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.

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.% H2SO4) at 40 °C under the influence of ultrasound.

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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
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 microalga strain that is fast-growing, highly efficient in starch production (precursor for bioethanol and biobutanol production), and tolerant to increased CO₂ 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% CO₂ (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 (NH₄)₂CO, 238 KH₂PO₄, 204 MgSO₄.7H₂O, 40 C₁₀H₁₂O₈N₂NaFe, 88 CaCl₂, 0.832 H₃BO₃, 0.946 CuSO₄.5H₂O, 3.294 MnCl₂.4H₂O, 0.172 (NH₄)₆Mo₇O₂₄.4H₂O, 2.678 ZnSO₄.7H₂O, 0.616 CoSO₄.7H₂O, and 0.0014 (NH₄)VO₃. The pH value was adjusted to 6.5–7.0 using 1 M KOH prior to inoculation from an inoculum mixture (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% CO₂ (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 (NH₄)₂CO, 238 KH₂PO₄, 204 MgSO₄.7H₂O, 40 C₁₀H₁₂O₈N₂NaFe, 88 CaCl₂, 0.832 H₃BO₃, 0.946 CuSO₄.5H₂O, 3.294 MnCl₂.4H₂O, 0.172 (NH₄)₆Mo₇O₂₄.4H₂O, 2.678 ZnSO₄.7H₂O, 0.616 CoSO₄.7H₂O, and 0.0014 (NH₄)VO₃. The pH value was adjusted to 6.5–7.0 using 1 M KOH prior to inoculation from an inoculum mixture (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 FeSO₄. 7 H₂O 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 (Branyikova et al., 2011).

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: \[ E = \left[ \frac{A_0 - A_1}{A_0} \right] \times 100 \] where A₀ is the initial absorbance of the microalgal suspension before separation and A₁ 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 (MgSO₄, 7 H₂O), nitrogen (NH₄)₂CO, iron (C₁₀H₁₂O₈N₂NaFe), phosphorus (KH₂PO₄), calcium (CaCl₂) 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.% H₂SO₄ (~1.9 mol/L H₂SO₄) 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.% H₂SO₄ 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: \[ R = \left( \frac{A_2}{A_1} \right) \times 100 \] where A₂ is the absorbance of the appropriate blank and A₁ 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 (MgSO₄, 7 H₂O), nitrogen (NH₄)₂CO, iron (C₁₀H₁₂O₈N₂NaFe), phosphorus (KH₂PO₄), calcium (CaCl₂) or microelements, with the pH value adjusted to 6.5. All samples were measured ten times. Presented results are mean values ± standard deviation.
3. Results and discussion

3.1. Magnetic microparticles

Iron oxide magnetic microparticles (IOMMs) tested in this work (consisting of nanoparticle-clusters with a broad size range, typically 0.15–20 μm, Fig. 1) can also be regarded as so called naked, uncoated magnetic particles, such as described by Xu et al. (2011). Nevertheless, the IOMMs have been prepared using a very simple and inexpensive procedure based on microwave treatment of Fe(OH)₂ for a short period of time (ferrous hydroxide formed from a very cheap precursor, namely ferrous sulfate, after hydroxide treatment). Such synthetic conditions are much simpler in comparison with regularly used approaches, where these hydrothermal methods of synthetic magnetic particles preparation employ both Fe(II) and Fe(III) precursors, hence the operation procedure is more complicated and the quality of the prepared particles is affected by the initial stoichiometric ratio of Fe²⁺/Fe³⁺. In addition, usually inert gases must be used to eliminate the oxygen in the reaction system in order to protect the magnetic precipitates. Such measures then lead to a complex synthesis and high operation costs (Zheng et al., 2010). In case of omitting the inert gas, an oxygen-resistant reagent must be present (Hong et al., 2008). Furthermore, the microwave method needs only a few seconds or minutes, whereas other reported procedures require hours or days to prepare a sufficient amount of magnetic particles (Zheng et al., 2010). In our laboratory it has been verified that a cheap technical ferrous sulfate (e.g. obtained from garden centers) can also be used for microwave synthesis and so the production costs of the magnetic iron oxides can be further lowered (data not shown). In a slightly modified form the proposed synthetic method can also be successfully applied for obtaining various so called magnetic biocomposites, i.e. biological material-IOMMs complexes (Safarik and Safarikova, 2011).

3.2. Harvesting in model environment

A model environment of 10 mM KCl was chosen for testing the sole effect of a wide pH range on magnetic harvesting of C. vulgaris. This solution had an ionic strength of freshwater microalgae culture media and simultaneously contained no ions (e.g. calcium, magnesium, phosphate) known to cause inorganic precipitate formation with subsequent cell flocculation at higher pH values (Uduman et al., 2010; Vandamme et al., 2012).

The effect of pH on separation efficiency of microalgae at two different IOMMs to microalgal mass ratios is depicted on Fig. 2A. The most effective cell harvesting was achieved at pH 4, while at higher pH values the separation efficiency significantly decreased. This pronounced effect of the model environment’s pH value on the separation efficiencies can be due to potential surface interactions of the naked (uncoated) IOMMs, which consist mainly of magnetite (Fe₃O₄). In acidic environment Fe₃O₄ can undergo certain surface reactions, transforming thus into maghemite and releasing ferrous ions (Sun et al., 1998). Subsequently, Fe²⁺ ions can potentially oxidize into Fe³⁺ ions, which can act as flocculants. Sun et al. (1998) described the solubility of magnetite (2 g/L solid concentration) at pH 4.5 as a function of equilibrium time, where an equilibrium was reached after 20 days of conditioning (final Fe ions concentration 1.4 10⁻⁴ M). Under the model conditions (10 mM KCl, pH 4; the mixing time 10 min; IOMMs concentration 0.9–2.4 g/L), an equilibrium cannot be expected. In order to verify the effect of Fe⁴⁺ ions (potentially released by surface reactions of IOMMs) on microalgal cells, the initial Fe ion concentration of the equilibrium experiments reported by Sun et al. (1998) was chosen, i.e. 0.4 10⁻⁴ M. Thus, cells were contacted with an appropriate amount of FeCl₃·6H₂O in 10 mM KCl of pH value 4 and subjected to the same standard procedure as in the case of magnetic separation. It was observed that such an amount of Fe⁴⁺ ions does contribute to microalgal floc formation and results in a separation efficiency of 62% after 5 min of sedimentation (data not shown). Nevertheless, the formed flocs were very fragile and broke upon agitation. In the case of IOMMs the formed aggregates were robust, compact and concentrated in one place when an external magnetic field was applied.

As shown in Fig. 2B the harvesting efficiency of algae in model environment is dependent also on the IOMMs to biomass ratio. The mass ratio of IOMMs to microalgal biomass equal to 0.4:1 (g/g) was sufficient to achieve a separation efficiency of 95%. Although different media compositions and microalgal species were used,
this is in accordance with literature (Cerff et al., 2012; Xu et al., 2011).

In order to further understand the impact of pH on IOMMs–microalgae interaction, zeta potential measurements of the involved particles was carried out (Fig. 3). It was found that C. vulgaris cells maintain predominantly a negative surface charge over a wide pH range, as previously already reported (Hadjoudja et al., 2010; Henderson et al., 2008; Xu et al., 2011). Simultaneously the micro-wave synthesized IOMMs were positively charged under acidic conditions with an isoelectric point at pH 6.2 (Fig. 3). These findings confirm the ion exchange character of (naked) iron oxide particles (Gregory et al., 1988; Hencl et al., 1995; Sun et al., 1998; Xu et al., 2011). Attachment of IOMMs to microalgal cells is favorable at pH values below the magnetic particle’s point of zero charge, ensuring a strong electrostatic attraction and subsequently high separation efficiencies. The most pronounced difference between zeta potentials of the interacting cells and IOMMs occurred in model environment at pH 4 (67 mV), which results in the strongest electrostatic interactions and so contributed to the highest separation efficiencies compared to other pH values (Fig. 2A). Thus, it can be concluded that under acidic pH two phenomena can contribute to high separation efficiencies: (i) pronounced differences in zeta potential values of the interacting particles, (ii) Fe ions that can be released from the IOMMs surface and act as flocculating agents.

The magnetic separation phenomenon based on different surface charges was also described in the case of bacteria (MacRae and Evans, 1983). Nevertheless, attachment of IOMMs to microalgae occurred also at higher pH values (Fig. 2A) even though the surface charges of both cells and IOMMs were negative (Fig. 3). According to Bos et al. (1999) it should not be a priori assumed that the electrostatic interaction is repulsive, simply because the zeta potentials of the interacting surfaces are both negative. At the molecular level microbial cell surfaces may have positively charged domains mediating attachment through local electrostatic attraction despite overall repulsion.

### 3.3. Harvesting in culture medium

Culture medium composition greatly effects the formation of IOMMs-microalgae aggregates and the amount of magnetic agent needed for efficient biomass separation (Fig. 4). The highest separation efficiencies were achieved when excluding the main phosphorus source (KH₂PO₄) from the mineral medium (pH 6.5). Separation efficiencies close to 100% were achieved at a mass ratio of IOMMs to microalgae biomass equal to 3:1 (Fig. 4), which is almost ten times higher when compared to separations under optimal model conditions (Fig. 2A). However, Cerff et al. (2012) achieved the same ratio (i.e. 3:1) in the case of separating C. vulgaris cells from IGV-medium at pH 8 and 90% separation efficiency using hydrophilic silica-coated magnetic particles MagSilica 50–85 (5 μm diameter). The authors performed the same experiments also in TAP-medium, obtaining 20% separation efficiencies at a mass ratio of MagSilica to microalgal biomass equal to 1:6:1 at pH 8. On the other hand, Chlamydomonas reinhardtii was separated at 90% separation efficiency from TAP-medium (pH 8) at ratios close to 0.8:1 (g/g). Nevertheless, the authors stress the negative effect of high pH on cell viability. Xu et al. (2011) applied synthetically prepared, naked magnetic particles to harvest Chlorella ellipsoidea and Botryococcus braunii from modified Chu 13 medium. The authors achieved separation efficiencies close to 90% for C. ellipsoidea at a mass ratio of magnetic particles to microalgal biomass equal to 0.38:1 (g/g) at a wide pH range (4–9). In the case of B. braunii the highest separation efficiencies (close to 100%) were at acidic pH values (4–7) with a ratio equal to 0.02:1 (g/g). The given differences in the results could be caused by the use of different mixing systems. In our case, the tests were performed in test tubes at 15 rpm, whereas Xu et al. (2011) contacted the microalgae with the magnetic particles at 120 rpm in Erlenmeyer flasks. Furthermore, the tested culture conditions (media) and microalgae species were different, which can result in different surface properties of the given microalgae as demonstrated in the case of Microcystis aeruginosa and C. vulgaris (Hadjoudja et al., 2010). Simultaneously, different magnetic agents (prepared by different synthetic procedures) were applied, thus also contributing to different surface interactions.

Zeta potential measurements (Fig. 5) shed some light into the observed phenomena. Microalgae C. vulgaris maintained a negative surface charge in all media, but the surface charge of magnetic particles was found to be dependent on the presence/absence of certain ions. When excluding KH₂PO₄ from the culture medium, IOMMs had a positive charge and therefore were able to interact with negatively charged cells (Fig. 5). However, the difference between the zeta potential of algae and magnetic particles (15 mV) was lower than in the case of model environment at pH 4 (67 mV). This led to weaker electrostatic interactions and higher IOMMs consumption. As K⁺ does not affect the separation procedure, as can be seen from experiments in model environment (KCl), the medium component interfering with cell–IOMMs interaction must be the negatively charged phosphate ions. It can be assumed that these bind to positively charged locations on IOMMs, thus blocking the potential binding sites for C. vulgaris cells. This is in accordance with other findings, where iron oxides serve as a very effective absorbent of phosphate ions in wastewater.

![Fig. 3. Zeta potentials of iron oxide magnetic microparticles (IOMMs) and C. vulgaris cells (CV) as a function of pH. The measurements were carried out in model environments (10 mM KCl, pH 2–12).](image-url)
treatment (Chittrakar et al., 2006; Kang et al., 2003). Therefore, the absence of competing ions is crucial for effective magnetic harvesting of microalgal biomass. This can be beneficial in the case of nutrient limited cultivations such as phosphorus-deprivation induced starch accumulation in C. vulgaris (Branyikova et al., 2011). Good timing of the harvesting is therefore essential and a balance between maximum productivity and separation efficiency at the moment of the depletion of interfering ions has to be carefully considered.

The obtained results confirm that the presence of specific ion types contribute significantly to the adhesion between microalgae and IOMMs. This has also been observed when Escherichia coli cells were harvested with magnetite (MacRae and Evans, 1983). Similar phenomena are observed also in the initial phase of the microbial biofilm formation, when a so called conditioning film is formed on abiotic surfaces upon submersion into an aqueous environment (Shi and Zhu, 2009), which influences the subsequent cell adhesion (Bos et al., 1999).

The applications of inexpensive, non-toxic IOMMs in microalgal biotechnology are far from being exhausted. For example, the formation of IOMMs-microalgae aggregates is likely of a non-selective (non-covalent) nature, so the proposed harvesting procedure can be successful for any other microalgal (microbial) species displaying similar cell surface properties as presented by C. vulgaris cells, being beneficial not only for cell separation of industrially important microalgae strains but also in the case of removing harmful microalgae (e.g. algae blooms, eutrophication). In the case of cells displaying different surface properties, the combination of IOMMs with bio-flocculation (Salim et al., 2010) could be an option as the potential to magnetize biological materials using microwave irradiation has also been successfully recognized (Safarik and Safarikova, 2011). In addition, IOMMs could also be combined with other harvesting agents that are environmentally friendly, e.g. cationic starch (Vandamme et al., 2010) or chitosan (Divakaran and Sivasankara Pillai, 2002), and by doing so, a significant decrease in the amounts of needed IOMMs can be expected.

3.4. Cell recovery

Magnetic separation of microalgal cells results in an intensive attachment of IOMMs to cell surface structures. Further processing of magnetically separated algae is dependent mainly on the applied downstream processes and on the character of the desired end product. In the case when the target compound is intracellular (e.g. algal starch of C. vulgaris) and the presence of IOMMs is not interfering with the processing technology, the treatment steps can be adjusted to the presence of magnetic agents. If magnetic microparticles remain attached to the cell walls, after e.g. cell disintegration and starch hydrolysis, they can serve as a means for the easy disposal of cell debris, thus leaving a clean supernatant rich in fermentable sugars for further procedures. In addition, the magnetic cell debris could be used as a low-cost, efficient adsorbent for removal of xenobiotics (Patzak et al., 1997; Safarikova et al., 2008). However, in situations when pure algal cell population free of IOMMs is required, the IOMMs have to be dissolved with acids under appropriate conditions. When applying 10 vol.% H2SO4 and mixing at room temperature, the cell recovery was very poor (Fig. 6). Upon introducing more energy into the system, i.e. heating (40 °C) or ultrasound + heating (40 °C), the recovery of demagnetized microalgae was close to 100% within 180 and 60 min, respectively (Fig. 6). The obtained cells could then be filtered as proposed by Xu et al. (2011). Nevertheless, it must be highlighted that the application of acids may not be suitable for all microalgal species and target compounds (Xu et al., 2011).

4. Conclusions

Harvesting C. vulgaris by iron oxide magnetic microparticles (IOMMs) prepared from Fe(II) precursors by microwave treatment was successful both in model environment and culture medium, after identifying the interfering effect of phosphorus ions. The efficiency of IOMMs-harvesting is comparable with other magnetic microalgae separation procedures. From the highly diluted culture broth, thick magnetized microalgal slurry was obtained within minutes, while a complete demagnetization of cells was achieved within one hour, if necessary. The results are in accordance with literature, emphasizing the role of electrostatic interactions. Consequently, the separation efficiencies depend upon culture medium composition and microalgae species.

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