- **5 min extraction in ultrasonication bath at 35 kHz (max 320 W)**
- **Use of NaCl for dilution of samples and as buffer for tests after [1]**

ACCOMPANYING MEASUREMENTS

- **SKALAR SAN^{++} Continous Flow Analyzer for determination of NH_4^+, NO_3^- + NO_2^-, N_{TOT}, PO_4^{3-}, P_{TOT}**
- **Protein measurement according to Bradford 1951**
- **pH, sludge volume index, total solids, volatile solids, sludge retention time**
- **BOD, COD, catalase activity and TTC dehydrogenase activity**

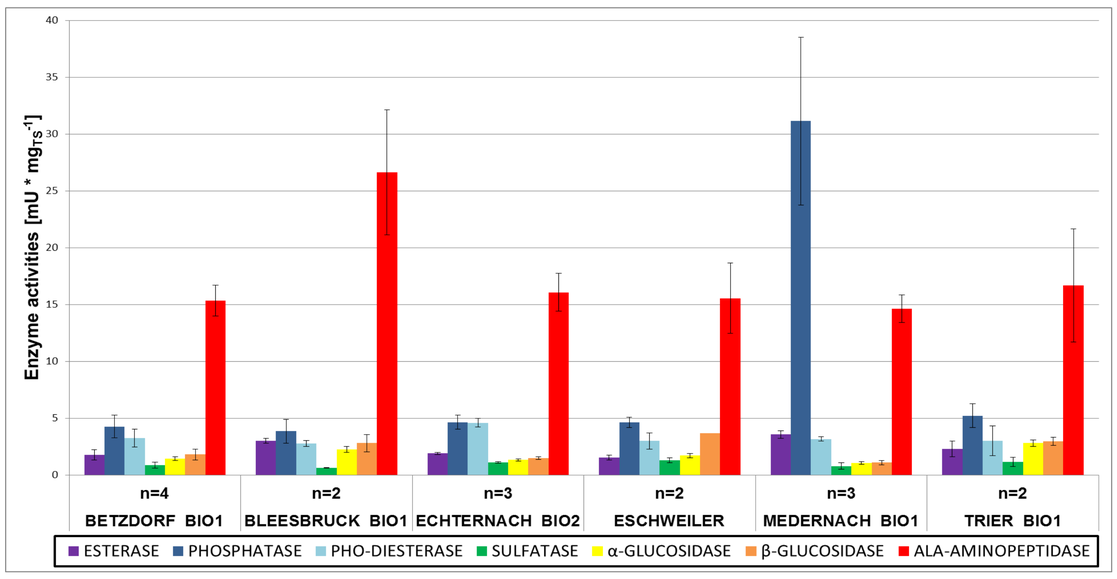
ENZYME ACTIVITY	EC N:	ROLE	SUBSTRATE	METHOD	INCUBATION
Unspecific esterase	3.1.1.1.	sum parameter	Fluoresceine diacetate	Obst 1995 [1]	1 h at 20°C
Alkaline phosphatase	3.1.3.1.	phosphorus metabolism	4-Nitrophenylphosphate disodium salt	Obst 1995 [1]	15 min at 30°C
Cyclic phosphodiesterase	3.1.4.1.	phosphorus metabolism	Sodium Bis(4-nitrophenyl)phosphate	analogous to [1] for PHO	15 min at 30°C
Aryl-sulfatase	3.1.6.1.	sulfur metabolism	Potassium 4-nitrophenyl-sulfate	analogous to [1] for PHO	15 min at 30°C
Phosphotriesterase	3.1.8.1.	phosphorus metabolism	Tris(4-nitrophenyl)phosphate	Eivazi &Tabatabai 1977	1 h 30°C
L-Alanine-aminopeptidase	3.4.11.14.	proteolytic	L-Alanine-4-nitroanilide-hydrochloride	Obst 1995 [1]	15 min at 30°C
α-glucosidase	3.2.1.20.	glycolytic	4-Nitrophenyl-α-D-glucopyranoside	Obst 1995 [1]	30 min at 30°C
β-glucosidase	3.2.1.21.	glycolytic	4-Nitrophenyl-β-D-glucopyranoside	Obst 1995 [1]	30 min at 30°C

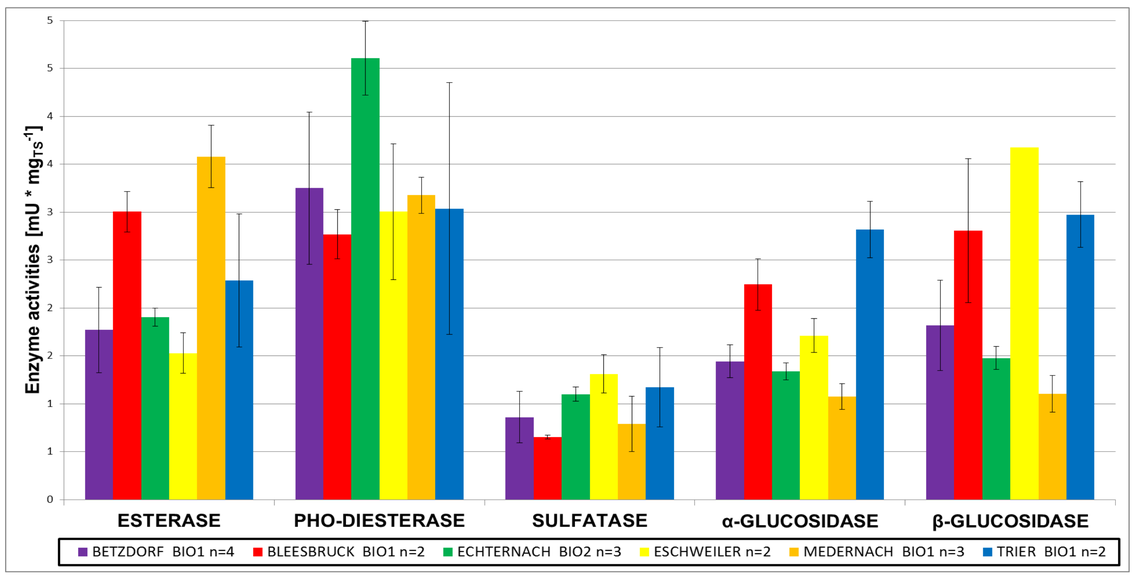
SAMPLING

Grab samples were taken during a prolonged dry weather period from aeration bassins from 8 a.m. to 10 a.m.. Activated sludge samples were transported within 2 hours on ice to the laboratory and assayed the same day.

RESULTS & DISCUSSION

Enzyme activities are expressed as mU [µmol substrate *min^{-1} *10^3] per mg$_{Total\ Solids}$





- **Esterase activity using fluoresceine diacetate as a substrate does not provide information on degradation of specific substances and can rather be seen as a sum parameter [1].**
- **Highest (alkaline) phosphatase activities were observed at WWTP Medernach, a plant without a phosphate precipitation step. Phosphorus degrading enzymes might be inhibited by precipitating agents.**
- **L-alanine-aminopeptidase is generally the most dominating enzyme activity (in accordance with [2]). One exception is WWTP Medernach, where proteolytic activity is lower (protein content is half of the content found at WWTP Bleesbrück). Proteolytic activity is highest for WWTPs Bleesbrück and Eschweiler. Both WWTPs have parts of influents coming from milk processing industry (also meat- and beer- processing factories linked to Bleesbrück).**
- **Aryl-sulfatase activities were among the lowest detected in this study.**
- **Phosphodiesterases are suspected to play a role in degradation of organo-phosphate xenobiotics like flame retardants and pesticides. Slight activity differences can be observed. WWTP Echternach shows phosphodiesterase activity almost reaching the level of phosphatase. High phosphodiesterase activities were observed in the effluent of a sewage plant (data not shown).**
- **α-glucosidases are involved in degradation of starch. They show a higher activity at the larger WWTPs Bleesbrück (100000 E.P.) and Trier (127000 E.P.). The high α-glucosidase of sludges from WWTP Eschweiler might be induced by the discharge from a milk processing factory. This discharge explains also the highest β-Glucosidase activity for WWTP Eschweiler.**
- **β-glucosidases are inducible enzymes that are released by microorganisms, when no readily biodegradable carbon sources are available. A higher return of recirculation sludge modifies the ratio of readily towards harder utilizable carbon sources and results in a higher activity in aeration bassins [3].**
- **The activity range of both glycolytic enzymes is similar within different WWTPs.**

CONCLUSIONS

Some remarkable variations in enzyme activities of activated sludges have been found. The composition of the inlet (e.g. starch- or protein- rich waste) seems to influence and modify specific enzyme activities. The broad range of data collected will be correlated to enzyme activities and might help to detect less obvious relationships. Further studies are devoted to link specific enzyme activities to the degradation of micropollutants by activated sludge consortia.

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