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IMOBILIZATION OF ENZYMES INTO NANOFIBER STRUCTURE FOR WASTEWATER TREATMENT

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Abstract

Wastewater treatment technology must deal with problematically cleanable substances. These substances include fats, proteins and endocrine disruptors, which can be partially or completely hydrolysed by enzymes.

Direct application of biologically active substances has several disadvantages. These drawbacks can be reduced by immobilization of biologically active enzymes into suitable inert structure like nanofibers, thanks to their high specific surface. Immobilized enzymes are active, stabilized and thus advantageous for long-term operation in cleaning process.

Actual activity of immobilized enzymes can be measured using fluorescence microscopy followed by image analysis. Moreover, dissolved and nanofiber-enzyme activity will be detected using standard methods. Efficiency of selected enzymes will be tested on concrete pollutants. Finally, we will test durability of carriers in unfavourable conditions directly in wastewater treatment plant.

Introduction

Over past century there has been an increasing amount of wastewaters, which cannot be cleaned by conventional wastewater processes. Among problematical substances (pollutants) are listed oils, fats, proteins and endocrine disruptors. Oils, fats, proteins are difficult to clean through conventional biological treatment due to their slow biodegradability. [1, 2]

Endocrine disruptors are chemicals that can or are suspected to mimic, or interfere with the action of endogenous hormones. [3]

Enzymes catalyse specific reaction under moderate conditions (temperature, pH), and mainly without undesired side-reactions, which would otherwise increase reactant consummation and raise the cost of treatment. [4]

Huge development of nanotechnology enables immobilization of enzymes on nanofibers. The premise of nanomaterials is to maximize functional surface area to increase enzyme loading resulting in higher enzyme activity. [5]

Experiment and methods (1)

- Aim to evaluate activity of immobilised enzymes.

Table 1. Pollutants and selected enzymes

Pollutant	Selected enzyme
Oils and fats	Lipase
Proteins	Protease
Endocrine disruptors	Laccase, tyrosinase

Experiment and methods (2)

- Enzymes covalently binden on carriers.
- Carrier = fiber with surface covered with nanofibers.
- Different types of nanofibrous carriers will be tested: PES, PUR, PVB.

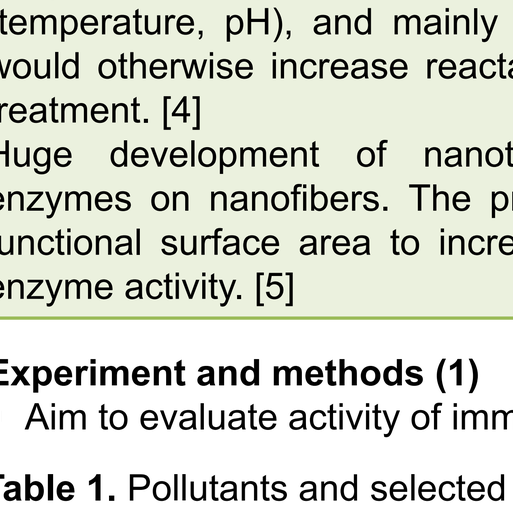

Figure 1. Structure of PES carrier (BW camera fluroscence microscope)

Activity (1)

Immobilized enzymes will be activated using:

- suitable buffer (Potassium phosphate, or Tris-HCl),
- fluorogennic substrate (1,2-Di-O-lauryl-rac-glycero-3-(glutaric acid 6-methylresorufin ester) (DGGR), or EnzChek® Lipase Substrate.

Activity (2)

- Activity of enzymes will be measured by:
  - fluorescence microscopy,
  - methods of image analysis,
  - spectrophotometric methods.

Figure 2. Structure of PES carrier (RGB camera fluroscence microscope)

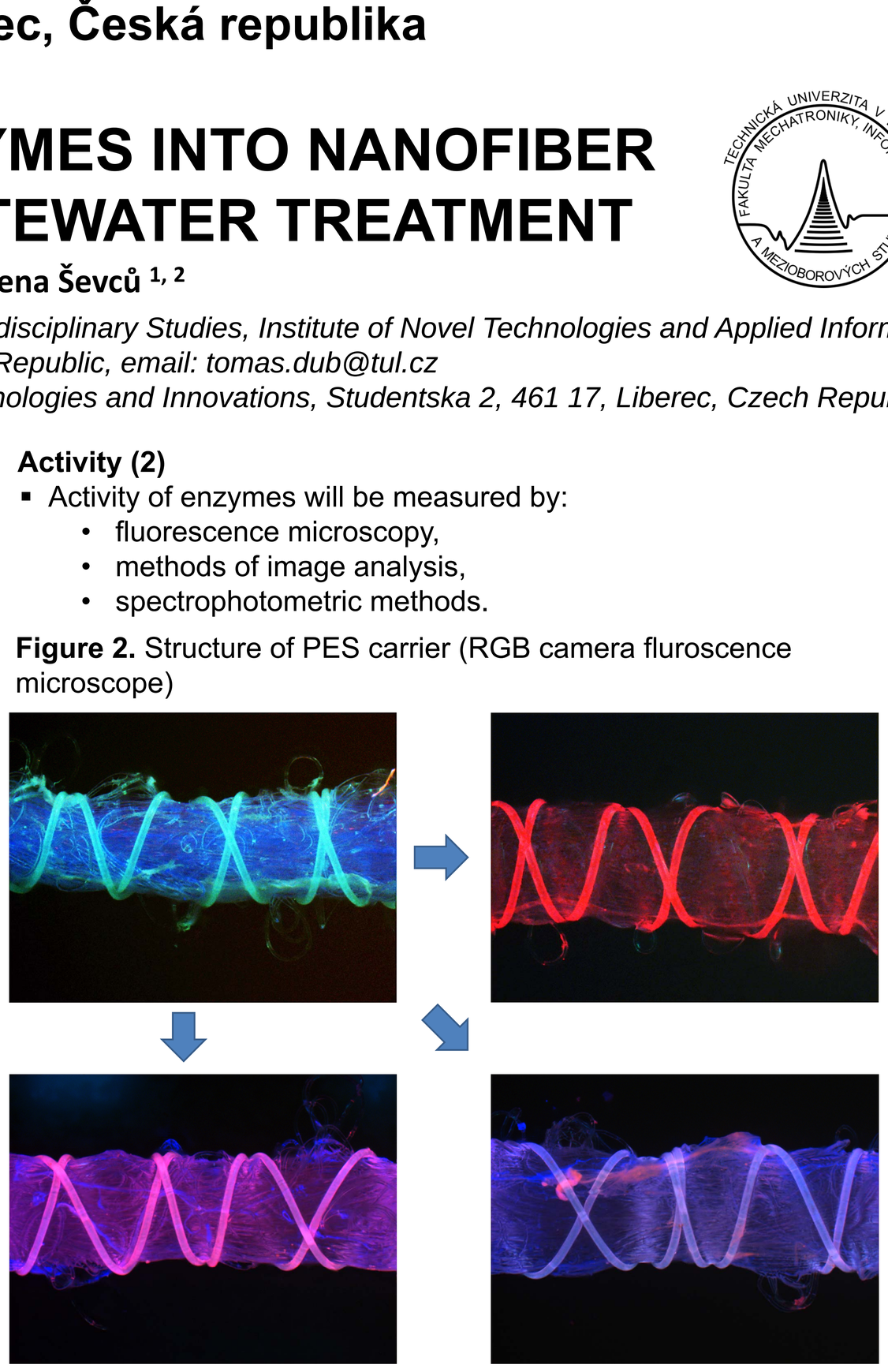

Figure 3. Structure of PES carrier with activated enzymes (RGB camera fluroscence microscope) same filter, different setting

Effectiveness (1)

- Effectiveness of selected enzymes – will be tested on concrete pollutants – concentration measured by chromatographic methods:
  - HPLC for proteins,
  - GC MS MS for endocrine disruptors.

Discussion

- Goal – find most suitable nanofiber carrier that will:
  1) carry optimal amount of covalently bind enzymes,
  2) be sufficiently resistant in hostile environment in application,
  3) have moderate antibacterial effect.

Conclusion

- Research is aimed to optimize new wastewater cleaning technology for degradation of problematically cleanable substances.
- Technology is based on enzymes immobilized on nanofibrous carriers.
- Most important goal of research is to:
  1) describe application of enzymes for concrete pollutants,
  2) find suitable conditions of usage in wastewater cleaning processes.

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