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Physicochemical approach to freshwater microalgae harvesting with magnetic particles

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ABSTRACT

Magnetic harvesting of microalgal biomass provides an attractive alternative to conventional methods. The approach to this issue has so far been pragmatic, focused mainly on finding cheap magnetic agents in combination with harvestable microalgae species. The aim of this work was to study experimentally and theoretically the mechanisms leading to cell-magnetic agent attachment/detachment using real experiments and predictions made by colloidal adhesion (XDLVO) model. Two types of well defined magnetic beads (MBs) carrying ion exchange functional groups (DEAE – diethylaminoethyl and PEI – polyethylenimine) were studied in connection with microalgae (*Chlorella vulgaris*). Optimal harvesting efficiencies (>90%) were found for DEAE and PEI MBs, while efficient detachment was achieved only for DEAE MBs (>90%). These findings were in accordance with the predictions by XDLVO model. Simultaneously there was found a discrepancy between the XDLVO prediction and the poor detachment of PEI MBs from microalgal surface. This can be ascribed to an additional interaction (probably covalent bonds) between PEI and algal surface, which the XDLVO model is unable to capture given by its non-covalent nature.

1. Introduction

Magnetic modification of diamagnetic biomass, bioactive compounds and biomacromolecules using magnetic nano- and microparticles maintains an important position in many biotechnological branches. It has found applications in protein or cell separations and purifications from complex mixtures (blood, serum, culture medium), flow cytometry, magnetic drug targeting and delivery, immobilization (enzyme- or whole cell-based biocatalysis, biosensors), preparation of adsorbents for organic or inorganic xenobiotics removal, etc. [1]. Very attractive is the use of magnetic beads composed of a magnetic core, that consists of natural materials (such as magnetite, Fe_3O_4, or maghemite, γ-Fe_2O_3), coated with a protective layer carrying specific, functional groups ensuring selective separations and/or targeting. The method is quick and efficient, the magnetic agents are biocompatible, and the harvesting of the magnetically modified product is fully controllable via an external magnetic field (using an appropriate magnetic separator, permanent magnet, or electromagnet) [1,2].

Magnetic modification of microalgae has been applied already in the 1970s for removing harmful algae from lakes/ponds [3]. Nowadays the effort is focused on studying magnetic agents (commercial beads, synthesized nano/microparticles) for harvesting biotechnologically attractive microalgae species [4–7], whereas cost-effective magnetic agents are sought [8]. As harvested microalgae display slow sedimentation rates, colloidal stability and low cell densities, the use of magnetic particles adherent to the cell surface is very attractive. In addition, the external magnetic field enables to concentrate the magnetically modified cells into compact slurry and remove large amounts of the bulk liquid in a short time. However, apparently the recovery of pure algal biomass by detachment and subsequent re-utilization of applied magnetic agents is difficult and therefore scarcely mentioned in literature. If mentioned, then focus is on the removal of magnetic particles by dissolution under acidic conditions, which may not be desirable for all target compounds or downstream processing [4–8].

So far there has been no explanation why the regeneration (e.g. by detachment) of magnetic agents is problematic. Thus, this paper aims at explaining the mechanism of microalgae harvesting with magnetic beads (MBs) and the subsequent recovery of algal cells by bead detachment under model conditions. The surface interactions of algae with model MBs is described with the use of a physicochemical approach, i.e. the extended Derjaguin–Landau–Verwey–Overbeek (XDLVO) theory [9] and the experimental campaign was carried out with an industrially attractive microalgal strain, *Chlorella vulgaris* P12 [10–12].

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2. Materials and methods

2.1. Microorganism, cultivation and preparation of algal suspension

Chlorella vulgaris Beijerinck strain P12 was obtained and maintained according to previously described procedures [10,13]. Batch cultivation in the photobioreactor proceeded as reported in literature [11], i.e. glass tubes were situated in a water bath (30 °C) under continuous illumination with incident light intensity 100 μE/m^2/s (PAR sensor QSL-2101, Biospherical instruments Inc., USA) and feeding of air enriched with 2% CO_2 (v/v) at 15 L h^{-1} per tube. Each tube contained 300 mL of mineral medium, having the initial composition (mg L^{-1}): 1100 $(NH_2)_2CO$, 238 KH_2PO_4, 204 $MgSO_4{\cdot}7\ H_2O$, 40 $C_{10}H_{12}O_8N_2NaFe$, 88 $CaCl_2$, 0.832 H_3BO_3, 0.946 $CuSO_4{\cdot}5\ H_2O$, 3.294 $MnCl_2{\cdot}4\ H_2O$, 0.172 $(NH_4)_6Mo_7O_{24}{\cdot}4\ H_2O$, 2.678 $ZnSO_4{\cdot}7\ H_2O$, 0.616 $CoSO_4{\cdot}7\ H_2O$, and 0.0014 $(NH_4)VO_3$. The pH value was adjusted to 6.5–7.0 using 1 M KOH prior to inoculation from an agar plate. The medium was treated as for outdoor culture so it was not sterilized, but distilled water was used nevertheless. After 144 h of cultivation a biomass concentration of 5 g L^{-1} was obtained, then the microalgal cells were centrifuged and washed twice with distilled water (4000 rpm, 5 min). Subsequently, they were used for image analysis and to prepare algal suspensions of a defined concentration for physicochemical surface characterizations (contact angle and zeta potential measurements) or studies of microalgae–magnetic beads (MBs) surface interactions in test tubes.

2.2. Image analysis

Image analysis was applied to determine the size of *C. vulgaris* cells. After the appropriate cultivation time a small amount of cell suspension was placed on a microscopic slide, the cells were photographed (microscope Nikon Eclipse E400, digital camera Nikon D300s) and subsequently analyzed with the commercial software NIS Elements (Nikon Instruments Inc., USA).

2.3. Physicochemical surface characterization

2.3.1. Zeta potential measurements

Two types of magnetic beads (SiMAG-ionex, 0.5 μm diameter, Chemicell, Germany) carrying defined functional groups, i.e. DEAE (diethylaminoethyl) and PEI (polyethylenimine), respectively, were used as model agents to contact *C. vulgaris* cells. Zeta potentials of *C. vulgaris* cells (50 mg L^{-1}) or magnetic beads (20 mg L^{-1}) were measured in model environments (10 mM KCl, pH 2–12) at 25 °C using the Zetasizer Nano-ZS (Malvern, United Kingdom) and calculated according to the Smoluchowski equation. Each sample was measured ten times. Presented results are mean values ± standard deviation.

2.3.2. Contact angle measurements of cells and magnetic beads

For contact angle (CA) measurements *C. vulgaris* cells and beads had to be prepared in the form of a flat surface, i.e. suspensions of the tested particles were deposited on a filter (nitrate cellulose membrane, 0.45 μm pore size, 47 mm diameter, Whatman, USA) under negative pressure. In the case of *C. vulgaris* the suspension was highly concentrated (cell concentration determined with a Bürker chamber) in order to gain 7.10^6 cells mm^{-2} on the filter. The obtained microbial lawns were then deposited on agar plates to stabilize moisture content [14], fixed to a microscopic glass slide, allowed to dry for 50 min to reach the plateau region [15], and subjected to CA measurements by the sessile drop technique (volume of 3 μL) using the CAM 200 goniometer (KSV Instruments, Finland). Measurements were performed at 25 °C with three test liquids (water, formamide, 1-bromonaphtalene), readings were taken after 0.5 s of deposition, and each sample was tested nine times. In the case of the magnetic beads, each bead type was diluted to reach 1.3 g L^{-1} and 14 mL were deposited on a filter under negative pressure. The subsequent procedure was the same as in the case of the microalgal cells, with the exception that the placing of the samples on an agar plate was excluded and the plateau region was reached already in 30 min. Presented results are mean values ± standard deviation.

2.4. Harvesting experiments

Harvesting of magnetically modified microalgae was tested in a defined model environment, where prepared microalgal suspensions (10 mL, 10 mM KCl, pH 4–12) of a defined concentration (0.2 g L^{-1}) were mixed (15 rpm, orbital mode, Hulamixer Sample Mixer, Invitrogen, USA) with specific amounts of beads (DEAE or PEI) for 10 min in plastic test tubes. Formed MBs-microalgae aggregates were then exposed to an external magnetic field (cylindrical NdFeB magnets, 25 × 10 mm, Neomag, Czech Republic) for 30 min and subsequently the absorbance of the obtained supernatant (3 mL) was measured at 750 nm. The efficiency of microalgae harvesting with MBs (E, %) was calculated as follows: $E = [(A_0 - A_1)/A_0] \times 100$, where A_0 is the initial absorbance of the microalgal suspension and A_1 the absorbance of the supernatant after harvesting of the magnetically modified microalgae. Due to the small cell size of *C. vulgaris*, the self-sedimentation of microalgae cells was neglected. All experiments were performed in duplicate and presented results are mean values ± standard deviation.

2.5. Detachment of magnetic beads

After achieving separation efficiency above 90% in the appropriate model environments, the bulk liquid was removed and magnetically labeled *C. vulgaris* cells were resuspended in 10 mM KCl pH 12. Samples were mixed continuously at room temperature (15 rpm, orbital mode, Hulamixer Sample Mixer, Invitrogen, USA) and analyzed at regular time intervals with appropriate blanks after a 30 min exposure to an external magnetic field (cylindrical NdFeB magnets, 25 × 10 mm, Neomag, Czech Republic). The absorbance of the supernatant (3 mL) was measured at 750 nm and the detachment efficiency (R, %) was calculated as follows: $R = (A_3/A_2) \times 100$, where A_2 is the absorbance of the appropriate blank (i.e. cell suspensions that are without MBs but otherwise of same composition, and underwent the procedure, as the tested samples) and A_3 the absorbance of the tested sample. All experiments were performed in duplicate and presented results are mean values ± standard deviation.

3. Results

3.1. Physicochemical surface properties of cells and magnetic beads

The dependence of the average zeta potential (ZP) of algae on the pH of the symmetrical model electrolyte (10 mM KCl) is shown in Fig. 1. On the one hand, the electrophoretic mobility data indicates that the surface of *C. vulgaris* (CV) was electronegative in the whole range of studied pH values. On the other hand, the magnetic beads (MBs) showed an ionex character that was dependent upon the functional group present on their surface (Fig. 1). Strong anion exchange groups (PEI) gave a high isoelectric point (pI) to the MBs ($pI_{PEI} = 9.0$) while the beads bearing the weak ionex group (DEAE) had a pI_{DEAE} equal to 6.3. However, all tested MBs showed a