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Effect of voltage and electrode material on electroflocculation of *Scenedesmus acuminatus*

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Abstract

Background: Microalgae are a promising new source for biomass production. One of the major challenges in regards to cost effectiveness is the biomass harvest. High energy input is required for the separation of the small algal cells from a large volume of surrounding media. Electroflocculation is reported as a promising harvesting technique to improve cost effectiveness within the downstream process. In the present study, six electrode materials were tested for electroflocculation of *Scenedesmus acuminatus*. Besides the commonly used aluminum and iron electrodes, magnesium, copper, zinc and brass electrodes were tested for biomass harvest and compared. The influence of four different voltages (10, 20, 30 and 40 V) was investigated and evaluated.

Results: Electroflocculation was applicable with all tested electrode materials. The highest flocculation efficiency was achieved using magnesium electrodes followed by Al, Zn, Cu, Fe and brass. Using magnesium, 90% of the suspension was clarified at 40, 30, 20, and 10 V after 9.2, 12.5, 18.5, and 43 min, respectively. All electrode materials showed the fastest flocculation at 40 V and the lowest at 10 V. The pH increased from 7.5 to values between 9.3 and 11.9 during the flocculation processes. Reuse of the supernatant showed no adverse effect on algal growth. The highest cell counts after 12 days of incubation were achieved with iron at 1.86×10^7 cells ml^{-1} and the lowest with copper at 1.23×10^7 cells ml^{-1}.

Conclusion: Besides the commonly used iron and/or aluminum electrodes, other materials like magnesium, copper, zinc and brass can be successfully used for microalgal biomass harvest. For special biomass applications like food or feed additives, metals like magnesium have other advantages besides their high flocculation efficiency such as their low toxicity at high concentrations. Higher voltages increased the maximum flocculation efficiency but also increased the required energy input.

Keywords: Electroflocculation, Microalgae, Biomass harvest, *Scenedesmus acuminatus*, Flocculation

Background

Microalgae are eukaryotic, photosynthetic microorganisms that convert sunlight into chemical energy. The produced biomass can be used as food, feedstock or as potential substrate for biofuel production (Chisti 2007; Mata et al. 2010). They show a broad application in biotechnology since they grow fast at low nutritional and environmental requirements (Chisti 2007; Mallick 2002; Mata et al. 2010; Wang et al. 2008). Although they are easy to cultivate, the bottleneck which often makes microalgal cultivation uneconomical is the downstream processing which contributes to 20–30% of the biomass production costs (Grima et al. 2003; Mata et al. 2010; Uduman et al. 2010). Separation of the cells (2–10 μm) from the surrounding growth media requires high energy inputs (Grima et al. 2003). Large volumes must be processed, since the concentration of cells is very low at around 0.5–2.5 g l^{-1}. Common separation processes combine filtration or flotation with a final centrifugation step (Grima et al. 2003; Uduman et al. 2011), often exceeding the energy content of the harvested biomass

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(Wijffels and Barbosa 2010). Increasing the efficiency at low energy demands within the harvesting process is a major challenge. By flocculation, the cells coagulate and larger particles are produced with a higher settling velocity.

Several studies are available on the use of chemical flocculation using metal salts or polyelectrolytes (Gerde et al. 2014; Granados et al. 2012; Papazi et al. 2010; Tenney et al. 1969), pH induced flocculation (Vandamme et al. 2012; Wu et al. 2012; Zheng et al. 2012), and bioflocculation using bacteria or filamentous fungi for biomass harvest (Zhou et al. 2013). In electroflocculation, the flocculant is produced by releasing metal ions from a sacrificial electrode (Vandamme et al. 2011). Numerous studies have been published on the use of electroflocculation for algal biomass harvest (Lee et al. 2013; Uduman et al. 2011; Vandamme et al. 2011); however, these experiments used primarily aluminum electrodes (Kim et al. 2012; Lee et al. 2013; Vandamme et al. 2011; Xu et al. 2010) and/or iron electrodes (Uduman et al. 2011; Vandamme et al. 2011). Very little information can be found on the use of other electrode materials like Mg, Zn, Cu or brass for electroflocculation of microalgae.

The microalgae *Scenedesmus* is one of the most common genera found in freshwater ecosystems. These polymorphic chlorophytes show a broad application in wastewater treatment (Chinnasamy et al. 2010; Hodaifa et al. 2008; Martinez et al. 2000), biodiesel production (Mandal and Mallick 2009; Tang et al. 2011) and in the production of high value pigments like lutein (Sanchez et al. 2008). Flocculation of *Scenedesmus* by metal salts, bioflocculants, and cationic polymers has been tested in prior experiments, but little research has been done on electroflocculation of *Scenedesmus* (Mallick 2002; Uduman et al. 2010; Vandamme et al. 2011, 2012; Wang et al. 2008).

In the present study, six electrode materials were tested at four different voltages. It was verified whether metals other than aluminum and iron are suitable for the electroflocculation process (H1) and if the flocculation efficiency increases at higher voltages applied (H2).

Methods

Culture media and microalgae cultivation

All experiments were carried out using the freshwater chlorophyte *Scenedesmus acuminatus* from the culture collection of the University of Applied Sciences Bremen. The cells were grown in 2 l of liquid Wuxal medium (WM) (Winckelmann et al. 2015). Aeration was provided by compressed air, which was introduced into the culture by aeration hoses. Light was emitted by fluorescent lamps (OSRAM L 30W, warm white) placed in front and behind the algae cultures for 24 h per day.

Electroflocculation

The flocculation experiments were carried out at room temperature in 100 ml beakers filled with 90 ml of algae suspension. The initial cell concentration of the algae was 1×10^7 cells ml^{-1}. The electrode plates were cut from commercial grade metal sheets. Before use, they were mechanically polished using abrasive paper and placed parallel and vertically into the algae suspension. The distance between the cathode and anode was 2.5 cm and the depth of immersion was 4 cm resulting in a submerged area of 51.2 cm^2 (40 mm × 32 mm × 4 electrode sides) for both electrodes. All experiments were carried out with the same area immersed in water and the same electrode distance. During the flocculation process, the culture was gently stirred at 100 rpm using a magnetic stirrer.

The electrodes were connected to a DC power supply (Hewlett Packard, Model 6205b, USA) and the voltage was adjusted to 10, 20, 30 or 40 V.

To determine the flocculation efficiency (FE), 1 ml samples were taken before the flocculation and every 2.5 min during the process. Samples were taken from 3 cm below the surface. Optical densities for each sample were measured at a wavelength of 750 nm in a Genesys 20 (Thermo scientific, Walthman, USA) photometer and pH values were recorded.

FE was calculated as follows:

$$Flocculation\ efficiency = \left(\frac{OD_{t0} - OD_{t1}}{OD_{t0}}\right) \times 100$$

OD_{t0} is the initial optical density before starting the flocculation and OD_{t1} is the optical density of the sample at a certain point of time during the process.

After the flocculation, the biomass and supernatant were separated and kept at −20°C for recycling experiments. Before and after each flocculation, the electrodes were dried at 60°C for 2 h.

Iron, magnesium, aluminum, zinc, copper and brass electrodes were tested and compared. Each material served as both the anode and cathode at the same time. For comparison, a continuous function of the flocculation efficiency depending on the flocculation time, an interpolation, was used based on the following assumption:

The flocculation process can be described by a sigmoid function with an upper limit of 100%, leading to the following function:

$$Efficiency(t) = \left(\frac{100}{1 + a^{b-t}}\right)$$

The coefficients a and b were determined for each voltage and electrode material, respectively, using a numerical curve fitting tool. By determining the coefficients a and b, it was possible to solve the efficiency function for the time when the curve reached values of 90%. Those values were used in order to compare the various flocculation experiments.

The influence of each electrode material was tested and the best material regarding FE at 90% was determined.

The mass loss as a function of time was assumed to be linear. This assumption allowed calculating the amount of mass lost during the time until a flocculation efficiency of 90% was reached. This mass was multiplied by the exchange prices of the corresponding electrode materials in order to estimate the costs of each flocculation process (London metal exchange 2015).

Media reuse after flocculation

A batch-recycling experiment was conducted over 12 days in 100 ml Erlenmeyer flasks, filled with 50 ml of algae cells incubated in flocculation supernatant. Cell growth was monitored every other day by optical density and cell count measurements. Cell number was determined using the a Thoma counting chamber (Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany) and adjusted to 2×10^6 cells ml^{-1} by the addition of concentrated cells to the cell-free supernatant. For positive control, the cells were diluted in fresh Wuxal Media. All experiments were carried out in triplicate.

Results and discussion

Iron, magnesium, aluminum, zinc, copper and brass electrodes were tested and their corresponding FEs compared. Figure 1 shows an example setup of the flocculation experiment. The electrodes were connected to a DC power supply (Hewlett Packard, Model 6205b, USA) and the voltage was adjusted to 10, 20, 30 or 40 V depending on the recent experiment (a, b). During the flocculation process, the suspension became milky white and gas visibly formed at the electrodes resulting in foam formation on the top of the liquid surface (c, d). The flocculation was successful when the liquid phase was clear and algal cells were located in the foam on top (d).

In Fig. 2, the performance (FE) of the different electrode materials at different voltages over time is shown. All graphs show the fastest flocculation at 40 V and the slowest at 10 V; the higher the applied voltage, the faster the maximal flocculation efficiency. Similar results were published by other authors (Alfafara et al. 2002; Poelman et al. 1997; Vandamme et al. 2011; Xu et al. 2010; Zhang et al. 2015).

The highest FE was observed when using Mg electrodes (Fig. 2a). Ninety percent of the suspension was clarified at 40 V after 9.2 min (Table 1). When the voltage was decreased to 30 V, the graph leveled slightly, and the recovery time increased. At 30 V, a FE of 90% is reached after 12.5 min, at 20 V after 18.5 min and at 10 V only after 43 min (Table 1).

During the EF process, metal ions are continuously released from the anode by electrolytic oxidation. In H_2O these ions immediately react and form metal hydroxides. These metal hydroxides represent the flocculant which reacts with the algae cells. For the magnesium electrodes, the following reaction occurs:

$$\mathrm{Mg} \rightarrow \mathrm{Mg}^{2+} + 2\mathrm{e}^- \quad 2\mathrm{H_2O} \rightarrow 4\mathrm{H}^+ + \mathrm{O_2} + 4\mathrm{e}^- \quad \text{(Anode)}$$

$$\mathrm{M(aq)\ n}^+ + \mathrm{ne}^- \rightarrow \mathrm{M(s)} \quad 2\mathrm{H_2O} + 2\mathrm{e} \rightarrow^- \mathrm{H_2} + 2\mathrm{OH}^- \quad \text{(Cathode)}$$

At higher applied voltages, more ions are produced, and consequently more flocculant is available in the algal solution (Mollah et al. 2001, 2004). The positively charged metal hydroxides react with the negatively charged cell surface of the microalgae. The potential of the algal cell

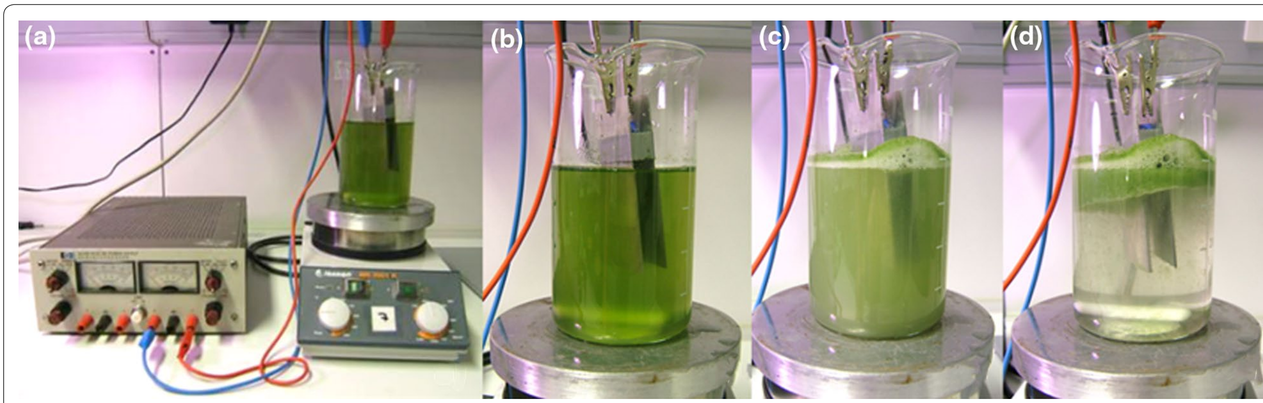

Fig. 1 Electroflocculation process. **a** Experimental setup: The flocculation experiments were carried out in 100-ml beakers filled with 90 ml of algae suspension. The electrodes were connected to a DC power supply (Hewlett Packard, Model 6205b, USA), **b** algae culture before flocculation, **c** during the flocculation process and **d** after the flocculation.

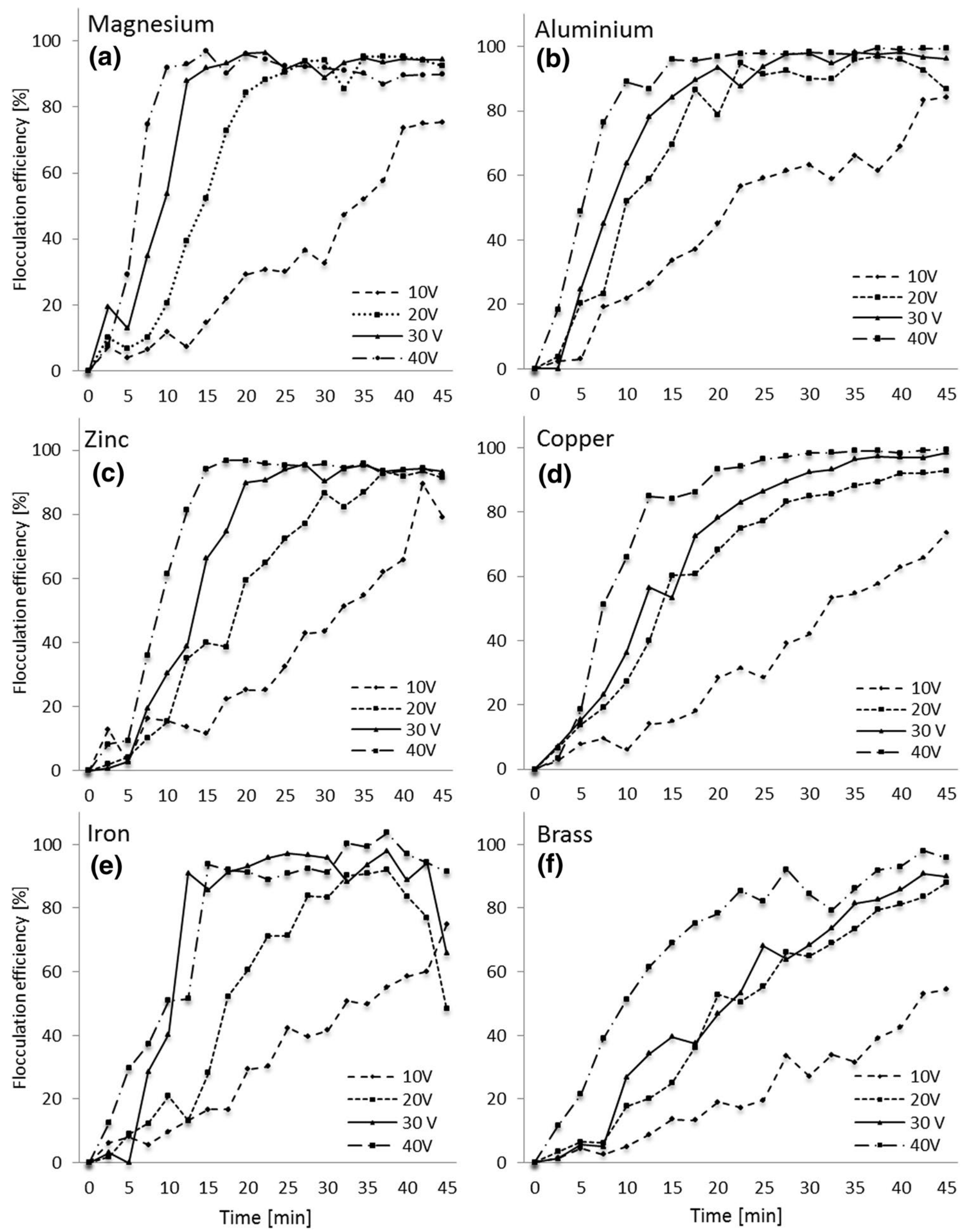

Fig. 2 Effect of electrode material and applied voltage on the recovery rate during the electroflocculation process. Flocculation experiments were conducted using different electrode materials: magnesium (**a**), aluminum (**b**), zinc (**c**), copper (**d**), iron (**e**) and brass (**f**). The graphs were arranged according to the calculated electrode efficiency from high (**a**) to low (**f**). The voltage was adjusted to 10, 20, 30 or 40 V and the flocculation efficiency was measured every 90 s.

Table 1 Calculated incubation time until a 90% flocculation efficiency is reached

	Magnesium	Aluminum	Zinc	Copper	Iron	Brass
10 V	43.2	42.1	53.6	61.0	53.7	71.1
20 V	18.5	20.6	30.6	31.3	27.9	43.9
30 V	12.5	11.4	21.7	20.3	16.0	40.7
40 V	7.3	9.0	14.2	14.6	46.9	30.9

The incubation time is given for each electrode material at 10, 20, 30 and 40 V.

is increased, and the surface charge is neutralized (Henderson et al. 2008). The suspension becomes destabilized, and flocs are formed. At 10 V a short lag phase could be detected in all experiments, which is assumed to be due to insufficient flocculent availability at the start the flocculation process. After 5–10 min, the values start to rise. The shortened lag phase present at higher voltages is due to the faster ion release from the electrodes. Magnesium is bivalent and therefore forms extra stable hydrogen bonds, resulting in an effective floc formation.

The contamination of the harvested biomass and the remaining media with metal particulates might interfere with further processing steps or the use of the biomass as food or feed additive. Here the use of magnesium shows another advantage. Accepted magnesium limits are higher when compared to aluminum, iron, copper, or brass. In the German Drinking Water Ordinance, for example the limits for magnesium and also for zinc were removed since a negative impact for human health was considered as very low, whereas concentrations of 0.2 mg l^{-1} for aluminum and iron and 2 mg l^{-1} for copper are not to be exceeded (Ordinance 2001).

Extensive research has been conducted regarding the use of aluminum electrodes (Chen et al. 2009; Kim et al. 2012; Lee et al. 2013; Vandamme et al. 2011; Xu et al. 2010) and iron electrodes (Uduman et al. 2011; Vandamme et al. 2011). Aluminum was found to have the second highest FE (Fig. 2b). Ninety percent FE was reached after 9, 11.4, 20.6 and 42 min at 40, 30, 20 and 10 V (Table 1). In literature the use of aluminum electrodes is compared to the use of iron electrodes in coagulation processes with (Vandamme et al. 2011) or without algae (Zongo et al. 2009) involved. The results of this present study agree with the data reported that the use of aluminum is more efficient than the use of iron electrodes (Fig. 2e). The lower efficiency of the iron electrode compared to aluminum might be explained by the lower current efficiency of the iron electrode (Zongo et al. 2009).

The lowest recovery efficiency was found using brass electrodes (Fig. 2f). After 15 min at 40 V only approximately 70% of the biomass was recovered. Ninety percent was reached after 30 min which is believed to have been caused by the high pH value during the process (Table 2). Figure 3a shows that after 11.5 min, a pH of 11.8 was observed. It is suspected that algal recovery efficiency decreases at pH values of 12 because of algal lysis (Contreras et al. 1981; Xu et al. 2010).

Electroflocculation technologies are currently in use at wastewater treatment facilities (Mollah et al. 2001). In industrial wastewater cleaning, the initial pH is one of the most important factors influencing the EF process (Mouedhen et al. 2008). In Fig. 3, the pH evolution during the EF with different electrode materials at 40 V is shown. Figure 3a shows the data for Al, Mg and Zn while Fig. 3b shows the values monitored with Fe, Cu and brass. In all of the experiments, the pH increased during the flocculation process.

A fast pH increase up to 10–12 was monitored in all samples except for Al (Fig. 3; Table 2). The highest pH value was reached during the flocculation using brass electrodes. The lowest pH was recorded in the samples using Al electrodes. Since the pH is a measure for the hydrogen and hydroxide concentration, it is expected that the produced metal hydroxides were immediately bound and thereby not contributing to pH increase. During the flocculation process, the algae recovery rate increased in the beginning but started to decrease after 20 min of flocculation (Fig. 2e). Similar effects were observed by other authors. Xu et al. (2010) described a decrease in recovery time and efficiency from pH 7 to 11 and an increase of recovery time at pH 12 which is expected to be because of algal lysis (Contreras et al. 1981; Xu et al. 2010). The fluctuations in the Al and Fe graphs might be due to insufficient mixing during sample taking.

Figure 4 shows the mass loss and the calculated material costs until a flocculation efficiency of 90% is reached. All tested electrodes lost weight between 1.1 g for magnesium and 101 g for iron. The highest mass loss was recorded for Fe with 101 g at 40 V and for brass at 20 and 30 V. Although the mass loss of the iron electrodes is high, the costs for the flocculation with iron are comparably low. Since brass is the most expensive electrode material showing the lowest FE, it cannot be recommended for algal harvest using electro flocculation. The most economical efficient flocculation was achieved with Mg, Al and Fe.

Table 2 pH values recorded after 30 min of electroflocculation

	Aluminum	Brass	Magnesium	Zinc	Copper	Iron
10 V	9.3	10.7	11.4	11.5	11.5	11.6
20 V	10.3	11.5	11.5	11.3	11.6	11.7
30 V	9.7	11.9	11.4	11.6	11.7	11.6
40 V	10.4	11.9	11.5	11.7	11.6	11.6

The table lists the pH values gained with aluminum, brass, magnesium, zinc, copper, and iron electrodes at 10, 20, 30 and 40 V starting with the lowest values (left) to the highest (right).

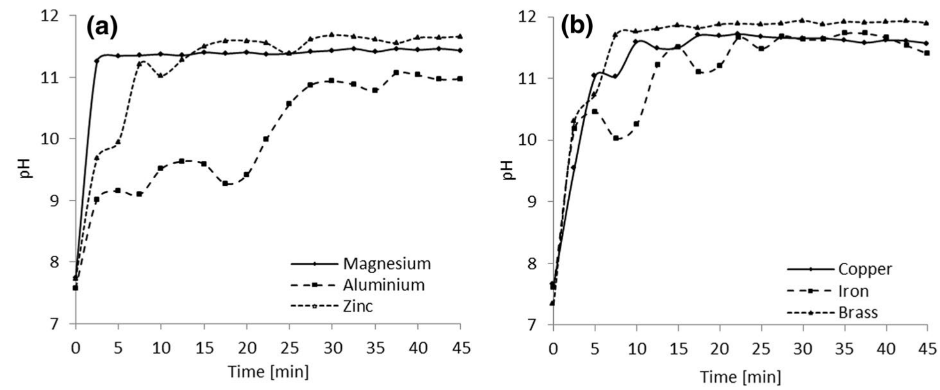

Fig. 3 Effect of electroflocculation on the pH value using aluminum, iron and brass (**a**) and zinc, copper and magnesium (**b**) for electrode material. Measurements were recorded during a flocculation at 40 V.

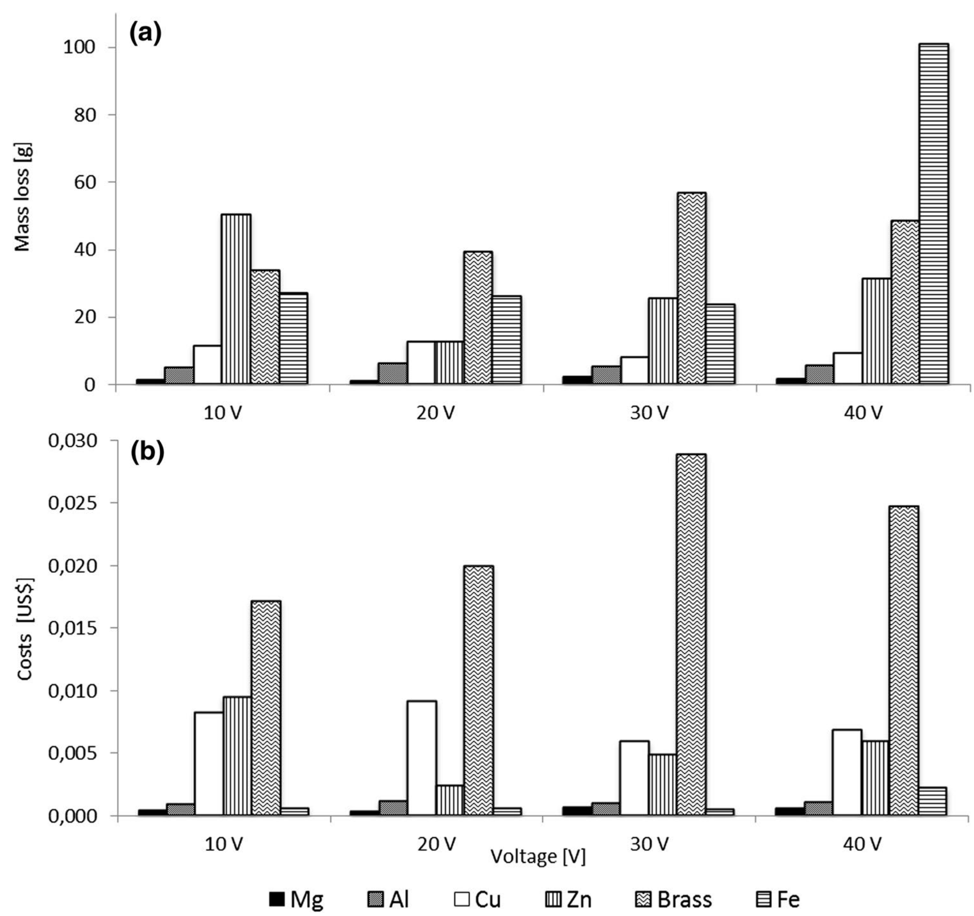

Fig. 4 Mass loss and electrode costs. Mass loss of the electrodes during a flocculation experiment (**a**) and the corresponding metal costs (**b**). The metal prices were stated by the London metal exchange and are subjected to daily fluctuations.

For industrial applications, reuse of the growth media after cell separation is an important factor regarding cost effectiveness. A recycling experiment was conducted to investigate if the high pH value and/or remaining metal residues negatively affect cell growth if the media is led back into the culture vessel after cell harvest. The growth of the *Scenedesmus* cells was monitored in a batch experiment over twelve days of cultivation.

Cell growth was monitored in all of the experiments (Fig. 5). The best result was achieved with Iron

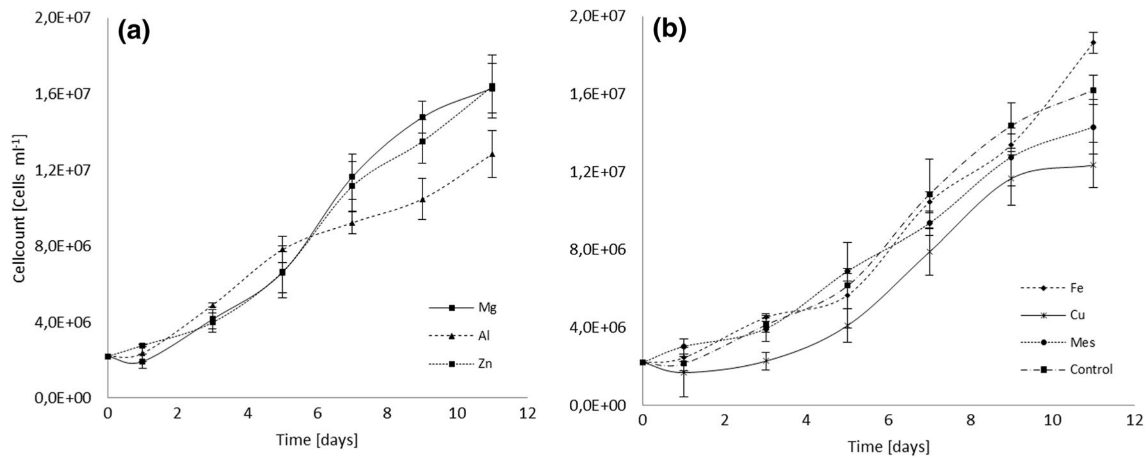

Fig. 5 Media reuse after flocculation. For 12 days *Scenedesmus* cells were incubated in magnesium, aluminum and zinc (**a**) as well as iron, copper and brass (**b**) flocculation supernatant enriched with nutrients. The graphs show the cell growth measured in cells ml^{-1} monitored every other day. For positive control the cells were incubated in WM.

supernatant and a final cell concentration of 1.86 × 10^{7} cells ml^{-1} (Fig. 5b). The control experiments with fresh media resulted in final cell concentration of 1.62 × 10^{7} cells ml^{-1}.The lowest cell concentration was reached with the copper supernatant with 1.23 × 10^{7} cells ml^{-1}. Although copper is an essential micronutrient for plants, higher concentrations are known to inhibit photosynthetic reactions (Kupper et al. 2009). The reuse of all supernatants showed similar growthbehavior within the 12 days of cultivation.

Several experiments have been published on the use of electroflocculation for algal biomass harvest (Lee et al. 2013; Uduman et al. 2011; Vandamme et al. 2011) mainly using aluminum electrodes (Kim et al. 2012; Lee et al. 2013; Vandamme et al. 2011; Xu et al. 2010) and/or iron electrodes (Uduman et al. 2011; Vandamme et al. 2011). Magnesium, copper, zinc or brass electrodes have so far not been used for this purpose. This study revealed that besides iron and aluminum, magnesium shows high potential for algal harvest by electroflocculation. Besides a high FE and cost effectiveness, magnesium is non-toxic and can be utilized in a broad range of application.

Conclusion

Besides the commonly used iron and or aluminum electrodes other materials like magnesium, copper, zinc and brass can be successfully used for microalgal biomass harvest by electroflocculation. The most cost effective flocculation was achieved with Mg, Al and Fe as electrode material. For special biomass applications like food or feed additives metals like Magnesium have other advantages besides their high flocculation efficiency such as their relative harmlessness even at higher concentration. A higher voltage increased the maximum flocculation efficiency but also increased the energy input needed. Recycling of the supernatant was shown to be possible but should be repeated in a long term experiment comprising several harvesting steps.

Authors' contributions

FB contributed to experimental design, data acquisition, result analysis and manuscript preparation. GQ contributed to result analysis and manuscript preparation. DW contributed to experimental design and data acquisition. GK contributed to experimental design, result analysis and manuscript preparation. All authors read and approved the final manuscript.

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Compliance with ethical guidelines

Competing interests

The authors declare that they have no competing interests.

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