Polyethylenimine-based polymers for harvesting and immobilization of microalgae and cyanobacteria

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Microalgae bloom in a natural water body
http://lovlyavsem.ru/ryby/karas/pogoda-dlya-lovli.html

Introduction

Phototrophic microorganisms (PM) - microalgae and cyanobacteria can produce toxins harmful to humans and animals, thus, there is a need for control of harmful PM blooms in water bodies. At the same time PM can produce a great variety of useful secondary metabolites and are potentially useful as treating agents for wastewater. One of the major practical limitations of PM application is the harvesting of biomass from treated water or cultivation systems. All these problems can be solve using the new polyethylenimine-based (PEI) polymers presented below combining a high affinity to the cell surface and capable of preserving of the PM cell viability.

METHODS

- ***Sorbent synthesis***

The solid and porous polymers were obtained by cross-linking of highly-branched polyethylenimine (PEI) with epichlorohydrin (ECH) and diethylene glycol diglycidyl (DEG).
The sorbents with DEG were synthesized from aq. solution of PEI and various content of DEG (7.5-120% by wt) using a cryopolymerization technique [1]. The sorbents with ECH were cross-linked by different quantities of ECH (1.8-30% by wt) at heating [2].

- ***Model microalgae and cyanobacteria strains***

Chlorella vulgaris (IPPAS S-2014) from the microalgal culture collection of Timiryazev Institute of Plant Physiology (IPPAS), *Desmodesmus sp.* 3Dp86E-1, *Anabaena variabilis* ATCC 29413, *Anabaena variabilis* 7120, *Nostoc muscorum* PCC 6310.

- ***Kinetics of microalgae and cyanobacteria immobilization***

The amount of the microalgae and cyanobacteria cells remaining unattached was monitored via chlorophyll (Chl) content [3, 4] with an Agilent Cary 300 spectrophotometer.
As a measure of the cell immobilization efficiency, the completeness of the immobilization was calculated as:

$$A\ (\%) = (Chl_0 - Chl_{t1}) \times 100\ \% / Chl_0 \qquad (1)$$

where A— completeness of culture immobilization, %; Chl_0—Chl content in the control suspension, mg l^{-1}; Chl_{t1}—residual Chl content in cell suspension after incubation with the sorbent for the time t_1, mg l^{-1}.

- ***Chlorophyll fluorescence***

Viability and photosynthetic activity of immobilized *C. vulgaris* cells were checked via variable chlorophyll fluorescence measurements on Walz Dual PAM 100 pulse-amplitude modulated fluorimeter [5]. Sorbent (0.1–0.15 mg DW) with the attached microalgal cells were withdrawn after 3, 24, 72 and 168 h of immobilization. Maximal photochemical quantum yield of dark adapted cells (Fv/Fm) was calculated as:

$$Fv/Fm = (Fm - Fo) / Fm \qquad (2)$$

where Fo and Fm are minimal and maximal Chl *a* fluorescence intensity of dark adapted cells during saturation pulse exposition.
The photon flux density (PFD) of measuring light and saturation pulse (620 nm) were 3 and 10000 мmol quanta/m2/s respectively. The saturation light pulses were 0,6 s long.

Electron microscopy

The fixed samples examined under JSM-6380LA scanning electron microscope (JEOL, Japan).

RESULTS AND DISCUSSION

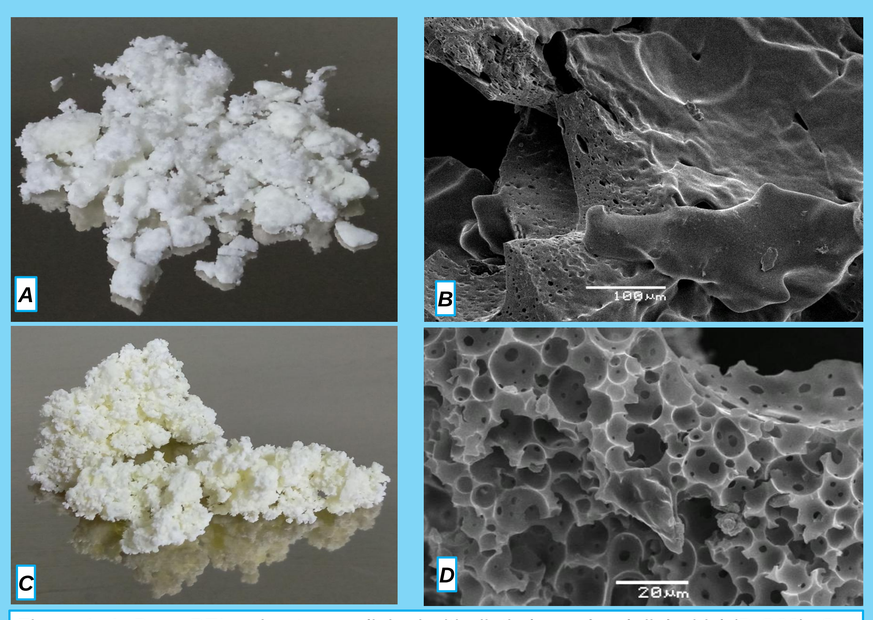
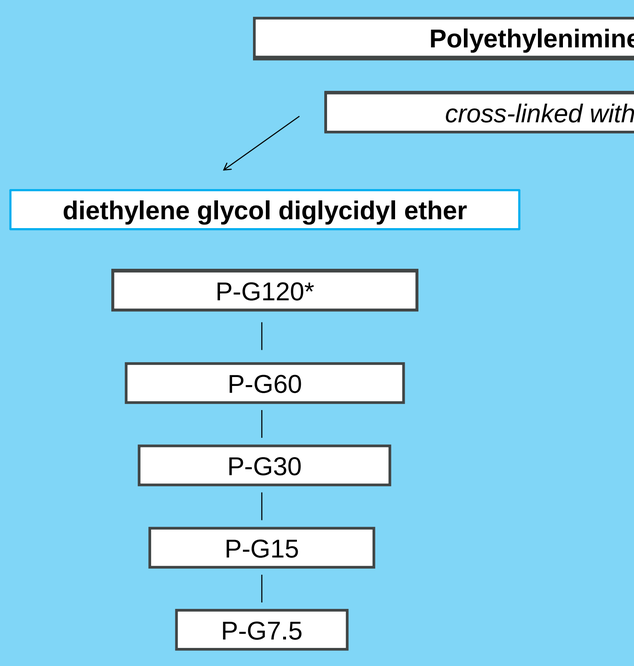


Figure 1. A, B – a PEI sorbent cross-linked with diethylene glycol diglycidyl (P-G30); *C, D* – a PEI sorbent cross-linked with epichlorohydrin (P-E15).



* Percentage of the cross-linker in the sorbent

Completeness of immobilization

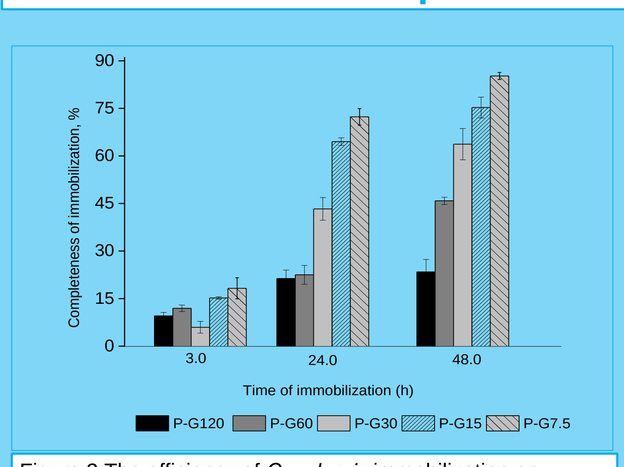


Figure 2.The efficiency of *C. vulgaris* immobilization on polyethylenimine sorbents cross-linked with diethylene glykol diglycidyl ether.

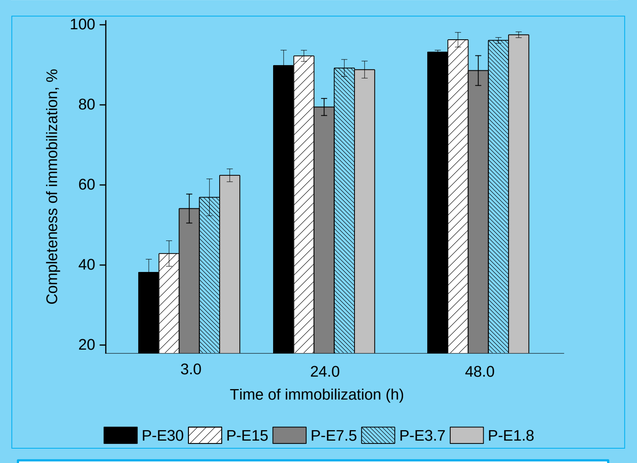


Figure 4. The efficiency of *C. vulgaris* immobilization on polyethylenimine sorbents cross-linked epichlorohydrin.

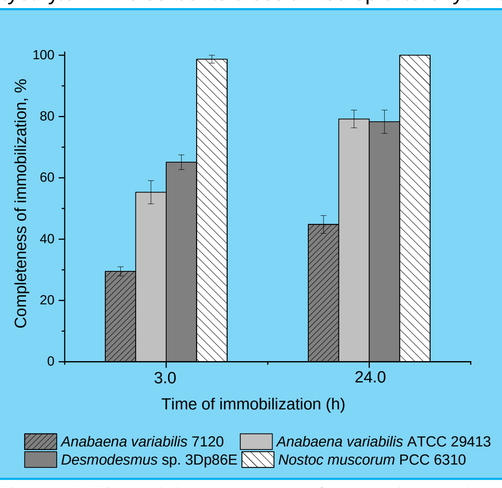


Figure 6. The efficiency of microalgae and cyanobacteria immobilization on P-G60.

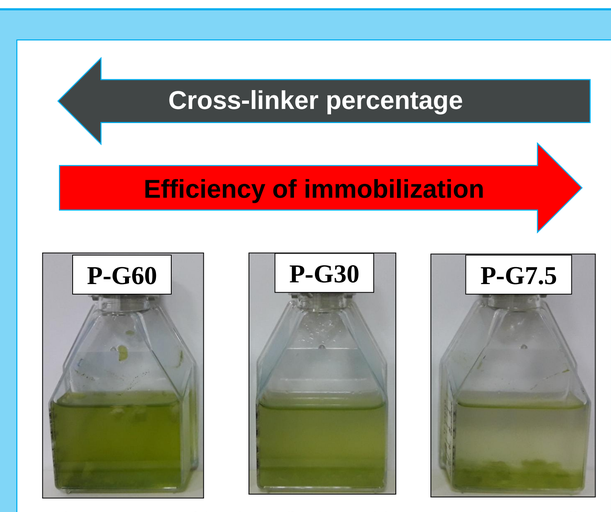


Figure 3. Cultivation flasks with *C. vulgaris* cells immobilized on sorbents cross-linked with different percentage of diethylene glycol diglycidyl ether.

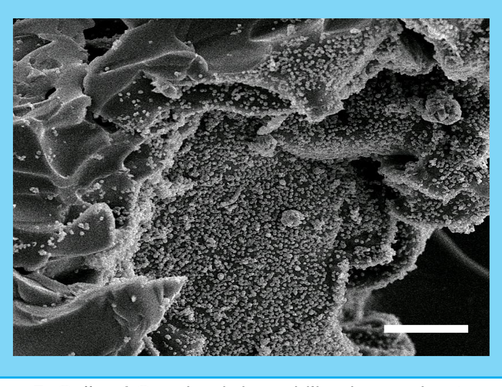


Figure 5. Cells of *C. vulgaris* immobilized on sorbent P-G60 during 24 hours (scale bar 50 µm) .

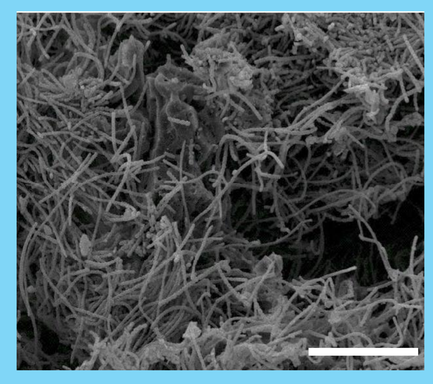


Figure 7. Cells of *Nostoc muscorum* immobilized on sorbent P-G60 during 3 hours (scale bar 100 µm) .

Photosynthetic activity of the immobilized microalgae cells

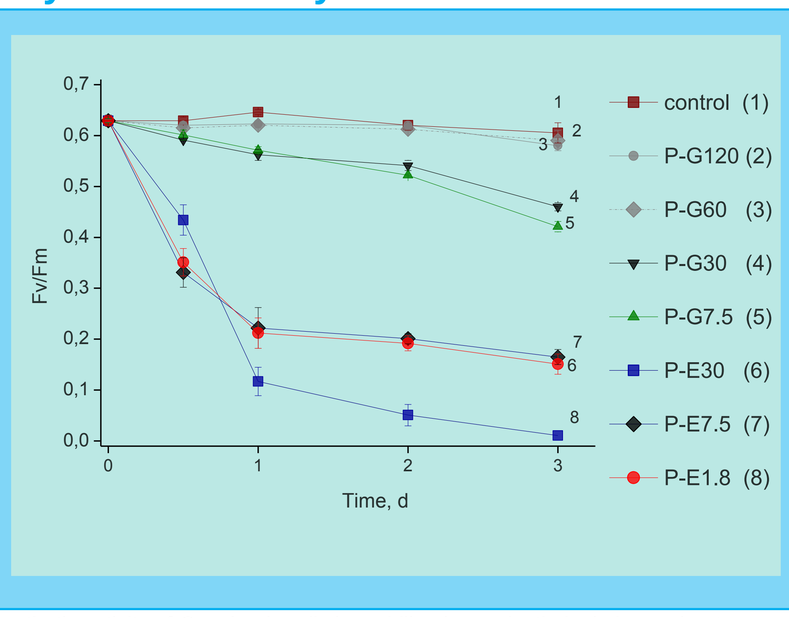


Figure 8. Photosynthetic activity of *C. vulgaris* cells immobilized on polyethylenimine sorbents.

CONCLUSIONS

- Sorbents cross-linked with epichlorohydrin exerted higher immobilization efficiency as compared to sorbents with diethylene glycol diglycidyl ether.
- In the most of the studied sorbents a decrease in the cross-linker percentage resulted in enhancement of the immobilization efficiency.
- Sorbents with higher percentages of diethylene glycol diglycidyl ether (60–120%) are suitable for prolonged cultivation of the immobilized PM since they did not affect the cell viability and kept the immobilized cells metabolically active.
- Sorbents with a lower percentage of DGDE (< 30%) and sorbents with ECH are suitable for harvesting of the PM cells for their subsequent utilization.

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