Bulgarian Journal of Agricultural Science, 19 (2) 2013, 132–134
Agricultural Academy

ENZYME ACTIVITIES AS A TOOL FOR BIOLOGICAL CONTROL IN DAIRY WASTEWATER TREATMENT

I. SCHNEIDER[1*] and Y. TOPALOVA
Sofia University "St. Kliment Ohridski", Department of General and Applied Hydrobiology, Faculty of Biology, BG – 1164 Sofia, Bulgaria

Abstract

SCHNEIDER, I. and Y. TOPALOVA, 2013. Enzyme activities as a tool for biological control in dairy wastewater treatment. *Bulg. J. Agric. Sci*., Supplement 2, 19: 132–134

The increased application of biofilm processes in the wastewater treatment requires development of new indicators for fixed biomass activity characterization. The evaluation of opportunity for application of enzyme activities of the total dehydrogenase, nitrate reductase, ß-galactosidase and phosphatase as biological indicators of well functioning biofilms in dairy wastewater treatment is the aim of this article. The *lab scale* wastewater treatment process was simulated in anaerobic biofilters in semi-continuous regime. The activity of biofilm and suspended biomass was studied in parallel. The enzyme activities were discussed with removal effectiveness for chemical oxygen demand, proteins and lactose at the 216th day, corresponding to the phase of stabilization of sequencing batch process. The obtained data showed two important tendencies for biofilm activity assessment: i/ the total dehydrogenase activity was appropriate indicator for assessment of total organic matter mineralization and ii/ the phosphatase and nitrate reductase activity were related to protein biotransformation. The ß-galactosidase activity of suspended biomass was more appropriate indicator for assessment of lactose biodegradation. The investigated enzyme activities can be used for quick, accuracy and easy applicable tool for control and management of dairy wastewater treatment.

Key words: anaerobic biofilm, ß-galactosidase activity, nitrate reductase activity, phosphatase activity index, total dehydrogenase activity
Abbreviations: AS – activated sludge; ASBB – anaerobic sequencing batch biofilm; COD – chemical oxygen demand; DHA – total dehydrogenase activity; GAL – ß-galactosidase activity; NRA – nitrate reductase activity; PAI – phosphatase activity index

Introduction

The selection of biological indicators for assessment and management of wastewater treatment processes requires the methods for it measurement to be quick, precise, cheap, easy applicable and to not require special, expensive equipment. The enzyme activities are an example for such indicators. The methods for their determination require less time for evaluation and they are more accurate in comparison to the microbial quantity (Lazarova and Manem, 1995; Schneider and Topalova, 2011). The activities of hydrolytic enzymes or of total enzymes (dehydrogenases and the phosphatases) were proposed in the scientific literature (Gabbita and Huang, 1984; Lazarova and Manem, 1995; Matavuly et al., 1990; Li and Chrost, 2005; Topalova, 2009). However, information about the enzymological control of anaerobic processes with fixed biomass for dairy wastewater treatment is missing. The evaluation of opportunity for application of enzyme activities of the total dehydrogenase, nitrate reductase, ß-galactosidase and phosphatase as biological indicators of well functioning biofilms in dairy wastewater treatment is the aim of this article.

Materials and Methods

A dairy wastewater treatment process was carried out in anaerobic sequencing batch biofilm (ASBB) reactors with

**E-mail: i.schneider@abv.bg*

gravel carrier and model wastewater (Schneider and Topalova, 2011). The inocula for ASBB reactors were prepared from specially treated and acclimated activated sludge (AS) enriched with microbial preparations HydroPacks and Laktazym (Schneider and Topalova, 2011). The ASBB reactors were inoculated with: 1) 10 $g.l^{-1}$ acclimated AS as dry weight (control); 2) 10 $g.l^{-1}$ acclimated AS as dry weight with 3 $g.l^{-1}$ HydroPacks (H); 3) 10 $g.l^{-1}$ acclimated AS as dry weight with 3 $g.l^{-1}$ Laktazym (L).

The chemical oxygen demand (COD), protein and lactose concentration were measured (Miller, 1959; Kochetov, 1980; APHA, 1989) and the removal effectiveness was calculated for it (Tshachev, 2001). The immobilized and suspended biomass for the enzyme activities was harvested on 216th day at the stable biofilm development (Schneider and Topalova, 2011). The activities of acid, neutral and alkaline phosphatases, nitrate reductase (NRA), total dehydrogenase (DHA) and ß-galactosidase (GAL) were investigated (Miller, 1972; Gabbita and Huang, 1984; Matavuly et al., 1990; Topalova, 2009). The phosphatase activity index (PAI) is an average value from the three phosphatases (Matavuly et al., 1990). The data for the enzyme activities were presented for unit protein (Kochetov, 1980).

Results and Discussion

The alternation of activities of DHA, NRA and PAI followed similar tendency (Figures 1 and 2): the organic matter biotransformation processes were accomplished with higher rate in biofilm in comparison to suspended biomass because the enzyme activities of fixed film biomass were higher. The retention of the enzymes (including of extracellular enzymes) in biofilm by a matrix from extracellular polymeric substances was a possible reason. The concentration of substrates, co-factors and other modulators of enzyme activities on inert carrier were other positive factors for this tendency.

The alternation of the DHA for the three immobilized communities was similar with the alternation of effectiveness of COD decreasing (Figure 1). Highest effectiveness (90%) and highest DHA was ascertained for the variant with Laktazym. Lowest activity was ascertained for the variant with HydroPacks. The organics removal effectiveness for this variant was lower with 26% and DHA was fifteen times lower. Moreover, the DHA of biofilm was almost equal of the activity of the suspended biomass, which was probably related to deteriorated conditions and biofilm thinning. The detachment of microbial cells or clusters of cells, which continued to be active biodegradation zones in this biofilter, were a result of that. Significant differences

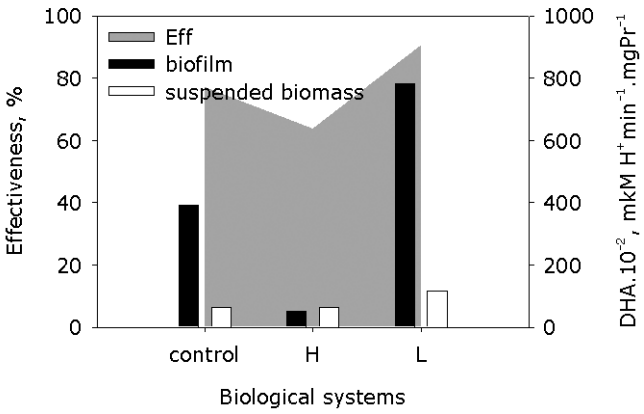

Fig. 1. Dehydrogenase activity (DHA) of biofilm and suspended biomass and effectiveness of COD decreasing (Eff) for the three biological systems

in the activity of the three model systems were ascertained although the same initial inocula (10 $g.l^{-1}$ AS). These differences were related to the different microbial species of both preparations, which were added to the acclimated AS, their different opportunity for inclusion in biofilm and retention in the biofilters.

The alternation of the NRA and the PAI was similar with the alternation of the protein biodegradation effectiveness (Figure 2). This trend confirmed the relation between carbon and nitrogen biotransformation as well as between carbon and phosphorous biotransformation. This dependence was related to the fact that the removal of 1 $mg.l^{-1}$ $N\text{-}NO_3$ requires 8.60 $mgO_2.l^{-1}$ COD and the removal of 1 $mg.l^{-1}$ phosphorus requires 54 $mgO_2.l^{-1}$ COD (Tshachev, 2001). Highest effectiveness of protein hydrolysis (65%) was ascertained for the control variant (Figure 2). In the same time PAI (Figure 2a) and NRA (Figure 2b) were highest. The lowest effectiveness of protein removal (56%) was ascertained for the variant with HydroPacks. The most possible reason was the decreased enzyme activity.

The GAL followed a different tendency in comparison to DHA, NRA and PAI. The GAL of the suspended biomass was higher in comparison to biofilm and the alternation of lactose removal effectiveness was similar with the alternation of GAL for the suspended biomass (Figure 3). This leads to conclusion that lactose biodegradation was accomplished with higher rate in the liquid. Highest GAL was measured for the control variant and lowest activity was ascertained for the variant with Laktazym. However, the lower GAL of the suspended biomass for this variant was compensated with higher GAL of biofilm.

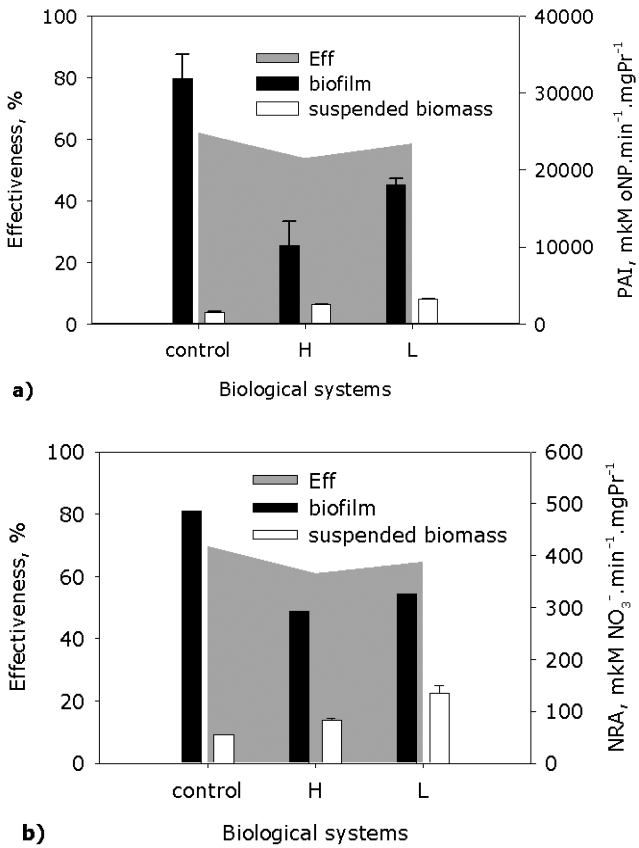

Fig. 2. Effectiveness (Eff) of protein removal and: a – phosphatase activity index (PAI) and b – nitrate reductase activity (NRA) of biofilm and suspended biomass

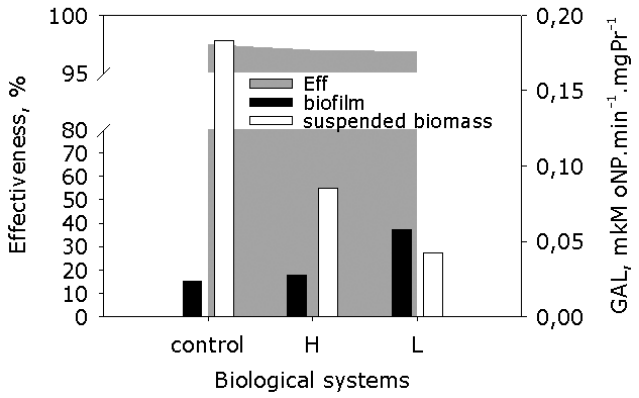

Fig. 3. ß-Galactosidase activity (GAL) of biofilm and suspended biomass and lactose removal effectiveness (Eff) for the three biological systems

Conclusion

The low enzyme activities of suspended biomass in comparison to biofilm samples for DHA, NRA and PAI indicated that majority of the activities of these enzymes was associated with the microbial cells and/or immobilized within the extracellular polymer matrix of the biofilm. This immobilization of enzymes on inert carrier provided higher operational stability in the semi-continuous flow reactors. This tendency was clearly ascertained for the control and for the variant with Laktazym. The summarized data bring to the following important conclusions: i/ the DHA of biofilm was appropriate indicator for assessment of total organic matter mineralization; ii/ the NRA and PAI corresponded with the protein biotransformation by fixed biomass; iii/ the GAL of suspended biomass was more appropriate indicator for assessment of lactose biodegradation in comparison with the GAL of biofilm. The combination DHA, NRA, PAI, GAL is quick, applicable and informative tool for the effective control and management of dairy wastewater treatment.

Acknowledgements

This work was supported financially by the Ministry of Education, Youth and Science – Project DMU03-56: "Innovative ecological approaches for dairy wastewater treatment".

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