Evaluation of the catalytic properties of cyanobacteria

Abstract

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Cyanobacteria, as an extremely diverse group of microorganisms, represent an interesting research object. The multiplicity of morphological forms, occurrence in almost all conditions and all over the globe means that their potential is not fully discovered. Holistic and comprehensive studies of the activity of selected photobiocatalysts towards model substrates have provided useful knowledge for the application of these biocatalysts in the field of sustainable biotechnology. Biotransformations carried out using cyanobacteria are an alternative to chemical synthesis, not only because they are environmentally friendly processes, but are also economically viable. The study focuses on the development of effective methods for the assessment of the hydrolytic activity of photobiocatalysts and attempts to check whether they are capable of carrying out redox reactions. The research was carried out on seven strains belonging to different taxonomic groups and differing in morphological structure. Three model substrates were used, including racemic mixtures of the chiral: 1-phenylethyl acetate and epoxystyrene to test hydrolytic activity, and styrene to test the ability of cyanobacteria to carry out redox reactions (hydrogenation, hydroxylation, or epoxidation). It has been proved that the enzymatic apparatus of cyanobacteria is active against the ester bond of the xenobiotic used, which may result from the activity of carboxylic ester hydrolases – (CEHs, EC 3.1.1). A new method of obtaining the chiral alcohol, acetophenone and the enantiomer of the unreacted substrate has been proposed. Strains with the highest biocatalytic activity were indicated: *Nostoc cf-muscorum* CCALA 129, *Leptolyngbya foveolarum* CCALA 76, *Synechococcus bigranulatus* CCALA 187 and *Limnospira indica* PCC8005. The study of morphology variability provided information about trichome rotation within the same strain over the years (dimorphism), which has not been described in the literature (in the case of strains other than the genus *Spirullina*), which is related to the cultivation of these organisms in non-physiological – laboratory conditions. In the case of the *Nostoc cf-muscorum* CCALA 129 strain, the trichomes from the new culture are definitely more twisted and shorter. A straightening of the trichomes and their elongation can be observed over time. In the case of a strain that has been in culture for many years, numerous heterocysts unrelated to filaments (trichomes) are visible, which are not seen in such large numbers in the case of the same strain cultured for a shorter time. The differences in morphology influenced the results of biotransformation processes in various ways, depending on the strain - they accelerated or slowed down the biotransformation process, which was also reflected in the biotransformation results. In the case of the *Kamptonema animale* strain, the new version of the strain conducts biotransformation reactions with higher enantioselectivity (E = 5.2) than the old strain (E = 2.0) for a conversion degree of approx 50%. For all the processes carried out, the E parameter was calculated, which is extremely important in the case of kinetic separation. It has been shown that parameters such as the duration of biotransformation, substrate concentration or the method of cultivation have a significant impact on its value. In the case of the *Nostoc cf-muscorum* CCALA 129 strain, pure alcohol was obtained: 1-(*R*)-phenylethanol with an excellent enantiomeric excess

to ee> 99% for the following substrate concentrations: 2mM, 4mM to 6mM with high enantioselectivity (E = 275). These results are promising for the upscaling of the process and the use of this strain for the production of chiral alcohols. By examining the course of the kinetic resolution reaction below and above the value of 50% conversion, it was observed that some strains also oxidize the ester bond hydrolysis product – phenylethanol, to acetophenone. The *Limnospira indica* PCC8005 strain was the most effective in oxidizing alcohol to acetophenone – the conversion rate after 72 hours of the process was 29%, while in the case of the *Nostoc cf-muscorum* CCALA 129 strain, the conversion rate was 2% after the same duration of the process. This is an extremely important observation and may indicate that under certain reaction conditions, the alcohol appearing in the culture environment seems to be an external source of protons and electrons, which allowed to maintain the redox balance important for photobiocatalysts. The rate of the oxidation process varied, indicating that separate studies must be conducted for each strain to investigate the formation of biotransformation by-products. The survival of the biocatalyst under biotransformation conditions in a wide range of concentrations (1 mM – 50 mM) was also tested. The studies with the use of flow cytometry showed a very high tolerance range of the tested biocatalyst towards the substrate, which is promising not only for increasing the scale of the process, but also for the use of cyanobacteria in continuous processes. Studies were carried out to check the speed of the biotransformation process in relation to the area of light incident on the culture – these studies were also correlated with the scaling up of the process by increasing the substrate concentration to a maximum of 50 mM. This is a critical element in planning the biotransformation process due to the fact that this effect differs for individual biocatalysts and should be checked each time for each strain. In the case of the strains used, these differences were important for the *Synechococcus bigranulatus* CCALA 187 strain and significantly accelerated the process – after 3 hours, the conversion rate of the process in the breeding bottle was 19% higher. The research approach described above made it possible to achieve the aim of the research through the appropriate selection of culture conditions and the development of biotransformation protocols for individual strains.