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Harvesting microalgae with microwave synthesized magnetic microparticles

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HIGHLIGHTS

- ▶ Successful harvesting of microalgae with new magnetic agent.
- ▶ Iron oxide magnetic microparticles prepared solely from Fe(II) precursors.
- ▶ High separation efficiencies (up to 99%) achieved in a matter of minutes.
- ▶ Non-covalent electrostatic interactions have great influence upon separation.

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ABSTRACT

To make magnetic harvesting a more viable option, a suspension of inexpensive iron oxide magnetic microparticles (IOMMs) prepared by microwave treatment is presented as a new agent for separating *Chlorella vulgaris* from a highly diluted suspension. Separation efficiencies were tested under various conditions (model environment, cultivation media, different pH), revealing not only a dependency on the pH and amount of IOMMs, but also the influence of the ions present in the culture medium. Phosphorus ions were identified as the medium component interfering with algae–IOMMs interactions that are essential for magnetic cell separations in the culture medium. Phosphorus limited *C. vulgaris* cells were magnetically separated from the medium at separation efficiencies of over 95% at a 3:1 mass ratio of IOMMs to microalgae. A rapid and complete demagnetization of harvested algae was achieved by acidic treatment (10 vol.% H_2SO_4) at 40 °C under the influence of ultrasound.

1. Introduction

Microalgae have received attention of the scientific community due to their biotechnological potential. Lipids and carbohydrates for biofuels, ω-3 fatty acids, proteins, pigments, food supplements or animal feed are only a few examples of their wide usability. Cost-effective, sustainable processing technologies of microalgal biomass are one of today's core challenges of algal biotechnologies, the harvesting being one of the main bottlenecks.

The cost of algae harvesting is usually high, since the cell concentrations in culture broth are generally low. The major strategies currently applied in the harvesting of microalgae include centrifugation, filtration, flocculation, sedimentation, and flotation (Chen et al., 2011; Christenson and Sims, 2011; Uduman et al., 2010). Among the numerous cell separation procedures for microalgae, magnetic nano- and microparticles draw an increasing attention in this field. Their application in bioseparation processes is characterized by biocompatibility, easy manipulation and regeneration, accompanied by the usage of simple devices and non-destructive nature of magnetic fields (Cerff et al., 2012; Lim et al., 2012; Prochazkova et al., 2012; Safarik et al., 2012; Safarik and Safarikova, 2009; Safarikova et al., 2008; Xu et al., 2011; Yavuz et al., 2009). Nevertheless, the application of large-scale magnetic harvesting of microalgae is yet to be optimized and several key factors clarified (e.g. the choice of an appropriate, cost-effective harvesting agent for the given strain under moderate/physiological conditions).

Generally, a microorganism tends to adhere to solid surfaces to minimize the free interfacial energy. In the course of algal adhesion to magnetic particles in an aqueous environment a whole range of interactions such as non-covalent Lifshitz van der Waals forces, electrostatic forces, and acid–base interactions have to be considered (Bos et al., 1999). Microalgae culture media can be divided into low ionic strength (<0.1 M), or high ionic strength environments (>0.1 M) (Bilanovic et al., 2009). In an aqueous environment surfaces tend to uphold various surface charges, which result in

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electric double layer formation and electrostatic interactions (EL), leading to attraction and/or repulsion, in between surfaces upon their encounter. The thickness of the electric double layer is strongly dependent on the ionic strength of the surrounding environment, thus determining the decay of EL with distance (van Oss, 2003). EL are prominent under low ionic strength conditions, thus they are likely to play an important role in the case of algal biomass harvesting with magnetic particles in freshwater culture media.

In this paper a novel contribution to the magnetic cell separation is presented by using non-toxic, inexpensive and easy to produce, high performance iron oxide magnetic microparticles (IOMMs) synthesized from Fe(II) precursors under microwave treatment. The IOMMs were tested as potential agents to harvest *Chlorella vulgaris* P12, an industrially attractive, freshwater microalgae strain that is fast-growing, highly efficient in starch production (precursor for bioethanol and biobutanol production), and tolerant to increased CO_2 concentrations (Branyikova et al., 2011; Douskova et al., 2009; Keffer and Kleinheinz, 2002). The goal of this work was to demonstrate the feasibility of harvesting microalgae by magnetic particles from culture media and to identify the main factors interfering with magnetic biomass separation.

2. Methods

2.1. Microorganism, cultivation and preparation of algal suspension

C. vulgaris Beijerinck strain P12 was obtained and maintained according to previously described procedures (Branyikova et al., 2011). Batch cultivation in the photobioreactor proceeded as reported in literature (Douskova et al., 2009), i.e. glass tubes were situated in a water bath (30 °C) under continuous illumination and feeding of a mixture of air 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): 1,100 $(NH_2)_2CO$, 238 KH_2PO_4, 204 $MgSO_4$. 7 H_2O, 40 $C_{10}H_{12}O_8N_2NaFe$, 88 $CaCl_2$, 0.832 H_3BO_3, 0.946 $CuSO_4$. 5 H_2O, 3.294 $MnCl_2$. 4 H_2O, 0.172 $(NH_4)_6Mo_7O_{24}$. 4 H_2O, 2.678 $ZnSO_4$. 7 H_2O, 0.616 $CoSO_4$. 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 ensuring linear growth a biomass concentration of 5 g/L was obtained. Subsequently, the microalgal cells were centrifuged and washed twice with distilled water (4000 rpm, 5 min.) and used to prepare algal suspensions of a defined concentration for subsequent magnetic cell separations and/or zeta potential measurements. Biomass concentrations were determined gravimetrically (Branyikova et al., 2011).

2.2. Synthesis of magnetic microparticles

The preparation of the iron oxide magnetic microparticle suspension (IOMMs) was performed in a similar way as described in Zheng et al. (2010). Shortly, 1 g of $FeSO_4$. 7 H_2O was dissolved in 100 mL of water in a 600–800 mL beaker and 1 M NaOH solution was added drop-wise under continuous stirring until the pH value 11–12 was reached. The volume of the solution was made up to 200 mL with water and the beaker was inserted in a regular kitchen microwave oven and heated at 700 W for 10 min. After treatment and cooling, the prepared magnetic material was washed several times with water to remove present ions and stored in water at room temperature. The concentration of IOMMs in suspension was determined gravimetrically.

2.3. Magnetic separation of microalgal biomass

Firstly, magnetic separation was tested in a defined model environment, where prepared microalgal suspensions (10 mL, 10 mM KCl, pH 4–12) of a defined concentration (DW = 0.3 g/L) were mixed (15 rpm, orbital mode, Hulamixer Sample Mixer, Invitrogen) with specific amounts of IOMMs for 10 min in plastic test tubes. After exposure to an external magnetic field (cylindrical NdFeB magnets, 25 × 10 mm, Neomag, Czech Republic) the formed IOMMs-microalgae aggregates settled within 1–2 min. The absorbance of the supernatant (3 mL) was then measured at 750 nm and the separation efficiency (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 before separation and A_1 the absorbance of the supernatant after the magnetic cell separation. Due to the small cell size of *C. vulgaris* and short separation time, the self-sedimentation of microalgae cells was neglected. Secondly, the effect of culture medium composition on magnetic separation efficiencies at a moderate pH value was tested. The same procedure was repeated as named above, only this time IOMMs contacted *C. vulgaris* cells (prewashed with distilled water after cultivation), which were suspended in mineral medium or mineral medium lacking the following components, respectively: the main source of sulfur ($MgSO_4$. 7 H_2O), nitrogen ($(NH_2)_2CO$), iron ($C_{10}H_{12}O_8N_2NaFe$), phosphorus (KH_2PO_4), calcium ($CaCl_2$) or microelements, with the pH value adjusted to 6.5. All experiments were performed in duplicate and presented results are mean values ± standard deviation.

2.4. Cell recovery

After removing the bulk liquid, magnetically labeled *Chlorella* cells were suspended in 10 vol.% H_2SO_4 (~1.9 mol/L H_2SO_4) in order to dissolve IOMMs and obtain clean cells. Three different modes of cell recovery were tested: (i) constant mixing at room temperature (15 rpm, orbital mode, Hulamixer Sample Mixer, Invitrogen); (ii) periodic manual agitation at 40 °C in water bath; (iii) constant agitation at 40 °C using an ultrasonic bath (SW3H, 280 W, Sono Swiss, Switzerland). Samples were analyzed at regular time intervals together with appropriate blanks, i.e. cell suspensions of a defined biomass concentration in 10 vol.% H_2SO_4 without IOMMs, after exposure to an external magnetic field (cylindrical NdFeB magnets, 25 × 10 mm, Neomag, Czech Republic) for several minutes. The absorbance of the supernatant (3 mL) was measured at 750 nm and the recovery efficiency (R, %) was calculated as follows: $R = (A_3/A_2) \times 100$, where A_2 is the absorbance of the appropriate blank and A_3 the absorbance of the tested sample. All experiments were performed in duplicate and presented results are mean values ± standard deviation.

2.5. Zeta potential measurements

The zeta potentials of *C. vulgaris* cells and IOMMs were measured at 25 °C using the Zetasizer Nano-ZS (Malvern, UK) and calculated according to the Smoluchowski equation. Suspensions containing *C. vulgaris* cells (50 mg/L) or IOMMs (11 mg/L) were tested in model environments (10 mM KCl, pH 2–12), mineral medium and mineral medium lacking the following components, respectively: the main source of sulfur ($MgSO_4$. 7 H_2O), nitrogen ($(NH_2)_2CO$), iron ($C_{10}H_{12}O_8N_2NaFe$), phosphorus (KH_2PO_4), calcium ($CaCl_2$) or microelements, with the pH value adjusted to 6.5. All samples were measured ten times. Presented results are mean values ± standard deviation.