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Magnetic Fe_3O_4-polyethyleneimine nanocomposites for efficient harvesting of *Chlorella zofingiensis, Chlorella vulgaris, Chlorella sorokiniana, Chlorella ellipsoidea and Botryococcus braunii*

Kristína Gerulová[a], Alica Bartošová[a,*], Lenka Blinová[a], Katarína Bártová[b], Mária Dománková[b], Zuzana Garaiová[c], Marián Palcut[b]

[a] Slovak University of Technology in Bratislava, Faculty of Materials Science and Technology in Trnava, Institute of Integrated Safety, Department of Environmental Engineering, Botanická 49, 917 24 Trnava, Slovakia
[b] Slovak University of Technology in Bratislava, Faculty of Materials Science and Technology in Trnava, Institute of Materials Science, J. Bottu 25, 917 24 Trnava, Slovakia
[c] Comenius University in Bratislava, Faculty of Mathematics, Physics and Informatics, Department of Nuclear physics and Biophysics, Mlynská dolina, 842 15 Bratislava, Slovakia

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ABSTRACT

A reduction in energy consumption at every stage of the down streaming process is needed to make 3rd generation of biofuels economically viable. In this study, magnetite (Fe_3O_4) nanoparticles were synthetized by coprecipitation of $FeCl_2$ and $FeCl_3$ in alkaline medium at two different temperatures. The particle sizes were 11.5 ± 4 and 9.5 ± 4 nm for the nanoparticles prepared at 80 °C and 25 °C, respectively. The adsorption of polyethyleneimine (PEI) onto Fe_3O_4 was studied by equilibrium batch measurements. A mono-layer absorption of PEI was found. The Fe_3O_4–PEI nanocomposites had a positive zeta potential, which decreased with increasing pH of the solution. The nanocomposites were used for magnetic harvesting of negatively charged *Chlorella zofingiensis*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Chlorella ellipsoidea* and *Botryococcus braunii* microalgae strains. Upon dosage of 200 mg/L of Fe_3O_4–PEI harvesting efficiencies of 68–97% were achieved at pH 4 within 1 min. The harvesting efficiency decreased with increasing pH of the suspension. The results demonstrate that Fe_3O_4 nanoparticles synthesized at the lower temperature (25 °C) could be used for an efficient magnetic harvesting of different microalgae strains. The lower synthesis temperature may thereby contribute to the cost reduction of microalgae harvesting.

1. Introduction

Algae are promising biomass resources as these photosynthetic organisms have a high growth rate, can be easily cultivated and accumulate large amounts of lipids compared to other feedstocks. One of the major obstacles in wider use, however, is the difficulty of their harvesting. The microalgae-based biodiesel production consists of 4 different stages: cultivation, harvesting, lipid extraction and lipid-to-biodiesel conversion. The harvesting step itself accounts for more than one quarter of the total biodiesel production cost [23,30].

The selection of the harvesting technique is given by the density and size of the microalgae and by the properties of desired products [19]. Current harvesting methods include chemical, mechanical and biological based methods [5,11]. In chemical harvesting, high doses of flocculants are required, which may affect the quality of by-products and pose further challenges to downstream processing in fuel production [19,22]. Furthermore, the chemicals may also contaminate the product [5]. Mechanical methods such as centrifugation and filtering are energy intensive and require a large capital outlay. Filters are prone to clog and foul and high costs of membrane replacement and pumping are major drawbacks [22,29]. Therefore, other harvesting methods are currently being explored.

Some novel low-cost harvesting methods include the utilization of non-sacrificial carbon electrodes [19], utilization of different types of carriers in traditional suspended culturing strategies [29], algal based biofilm technologies [1,6,20] and the separation after algae adsorption onto nanoparticles by magnetic field.

Magnetic harvesting has a low energy consumption, simple operation and low cost. In recent years, magnetite (Fe_3O_4) nanoparticles have been frequently used in harvesting. Naked magnetite NPs were studied

* Corresponding author.
E-mail address: alica.bartosova@stuba.sk (A. Bartošová).

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by a number of authors (e.g., [8,9,15,16,24,25,28]). For *Botryococcus braunii* and *Chlorella ellipsoidea*, the maximal recovery efficiency reached > 98% within 1 min at a mixing rate of 120 rpm [28]. There have also been many studies reporting the miscellaneous coatings of magnetic nanoparticles (MNP), as the coatings may prevent agglomeration. Fe_3O_4 coated with polyamidoamine or aminoacid [27], polydiallyldimethylammonium chloride (PDDA, [10,17,24]), cationic polyacrylamide [26], silica-coated Fe_3O_4 [4] and chitosan coated magnetite [14,24,25] have been investigated. In these studies, the removal efficiency was found to be in the range of 95–99%. Furthermore, the microalgal recovery was found to be strongly pH-dependent and varied with nanoparticle dose and algae species.

Fe_3O_4 nanoparticles functionally coated with polyethylenimine (PEI) have been studied by [7]. The authors studied a single microalgae strain, *C. ellipsoidea*. Using the nanocomposite dosage of 20 mg/ L a harvesting efficiency of 97% was achieved within 2 min. Polyethyleneimine (PEI) is an amine-based cationic polymer with good water solubility. It has a high number of functional groups, suitable molecular weight, and good physical stability and chemical compatibility [12,13]. The ratio of primary to secondary and tertiary amines is 1:2:1. In each PEI molecule, one nitrogen atom is protonated per two carbon atoms. Due to different pKa values of the primary, secondary and tertiary amino groups, PEI has the ability to capture protons at different pH, which is known as the “proton sponge” mechanism [18]. $-NH_2$ groups can easily react with −COOH and −OH groups of the microalgal cell surface, allowing functional magnetic particles with an amine-rich structure to assist with the microalgal recovery [7].

The main purpose of this work was to evaluate harvesting efficiencies of five different microalgae strains with magnetite nanocomposites (Fe_3O_4–PEI). The nanocomposites have been synthetized at low temperature (25 °C) compared to previously used 80 °C in order to reduce the total energy costs needed for harvesting.

2. Materials and methods

2.1. Synthesis of Fe_3O_4–PEI nanocomposites

The magnetite nanoparticles were synthesized by a previously reported method with some modification [3]. The most important difference was the temperature decrease from 80 °C to room temperature (25 °C). For one dose of Fe_3O_4 NPs 1.98 g $FeCl_2.4H_2O$ and 5.4 g $FeCl_3.6H_2O$ were dissolved in 200 mL distilled water and vigorously stirred in a three-neck flask vessel in a nitrogen atmosphere. Before the experiment the distilled water was deoxygenated by flowing nitrogen for 20–30 min. After the drop wise addition of 20 mL NH_4OH (25 wt%) and continuous stirring for 30 min magnetite was precipitated in the solution. After 1 h of sedimentation, the precipitate was three times washed with distilled water. The decantation process was aided by a neodymium magnet. One dose of decanted NPs was mixed with 250 mL of phosphate buffer (pH 7.3). After that 15 mL of PEI solution (MW 1.2 kDa, Sigma Aldrich) was added. The mixture was stirred for 1 h at laboratory temperature (25 °C). The prepared NCs were three times washed with distilled water and the decantation process was again aided by the use of a neodymium magnet. The nanocomposites were stored in distilled water for further use in a sealed glass bottle. All chemicals were analytical grade and used without prior purification.

2.2. Microalgae source and cultivation

Chlorella zofingiensis (SAG 211–14), *Chlorella sorokiniana* (SAG 211–32), *Chlorella ellipsoidea* (SAG 2111), *Chlorella vulgaris* (SAG 211-11b), and *Botryococcus braunii* (30.81) were obtained from the *Sammlung von Algenkulturen der Universität Göttingen* (Culture Collection of Algae at Göttingen University, international acronym SAG). The sterile cultivation was realized in 250 mL Erlenmeyer flasks with a Basal medium. For the experiments, the strains were cultivated in a BG-11 liquid medium. The cultures were illuminated at 35 μmol m^{-2} s^{-1} on a light/dark cycle of 16 h/8 h at 27 °C ± 1 °C.

2.3. Analytical methods

To measure the dry cell weight (DCW), the microalgal samples were diluted to various concentrations and subsequently the light absorbance was measured at 680 nm. The samples were then collected by centrifugation. After washing three times with distilled water, the samples were dried at 105 °C in pre-dried and pre-weighted evaporated dishes to a constant weight. The biomass concentration of samples (DCW in g L^{-1}) was calculated from the linear relationship between the optical densities (OD) and DCW. The optical densities were measured at 680 nm with UV-VIS spectrophotometer (Genesys 8) in a 2 cm cuvette, against a blank. PEI was assessed by the total organic carbon (TOC) measurement, utilizing a TOCV-CPN analyser (Shimadzu). The amount of PEI adsorbed onto magnetite nanoparticles (the sole source of organic carbon in the solution) was calculated as the difference between the starting and final concentration of PEI in the solution. Zeta potential of studied algae species and Fe_3O_4 uncoated/PEI coated nanoparticles was measured with a Zetasizer Nano ZS90 (Malvern, UK). As working buffer, Na-phosphate buffer was used (10 mM; NaH_2PO_4 and Na_2HPO_4) at pH (4–9), adjusted by adding small aliquots of 1 M HCl/NaOH. The specimens were prepared by dispersing 2 μL of magnetic nanoparticle stock solution into 998 μL of working buffer and 100 μL of algae stock solution into 900 μL of working buffer. Samples were measured in a clear disposable folded capillary zeta cell (DTS1070; Malvern, UK) at temperature 25 °C. The mean values were calculated as an average of three measurements following automatic measurement duration of 10–30 runs. The microstructure and particle size distribution of the nanocomposites were studied by high resolution transmission electron microscope Philips CM300 (TEM). The samples for TEM were prepared by placing a drop of the aqueous solution onto a carbon layer-covered copper grid and air dried. The particle size measurement was realized by ImageJ, a Java-based image processing program developed at the National Institutes of Health.

2.4. Algae harvesting

The harvesting was performed with two-weeks-old algae. The experiments were carried out in 250 mL glass beakers. The glass breakers were filled with 50 mL of algae suspension with adjusted pH. A specific amount of magnetite nanocomposites was added and the mixture was stirred for 60 s. After mixing the algal cell attached to magnetite, the magnetic nanocomposites were allowed to settle down by placing the glass beaker on the NdFeB permanent block magnet (permanent magnetisation 1.22–1.30 T, Magsy Ltd.). The concentration of the algae before and after harvesting was calculated from the experimental optical densities at 680 nm. The supernatant was carefully removed by pipetting while the glass beaker was kept on the permanent magnet. The harvesting efficiency was calculated according to the following equation:

$$R = \frac{(C_0 - C_e)}{C_0} \times 100\ [\%] \quad (1)$$

In this Eq. C_0 is the initial concentration of the algae suspension (g L^{-1}) and C_e is the concentration of algae in the supernatant after harvesting (g L^{-1}).

2.5. Adsorption isotherm experiments

The adsorption study of nanocomposites on microalgae (or PEI onto naked nanoparticles) was carried out by batch equilibrium experiments, in triplicate. A known mass of nanocomposite adsorbent (or naked nanoparticles) was suspended in 50 mL of algae solution (or PEI solution) of different initial concentration in stoppered 150 mL flasks and